**01-P001** Nasal cartilage reconstruction using decellularized extracellular cartilage matrix: Long-term comparison of subcutaneous and intranasal biocompatibility in a rabbit model

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Appropriate autologous cartilage as donor tissue for various surgical reconstructions such as nasal septum regeneration is limited and associated with donor site morbidity. Our goal was to evaluate a new resorbable, chondroconductive biomaterial made of decellularized cartilage invented and characterized by us compared with native cartilage as actual gold standard. In order to further examine the material and determine its long-term outcome, we used subcutaneous implantation as well as septal implantation in an orthotopic rabbit model. Besides non-seeded decellularized xenogenic cartilage, chondrocyte seeded decellularized xenogenic cartilage was implanted as septal replacement. After a three and a six months period formation of newly synthesized cartilage extracellular matrix was evaluated immunohistochemically, septal integrity and biocompatibility were evaluated histologically. The formation of the implanted neoseptum and form stability was analysed using 7-Tesla Magnetic Resonance Imaging.

Good biocompatibility without an excessive rejection reaction was demonstrated in all groups. Both in the study group with and without cell seeding a long-term stable and reliable septal reconstruction could be achieved. Autologous cell seeding was advantageous only with regard to septal perforations. Thus, cell seeding derives a benefit regarding long-term stability. However, because of slightly better biocompatibility, less pronounced septum deviations and the markedly lower effort, the non-seeded scaffold is to be favoured for a possible clinical application.

**01-P002** The acceleration of angiogenesis into inactivated dermis by high hydrostatic pressure using the sustained release basic fibroblast growth factor

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We have developed a novel skin regeneration therapy combining the inactivated nevus tissue by high hydrostatic pressure (HHP) in the reconstruction of the dermis with a cultured epidermal autograft. The issue of this treatment is the instable survival of inactivated dermis. In this study, we applied collagen-gelatin sponges (CGS) that can sustain the release of basic fibroblast growth factor (bFGF) on the inactivated skin to accelerate the angiogenesis into it. Murine skin grafts from C57Bl6/Jcl mice 8 mm in diameter were prepared and inactivated by HHP and cryopreserved. One month later, grafts were transplanted into the subcutis of other mouse back and covered by CGS impregnated with saline or bFGF. Grafts were taken after one week and two weeks, then those survival were evaluated histologically and the angiogenesis-related gene expression were also detected by real-time PCR. Histological sections showed that the dermal cellular density and newly formed capillaries in the bFGF group were higher than in the control group significantly. Relative expression of PDGF gene in the bFGF group were significantly higher 4 folds than in the control group after one week and two weeks. This study suggested that angiogenesis into grafts were accelerated and the survival could be improved in combination with the sustained release of bFGF.
Biologic scaffolds composed of extracellular matrix (ECM) have been developed as surgical mesh materials, comminuted forms (powders) for topical wound care, and hydrogels (currently in Phase I trial for intracardiac injection post MI); all of which have been approved for a large number of clinical applications including musculoskeletal reconstruction. We recently showed that an ECM-scaffold can facilitate a macrophage phenotype transition that leads to downstream site-appropriate functional tissue deposition and myogenesis as a treatment for volumetric muscle loss (VML) in preclinical animal models and in 13 human patients. However the molecular mechanisms that direct the switching of immune cell phenotype in both normal skeletal muscle regeneration and in the presence of an ECM-scaffold are only partially understood. A new perspective on the bioactivity of ECM-scaffolds is presented herein; specifically, that matrix bound nanovesicles (MBV) embedded within ECM-scaffolds are a rich source of extra-nuclear interleukin-33 (IL-33). IL-33 is typically found in the nucleus of stromal cells and generally regarded as an alarmin to alert the immune system to cell injury, resulting in production of pro-inflammatory mediators via poorly defined mechanisms involving the IL-33 receptor, ST2. However, emerging evidence suggests that IL-33 may function as a promoter of tissue repair. We show that IL-33 is stably stored within ECM and protected from inactivation by incorporation into MBV. Results of the present study show that MBV from IL33+/+ mice, but not IL33−/− mouse tissues, directs ST2−/− macrophage differentiation into the reparative, pro-remodeling M2 phenotype, and further suggest that MBV-associated IL-33 modulates macrophage activation through a novel ST2-independent pathway. The discovery of IL-33 as an integral component of the ECM provides mechanistic insights into the regulation of immune-driven pathological fibrosis. Furthermore, the use of ECM-scaffolds for musculoskeletal reconstruction can now be examined with new insights that will help guide the design of next generation products, diagnostics and therapeutic applications.
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Therefore, emulsion templating offers a novel rapid method of fabricating nano-structured fibrous protein scaffolds with micro-scale pore dimensions. The manufacture process enabled control of key scaffold parameters: nano-fibrous structure, pore diameter, mechanical strength, and the degradation rate of scaffold. Each EmDerm type supported efficient cell ingress and proliferation compared to commercial scaffolds.

There are a large number of commercial acellular scaffold biomaterials for soft tissue reconstruction, mostly made from decellularised tissue or fabricated from collagen into porous sponge structures. These materials often lack the nano scale pore-structure of native extra-cellular matrix and have limited efficacy for regenerative tissue reconstruction.

There are significant challenges arise because of protein denaturation by absorption at hydrophobic interfaces and by surfactants and instability of macro-emulsions. We investigate an oil/aqueous macro-emulsion stabilised with a surfactant blend, for forming protein-based nano-structured bio-intelligent scaffolds (EmDerm) with tuneable micro-scale porosity for tissue regeneration without protein denaturation.

Porcine aorta was decellularized by using HHP and SDS methods. PDMS replicas of their acellular aortas were obtained by two-step transferring method. Surface topology of them was investigated by FE-SEM and 3D laser microscope observation. For HHP decellularized aorta, the striated structure of micro-scaled fibers was observed along with the longitudinal direction, while, for the SDS decellularized aorta, the striated structure disappeared and the surface became flat. These results indicated that the surface topology of acellular aorta differed by decellularization method. The replicas of HHP and SDS decellularized aortas had similar topological structure to their acellular aortas, respectively, suggesting that the surface topology could copied by this transferring method. HUVEC were seeded on decellularized aortas and replicas. For HHP decellularized aorta and HHP replicas, HUVECs adhered with cobblestone morphology and proliferated. On the other hand, for SDS replicas, HUVECs adhered with spindle shape likewise flat PDMS. These results suggest that the surface topology of basement membrane affected cell adhesion and morphology and this transferring method could be applicable as surface processing method for regenerative medicine device.

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**Development of the optimal decellularization protocol for respiratory mucosa and evaluation of its feasibility for treating partial mucosal defect**

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**Objectives:** The partial mucosal defect is common in the otolaryngology clinic. The conventional treatment options are observation or autologous skin graft. However, these treatment modalities require a long time for the wound to completely be healed and cause pain and discomfort to the patient. Lately, acellular dermal matrix (ADM) was developed and clinically used to cover the mucosal defect. However, since ADM was derived from the dermis, it is too thick and tough to be applied in oral cavity and pharynx. To overcome these limitations, authors developed the acellular mucosal matrix by using decellularization protocol for respiratory mucosa. The aim of this study was to develop the decellularization protocol for sheet-type respiratory mucosa and evaluate its feasibility.

**Materials and Methods:** Porcine tracheas were obtained at a local abattoir from market sized pig. The 15 cm-sized trachea was frozen at -80°C. After thawing, the respiratory mucosa was separated from the tracheal cartilage. The two detergent (A: Trypsin + Triton X-100+Deoxycholic acid, B: Trypsin+Triton X-100+Perasafe)-based decellularization protocols were applied with agitation. DNA quantification, histological evaluation, mechanical test, and collagen assay were performed.

**Results:** The protocol B (protocol with perasafe) took 120 hours to reach the adequately decellularized level. However, protocol A was shown to be unsuitable methods which was poorly decellularized. The decellularized respiratory mucosa by protocol A showed proper mechanical property to be used in oral cavity reconstruction. It was elongated to 150% of its original length and restored to original shape.

**Conclusion:** The results indicated that these decellularized respiratory mucosa had good biomechanical properties and could be the potential candidate for biological sheet to repair mucosal defect.
**Crosslinking of Collagen or Collagen-like Hydrogels by Fenton's Reagent for Ocular Applications**

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Chemical crosslinking of hydrogels can be achieved by several different mechanisms: Light-, chemically- or enzymatically-initiated crosslinking have their own advantages and limitations. Among these crosslinking methods, Fenton’s reagent (Fe (II) & \( {\text{H}_2\text{O}_2} \)) is known to generate hydroxy and peroxy radicals which will initiate the crosslinking of polymer chains. By changing the concentration of either Fe (II) or \( {\text{H}_2\text{O}_2} \), the gelling time and the degree of crosslinking of the polymers can be fine-tuned. Compared to other chemical cross-linkers, \( {\text{H}_2\text{O}_2} \) has a lower toxicity on cells. The low toxicity of the method as well as the high availability of the materials provides a valuable opportunity for the tissue engineering applications. Moreover, as it doesn’t require any UV-light or thermal initiation for the radical generation, practical usage of Fenton’s reagent has a great versatility. This approach can be applied to acrylate-functionalized gelatin-like or collagen-like materials to synthesize radical-initiated chemically cross-linked hydrogels. Due to fast kinetics and possibility of fine-tuning this process, in situ cross-linking of these materials would provide an alternative to different applications of hydrogels. As the reaction between Fe (II) and \( {\text{H}_2\text{O}_2} \) occurs at room temperature or at body temperature and at pH 7.4, this method can be applied for injectable hydrogels, for cell encapsulation or for hydrogel patches for several different purposes, one of which would be serving as filler for sealing corneal perforations. The current treatment is performed via cyanoacrylate glue, which has been shown to have proinflammatory effects as well as hindering the corneal regeneration[1]. The preliminary data that we have on Fenton’s reagent in acrylate-modified hydrogel cross-linking demonstrates the tailoring of gelling time by modifying the total Fe (II) or \( {\text{H}_2\text{O}_2} \) concentration. By employing Fenton’s reagent for the initiation of the cross-linking of a collagen-like material for an injectable patch to treat the corneal perforations would provide a feasible, fine-modified, biocompatible and easily-available alternative to the current treatment strategy.

References:  

**3D printing of highly stable hydrogels composited of gelatin and dopamine-modified alginate**

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Extrusion-based bioprinting of alginate hydrogels has been one of the most popular techniques in the bioprinting community. However, it is not easy to control swelling and dissolution of calcium ions crosslinked alginate hydrogels, which prevents its widespread use for tissue engineering scaffolds. Here, we have fabricated a printable and stable hydrogel comprised of dopamine-modified alginate and gelatin that can be used for cell encapsulation. Dopamine-modified alginate (D-Alg) is synthesized through carbodiimide coupling reaction, using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and N-hydroxysulfosuccinimide (NHS). A suitable concentration of D-Alg and gelatin is dissolved in deionized water under stirring, until a homogeneous bioink with suitable viscosity for bioprinting is achieved. The printed structure is further crosslinked with \( {\text{FeCl}_3} \) solution, which shows significant enhancement of stability and mechanical strength. The \( \text{Fe}^{3+} \) crosslinked D-Alg hydrogel shows higher mechanical properties compared with conventional Ca-alginate hydrogels. On the other hand, adding \( \text{Fe}^{3+} \) metal ions to gelatin from hexavalent Fe complexes, enhancing cohesion ability of the hybrid hydrogel. The printing process shows little viability effect to the encapsulated cells. The bioprinted hybrid hydrogel shows extremely high stability under physiological conditions, without significant cytotoxicity. The adhesive properties of catechol groups in D-Alg also enhances tissue attachment. The results demonstrate the potential use of D-Alg/gelatin hybrid hydrogels for 3D bioprinting of living tissues.
01-P011 Investigation of Bone Reconstruction Using Attenuated Immunogenicity Xenobone Composite Graft Fabricated by 3D Printing

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Most current bone graft strategies including autograft and allograft are limited in supply and suffer high donor site morbidity rate [1]. We fabricated a novel bone graft composed of decellularized porcine bone (DPB) matrix and polymeric materials through 3D printing to provide an alternative regeneration solution for bone defect clinical solution. The natural DPB component in our bone graft derived from α, 1,3-galactosyltransferase gene knockout (GTKO) porcine [2] bone with attenuated immunogenicity, was used to initiate bone regeneration. The polymeric material of polycaprolactone (PCL) enables the grafts to be 3D printable and have controllable precise structures. Hybrid scaffolds displayed robust mechanical properties. Assessments of surface features revealed both collagenous and mineral components of bone were present. Qualitative and quantitative assessments showed increased ALP expression and osteogenic differentiation of hMSCs on those grafts. The bone graft developed is intended for use in patients undergoing bone defect healing to expedite the bone defect reconstruction and reduce morbidity as harvesting bone from the donor site is no longer required.

References:

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01-P012 Nanomaterials for tissue engineering of heart valve bioprosthesis – the use of acoustic wave and laser ablation methods for the preparation of bioactive scaffold

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The surgical procedure for the replacement of diseased heart valve is a commonly accepted method of treatment in situations where the conventional pharmacological treatment is exhausted. For the replacement the mechanical or biological prostheses can be used, however both of them have limitations. Because of this, there is still a need to optimize their methods of bioprosthesis creation to increase their durability while maintaining high biocompatibility and hemocompatibility. In the recent years a development of issues related to the use of tissue engineered techniques are developed and the methods should meet clinical needs. The goal of our study was to develop a new method for the preparation of acellular scaffold, to improve the scaffold porosity, cell growth and migration and the efficiency of cell removal, with maintaining the intact morphology and mechanical stability. The goal is to obtain series of clinically available organs “from the shelf”. For the scaffold preparation we use the new techniques: laser ablation and acoustic waves. The acoustic methods work in the pulse reflection mode. The most important component in the scanning acoustic microscopy is a high frequency piezoelectric sound transducer. In the acoustic method, the cells were rejected by the activation of programmable cell death through the use of an appropriate frequency of acoustic waves. The second methods laser ablation was realized using two techniques, namely, direct laser writing and direct laser interference lithography. Direct laser writing involves focusing a laser beam on the target surface, creating predefined points or lines in a continuous manner, using continuous wave lasers or quasi-continuous regime pulse, high repetition lasers. Direct laser interference lithography (DLIL) involves the irradiation of virtually any solid surface with a proper interference pattern by means of a high-power density pulse laser (from MW/cm² up to GW/cm²). The multi-scale advanced biological, molecular, biomechanical studies have been used for the scaffold characterization, including preclinical studies. The issue of the formation of new tissue on the basis of the extracellular matrix is of strong clinical interest.

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Novel Approach for Quantification of Pore-size for Scaffolds (>16 cm²) Using ImageJ Software for Quality Control

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With the advancement of digitalisation and computer programming, image processing has become the latest tool to understand and quantify pore size, pore size distribution, porosity and microstructure of bio-aggregates. Techniques available to analyse the microstructure of biomaterials include optical light microscopy, scanning electron microscopy, X-ray spectroscopy and field emission scanning electron microscopy along with image processing softwares like MATLAB, ICY, Avizo, Image Pro and others. However, these techniques are widely used to quantify pores over small sample areas that have a homogenous pore structure. The analysis becomes difficult when the size of the biomaterials increases (>16 cm²) and if the pore distribution is gradient. In this study, we present a method using ImageJ software to quantify pore size distribution of porous materials with gradient porosity distributed over an area of 1 – 100 cm² by utilising laser scanning confocal microscopy images. A macro was created in ImageJ to test this method. A plugin called tubeness is used to perform the analysis. After tubeness processing to identify pore boundaries, images are thresholded then skeletonized. A watershed is run to separate overlapping pores, before measurement of pore area and shape descriptors. Using this method, pore distribution of a fibrin-alginate scaffold (49 cm² > 1x10⁶ pixels) was quantified within three minutes on a standard PC and measured over 227,000 pores. Such tools offer the potential of repeatability, accuracy and effectiveness in data quantification which will be valuable for quality control during biomaterial manufacturing processes.
By providing natural biologic cues and three-dimensional microstructure, decellularized tissues are emerging as effective scaffolds in regenerative medicine. For myocardial regeneration, different approaches are based on decellularized myocardium, however this latter is characterized by poor mechanical properties with respect to the native tissue, where the extracellular matrix represents a relatively small mass fraction. As innovative and easily accessible alternative, the human decellularized dermis (HDD) has been proposed [1]. Preliminary tests demonstrated that cardiac primitive cells engrafted onto HDD, survived and retained expression of markers specific for cardiac myocytes, at gene and protein level [1]. In this study we investigated the mechanical behaviour of the HDD in comparison with the human myocardium.

Four samples of dermis, collected from a plastic surgery patient marking the Langer’s lines orientation, were decellularized following a novel, fast protocol [1]. Sample specimens, cut in parallel and perpendicularly to the Langer’s lines, were measured by photogrammetry (width = 5.25 ±0.71 mm, thickness = 3.62±0.45 mm), and mechanically characterized by uniaxial tensile tests (strain rate = 3.2% s⁻¹, gauge length = 5 mm, MTS Qtest/10 [2]). The elastic modulus maximum (E_{max}) at 10% (E_{10%}) and 20% (E_{20%}) of strain were calculated. A multivariate variance analysis was adopted (α = 0.05).

Specimens oriented along the Langer’s lines presented significantly higher elastic moduli (+64.5% ±6.2% for E_{max}, +48.2% ±19.7% for E_{10%}) than the perpendicularly-oriented ones (p<0.0001). The average E_{max} values of parallel (9.99 ± 1.91 MPa) and perpendicular (3.52 ± 0.29 MPa) specimens fitted with elastic modulus values of porcine acellular myocardium subjected to uniaxial tests along (9.50±1.50 MPa) and cross (3.27 ± 0.80 MPa) fiber directions [3]. Moreover, the average E_{10%} values of parallel (0.32 ± 0.08 MPa) and perpendicular (0.15 ± 0.04 MPa) specimens fell in the human myocardium value range (0.01-0.5 MPa) [4]. Preliminary results demonstrate, in terms of both directionally dependent anisotropy and elastic modulus values, the mechanical suitability of the HDD as scaffold for myocardial regeneration.

Determining a reconstruction method for lower limb skin and soft tissue defect has always been a problem. In the case of full-thickness defect, full-thickness skin grafts or local flaps are generally used for reconstruction. The objective of the current study is to use CGDerm and skin grafting for improved skin grafting without contracture.

Between September 2012 and June 2014, 27 patients received CGDerm and split-thickness skin grafting (STSG) simultaneously for lower limb full-thickness skin defects. The researchers performed chart reviews retrospectively and examined the patients based on the following factors: gender, age, injury mechanism, size, exposed structure, pre-coverage dressing method, coverage method, post-operative engraftment and total healing period, contracture development, elasticity, and infection development.

Of a total of 30 cases, 29 showed successful engraftment without infection or contracture. In one case, continued seroma was observed. Engraftment was successful in this case following new coverage using both CGDerm and STSG.

Human allodermis can play a ground-breaking role in securing the availability of surrounding tissue and in contracture prevention, both of which are key to lower limb reconstruction. Of the types available, the cell-free human dermis, CGDerm, showed lower infection rates than other human dermis types, and its engraftment rate was higher than in STSG-only cases. These findings suggest that CGDerm adoption in STSG is more effective and safer in lower limb reconstruction, and has lower contracture and infection rates.
Melanin pigments in the melanocytic nevus regress spontaneously after inactivation by high hydrostatic pressure

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Giant congenital melanocytic nevi (GCMN) are large brown-to-black skin lesions that appear at birth, and are associated with the risk of transformation to malignant melanoma. Therefore, the entire nevus tissue should be removed to prevent the emergence of melanoma. We have reported a novel treatment for GCMN that re-uses the autologous nevus with high hydrostatic pressurization (HHP). The inactivated nevus is autografted to the original site and a cultured epidermal autograft is applied 2 or 3 weeks later. We have shown that all kinds of cells in the nevus tissue were completely inactivated after HHP and that the cultured epidermis survived on the inactivated nevus. An important issue with our novel treatment involves the remaining melanin pigments in the inactivated nevus tissue. In this study, we inactivated nevus tissue at 200 MPa and implanted it subcutaneously in nude mice and observed color changes and histology.

Resected nevus tissue specimens were pressurized at 200 MPa for 10 minutes. Pressurized nevus specimens (200 MPa group, n=9) and non-pressurized nevus tissue (control group, n=9) were implanted in the subcutis of nude mice and harvested 3, 6, and 12 months later. Color changes of the nevus specimens were evaluated using the brightness parameter of the HSB color model. Nevus specimens were evaluated histologically with hematoxylin and eosin-stained sections and immunohistochemically stained sections of CD31 or human vimentin.

In the 200 MPa group, colors of the nevus specimens gradually regressed and turned white, brightness values increased after 6 months and were significantly higher than those of the control group, and melanin granules decreased in size and nearly disappeared at 12 months. Immunohistochemical staining for human vimentin showed abundant remaining human cells in the dermal part for the control group; however, no human cells were confirmed for the 200 MPa group at 3, 6, and 12 months. Immunohistochemical staining for CD31 showed regenerated capillaries in the dermal parts of nevus specimens for both groups after 3 months.

In this study, it was determined that melanin pigments of nevus tissue regress spontaneously in vivo after inactivation by HHP. This suggests that we can reimplant the removed and inactivated nevus without removing melanin pigments in combination with consecutive grafting of CEA. Then, the remaining melanin will regress spontaneously after grafting. Our novel HHP treatment could be promising for GCMN.

Bioinspired scaffolds for tissue engineering applications

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In the context of Tissue Engineering and Regenerative Medicine [1;2], we developed bio-inspired scaffold from renewable resources with controlled external shape and internal architecture. Different homemade fabrication apparatuses have been developed to produce controlled systems inspired by the nature. Mechanical, physico-chemical and biological characterizations assess the therapeutic potential.

This presentation will report our recent advances on bioinspired scaffolds such as:

1) Electrospun fibers of chitosan and crosslinked chitosan which has been inspired by the hierarchical structure of Dynastes hercules elytra [3], and can be seen as new architecture to TE.

2) Free standing membranes functionalized in volume by incorporating nanoliposomes based on marin origin lecithin [4].

3) Cicada wings Bioinspired Membranes for Tissue Engineering

Perfusion Decellularization of Cadaveric Porcine Kidney Using Chemical Methods with Sonication

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Renal transplantation is the only definitive treatment to End Stage Renal Disease (ESRD), which has become a growing concern worldwide because of a rapid increase in the number of people suffering from it. However, this treatment is continuously challenged with the organ supply shortage and the incompatibility of the transplanted organ to the patient. Production of acellular extracellular matrix (ECM) scaffolds from whole organs aims to solve these problems using perfusion decellularization. Current perfusion decellularization techniques are performed using chemical methods where detergents are perfused into the organ to remove cellular components. But these usually require long incubation times, repeated treatment cycles and intensified washing steps which affects the integrity of the scaffold and the retention of essential ECM components which are vital for a successful recellularization. Therefore, these protocols are usually combined with physical methods to achieve a more efficient method of decellularization of organs. Sonication treatment is an example of physical method used to improve decellularization because it helps to penetrate chemical detergents into cells membrane and disrupt it and its components leading to require lesser amount of chemicals and faster removal of cellular debris while preserving the ECM structure. Until now, sonication studies have focused only on tissues and not whole organs.

In this study, sonication treatment was used in addition to the perfusion of detergents, sodium dodecyl sulfate and Triton X-100, to improve efficiency in producing cadaveric porcine ECM scaffolds in terms of the percentage of cell removal, retention of essential ECM components and the preservation of the ECM structure and vascular network. Decellularized scaffold was evaluated using hematoxylin eosin (H&E) and diamidino-2-phenylindol (DAPI) staining for cellular content. The integrity of scaffold was then analyzed by transmission electron microscopy (TEM) and lastly, vital ECM components were analyzed using enzyme linked immunosorbent assay (ELISA) and immunohistochemical (IHC) staining. Results showed that the sonication treatment was able to improve the decellularization method and was able to produce a cadaveric porcine kidney ECM scaffold that was successfully devoid of cellular components while preserving the structure and essential components of the ECM.

Preparation of neural stem cell-derived decellularized matrices as a stem cell niche model

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Neural stem cells (NSCs) are one of the important cell sources for neural tissue engineering. NSCs lose their stemness during their in vitro culture, which is one of the barriers for neural tissue engineering with NSCs. NSCs can keep their stemness in vivo in stem cell niche. Particularly, it is reported that there is special extracellular matrix (ECM) architecture which are called as “fractones”, in NSC niche. And the fractones are reported to be required for NSC growth and maintenance. However, the mechanisms are still unclear. Here, we proposed cultured NSC-derived decellularized matrix as a new in vitro “fractones” model. MEBS cells, a murine NSC line, were cultured for a week in the monolayer culture on laminin-coated substrates. During this monolayer culture, MEBS did not differentiate into neural cells (neurons and astrocytes). And the cells expressed basement membrane components to deposit them beneath the cells. After the culture, MEBS cells were specifically removed from the culture by the treatment with Triton X-100 and NH₄OH, followed by the treatment with DNase and RNase to obtain MEBS-derived decellularized matrices. No F-actin and cell nuclei were observed in the samples after decellularization, indicating that decellularization was successfully performed. The newly prepared MEBS-derived decellularized matrices can support the adhesion and growth of freshly harvested MEBS cells. Finally, MEBS cells were cultured under differentiation condition. MEBS cells rapidly formed neurites on laminin-coated substrates, indicating that the cells differentiated into neural cells. On the other hand, MEBS cells kept a round shape on MEBS-derived decellularized matrices, suggesting that the cells suppressed their differentiation. Conclusively, MEBS-derived decellularized matrices might be useful as an in vitro “fractones” model for making it easy to analyze their roles in vitro.
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In in-vivo tests, spermatogonial stem cells (SSCs) are localized and infinitely self-renew on basement membrane of seminiferous tubule. Therefore, reconstruction of a three-dimensional (3D) niche mimicking basement membrane is important for conducting effectively in-vitro maintenance of SSCs. In this study, in order to promote self-renewal of SSCs derived from pig, we tried to develop a 3D non-cellular niche engineering signaling of integrin α5β1 or αVβ1 interacting with fibronectin observed in basement membrane. For these, we investigated the presence of integrin α5β1 or αVβ1 on the surface of porcine SSCs in the undifferentiated state through immunocytochemistry and attachment and antibody inhibition assay, and effects of extracellular signaling derived from RGDSP activating integrin α5β1 or αVβ1 in 3D PEG-based hydrogel on maintaining self-renewal of porcine SSCs through analysis of morphology and self-renewal-related gene expression post-culture of porcine SSCs inside 3D PEG-based hydrogel combined with 0, 400, 800, 1200 or 1600 μM RGDSP. As the results, on the surface of the undifferentiated porcine SSCs, the localization of integrin α5, αV and β1 subunit proteins was identified and the integrin heterodimers α5β1 was present in active form. Subsequently, porcine SSCs cultured in 3D PEG-based hydrogel incorporating RGDSP activating integrin α5β1 formed colonies of round or grape-like shape, regardless of RGDSP concentration. However, porcine SSCs cultured inside 3D PEG-based hydrogel combined with 800 μM RGDSP showed higher transcriptional level of most of self-renewal related genes than the other groups. Accordingly, these results suggest that extracellular signaling derived from 800 μM RGDSP provide beneficial effects on maintenance of the porcine SSC self-renewal in in-vitro culture of porcine SSCs in 3D PEG-based hydrogel.

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In in-vivo tests, spermatogonial stem cells (SSCs) are localized and infinitely self-renew on basement membrane of seminiferous tubule. Therefore, reconstruction of a three-dimensional (3D) niche mimicking basement membrane is important for conducting effectively in-vitro maintenance of SSCs. In this study, in order to promote self-renewal of SSCs derived from pig, we tried to develop a 3D non-cellular niche engineering signaling of integrin α6β1 interacting with laminin observed in basement membrane. For these, we investigated the presence of integrin α6β1 on the surface of porcine SSCs in the undifferentiated state through immunocytochemistry and attachment and antibody inhibition assay, and effects of extracellular signaling derived from TTSWSQ activating integrin α6β1 in 3D PEG-based hydrogel on maintaining self-renewal of porcine SSCs through analysis of morphology and self-renewal-related gene expression post-culture of porcine SSCs inside 3D PEG-based hydrogel combined with 0, 400, 800, 1200 or 1600 μM TTSWSQ. As the results, on the surface of the undifferentiated porcine SSCs, the localization of integrin α6, αV and β1 subunit proteins was identified and the integrin heterodimers α6β1 was present in active form. Subsequently, porcine SSCs cultured in 3D PEG-based hydrogel incorporating TTSWSQ activating integrin α6β1 formed colonies of round or grape-like shape, regardless of TTSWSQ concentration. Moreover, porcine SSCs cultured inside 3D PEG-based hydrogel combined with 800 μM TTSWSQ showed higher transcriptional level of most of self-renewal-related genes than the other groups. Accordingly, these results suggest that extracellular signaling derived from 800 μM TTSWSQ provide beneficial effects on maintenance of the porcine SSC self-renewal in in-vitro culture of porcine SSCs in 3D PEG-based hydrogel.
**01-P025**

Effects of PEILDVPSTV-derived Extracellular Signaling in Three-dimensional Polyethylene Glycol (PEG)-based Hydrogel on Maintaining Undifferentiation of Spermatogonial Stem Cells in pig

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In *in-vivo* testis, spermatogonial stem cells (SSCs) are localized and infinitely self-renew on basement membrane of seminiferous tubule. Therefore, reconstruction of a three-dimensional (3D) niche mimicking basement membrane is important for conducting effectively *in-vitro* maintenance of SSCs. In this study, in order to promote self-renewal of SSCs derived from pig, we tried to develop a 3D non-cellular niche engineering signaling of integrin α₄β₁, interacting with fibronectin observed in basement membrane. For these, we investigated the presence of integrin α₄β₁ on the surface of porcine SSCs in the undifferentiated state through immunocytochemistry and attachment and antibody inhibition assay, and effects of extracellular signaling derived from PEILDVPSTV activating integrin α₄β₁ in 3D PEG-based hydrogel on maintaining self-renewal of porcine SSCs through analysis of morphology and self-renewal-related gene expression post-culture of porcine SSCs inside 3D PEG-based hydrogel combined with 0, 400, 800, 1200 or 1600 μM PEILDVPSTV. As the results, on the surface of the undifferentiated porcine SSCs, the localization of integrin α₄ and β₁ subunit proteins was identified and the integrin heterodimers α₄β₁ was present in active form. Subsequently, porcine SSCs cultured in 3D PEG-based hydrogel incorporating PEILDVPSTV activating integrin α₄β₁ formed colonies of round or grape-like shape, regardless of PEILDVPSTV concentration. However, porcine SSCs cultured inside 3D PEG-based hydrogel combined with 1200 μM PEILDVPSTV showed higher transcriptional level of most of self-renewal-related genes than the other experimental groups. Accordingly, these results suggest that extracellular signaling derived from 1200 μM PEILDVPSTV provide beneficial effects on maintenance of the porcine SSC self-renewal in *in-vitro* culture of porcine SSCs in 3D PEG-based hydrogel.

**01-P026**

Degradation rate defines *in situ* cell reprogramming efficiency of the active chitosan implants

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Reprogramming somatic cells directly at the site of lesion or trauma is a promising approach to reduce the time of recovery and the labor load. However, optimal pharmacokinetic parameters for the release of reprogramming factors are not known. Here we compare two designs of reprogramming factor releasing biodegradable chitosan implants. After implantation, both designs are efficient in transfecting the surrounding cells. Slower degrading implants pretreated by freeze-drying produced a scattered distribution of CD9-positive cells, while faster cell-infiltrated implants having tendency to promote cluster distribution of CD9-positive cells. Cell reprogramming *in situ* is transient, providing the cells ready to reenter differentiation into cell type repertoire necessary for local tissue repair. We employed an animal model allowing to back-trace the reprogramming events, and quantitatively estimated the efficiency of local *in situ* reprogramming. Several biomarkers of tissue response to the induced cell reprogramming were identified, with Chemokine ligand 6 (CXCL6) among the most prominent. We propose that neutrophilic granulocytes are the conduits for checkpoint mechanism controlling the quality of *in situ* cell reprogramming and eliminating the erroneously/incompletely reprogrammed cells.
Sterile acellular tissues developed by a CO$_2$-philic detergent and supercritical carbon dioxide

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Decellularization of tissues has attracted much attention in regenerative medicine as an alternative to synthetic materials. However, most decellularization techniques are time-consuming and compromise the structure and mechanical properties of the tissue. Here, we report a cost-effective and rapid decellularization method based on a CO$_2$-philic detergent and supercritical CO$_2$. Due to its dual-character as a gas and a liquid, supercritical CO$_2$ has a substantially enhanced diffusion and simultaneously sterilizes the sample which therefore reduces the treatment time significantly.

We have successfully tested this method on cartilage, tendon and skin tissues and have found that it has removed the majority of the cellular material. DNA content was substantially reduced by 81.78 ± 3.40% for cartilage, 66.73 ± 6.03% for tendon, and 86.73 ± 0.004% for skin compared to native tissue (p<0.05). Simultaneously, GAG content was reduced by 87.00±6.12% for cartilage, 69.88±6.23% for tendon and 65.71±0.43% for skin compared to the native tissue (p<0.05). The lower GAG content has effectively reduced the elastic modulus of articular cartilage by 85.56±2.81% (p<0.0001), but not significantly reduced the elastic modulus of tendon.

The removal of GAGs was also confirmed by the ultrastructure of decellularized articular cartilage associated with a looser collagen fiber arrangement. Finally, we have shown that acellular tissues were non-cytotoxic to bovine chondrocytes. Through further optimization of the processing parameters, this method has the potential to be cost-effective, one-step procedure for the decellularization of organs and tissues.
Collagen materials are extensively used in regenerative medicine and clinics. However, they still present limitations such as a monodomain composition, poor mechanical properties, and the need of crosslinking. On the other hand, tissue grafts overcome most of these limitations. In addition, although numerous tissue grafts are employed in hernia repair and wound care, their potential in musculoskeletal tissue engineering has not been fully investigated. Herein, we ventured to assess the potential of a bi-phasic porcine peritoneum for musculoskeletal regenerative medicine by comparing its characteristics with a commercial collagen scaffold employed in tendon.

Results indicated that the porcine peritoneum had higher mechanical properties and a lower crosslinking ratio while maintaining stability at human body temperature. The porcine peritoneum was almost completely degraded by MMP-1 and MMP-8 after 24h, contrary to the collagen matrix, which suggests a faster remodelling in vivo of the tissue graft. The histology and immunohistochemistry analysis showed a multicomponent and organized structure in the porcine peritoneum, including basal membrane markers, elastin, fibrin, and collagen; compared to only collagens type I/III and fibrin in the collagen matrix. These results confirm the multifunctional nature of the peritoneum tissue graft. Cell studies on human tenocytes and fibroblasts showed the capability of the peritoneum to support cell growth. In addition, tenocytes had a slight higher proliferation on the basal membrane, meanwhile they did not proliferate on the collagen matrix. ADSCs were able to grow on both materials, however, proliferation was enhanced by the porcine peritoneum (p<0.01). Immune response by THP-1 showed an acute inflammatory response by macrophages to the collagen matrix, contrary to the porcine peritoneum which triggered a mild reaction. Currently an in vivo study in a rabbit flexor tendon model is being carried out to assess the potential of the graft for tendon repair.

This study shows the suitability of porcine peritoneum as an implantable device and its multifunctionality due to its heterogeneous composition and structure. In addition, its multifunctionality provides higher cytocompatibility than a mono-domain collagen matrix with human tenocytes and ADSC. Besides, its lower immune response in vitro suggests better remodelling in vivo.

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Cardiovascular disease and stroke still have a huge health, economic and social impact in the world [1]. Cardiac tissue engineering has been studied for almost 20 years, nevertheless, engineered products have not entered into clinical yet due to the lack of functional cardiac tissue. Recently, stem cells and acellular scaffolds have got so much attention in cardiac tissue engineering. However, in vitro microphysiological systems are needed with chemical, electrical or mechanical stimuli to model functional cardiac tissue from stem cells. The aim of the study was to engineer a cardiac patch by using acellular bovine scaffolds and stimulating the cardiomyocyte differentiation of MSCs with different chemical, mechanical and electrical stimulations. With this purpose, decellularization procedure of bovine pericardia was carried out by using different detergents and chemicals to remove the cells and optimize the pore sizes. On the other hand, isolated and characterized rat bone marrow mesenchymal stem cells (MSCs) were seeded on sterilized acellular scaffolds and differentiated into cardiomyocyte cells inside the bioreactor system simulating the beating heart by mechanical and electrical stimulations. To determine the effect of developed patches on tissue repair, rat myocardial infarction model (MI) was developed and implantation was carried out. It was determined that early stage cardiac specific transcription factors were expressed in bone marrow MSCs, seeded on 3-dimensional tissue scaffolds in optimal chemical concentration (10 µM, 5-Azacitidine) and physical stimulation parameters (Voltage: 5V/cm, 3 ms; and strain: (% 5, 1 Hz). It was concluded that, chemical stimulation is not merely enough for cardiomyogenic differentiation of rMSCs. Hence, cardiomyogenic differentiation of MSCs and the expression of cardiomyocyte biomarkers can be increased when synchronous mechanical and electrical stimulations are applied. It was observed that in rat MI model, in compared to patches cultured in static culture condition, successful results were obtained and heart muscle features were retrieved with low inflammation response for patches which were exposed to electromechanical stimulation. With respect to neovascularization, there was no difference between the groups.

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Reference

Engineering of Cardiac Patch By Using Acellular Scaffolds To Repair A Myocardial Defect

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Extracellular matrix from the biological scaffolds can be used in regenerative medicine for organ replacement. The preservation of ECM components has been shown to provide cues for cell differentiation and proliferation. Decellularisation of tissues is one of the techniques for the development of a biological scaffold with preserved architecture and vasculature for bioengineered organs. Our study involved the perfusion of rat liver through the portal vein for its decellularization. We used serially increasing concentration of SDS (0.1%-0.5%) and TritonX100 for the complete removal of the cellular components. The resultant decellularised liver matrix was white and translucent with visible vasculature. The Haematoxylin and Eosin staining showed no residual cells in the decellularized liver matrix. Whereas, the Masson’s Trichrome staining showed the collagen fiber components with tubular structure of the liver ECM. These findings were confirmed by SEM where no cells could be observed within the decellularised liver matrix. The extracellular matrix showed arrangements of collagen fibers with lamellar structures with intact large and small vessels. Immunohistology revealed the presence of major extracellular matrix proteins like Collagen type I, Laminin and Reticulins well indicating the preservation of structural and basement membrane components of the ECM in the decellularised liver scaffolds. The residual DNA content was significantly decreased in the decellularized matrix as compared to normal untreated rat liver tissues, 20±8 and 1896±224 ng/mg wet tissue respectively. Furthermore, collagen content was significantly high indicating the development of an ideal biological scaffold. Hence, this study provides an innovative breakthrough in tissue reconstruction. In this framework, the key challenge will be to repopulate the liver matrix with functional cells, along with its maintained vasculature to develop a substitute for diseased non-functional livers.

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A Word of Caution – Challenging Decellularization with Established and Modified Treatment Protocols

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Objectives:
Decellularization (DC) is a widely applied technique to produce acellular pulmonary artery roots for cardiac surgery. The aim of this study was to evaluate four published detergent based DC procedures. Process modifications should help to optimize the protocols, if necessary.

Methods:
4 Detergent based DC protocols for pulmonary valves (each n=5) were reproduced exactly as described in previous publications1-4. After evaluation, processes were physically and procedurally modified (e.g. cyclic DC, circulatory system, ultrasonic exposure) in order to optimize DC outcomes. An enzymatic (DNase and α-galactosidase) incubation step was added after the detergent incubation. Native and treated samples were analyzed by scanning electron microscopy, histological evaluation, immunological staining, quantification of DNA and glycosaminoglycan as well as tensile tests.

Results:
All reproduced protocols revealed unsatisfactory results and modifications were necessary for a successful DC. The scaffolds of the SD/SDS treated group were completely decellularized, however showed severe ECM damages. Therefore, the length of the procedure was halved, which resulted in successful DC and a preserved ECM. The published SD based procedure was not able to produce acellular valves. A changed DC setup with a cyclic incubation scheme was necessary for a successful procedure. The published SD/Tx treatment did not lead to full DC as well. Alterations to a cyclic protocol and a circulatory DC setup resulted in degeneration of the ECM. Further modifications and reduction of the detergent concentration, finally resulted in acellularity while the ECM was preserved. All newly developed protocols showed significantly reduced DNA concentrations - especially after nuclease treatment. The protocol using low concentration SD/Tx/IGEPAL was not able to produce acellular scaffolds. As it showed only minimal treatment effects, no modifications were investigated.

Conclusion:
None of the published protocols led to a successful DC after exact reproduction of the described processes and modifications were necessary. Therefore, we strongly recommend a careful interpretation of published protocols and a stringent quality control for all DC processes.

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Decellularized Fetal Membranes: Naturally-derived materials improve ex vivo expansion of mesenchymal stem cells and form injectable gels for cell delivery

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The use of mesenchymal stem cells (MSCs) in clinical therapies is limited by the inadequate number of functional MSCs that can be delivered to the injury site. Extracellular matrix (ECM) is a major component of the MSC niche and is critical for maintaining MSC functions. We produced decellularised ECM (dECM) from human fetal membranes (amnion and chorion) and after solubilising the dECM, we coated tissue culture plastic and tested if MSC functions were improved. Furthermore, we tested if the solubilised dECM (sdECM) could form a thermoreversible hydrogel that can be used as a carrier system for MSC delivery.

We showed that cell culture substrates produced from amnion-derived sdECM improved several key functions of placental MSCs (pMSCs). pMSCs were more proliferative on amnion-derived sdECM, than tissue culture plastic alone, and Matrigel (~1.7x and 1.3x higher, respectively). pMSCs also were smaller in size and exhibited greater adipogenic differentiation capacity (~3.5x and 2.5x on sdECM compared to TCP and Matrigel).

Additionally, sdECM formed injectable and thermoreversible hydrogels. Hydrogels produced from amnion-derived sdECM (8 mg/mL) improved the proliferation of pMSCs (~2x compared to Matrigel). Additionally, pMSCs cultured on these gels exhibited greater adipogenic differentiation efficiency as they contained a larger number of lipid deposits that were also larger in size.

In conclusion, we produced sdECM bioactive materials and showed they are promising cell culture substrates for ex vivo expansion of pMSCs, and can act as a bioactive carrier system for the delivery of MSCs.
**The Development of a Decellularisation Protocol for the Intervertebral Disc for Disc Replacement**

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Back pain places a huge burden on economies and negatively impacts quality of life. A major cause of back pain is the degeneration of the intervertebral disc (IVD). Current treatments involving artificial replacements or vertebral fusion have poor outcomes. Decellularisation technologies aim to remove DNA and other cellular components from tissues to produce an acellular scaffold. A decellularised IVD could be used to replace degenerated IVDs and would not illicit an immune response upon implantation.

Protocol development was carried out on bovine tail IVDs. Decellularisation protocols, based upon the use of hypotonic low concentration sodium dodecyl sulphate plus proteinase inhibitors with freeze/thaw and nuclease treatments, were applied to IVDs with vertebral bone (VB) attachments. Changes were made to the basic protocol to improve method effectiveness of DNA and cell removal. Acetone washes, sonication, increased solution volumes and reduction of VB volume were introduced incrementally. Method effectiveness was assessed histologically (H&E, DAPI and Safranin O straining) and biochemically (DNA and glycosaminoglycan (GAG) assays).

Almost complete removal of whole cell nuclei from the inner and outer annulus fibrosus (iAF/oAF) was achieved. The region where the IVD integrates into the VB (endplate/EP) and the notochord cell areas of the nucleus pulposus (NP) within the IVD, proved challenging to decellularise; whole nuclei persisted in these areas. This is thought to be due to the dense collagen structure at the EP and the deep location of the notochord cells which made penetration of the decellularisation solutions challenging. Introduction of sonication to the protocol and the removal of excess VB resulted in a 91% decrease in the total DNA content of the EP region to 29.3 ng.mg^-1 dry weight tissue. The iAF and oAF regions of the IVD were also decellularised to within the target value of 50 ng total DNA.mg^-1 dry weight tissue. Encouragingly, the GAG content (important for IVD compressive properties) was preserved in the NP.

Future work will concentrate upon applying the decellularisation protocol to human IVDs which are less cellular than the immature bovine tissue used for protocol development and which are unlikely to contain notochord cells. Testing the effects of the decellularisation process on the biomechanical performance of decellularised IVDs will also be investigated.

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### Background:
ADM has been extensively used in plastic surgery. However, previous studies have shown that allogeneic and xenogenic ADM still have immune responses after transplantation. To improve the repair quality of ADM, Q Liang, Tejaswi S et al researchers used ADM as the carrier of ADSCs(adipose derived stem cells), taking advantage of its paracrine effect to pruduce immunomodulatory cytokines so as to attenuate the immunological rejection of allogeneic or xenogenic ADM. Yet, the existing studies only focused on the properties of ADM as a scaffold to regulate cell viability, proliferation or differentiation, the impact of ADM microenvironment on the paracrine effect of ADSCs has not been reported so far.

### Object:
Here, porcine acellular dermal matrix(PADM) was used, and the paracrine behavior of ADSCs on the scaffold were investigated in comparison to cell culture via conventional microplates.

### Methods:
Inguinal adipose was harvested for ADSC extraction. 100,000 ADSCs were seeded on polystyrene 24-well plates or on circular scaffolds trimmed with the same size as the microwell. After cultivation for 24h, the total RNA of these samples was extracted and the gene expression levels were analyzed using qRT-PCR. On the other hand, the supernatant was collected and the cytokines within was analyzed by ELISA. The same supernatant was used to do the HUVEC proliferation assay.

### Result:
It was found that the ADSCs on the PADM produced significantly higher levels of anti-inflammatory and pro-angiogenic cytokines compared to those cultured on microplates. At the same time, ADSCs cultured on PADM had higher stem gene expression than on microplates. The enhanced modulatory effects of secreted products of ADSCs on PADM were also proven in the cultures of endothelial cells, with PADM and microplates showing distinct influences on the paracrine function of ADSCs.

### Conclusion:
Our study demonstrates that PADM has the potential of modulating the paracrine function of cells. The discovery reveals a new aspect of material functions, laying the foundation for further study of interaction between ADM and cells.
**01-P040** Decellularized Extracellular Matrix Based Bio-ink having an enhanced 3D printability

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Bio-ink needs to safely deliver living cells to a desired area and provide biological environments for regeneration of artificial tissue or organ. These bio-inks should have proper rheological properties for precise patterning with living cells. It should also possess good cytocompatibility to achieve a desired artificial tissue or organ. One of the methods for getting good biological properties is a mimicking biochemical compositions of target tissue. In this aspect, tissue decellularization technic is actively applied to produce bio-ink. The decellularized ECM (dECM) based bio-ink contains various bioactive molecules including collagen, GAGs and growth factors. Recent advances have showed that the dECM based bio-ink has beneficial in the study of tissue engineering.

Generally, dECM bio-ink having hydrogel form was used for bioprinting. Its rheological properties are affected by various factors such as concentration, tissue source and decellularization protocol. Because of these reasons, its rheological properties were unstable and its mechanical property was too weak to use as bio-ink. Here, we introduce new strategy to prepare dECM bio-ink. A dECM powder was prepared instead of hydrogel form. Then it was mixed with gelatin based bio-ink to apply into bioprinting process. Cytocompatibility and patterning tests were conducted to evaluate the new material. Primary hepatocyte was encapsulated in the dECM powder based bio-ink then viability and functionality were evaluated. The results showed that dECM powder based bio-ink has proper cytocompatibility as a novel bio-ink for hepatocyte. Also, various 2D and 3D patterning and mechanical test conducted that our new bio-ink had an enhanced printability. Our results successfully demonstrated that the new strategy can be applied in developing various types of dECM based bio-ink that supports good biocompatibility and printability.

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**01-P041** An adipose tissue extraction method from decellularized cancerous bone for exploring hematopoietic-related extracellular matrix

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It is known that hematopoietic stem cells (HSCs) reside in the microenvironment, and they are regulated their maintenance, proliferation and differentiation by the microenvironment. The preparation of artificial microenvironment for hematopoiesis is needed as one of elements for producing the blood. As the native microenvironment has complicated structure, it is difficult to construct the appropriate matrix artificially. Therefore, we have focused on decellularized cancellous bones (DCBs) as an original matrix structure to find out the role of microenvironment. Previously, we reported that the DCBs could induce the ectopic hematopoiesis in vivo, indicating that they act as the microenvironment for hematopoiesis. DCBs consist of trabecular bone, adipose and reticular tissue. We hypothesized that the adipose tissue and reticular tissue play important roles in hematopoiesis. In the present study, in order to clarify which is contributing as hematopoietic microenvironmental component, we tried to remove adipose tissue from DCB with keeping reticular tissue. DCBs were perfused with hydrophobic solvent under various conditions and were evaluated by mass measurement and histology. From mass measurement, adipose tissues were included in DCBs at 30 w/w%. From H-E and oil red o staining, DCBs without adipose tissue could be prepared with keeping the 3D structure of trabecular bones and reticular tissues. Therefore, it is revealed that ECM components separation would be applied for preparing the several scaffolds for clarifying the ECM involved in hematopoiesis.
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Scaffolds with cartilage-like environment and suitable physical properties are critical for tissue-engineered cartilage repair. In this study, decellularized porcine cartilage-derived extracellular matrix (ECM) was utilized to fabricate ECM scaffolds. Mechanically reinforced ECM scaffolds were developed by combining salt-leaching and crosslinking for cartilage repair. The developed scaffolds were investigated with respect to their physicochemical properties and their cartilage tissue formation ability. The mechanically reinforced ECM scaffold showed similar mechanical strength to that of synthetic PLGA scaffold and expressed higher levels of cartilage-specific markers compared to those expressed by the ECM scaffold prepared by simple freeze-drying. These results demonstrated that the physical properties of ECM-derived scaffolds could be influenced by fabrication method, which provides suitable environments for the growth of chondrocytes. By extension, this study suggests a promising approach of natural biomaterials in cartilage tissue engineering.

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In situ gel-forming injectable scaffolds have been used for several tissue engineering applications such as drug delivery, injured tissue treatment, and tissue reconstruction. Injectable hydrogels are promising substrates for therapeutic applications, owing to their targeted delivery by minimally invasive techniques and their ability to easily fill defects in tissues. Natural materials derived from porcine articular cartilage are composed of an extracellular matrix. The extracellular matrix provides not only high biocompatibility but also an environment in which the cells can survive, supplying the growth factors necessary for cell growth and differentiation. Because of these features, immune responses hardly occur. However, porcine articular cartilage has a short biodegradation period and poor mechanical properties. To overcome these drawbacks of the naturally derived material, we prepared the porcine articular cartilage hydrogel as mechanical property and degradation period controllable carriers through a biochemical cross-linking reaction.
Fabrication and anti-adhesion feasibility test of cross-linked cartilage acellular matrix film with adjustable mechanical properties

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Due to the large number of surgical procedures in recent years, adhesion prevention techniques and materials have been developed. For this reason, many anti-adhesion materials have been developed, but these products are difficult to apply in precise locations or to control the biodegradation period. In this study, a biodegradable polymer is synthesized to produce a cross-linked cartilage acellular matrix (Cx-CP) film capable of controlling biodegradation period and mechanical properties. It was confirmed that the Cx-CP films can control the physical properties through the tensile strength and the contact angle. We could confirm that the cells were hardly adhered through in vitro and in vivo experiments for the evaluation of anti-adhesion test. In conclusion, we developed a Cx-CP film with adjustable mechanical properties for anti-adhesive film.
Introduction:

Surgical resection is the gold standard for treatment of renal cell carcinoma, and partial nephrectomy (PN) is the treatment of choice for tumors smaller than 4 cm in size.

However, chronic kidney disease (CKD) has always a considerable risk after PN and there is no definitive therapy to recover and reconstruct resected kidney after surgery to avoid further renal dysfunction. In this study, we focused on the organ specific 3D structures, obtaining from native porcine kidney by decellularization, which is a fundamental factor for cell recruitment, adhesion and proliferation after organ injury.

Methods:

The pigs weighing 15 kg were used for kidney harvest, and the renal arteries were catheterized. Subsequently, phosphate buffered saline and sodium dodecyl sulfate solutions were perfused from the catheter for 24 hours, and finally acellular bio-scaffold of porcine kidneys were secured. At mean time, PN were performed under general anesthesia, and the partially dissected acellular bio-scaffold was simply sutured onto the surface of the resected kidneys. One month after surgery, surgical functional analysis (angiography and urography) was performed, while pathological study such as H.E. staining, immunohistochemistry and scanning electron microscopy were evaluated.

Results: One month after the surgery, the bio-scaffold has kept its 3D structure with massive cell infiltration. Angiography and urography demonstrated that partly arterial blood flow and urinary tract was patent in the scaffold. H.E. staining showed that glomerular and tubules structures in the bio-scaffold, and CD31 positive endothelial cells were observed around the glomerular structures. In addition, immature and mature nephrin structures were observed in the scaffold, suggesting that the native kidney scaffold promoted nephrogenesis. Immunohistochemistry showed that there were some nephrin or AQP1 positive cells were observed. In addition, scanning electron microscopy revealed the podocyte like cells morphologically in the glomerular-like structures by showing foot processes, and also brush borders, which is specific structure for proximal tubule were observed in some tubule-like structures in the scaffold.

Conclusions: Our results demonstrated that the organ derived acellular bio-scaffold may have a potential to support kidney regeneration after PN in construction and function. Further study is needed to clarify the specific mechanism of kidney regeneration in this bio-scaffold.
We noticed the document contains references and affiliations for two different abstracts. Here is the text for the first abstract:

**A Novel Pro-Angiogenic Fibrin-Alginate Technology for Repair and Regeneration of Multiple Tissues**

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In this abstract we would like to introduce a novel patented technology developed in our laboratory based on a fibrin-alginate mesh that is pro-angiogenic and shows excellent cell attachment and infiltration properties, making it an ideal technology for repair and regeneration of multiple tissues. The first product developed using this technology is a dermal replacement scaffold called Smart Matrix® that is under clinical trial. The clinical trial will assess its performance in treating full thickness skin wounds without the use of a skin graft. Advantageously, the fibrin-alginate mesh can also be combined with synthetic polymers, either inert (i.e. silicones) or bioactive (i.e. polycaprolactone, PCL) in various shapes (sheets, 3D structures). A novel two-component dermal scaffold for the treatment of pressure sores was designed using a polydimethylsiloxane backing membrane to make the fibrin-alginate dermal scaffold more robust (i.e. polycaprolactone, PCL) in various shapes. The fibrin-alginate mesh can be combined with 3D electrospun PCL structures for the treatment of non-union fractures: the pro-angiogenic properties of the resulting PCL/fibrin-alginate composite scaffold were increased compared to PCL alone as shown by an ex ovo chorion allantoic membrane (CAM) assay. PCL/fibrin-alginate scaffolds are currently being tested in a rat model. Moreover, the fibrin-alginate mesh has been modified by introducing an osteogenic element into the mesh to be used as a bone void filler at spinal fusions, after bone cancer resection or to fill alveolar bone defects. Future development of the fibrin-alginate technology will see its combination with 3D printing technology for development of custom-made implants. All the work described in this abstract has been done in collaboration with scientists from renowned academic institutions from all over the world, clinicians, and industry.

**References:**


**Acknowledgements:**

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An Ultrapurified Alginate Gel on an Acellular Scaffold for Cartilage Regeneration: A Pre-clinical Animal Study

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We aim to evaluate the repair of articular cartilage defects by using ultrapurified alginate gel (UPAL gel) in canine as a pre-clinical animal study. Forty osteochondral defects were created in 10 beagle dogs. The defects were divided into four groups; a 3.0 mm diameter defect group, a 3.0-mm diameter defect with UPAL gel group, 5.0-mm diameter defect group and 5.0-mm diameter defect with UPAL gel group. The reparative tissues at 27 weeks postoperatively were assessed through gross and histological analyses. The total histological score in the UPAL group was significantly higher than that in the control in 3.0-mm diameter group. Regarding the 5.0-mm defect groups, the macroscopic score and histological score in the UPAL gel group were significantly higher than those in the control group. The implantation of UPAL gel enhanced a cartilage repair in canine. Although UPAL gel significantly enhanced osteochondral repair, the reparative tissues of the large defects with UPAL gel showed fibrocartilage tissue.

In order to improve the reparative tissue, we subsequently evaluated the effects of a bone marrow stimulation technique augmented by UPAL gel in a canine osteochondral defect model. 108 osteochondral defects were created in 27 beagle dogs (two defects in each knee). For the bone marrow stimulation technique, five holes (1.0 mm in diameter) were drilled into the defect. The total 108 defects randomly assigned to one of three groups (n = 36 defects in each) as follows: defects without intervention; defects with bone marrow stimulation technique; and defects with bone marrow stimulation technique augmented by UPAL gel. At 27 weeks postoperatively, macroscopic and histological evaluation including collagen orientations, micro-CT evaluations for subchondral bone repair and mechanical testing were carried out. Compared with a bone marrow stimulation technique alone, this technique significantly improved the histological findings and the mechanical property. Our results suggested that the augmentation by UPAL gel would enable to expand an operative indication of a bone marrow stimulation technique for larger defects in clinical situation. The obtained data will give support to the clinical reality of a 1-step, minimally invasive, cartilage tissue reparative medicine without harvesting donor cells.

Histological Analysis of Acellular Dermal Matrix After Implantation in the Human Body

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Acellular matrices are used for various purposes and they have been studied extensively for their potential roles in regenerating tissues or organs. The acellular matrix generates physiological cues that mimic the native tissue microenvironment. Acellular dermal matrix (ADM) is a soft connective tissue graft generated by a decellularization process that preserves the intact extracellular skin matrix. Upon implantation, this structure serves as a scaffold for donor-side cells to facilitate subsequent incorporation and revascularization. In breast reconstruction, ADM is used mainly for lower pole coverage and the shaping of a new breast. It helps control the positioning of the implant in the inframammary fold, and prevents the formation of contractile pseudocapsule around the breast implant. In our study, we provide a comprehensive histological description of ADM used for human breast reconstruction over the course of several months following implementation. Using immunohistochemical methods (a panel of 12 antibodies) coupled with optical and transmission electron microscopy, we confirmed that the original acellular dermal matrix became recolonized by fibroblasts and myofibroblasts, and also by various other free cells of the connective tissue (lymphocytes, macrophages and multinucleated giant cells, granulocytes, mast cells) after implantation into the patient's body. Within the implanted ADM, there was a relatively rapid ingrowth of blood vessels. Lymphatic vessels were only detected in one case 9 months after the implantation of the ADM. These results suggest that lymphangiogenesis is a longer process than angiogenesis.
01-P052  Mid- and long-term evaluation of REDV-modified small-diameter acellular grafts

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[Introduction] Our research group demonstrates an excellent patency of the peptide-modified acellular grafts measuring 20-30 cm in length and having 2 mm inner diameter as femora-femoral crossover bypass in minipig transplantation model1. We revealed that microthrombus formation on the graft surface was suppressed by the REDV peptide-modification. Moreover, the endothelialization was observed in one week after transplantation. These features might contribute to not only graft patency but also initial tissue regeneration process of the vascular grafts. However, the recellularization process of the intima and tunica media of the decellularized graft by using short and long-term transplantation model. [Materials and Methods] The vascular graft was transplanted into minipig and goat femoral artery. After the transplantation tissue regeneration was evaluated by immunostaining with antiCD31, CD105, CD34, and Flk-1 antibody. [Results] After 3-month transplantation, cells were detected in the medium layer of the graft. These cells expressed the αSMA but the expression level was low as compared with the native blood vessel. Endothelial layer expressed CD31 and CD105 but not CD34 and Flk-1 which are endothelial progenitor markers. Twelve-months later, αSMA-positive cells were uniformly aligned in the tissue, and stable vWF positive layer was observed in intimal layer. [Conclusion] The endothelial layer was formed on the graft by the action of endothelial progenitor cells, and tunica media were formed in three months. [Acknowledgements] This research was supported by the Intramural Research Fund of National Cerebral and Cardiovascular Center (22-2-4) and the S-Innovation Project of AMED. [References] 1. Mahara A, Somekawa S, Kobayashi N, Hirano Y, Kimura Y, Fujisato T, and Yamaoka T. Biomaterials 58, 54, 2015. [Conflict of interest] The authors declare that they have no conflict of interest.

01-P053  Investigate biocompatibility of decellularized porcine coronary artery for vascular tissue engineering

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Decellularized xenogenic vascular tissue has potential in vascular surgery application. Theoretically, decellularization removes most of xenogenic antigen and leave intact extracellular matrix for cell adhesion, proliferation and migration. There are several methods for vascular tissue decellularization and the most common is enzyme-detergent method. Both mechanical property and biocompatibility are crucial and should be evaluated. In this study, we analyzed biocompatibility of decellularized porcine coronary artery both in vitro and in vivo. The porcine artery was decellularized with trypsin and Triton X-100. In vitro, the result showed human adipose stem cells and umbilical vein endothelial cells could adhere and proliferate on the decellularized porcine coronary artery scaffolds. Furthermore, ASCs could successfully differentiate to smooth muscle cell in the scaffold. In vivo, rat abdominal aorta repair model showed that decellularized porcine vascular tissue could achieve about 50% successful rate. Macroscopically, the lumen was patent with slight dilatation at patch site. The Doppler ultrasound confirmed patent flow in repair site. Gross examination showed thin layer of mural thrombi coating at luminal side. But there was poor endothelialization at luminal side of patch at Day 30. For adipose stem cell seeded scaffolds, the animals all survived and there was partial endothelialization at luminal side at Day 30. Preliminarily, we found that decellularized porcine coronary artery could provide human cell proliferation in vitro and showed biocompatibility in vivo. Future study will focus on improving endothelialization and tissue remodeling.
The Effect of Gamma Irradiation Dose on the Histoarchitectural and Physical Properties of Decellularised Heart Valve Roots

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Decellularisation has emerged as a promising approach for generating biological scaffolds for heart valve replacements that provide close to native properties, reduced immunogenicity, and have the potential for cellular regeneration and tissue growth. Current decellularised heart valve options are produced using aseptic techniques. Introduction of a robust terminal sterilisation process would provide a superior sterility assurance level and improved production efficiency; consequently enhancing patient safety; facilitating translation to commercial production, and promoting clinical adoption of decellularised heart valves.

This study investigated the effect of a range of industry relevant gamma irradiation sterilisation doses (15 kGy, 25 kGy and 50 kGy) on decellularised porcine pulmonary heart valve root (dPHV) histoarchitecture and physical properties using histology (n = 3); differential scanning calorimetry, collagenase resistance assay, and denatured collagen quantification (n = 6). Non-irradiated dPHV were used as the control group. Specimens analysed were taken from valve cusps and pulmonary conduit wall.

Scaffold resistance to digestion by collagenase decreased with increased irradiation dose. The concentration of protein released from dPHV cusp and conduit specimens was significantly higher in all irradiated groups compared to controls. Denatured collagen content of cusps was significantly higher in the 25 kGy and 50 kGy groups. Thermal transition temperatures were significantly decreased for cusp and conduit in all irradiated groups, in a dose dependant manner for conduit. Histologically, reduced staining of glycosaminoglycans was observed following irradiation, with variable disruption of collagen and elastin fibres in both cusps and conduit.

The biological and functional consequences of changes in dPHV properties induced by irradiation remain to be elucidated. These preliminary data highlight the importance of using multiple analysis techniques to interrogate the impact of sterilisation techniques on properties of complex decellularised biological scaffolds. On-going studies are aimed at increasing the understanding of dPHV physical, biological and ultrastructural properties to allow novel sterilisation strategies and mitigation measures to be developed.
**A toolbox of single factors and their influence on successful decellularization processes**

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**Introduction:**

Today decellularization (DC) is one of only a few approaches in regenerative medicine already used for clinical implants. DC is a multifactorial process with physical, biological, chemical and procedural influences. In this study, we identified for the first time the impact of single factors on the complex DC process.

**Methods:**

Different detergents (SD, SDS, Triton-X; individually and combined), treatment procedures (time, cyclic vs. continuous, washing steps), physical factors (shaking, flow, perfusion, temperature, ultrasound) and various sterilization methods (5 chemical, one biological and 2 physical) were evaluated. Additionally, enzymatic incubation (α-Galactosidase, DNase) was evaluated. By changing only one factor at a time, their individual influence on the DC process was measured. As test materials (n=5/group), porcine valves and vessels, bovine pericardium as well as homograft valves were taken. Finally, the most effective factors were combined to create a progressive and individual DC protocol for every material. Scanning electron microscopy, standard and immunological staining methods, tensile testing, nucleic acid and glycosaminoglycan quantification as well as biocompatibility assays were used for scaffold evaluation. For sterility assessment samples were incubated in 2 specific microbiotic media.

**Results:**

- Vectored flow (if possible by perfusion), ultrasonic exposure and treatment at room temperature are physical factors with a highly significant influence on DC. Cyclic procedures and enforced washing are procedural methods supporting DC efficiency significantly. A combination of SD/SDS (each 0.5%) is the most advisable detergent. Enzymes showed supporting effects depending on the material. Sterilization of the biologic scaffolds was challenging and only a combination of peracetic acid and octenidine resulted in sterile and intact samples. Tensile tests revealed high variations within the native and processed biologic scaffold groups. Reasonable combination of the factors resulted in a significant increase of DC efficiency – specific protocols for the investigated materials were established.

**Conclusion:**

By a comprehensive evaluation of single DC factors we established a toolbox to create individual, material specific DC protocols. However, natural variation and final sterilization of DC tissues are big challenges for a successful scaffold production.

**Designer Leucine-Zipper hydrogel for Tissue Repair and Regeneration**

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A common theme in biomineralization is the intimate interaction between the organic and inorganic phases and this leads to the unique properties seen in biological materials. ECM of mineralized matrix not only provides the structure but also signaling cues for cellular proliferation and differentiation. Molecular self-assembly presents a very attractive strategy to construct nanoscale materials. The DNA binding leucine zipper proteins contain a self-assembling leucine zipper (LZ) domain. Upon elevation of the pH, temperature or ionic strength the LZ domains reversibly dissociate and create a viscous solution. The reversible assembly makes the leucine zipper domain to facilitate the formation of physical crosslinks in hydrogel structures. In designing the second-generation chimeric LZ protein, we have introduced several cysteine residues in the polypeptide to enable the formation of intermolecular disulphide bonds, which would effectively cross-link the nanofibers. Cryo-SEM showed that the introduction of cysteines were effective in promoting nanofiber networks. In order to exploit the use of LZ polypeptides in hard tissue engineering we have designed a LZ polypeptide with the hydroxyapatite nucleating domains from dentin matrix protein 1 (DMP1). Two HAP nucleating domains (ESQES and QESQSEQS) were incorporated into the LZ hydrogel. We report that HMSC cells on mineralized LZ-DMP1 hydrogel could undergo faster osteogenic differentiation when compared with the control. As the LZ hydrogel is tunable, we incorporated cues permitting angiogenesis during tissue regeneration. For this heparin-binding domain with MMP-2 cleavage site was introduced into the LZ-zipper backbone. The heparin-binding domain could bind growth factors such as VEGF, TGF-β1 and BMP-2 and controlled release of active growth factor could be anticipated both in vitro and in vivo. The growth factors could be released via proteolytic cleavage through the designated MMP-2 cleavage site. In this study two heparin-binding growth factors TGF-β1 and BMP-2 were evaluated in vivo. Results showed that both proteins remained active when released and influenced HMSCs fate showing the feasibility of this approach. Overall, we have shown that LZ, a self-assembling peptide can be utilized for enhancing mechanical property as well as provide signaling cues during the process of tissue regeneration or repair.
Engineered phage nanofibers induce Angiogenesis
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Angiogenesis can be considered a hallmark for healing wounds and curing various ischemic diseases because therapeutic opportunities can be envisaged by achieving a vascular niche. Recently, nanofibrous structures of M13 phages have been considered as ECM-mimicking nanofibers, and have been used after chemical or genetic modification with cell signaling peptides as novel tissue engineering matrices to direct the desired cellular functions. Here, we employed bioinspired M13 nanofibers comprising extracellular and vascular niches and investigated their potential in inducing neovascularization as a treatment for ischemic diseases. An engineered phage nanofiber (expressing SDKP and RGD) can act as an angiogenic factor and an extracellular component at the same time, if we display angiogenic and/or integrin binding peptides together on its body, thereby providing phage angiogenic niches, and interacting with endothelial cells to induce angiogenesis. We found that cell viability, migration, elongation, and angiogenesis were predominantly affected by nanofibrous structures (topological cues) of the engineered phages, providing a therapeutic platform for providing therapeutic biochemical cues using specific peptide expression on phage coat proteins. The phage structure has many advantages for creating a therapeutic platform, that is, "a niche. Our engineered phage nanofiber provides an angiogenic niche with therapeutic potential for future regenerative medicine applications.[This research was supported by a grant of the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (H16C1067)]

Hydrogel control the amount and distribution of host myeloid cells for vesculogenesis and angiogenesis
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In the last decade, different approaches have already been explored to date to improve vascularization. To decrease the time that is needed to vascularize an engineered tissue, the timeliest strategy is the uses of a suitable hydrogel as scaffold incorporated with endothelial colony-forming cells (ECFCs) and mesenchymal stem cells (MSC), but how to precisely and timely control the direction, width and length of bioengineered functional vasculature anastomosed with host is still a problem. Recent studies have shown that host non-inflammatory myeloid cells mediate the engraftment of bioengineered vascular networks, so developing a biomaterial to control and manipulate host myeloid cells into biomaterials could control of vascular network formation. In this study, we developed the hydrogel with different microstructures through different chemical bonds to control the amount and distribution of host myeloid cells. Results demonstrated that microstructure of hydrogel could induce the different degrees of hypoxia and stimulate cytokines and growth factor release of encapsulated ECFCs and MSC that further regulate the number and distribution of host myeloid cells infiltrating into the hydrogel. By using this inducible hypoxia hydrogel we can control the formation of bioengineered functional blood vessels in vivo.
**01-P061  Temperature-dependent Affinity Binding of Heparin-binding Growth Factors and Cells with a Heparin-immobilized Thermoresponsive Surface**

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Heparin-immobilized thermoresponsive poly(N-isopropylacrylamide) (PIPAAm)-grafted cell culture surface was designed for stimulating cellular receptors and nonenzymatic recovery of cultured cells as a contiguous sheet. Immobilized heparin molecules on shrunken PIPAAm were able to capture heparin-binding proteins such as heparin-binding EGF-like growth factor (HB-EGF). In addition, the cultured cells were recovered as a sheet when lowering temperature to 20°C. This was probably because HB-EGF penetrated grafted PIPAAm network with no steric hindrance regardless of the swelling/shrinking of the grafted PIPAAm. In addition, the inhibitory adhesion of hepatocyte with soluble EGF was conducted on the HB-EGF bound heparin-immobilized thermoresponsive cell culture surfaces. At 37°C, the adhesion of hepatocytes was inhibited with increasing soluble EGF. This result suggested that hepatocytes adhered partially via affinity between immobilized HB-EGF and EGF receptor. At 20°C, by contrast, hepatocytes hardly adhered regardless of soluble EGF, indicating that there was no affinity between immobilized HB-EGF and EGF receptor. Therefore, the affinity between the receptors and immobilized HB-EGF was considered to be attenuated by steric hindrance due to hydration and/or swelling of PIPAAm. When temperature was changed from 37°C to 20°C, however, a cultured hepatocyte sheet detached with HB-EGF, indicating that HB-EGF might bind to the EGF receptors, and affinity between HB-EGF and immobilized heparin was ruptured. Because the detachment of hepatocyte sheets was complex phenomena including non-steady changes in hydration of grafted PIPAAm and morphology of hepatocytes, further investigations into dynamic changes was required for revealing the detachment process triggered by lowering temperature.


**Acknowledgments:** Teruo Okano is a founder and a member of the board of CellSeed Inc., which has licenses for certain cell sheet-related technologies and patents from Tokyo Women’s Medical University. Teruo Okano and Masayuki Yamato are stakeholders in CellSeed Inc.
01-P065 Differential Release of BMP2 and VEGF from a Biomimetic Nanofibrous Scaffold Enhances Bone Regeneration in Critical Sized Calvarial Defects

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The natural process of bone tissue regeneration involves the co-ordination of osteogenic and angiogenic growth factors at the defect site. In this study, we aim to investigate the suitability of using a nanofibrous scaffold as a delivery system of growth factors like Bone Morphogenetic Protein (BMP2) and Vascular Endothelial Growth Factor (VEGF-2) for enhancing bone regeneration in critical sized cranial defects in rats. In vivo studies using radiolabelled growth factors demonstrated a differential release profile of VEGF and BMP2 from the carrier system, ie. a fast release of VEGF versus sustained and prolonged release of BMP2. In vitro studies with mesenchymal stem cells showed a higher alkaline phosphatase activity and calcium deposition on growth factor loaded scaffolds compared with the scaffold without growth factors. Similarly when the scaffold was implanted in calvarial defect, local delivery of VEGF and BMP2 increased vascularisation and bone formation at 3 months. While comparing, augmented osteogenic differentiation and tissue regeneration were observed on BMP2 loaded scaffolds than VEGF groups, suggesting that the prolonged released of BMP2 has a positive effect on bone healing. In short, the biomimetic nanofibrous scaffold can be used as a potential candidate for differential release of osteogenic and angiogenic growth factors for orthopedic application.

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Platelet Lysate-based Nanocomposite Bioinks for 3D Printing in Tissue Engineering

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Blood derivatives have attracted great attention as an inexpensive cocktail of growth factors, cytokines and extracellular matrix precursors with high potential in tissue engineering and regenerative medicine (TERM). In previous studies, platelet lysate (PL) was reinforced with cellulose nanocrystals (CNC) to promote the in-situ PL-clotting via thrombin and calcium activation along with the CNC-protein covalent crosslinking. This platform allowed the use of PL as stable injectable formulations for either the delivery of biological factors as well as a cell carrier matrix, thus, opening new avenues to explore PL based hydrogels in different TE applications, for example in 3D printing. In this context, we hypothesized that PL-CNC could be used as a nanocomposite bioink for printing personalized structures in combination with encapsulated cells to successfully apply 3D (bio)printing in TERM.

PL/CNC hydrogels were produced with double barrel syringes using optimized conditions for PL gelation (1 U/ml thrombin and 5 mM CaCl2). The effect of different CNC concentrations in hydrogel rheological properties was fully characterized. Then, a desktop 3D printer and its firmware were customized to hold the syringe and print the PL-CNC hydrogels in a gelatin supporting bath. Finally, the biological performance was assessed using encapsulated human adipose tissue derived stem cells (hASCs).

After showing that PL-CNC 0.6 wt% formulation had shear-thinning behaviour, which allowed extrusion at low printing pressures, 3D printing parameters (printing speed, flux, layer height and infilling) were optimized. The 3D structures printed using PL-CNC bioink showed good shape-fidelity and self-supporting characteristics after removing the gelatin bath. Furthermore, the 3D printing process did not affect the hydrogel degradation profile. In addition, 3D printed constructs showed cell supportive properties, since hASCs were viable, active, and in a proliferative state. Overall, the studied PL-based nanocomposite bioinks have a great potential in personalized TERM applications.

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Bioprinting Meets Macromolecular Crowding: A Facile Approach to fabricate 3D Hierarchical Collagen-based Hydrogels

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The spatial gradient in physical properties found in most native biological tissues imparts highly-specialized functions over hierarchical scales. Despite advances in material synthesis and assembly process, it is still highly challenging to emulate the hierarchical porous architecture found in native tissues. In this work, macromolecules were utilized to fabricate hierarchical collagen-based structures using our proposed bioprinting-macromolecular crowding process. The influence of macromolecular crowding on collagen fibrillogenesis remains poorly understood; hence an in-depth investigation on the mechanism of MMC phenomenon was conducted.

As a proof of concept, the printing parameters were first optimized to facilitate the pH-dependent crosslinking of thin collagen layers (~ μm) and discrete droplets of macromolecule-based bio-ink was deposited to fabricate hierarchical collagen-based hydrogel via drop-on-demand (DOD) bioprinting process. The presence of PVP macromolecules not only accelerates the collagen fibrillogenesis process but also tunes the collagen architecture in a controlled manner. The PVP macromolecules exert excluded volume effect (which is dependent on both electrostatic repulsions and non-specific steric hindrances) on the surrounding collagen molecules; an increasing PVP concentration results in formation of larger pores and increased porosity within the 3D collagen matrices. The uniform deposition of discrete droplets of cross-linkers (NaHCO3) and macromolecules (PVP) over each thin layer of printed collagen (~20 μm) facilitates the rapid and homogeneous cross-linking. Our work demonstrates a new printing strategy that facilitates the fabrication of hierarchical porous collagen-based constructs via drop-on-demand bioprinting process in a highly-controlled manner. As collagen is a widely used biomaterial, this facile single-step bioprinting process could be useful for fabrication of complex 3D tissue models for tissue engineering applications.
Dynamic modulation of hydrogels to mechanically manipulate cells in a reversible manner

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The extra-cellular matrix (ECM) offers different biochemical, mechanical, and structural signals to cells. These cues are highly orchestrated in space and time. Precise instructions from the ECM dictate cell fate processes, such as proliferation, differentiation and migration. The ECM exerts mechanical forces on cells, which are sensed by cells through different mechanisms, and are translated into biological outcomes. However, these mechanisms are not very well understood. One of the main limitations in deciphering this language of forces on cells has been the lack of in vitro systems, which can generate forces on cells that mimic natural stresses.

Here we demonstrate a novel hydrogel system, which can reversibly apply precise, user-defined mechanical forces on selected cells from a cell population. Our approach comprises of a smart ECM-mimic hydrogel system, which responds to a light trigger. This causes reversible local deformations of the cell growth substrate and leads to the generation of mechanical forces on cells. These forces are transient and can be controlled at a sub cellular and sub-population scale, in a wide range of time scales (up to ms), with pre-defined directionality.

Such a system for opto-mechanical stimulation of cells is an effective tool for investigating how repeated actuation of a soft hydrogel affects cells. This is experimentally demonstrated in a case study using fibroblast cells to show the proof-of-principle of the concept.

The dynamic hydrogel swelling/shrinking closely replicates the stretches experienced by soft tissues in the body during activities, such as movement, growth etc. We believe that this system bridges the gap between single cell manipulation techniques and cell sheet deformation techniques. This system shows great potential in fields of mechanobiology and in understanding cell-ECM interactions.
Surgical replacement of diseased heart valves at the end-stages has been widely performed with mechanical valves (MVs) or bioprosthetic heart valves (BHVs). All these current devices have significant limitations with risks of further morbidity and mortality. For example, MVs may cause hemorrhage and thromboembolism, and require anticoagulation for the lifetime of the patients. BHVs show better hemodynamic behavior due to the composition and structural similarity to native heart valves when compared to MVs, however, they do show limited durability because of calcification and progressive degeneration [1]. Thus, polymeric heart valve (PHV) prostheses with long-term durability and no necessity for permanent anticoagulation are of great interest and also show potential applications in advanced transcatheter devices.

In this study, novel PHVs were fabricated, consisting of natural protein fibers to mimic the fibrous networks in the fibrosa and ventricularis layers for stress bearing, as well as poly(ethylene glycol) (PEG) hydrogels to improve anti-fouling function [2,3]. These layered constructs showed mechanical properties, i.e., elastic modulus and elongation percentage, close to those of human aortic valve leaflets. The hemodynamic property of these PHVs can meet the requirements of the ISO-5840 standard. Furthermore, the presence of PEG hydrogels improved the resistance to progressive calcification of the embedded protein fibers in vitro, likely due to prevention of large-size hydrated ions to pass through by the polymeric networks. In addition, the fibrous structures retained in the PEG-protein fiber composites after subcutaneous implantation for four weeks, while those from natural protein samples showed early degradation to certain extent, suggesting the prevention of enzymatic degradation of protein fibers by PEG hydrogels in vivo [4]. Thus, this study lays down a basis for fabrication of novel PHVs to mimic the heterogenic structures, mechanical properties and biological functions of heart valve leaflets.

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References
**01-P072** Tissue-engineered Descemet’s stripping endothelial keratoplasty

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There are donor cornea shortages in many nations. A tissue-engineered corneal endothelium (TCE) could replace donor tissue for treatment of corneal endothelial dystrophies, a common indication for corneal transplantation.

A pre-clinical trial of a tissue-engineered Descemet’s stripping endothelial keratoplasty (TE-DSEK) was conducted. The TCE was assessed for ability to be implanted via a minimally invasive procedure, lack of toxicity and immunogenicity, biodegradation of the scaffold, and abatement of oedema in a model of corneal endothelial dystrophy.

A TCE was manufactured as previously described¹. Briefly, ovine corneal endothelial cell (CEC) monolayers were cultured on an novel thin (50 µm) poly(ethylene glycol)-based hydrogel film (PHF) with excellent physical properties¹. Culture conditions were adapted from those used for cultivated limbal epithelium transplantation (CLET)². Donors and recipients were outbred Merino-Dorset ewes of 12-14 months of age. A model of corneal endothelial dystrophy was created by surgical removal of CEC from a 7 mm diameter area of the central cornea. The TE-DSEK procedure was essentially the same as an existing surgical technique: Descemet’s stripping automated endothelial keratoplasty (DSEA). Negative control animals received a PHF without CEC, or a TCE not placed over the endothelial wound. Animals were observed for at least 21 d post surgery and scored for inflammation, corneal clarity, and oedema on a validated pro-forma³. Oedema was rated 0 – 4, with 0 being no oedema and 4 being maximally thick. Post mortem whole eyes were subjected to haemotoxylin-eosin histology.

TCE was found to be sufficiently robust for implantation by a DSAEK-like minimally invasive procedure. No evidence of toxicity or immunogenicity was observed in both clinical observations and histological sections. Allogeneic TCE was non-toxic and non-immunogenic for >20 d (n = 13). In animals receiving TCE the PHF completely degraded in <21 d. The TCE abated oedema (score 0 or 1, 70% n = 10).

References:

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**01-P073** A mechanobiologically inspired platform for therapeutic T-cell activation

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Adoptive T cell therapy is a cancer treatment that uses patient-specific T cells to seek and destroy cancer. A key step in this process involves ex vivo activation and expansion of T cells. However, it is often limited by low expansion rates and T-cell products of suboptimal functionality. Currently, there is a limited repertoire of biomaterials for T-cell activation and one of the most common methods involves the use of antibody-coated, rigid materials. Although the critical role of physical parameters in T-cell activation is increasingly recognised, it is often overlooked in the design of T-cell-activating biomaterials. Gaining the ability to harness T cell mechanobiology may therefore provide new ways of optimising and tuning T cells for therapy. In this work, we studied the impact substrate stiffness has on T-cell activation. To this end, antibody-coated polyacrylamide hydrogels were constructed as a stiffness-tunable platform to activate Jurkat T cells. Cells cultured on hydrogels of different elastic moduli exhibited a stiffness-dependent response, as indicated by their differential secretion of Interleukin-2. In addition, we also incorporated the biomaterial into a microfluidic device such that automation and modularity can be added to ex vivo cellular processing. Taken together, these findings suggest the feasibility of exploiting T cell mechanotransduction as a new strategy to optimise T-cell manufacturing for cancer immunotherapy.
**01-P074  Adhesive Hydrogel Sponge Based on Biomaterials Derived from Marine Life for Topical Hemostatic Dressing**

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Appropriate actions for hemostasis have great importance at emergent situation or surgical operation to minimize blood loss. Hemostatic action also helps surgeons to secure their visibility on wound site, reduce the amount of transfusion, and save operation time. Among them, topical hemostatic agents cover the bleeding site with less tissue damage than mechanical pressure or electrical/thermal devices. However, most of commercially available products have low clotting ability and inappropriate mechanical property to seal the wound site. In this work, we designed a novel hydrogel sponge as topical hemostatic dressing using biomaterials from marine life. Mussel adhesive proteins (MAPs) originated from the byssus of mussel retain strong underwater adhesiveness on account of their abundant 3,4-dihydroxyphenylalanine (DOPA), modified form of tyrosine residues. Aneroin, a silk-like protein derived from starlet sea anemone has high similarity to its repetitive amino acid sequence motives and mechanical strength to silk fibroin or spidroin. These structural proteins were fabricated to light-activated hydrogel and then lyophilized to raise its moisture content. The tensile strength was improved due to the nature of aneroin and even more increased after lyophilization. Blood cells and plasma proteins were attached to MAP which resulted in rapid clot formation, examined via thromboelastogram. The hydrogel sponge was tested in vivo to reveal its capability to save amount of bleeding and clotting time on damaged organ and blood vessels. Collectively, our developed hydrogel sponge would be outstanding topical hemostatic dressing to treat bleeding site quickly and safely.

**01-P075  Novel Porous Calcium Phosphate Nanoparticles As A Drug/ion Delivery Platform And Primary Precursor Of Bone Mineral Formation**

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Bone, a mineralised connective tissue acts as a supportive framework for the body and enters metabolic activities. About 30% of the dry weight of mature bone is organic in nature, mostly collagen, while the remainder is a complex inorganic calcium phosphate system. The mineral of bone has been shown to consist of two phases, a hydroxyapatite-like crystalline phase and an amorphous calcium phosphate. In early stages of bone formation, mineral precursors are transiently produced by osteoblast mitochondria, transported via intercellular vesicles and deposited within collagen fibrils, where they transform into semi crystalline apatite platelets or needles. In present study, we report on fabrication of porous calcium phosphate nanoparticles that chemically mimics bone mineral precursors with ability to be converted into apatite crystals with tuneable crystallinity and morphology, that highlights their potential to be used in bone regeneration application. Moreover, various bioactive elements can be incorporated into the structure of these precursor nanoparticles and their pores can be closed upon exposing nanoparticles to aqueous media, facilitating the encapsulation of various types of soluble biomolecules.
**01-P076 Natural polyphenol-based fast-forming hydrogel system for versatile biomedical applications**

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Nature-inspired biomaterials have been used for biomedical applications. Especially, exploitation of unique biochemical and biophysical properties of marine organisms has led to the development of functional biomaterials. Recently, ascidians have received great attention, owing to their extraordinary properties such as strong underwater adhesion and rapid self-regeneration. In this study, a hyaluronic acid (HA) hydrogel platform inspired by ascidian adhesion has been developed, demonstrating versatile applicability for tissue engineering and drug delivery. The synthesized conjugate can be rapidly crosslinked by dual modes of oxidative mechanisms, resulting in hydrogels with different mechanical and physical characteristics. The versatile utility of HA hydrogels formed via different crosslinking mechanisms has been demonstrated for different biomedical platforms, including microparticles for sustained drug delivery and tissue adhesive for noninvasive cell transplantation. With extraordinarily fast and different routes of oxidation, ascidian-inspired HA hydrogel system may provide a promising biomaterial platform for a wide range of biomedical applications, including tissue engineering, drug delivery, and stem cell therapy. This work was supported by grant (2017R1A2B3005994) from the National Research Foundation (NRF) of Korea funded by the Ministry of Science and ICT (MSIT), Republic of Korea. This work was also supported by grant (2016R1A5A1004694) from the Translational Research Center for Protein Function Control (TRCP) funded by the Ministry of Science, ICT and Future Planning, Republic of Korea.

**01-P077 Serum Derived Exosomes for the Delivery of Small Molecules and Peptides**

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To determine whether serum derived exosomes are efficient carriers for small molecules, nucleic acids, and peptides into lymph nodes, exosomes (EXOs) were purified and loaded with diverse small molecules and peptides. Serum-derived EXOs have several advantages e.g. a preferable size and generated higher yields than cell-derived exosomes. Fetal bovine serum-derived exosomes with a size below 50nm were delivered to whole region of lymph nodes, which provided an efficient location of immune stimulating molecules and following interaction with antigen presenting cells. The encapsulation of immune stimulating biomolecules and oligodeoxynucleotides within EXOs greatly improved intracellular delivery to macrophages via phagocytic pathways. Taken together, serum derived EXOs might serve as promising carrier systems of small molecules, nucleic acids, and peptides to lymph nodes.
**01-P078**  
Surface biofunctionalization through step-by-step build-up of covalent poly (ethylene oxide) nanogel films

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Hydrogels based on poly(ethylene glycol) (PEG) are commonly used for studies related to cell fate and tissue engineering. Here we present a new covalent layer-by-layer build-up process leading to PEG coatings of nanometer size called "nanogel films". Compared to macroscopic hydrogels, such nanogels should provide a fine control over the structure and the thickness of the coating. Alternated deposition of bifunctional and tetra functional PEG molecules reacting through thiol/maleimide click chemistry is evaluated by quartz crystal microbalance. We first study parameters influencing the build-up process of such coatings and demonstrate the importance of i) the nature of the first deposited layer, ii) the PEG concentrations and iii) the length of the PEG chains that appears to be the most significant parameter influencing film growth. It appears that the nature of the first deposited layer is crucial for the build-up and a linear build-up process can be obtained on gold when starting with bifunctional thiol-PEG chains. The steric hindrance between the molecules plays a key role in the build-up kinetics.

The build-up process can be extended to a large variety of substrates like SiO₂ or polymers by using an appropriate anchoring layer. Covalent functionalization of these nanogel films by proteins or enzymes is achieved by modifying the biomolecules with thiol or maleimide groups and immobilizing them during the build-up process. Activity of the embedded enzymes can be maintained. Moreover ligands like biotin can be incorporated into the film and recognition by streptavidin can be modulated by playing with the number of PEG layers covering biotin. Compared to well-known PEG hydrogels, these new coatings are promising as they allow to i) build thin nanometric coatings, ii) finely control the amount of deposited PEG and iii) organize the position of the embedded biomolecules inside the film layers. Finally this new coating based on covalent interactions of PEG chains could be applied to functionalize all kind of surfaces in a precise manner. In this way, antifouling and biocompatible surfaces could be easily designed or coating of nanoparticles for biomedical applications.

Reference:

**01-P079**  
Bioinspired Extracellular Matrix Hydrogel Doped with Polyelectrolyte Nanoparticles for Endogenous Stem Cell Regulation in Brain Tissue Regeneration

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Poor regenerative capability of stem cell transplantation in the injured central nervous system limits their therapeutic efficacy. The sustained inflammatory response, lack of structural support, and trophic factors deficiency limit the integration and long-term survival of stem cells. Instead of exogenous stem cell therapy, we describe the synthesis of nanohybrid hydrogel containing sulfated glycosaminoglycan-based polyelectrolyte complex nanoparticles (PCN) to mimic the brain extracellular matrix and control the delivery of stromal-derived factor-1α (SDF-1α) and basic fibroblast factor (bFGF) in response to matrix metalloproteinase (MMP) for recruiting endogenous neural stem cells (NSC) and regulating their cellular fate. Bioactive factors are delivered by electrostatic sequestration on PCN to amplify the signaling of SDF-1α and bFGF to regulate NSC in vitro. In vivo ischemic stroke model, the factors promote neurological behavior recovery by enhancing neurogenesis and angiogenesis. These combined strategies may be applied for other tissue regenerations by regulating endogenous progenitors through the delivery of different kinds of glycosaminoglycan-binding molecules.
Adhesive hydrogels with adequate tissue adhesiveness and excellent mechanical properties are attractive biomaterials for various applications such as wound dressing and scaffold implantation. However, fabricating such a hydrogel remains a challenge. Here we report the fabrication of an adhesive and stretchable hydrogel by incorporating polydopamine nanoparticles (PDAPs) into poly(vinyl alcohol) -tannic acid (PVA-TA) hydrogels. The strong multiple H-bonding formed between PVA and TA leads to easy formation of hydrogel network when they are physically mixed at room temperature or after freeze-thawing process. The amorphous structure, the strong H-bonding between PVA and TA, and the weaker H-bonding between PVA chains endow the PVA-TA hydrogels with excellent mechanical properties. The introduction of polydopamine nanoparticles further enhances mechanical properties by the formation of physically cross-linked networks from H-bonding between PVA and PDAPs. On the other hand, the reactive catechol groups on TA and PDAPs contribute to the cell-tissue adhesiveness. The as-prepared PDAPs-incorporated PVA-TA hydrogel is a pure physically cross-linking network and possesses adjustable tensile strength and adhesion strength, relying on the concentration of TA and PDAPs. In vivo full-skin defect experiments demonstrate the PDAPs-incorporated PVA-TA hydrogel and an immobilized growth factor has a synergistic effect on accelerating wound healing. In summary, this hydrogel should have great potential in wound dressing and artificial skin.

Background: Adipose based tissue engineered breast (TEB) represent a promising strategy for breast reconstruction. Nevertheless, the exorbitant mechanical strength of currently used scaffolds and the insufficient survival rate of grafted fat hamper its further development.

Method and Result: Herein we developed four series of 3D printed breast scaffolds using thermoplastic polyurethane (TPU). The computer designed internal structure of each scaffold was inspired by a kind of crystal lattice. These scaffolds have the similar strength to natural breast tissue and could rebound to its original shape after repeated compression. Bio-compatibility of the scaffold was confirmed by cell adhesion assay and in vivo tissue ingrowth assay. Finally, we implanted the scaffolds under the dorsal skin of immunodeficient mice for two weeks to allow the vessel ingrowth and then transplanted fat granule into the scaffolds in a multiple injection manner to create TEBs. Three months later the TEBs were harvested and analyzed. All the scaffolds and grafted fat integrated well with the adjacent tissue whereas the neo-vasculature density and survival rate of grafted fat was higher in diamond like internal structure group. Conclusion: Tissue engineered breast with similar mechanical properties to natural breast tissue could be manufactured by multiple injection of fat granule into pre-vascularized crystal lattice internal structure based TPU scaffold. The scaffolds with diamond like internal structure is superior to others in term of neovascularization and grafted fat survival. Tissue engineered breast could be a potential alternative for plastic surgeons to perform breast reconstruction.
Imagine that a shark bite can be not a traumatic event causing severe tissue loss but actually an approach for regeneration of osteoarticular tissues. It may be so if bite is actually taken as the acronym for Blue Inspired Tissue Engineering (B.I.T.E.) and sharks as the source for diverse chemical compounds with striking biomedical relevance. In the present work we addressed the isolation of collagen from shark skins (widely available by-products from fish processing) and the production of Fluor-rich calcium phosphates, which were further combined for the production of composite scaffolds capable to support the adhesion and proliferation of osteoblast-like cells, as well as the osteogenic differentiation of adipose derived stem cells (ASC). In addition, other examples will be given to underline the role of marine origin biopolymers on the development of other biomaterials for tissue regeneration, as well as advanced therapies to address chronic diseases. In particular, squid chitosan, with higher deacetylation degree, was proposed as component of porous sponges and hydrogels for the engineering of cartilage tissues, while seaweed sulfated polysaccharide fucoidan was studied not only for encapsulation of pancreatic cells and islets as diabetes therapeutic approach, but also as potential drug capable to tackle breast cancer as (selective) cytotoxic compound or anti-angiogenic agent. Moreover, marine biomimetic concepts will be also discussed, namely the use of marine sponges as Nature made scaffolds, as inspiration for the design of hierarchical structures by combining 3D printing of a support material with biocompatible biopolymer microfibers or as source of biochemical compounds for the development of biomaterials for regenerative medicine approaches or as 3D models of tissues and related diseases.

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Catechol-based freestanding multilayer membranes as an adhesive and functional scaffold to treat superficial cartilage damage

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Traumatic and degenerative cartilage damage cause serious clinical problems in millions of people, characterized by the progressive degradation, pain and loss of mobility. Due to the lack of vascularization and perichondrium, articular cartilage has a low capacity for spontaneous repair. Lot of effort has been done in the last decades to restore the structure and function of articular cartilage by tissue engineering (TE) strategies involving the combination of cells, scaffolds and bioactive agents. While several materials have been successfully applied to deal cartilage defects, superficial defects are still challenging in terms of fixation. Superficial glues and sewing of the material to the cartilage are either not strong enough or can damage the healthy cartilage. We suggest a layer-by-layer (LbL) technique as a bottom-up approach to produce self-adhesive freestanding multilayer membranes (FMM) for cartilage repair; for that, we chose natural components proven to work for cartilage regeneration (chitosan, alginate and hyaluronic acid, HA). Adhesion was addressed through the modification of HA backbone with catechol groups of dopamine (DN, the mussel foot protein amino acid) and their incorporation into the FMM. Effectively, the catechol groups increased the adhesion strength between the FMM and cartilage. We used FMM with different nanotopographical structure as delivery devices for therapeutic cells (adipose derived stromal cells, ASCs) and observed the interaction of the RFP-transduced ASCs with the FMM membrane and the cartilage surface. Fluorescence microscopy revealed that cells formed clusters on the membrane and that the presence of nanotopographical features increased the spreading of ASCs. After application on damaged cartilage samples, cells adhered to the cartilage, spread on the surface and bridged between the FMM membrane and the tissue. Despite the presence of the cells on the membrane surface, the catechol DN still showed its adhesive effect.

This shows that adhesive and functional LbL-engineered FMM membranes are promising materials for TE and reconstruction of superficial cartilage damage.

Acknowledgments

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Porosity-controlled Catechol Modified-Chitosan Matrices for Tissue Adhesives

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Various tissue adhesives have been developed but it is still difficult to satisfy both strong adhesion and safety onto bleeding wet tissues. To overcome disadvantages of conventional sutures and adhesives, biomimetic adhesives have been studied in recent years. Among the biomimetic adhesives, the mussel-inspired adhesives are of great interest due to their strong adhesion and biocompatibility in wet environments. However, mussel-inspired adhesives need to be prepared in a lyophilized form for commercialization because the catecholamines of the mussel foot protein, which cause tissue adhesion, are oxidized in aqueous solution and decreased its adhesiveness. The lyophilized catechol-modified chitosan (Chi-CA) matrix is the most commonly studied mussel-inspired adhesive and has excellent adhesion to tissues with easy of produce. Adhesion force of Chi-CA onto tissues is determined by the substitution degree of catecholamine to the chitosan backbone and by the cohesion of the matrix itself. So many have studied ways to increase the degree of substitution of catechol but have not tried much to improve the adhesion by changing the physical properties of the matrix.

Here, we demonstrate improve physical properties of the lyophilized Chi-CA matrix to enhance the adhesiveness by controlling porosity of the matrix through a simple co-solvent method. This method decreases pore size and porosity of the Chi-CA matrix, which reduces mechanical strength of bulk materials. We have not only controlled the surface energy between the polymer mold and the solution by adding solvents, which are miscible with water, to the aqueous solution of Chi-CA, but also gave the difference in sublimation speed during freeze drying. The solvent-added chitosan solution was lyophilized and made into a matrix with reduced porosity and pore size, resulting in a several times increase in adhesiveness to porcine skins. In addition, the matrix produced by this method will also increase the period of biodegradation, making it suitable for tissues that take a long time for tissue healing and will show stability at wound location.

Reference


Acknowledgments

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Bioactive Materials of Immobilized EphrinB2/EphB4 Signals Regulate Arterial Venous Differentiation of Pluripotent Stem Cells

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Vascular endothelial cells (ECs) differentiated from pluripotent stem cells have potential in a variety of therapeutic areas such as tissue engineered vascular grafts and re-vascularization of ischemic tissues. However, there remain limitations in the control of stem cell differentiation into the distinct functional phenotypes with current methods such as adding growth factors or exposing to hemodynamic flow, which up-regulates a few arterial markers but does not influence venous cell fate. Functional distinction between arterial and venous ECs was also not achieved with current methods. Here, we propose to more specifically control embryonic stem cell arterial venous differentiation by developing ephrinB2/EphB4 hydrogels to mimic its cell-cell bidirectional signaling during stem cell differentiation. Our data shows that we can differentially influence the stem cell EC phenotypic pathways using biomaterial based approaches. Specifically, ephrinB2 hydrogel showed an increase venous marker expression within the Flk1+ sorted population. In combination of immobilized and soluble factors we were able to achieve the distinct arterial venous cell populations. We have identified that EphB4 hydrogel with VEGF, bFGF2 and either BMP4 or chiral99021 media can create arterial-like and venous-like profiles, respectively. Furthermore, these two populations show differential functional gene expression including anti-thrombotic, pro-atherogenic and osteogenic markers, which are consistent with the in vivo functional differences in adult artery and vein. In conclusion, this project shows arterial venous stem cell differentiation can be controlled through immobilized ephrinB2 or EphB4 with the addition of specific soluble signals, VEGF, bFGF2 and either BMP4 or chiral99021, lead to two distinct groups of arterial venous and functional gene expression profiles.
Engineered phage matrices facilitate angiogenic differentiation of adipose derived stem cells

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Although stem cell niche plays a vital role in stem cell differentiation towards different lineages, an artificial stem cell niche achieved so far is not successful to fulfill the complex microenvironment of the stem cell. Here, we demonstrated engineered hybrid phage matrices that possess cell adhesive and angiogenic peptides with a suitable scaffold by formulating polyacrylamide hydrogel incorporating phage in different stiffness to guide adipose derived stem cells (ASC) and could achieve higher stiffness favoring osteogenesis and lower stiffness favoring adipogenesis. In this study, we present a specific phage based angiogenic matrices by modulating physical and biochemical cues in differentiation of ASC, providing convenient artificial stem cell niche. [This research was supported by a grant of the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (H16C1067)]
**Synthetic Coral Scaffold as a Microenvironment to Induce MSC Differentiation Into Osteoblast**

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Fundamental technology for tissue regeneration is preparation of biomaterials as an artificial scaffold to act as synthetic extracellular matrix. Synthetic coral scaffold was designed and its ability to induce MSC (mesenchymal stem cells) to differentiate into osteoblast both in vitro and in vivo were investigated. The study was divided into 3 phases. At first, fabrication of synthetic coral scaffold was done in various concentrations to be screened to achieve the most ideal one. For in vitro cell experiment, the most potential scaffold candidate was observed wherein 5 groups were investigated, i.e. S0P0M1-OM (MSC cultured in osteogenic medium), S1P1M1-OM (scaffold, PRP, MSC, with osteogenic medium), S1P0M1-OM (scaffold, without PRP, MSC, with osteogenic medium), S0P0M1-SM (MSC cultured in standard medium), S1P1M1-SM (scaffold, PRP, MSC, with standard medium). The cells were seeded and cultured for 7, 14 and 21 days. Runx, Osterix and Osteocalcin expressions were investigated by RT-PCR, gel agarose electrophoresis and Image-J software was used for measurement. To observe the success of the system in an animal model, implantation of the construct into subcutaneous tissue of Sprague dawley rats was done with the following system: S1P0M0 (scaffold only), S1P0M0 (scaffold incorporated with PRP), S1P1M1 (scaffold incorporated with PRP and MSC) and S1P0M1 (scaffold with MSC). After 7, 14, 21 and 28 days, ectopic bone formation was observed by osteocalcin expression. Based on the in vitro cell studies and in vivo studies using animal model, it was known that when the scaffold is combined with PRP and MSC, the system provides micro environment for MSC to generate and secrete bone extracellular matrix in osseous and non-osseous environment shown by the results of subcutaneous implantation. It is concluded that synthesis of calcium carbonate film has successfully resulted ideal coral-like scaffold to provide micro environment for MSC to generate and secrete bone extracellular matrix both in vitro and in vivo, in osseous and non-osseous environment.

**Surface-initiated atom transfer polymerization of glycidyl methacrylate on electrospun nanofibrils for surface-immobilization of decellularized extracellular matrix**

Hyuksang Yoo, Hyesung Kim, Yougju Son, Wei Mao, Sol Lee, Jiu Shin, Juwon Lee  
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Nanofibrilar scaffolds retains biomimetic morphology biomimicking natural extracellular matrix in tissues. Electrospinning is a versatile tool to fabricate nanofibrous structures, however, cells cannot freely migrate and infiltrate through the non-woven meshes, which prevents formation of 3-D tissue formation. We electrospin poly(caprolactone) nanofibers and mildly fragmented the electrospun meshes by milling and hydrolysis. Surface hydroxyl on the nanofibrils was initiated and employed for atom transfer polymerization of glycidyl methacrylate on the surface. NMR, XPS, and NMR spectroscopy revealed the morphology and surface chemistry of the surface-polymerized glycidyl methacrylate moieties on nanofibrils (PGMA@NF). Bovine articular cartilage was harvested and decellularized and the water-soluble fraction was obtained. PGMA@NF was reacted with the water-soluble extracellular matrix (dECM). dECM containing PGMA@NF was confirmed to significantly promote proliferation of adipocyte-derived stem cells and expressions of chondrogenic markers were highly elevated in comparison to cells in PGMA@NF without dECM. In vivo study also showed that dECM-transplanted on cartilage defects strongly promote recovery process, which was confirmed by μCT and histological examination.
Heart disease continues to be the leading cause of death worldwide and will continue for years to come with growing civilized populations and sedentary lifestyles. Cardiomyocytes are terminally differentiated cells with very limited native regenerative potential. Therefore, any loss of these cells is a loss of functional cardiac output and as a result a number of systemic disorders. Current therapeutics for ischemic cardiac tissue are mainly palliative and will never restore native function. While methods of reprogramming and differentiation through exposure of defined factors seem to be an effective means of generating cardiomyocytes, the process is time consuming and can vary greatly depending on the specific cell line.

Our approach to cardiac regeneration follows a biomimetic nature, and focuses on two aspects of regeneration. The first being a cellular based approach focusing on enrichment of cardiac cells using various nanotechnologies. Our lab has been able to specifically tune porous silica particles to release inhibitory small molecules in a spatiotemporal manner driving the differentiation of pluripotent stem cells into functional cardiomyocytes. We have also applied this nanoparticle delivery system to deliver growth factors for neovascularization in areas of cardiac ischemia. Another application of our nanotechnologies comes in the form of nanoneedles and their ability to transfect cardiac cells and stem cells with minimal cell damage. The advantages of controlling cellular response using nanoparticles are exponential in cardiac cell sourcing and applications in cardiac drug screening.

The second approach focuses on novel biomaterial platforms which provides structural and functional cardiac repair and regeneration. Translating our nanoparticle delivery systems, we are able to combine different release strategies with tunable biomaterials to create three-dimensional biomimetic cardiac patch solutions. Our lab is developing a cardiac patch that is able to drive differentiation of pluripotent cells into functional cardiac cells allowing for minimal human interactions and therefore a more clinically relevant product. These patches would be autologous to the patient, spontaneously contractile and able to be electrically paced. Together our biomimetic platforms have the potential to make significant advances in clinically relevant cardiac regeneration able to treat countless patients.

Synthetic biomaterials, which can act as extracellular matrices in regenerative medicine applications, are biologically inert and have to be functionalised with adhesive proteins or biomolecules to be recognized by cells. Certain materials, such as poly(ethyl acrylate) (PEA), are able to induce the organization of fibronectin (FN) in biomimetic (nano)networks, which have been shown to be recognized by cells [1-3]. The ability of PEA to organize FN has been demonstrated in 2D and 2.5D environments (fibres), but not yet in 3D scaffolds, which incorporate 3-dimensionality and chemical crosslinkers that may influence its fibrillogenic potential.

The aim of our work is to engineer PEA-based 3D scaffolds that sustain the organization of FN in their pores into physiological-like (nano) networks in the same way as in 2D and 2.5D environments.

2D substrates were obtained by radical polymerization with different amount of crosslinker and characterized by DMA, DSC and TGA. 3D scaffolds were prepared by combining radical polymerization and particle-leaching techniques. A polyvinyl alcohol template obtained by rapid-prototyping was used as porogen. Scaffold morphology was studied by SEM and the FN organization was analysed by AFM and ELISA assay.

We found that the organization of FN after adsorption and the availability of the FN cell-binding domain were dependent on crosslinking density and surface mobility was identified as a key parameter for FN organization. Scaffolds prepared by 2% of crosslinker showed FN (nano) networks assembled on the walls of the pores, demonstrating that PEA-based scaffolds are able to induce FN fibrillogenesis in 3D environments as long as the amounts of crosslinker is low enough.

Acknowledgements

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Knee is the second most common site of osteonecrosis after femoral head and its clinical management is demanding, often requiring invasive surgical procedures. The design of new material formulations for regenerative medicine, easier to use and combinable with biological adjuvants, might offer new therapeutic possibilities, but the relevant blank in preclinical models makes the development of innovative therapeutic strategies difficult. Aim of our work was to assess the osteogenic potential of injectable calcium phosphate pastes and to study the set-up of a preclinical model of osteonecrosis of the knee, both in vitro and in vivo. Bone marrow human mesenchymal stem cells were co-cultured with two calcium phosphate formulations for 2 weeks, showing that both can stimulate osteogenic differentiation, increasing the expression of RUNX2, COL1A1, VEGF (p<0.05). An in vitro culture model was set up with rabbit femoral condyles for 4 weeks to compare two methods for necrosis induction: ethanol injection or cycles of freezing/thawing. Both treatments induced a sharp drop of viability without recovery over time in comparison to control group (p<0.005), reduction in RNA yield with poorer A260/280 and A260/230 and RNA degradation, and histological signs of necrosis. In the light of in vitro results, a rabbit model of knee osteonecrosis was set up via ethanol injection. After a month, rabbits were randomly treated with the calcium phosphate material that resulted more promising after in vitro culture, with and without autologous bone marrow concentrate. Histological results after 8 weeks showed in treated groups bone metabolic activity, with the presence of a large number of osteoblasts along trabeculae. Lacunae were found generally filled with osteocytes and bone marrow appeared more reactive and healthy than untreated group, in which classical histopathological signs of bone necrosis were present, with empty lacunae in the trabeculae and signs of substitution of normal bone marrow with adipose tissue. Results suggest that materials tested have promising potentialities for regenerative medicine protocols, open up the possibility to apply a reliable in vitro model with long term bone culture for preliminary therapy evaluations and to successfully set up an in vivo model of knee osteonecrosis, never described in literature.

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Fujifilm Manufacturing provided experimental materials.
Surgical techniques, including marrow stimulation, autografts and allografts and allografts, result in production of fibrocartilage of inferior quality and lack of integration. A recent trend for treating cartilage lesions is based on the application of biomimetic materials. Biomimetic materials retain a unique repertoire of relevant cues involved in tissue regeneration, as chemical composition, physical ultrastructure and biological activity. In the design of these materials, the main challenge is to modulate the host’s reaction to the material, in particular the response to foreign bodies mediated by the macrophages. We think that an effective biomaterial-based strategy requires not only the matching of tissue specific mechanical and functional requirements, but also an in-depth understanding of the interactions between the host innate immune response to the biomaterial of choice. In particular, macrophages can play both beneficial and detrimental roles in tissue remodeling. Polarized macrophages can be classified as: (i) classically activated macrophages M1 and (ii) alternatively activated macrophages M2 (anti-inflammatory). The ability of a biomimetic material to control this switch would improve functional outcome and reduce scar tissue formation toward a better restoration of the native tissue. We study, in full chondral defect rabbit model, the regenerative effect of a porous collagen functionalized with chondroitin sulfate scaffold (CSCL) at early (7 days) and long time points (12 weeks) in order to compare the inflammatory reaction with the regeneration outcome. PCR arrays demonstrated that at early time points CSCL induced the down-regulation of inflammatory and detrimental genes such as IL-B1, TNF-alpha and MMPs which are correlated with post-traumatic osteoarthritis. CSCL induces, instead, the overexpression of anti-inflammatory cytokines such as IL-4 and IL-10. Histological and functional evidences after 12 weeks proved that the anti-inflammatory effect correlated with a better tissue restoration. RNA sequencing highlighted a total different molecular pathway activated by CSCL in comparison with the untreated defect. All together, these data suggest that the platform we propose is able to accelerate the biological processes in the chondral tissue. We believe this study described for the first time a chondral repair approach which aim to tune the inflammatory environment in order to enhance the tissue restoration.

Nature inspired chemistry and small molecules has led to the development in the field of the material science and biomedical engineering as it exhibited unique physicochemical properties. Recently, polyphenols extracted from green tea have been widely investigated, due to their intrinsic properties such as anti-inflammation and radical scavenging. Interestingly, 1,2,3-trihydroxyphenyl group in epigallocatechin gallate (EGCG) could mimic mussel inspired chemistry through oxidative reactions, and generate tissue adhesive nature. In this paper, we report a tissue adhesive and immune modulation hydrogel inspired by the mussel chemistry and polyphenol. We conjugated tyramine (HA_T) and EGCG (HA_E) into hyaluronic acid (HA), and the hydrogel (HA_TE) was fabricated by oxidative reaction using tyrosinase from Streptomyces avermitilis (SA_Ty). With strong oxidative nature of EGCG, the HA_TE hydrogel can be fast formed in a few seconds. We compared HA_TE hydrogel with commercial products (cyanoacrylate and fibrin glue) in the aspects of tissue adhesive and sealants. In the lap shear and burst pressure test, HA_TE exhibited the highest tissue adhesiveness regardless of wetness compared to commercial products. When HA_TE was applied as tissue adhesive into mouse wound closure, and it successfully closed wound and recovered damaged tissue. Additionally, due to EGCG naturally possesses anti-inflammation and minimize host recognition, HA_TE hydrogel produced little inflammatory cytokines in vivo that are comparable to PBS group. This demonstrates that polyphenol based hydrogel might provide a robust platform in the field of both material science and translational medicine.
Ultra violet radiation (UVR) induced skin damage and resultant presentation of skin cancer has grown into a major health problem in recent years. A key challenge for design and development of biomaterials with UV cell protectant features, intended for applications in skin tissue engineering and regenerative medicine is the presentation of UV-absorbing and tuneable bioactive modalities on the material with low impact on human health. In UV induced skin damage, the innate capacity of skin cells to survive, produce collagen matrix to maintain tissue homeostasis is severely hindered. In addition, DNA damage due to the deleterious effects of UVR trigger the formation of metastatic skin melanomas. This gives rise to the need for niche ‘smart’ materials that can provide preventive protection and guide regeneration of damaged skin tissue through the action of UV protective technologies such as natural UV-absorbing molecules known as mycosporines-like amino acids and mycosporines. Collagen from marine-based species such as jellyfish show promise for use in tissue-engineering applications for several years due to their amenability for chemical modifications, solubility, biodegradability and very low toxicity profile towards human tissue. In this study, we present a new engineering approach whereby the mycosporines-like amino acids and mycosporines are covalently bound to collagen (both jellyfish and rat tail) through simple chemistries. This allows the formation of collagen solutions, and films that can be used for the aforementioned applications based on strategies described previously. The resultant materials exhibit highly desirable properties such as chemical stability, water-solubility, thermal responsiveness, mechanical robustness, biocompatibility and offer protection for UVR induced damage in skin cells through the formation of a thin film. Another highlight of these materials is the use of marine mimesis approaches, green and environmentally sustainable methods for the development of the materials with wide ranging medical applications.

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References


Taking inspiration from fascinating biological systems, including the native extracellular matrix (ECM) surrounding the cells of tissues or organs, and making use of the pioneering concept of supramolecular chemistry, scientists have been boosted towards the fabrication of ECM-like supramolecular biomaterials aiming at recreating their dynamic, structural and functional features. Ultimately, those bioinspired artificial systems are intended to restore, regenerate and/or substitute damaged natural tissues and/or organs while avoiding adverse effects for healthy ones.

Although very promising for tissue engineering and regenerative medicine (TERM) purposes, current bioinspired supramolecular systems still lack key features such as control in thickness, composition, and structure, as well as the functional dynamic nature and structural complexity found in natural systems.

In this work, bioinspired supramolecular multilayered biomaterials comprising negatively charged (ALG) biopolymer and oppositely charged self-assembling peptide amphiphiles (K3PA) are successfully developed by combining the self-assembly strategy with the electrostatic-driven Layer-by-Layer (LbL) assembly approach. ALG, an anionic biocompatible polysaccharide extracted from brown algae, is used to trigger the self-assembling capability of oppositely charged K3PA molecule. Furthermore, the prominent LbL assembly approach is successfully employed to build-up supramolecular multilayered biomaterials with precisely controlled composition, structure and function by repeating the alternate deposition of both materials. The build-up process is monitored in situ by the quartz crystal microbalance with dissipation monitoring and the morphological properties analyzed using atomic force microscopy and transmission electron microscopy. The in vitro biological performance is studied using C2C12 myoblast cells, revealing an enhanced cell behavior on the K3PA-ended supramolecular biomaterials. The developed supramolecular biomaterials show immense potential for being used as bioinstructive matrices in TERM strategies, including those in muscle tissue regeneration.

Acknowledgments

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**01-P101** Bioinspired medical adhesives based on tough hydrogels

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Adhesion to wet and dynamic surfaces, including biological tissues, is important in many fields but has proven to be extremely challenging. Existing adhesives are cytotoxic, adhere weakly to tissues, or cannot be used in wet environments. We report a bioinspired design for adhesives consisting of two layers: an adhesive surface and a dissipative matrix. The former adheres to the substrate by electrostatic interactions, covalent bonds, and physical interpenetration. The latter amplifies energy dissipation through hysteresis. The two layers synergistically lead to higher adhesion energies on wet surfaces as compared with those of existing adhesives. Adhesion occurs within minutes, independent of blood exposure and compatible with in vivo dynamic movements. This family of adhesives may be useful in many areas of application, including tissue adhesives, wound dressings, and tissue repair.

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**Bio-inspired adhesive protein-based swellable microneedle patch for regenerative wound closure**

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There is still a significant medical demand for sutureless wound closure to simplify surgical/post-surgical procedures and to improve healing quality due to limited adhesion in wet environments, poor biodegradability, and biocompatibility of existing methods. Herein, inspired by the adhesion mechanism of endoparasitic worms to swell its proboscis for firm attachment to its host's intestinal tissue, we designed a double-layered biodegradable microneedle (MN) patch consisting of a non-swellable silk fibroin-based layer and swellable mussel adhesive protein (MAP)-based tip. With the use of a customized backside vacuum chamber and visible light-activated crosslinking system, a hydrogel-forming double-layered MN patch was successfully fabricated without separation between two layers with different mechanical properties. Because of its inherent surface adhesive property from MAP and its shape change-induced interlocking with tissues upon contact with body fluid after insertion, the bio-inspired MN patch achieved similar tissue adhesive strength to current medical tape on semi-dry porcine skin surface and was superior to the level of adhesion of the medical tape on wet porcine skin surface. In vivo studies demonstrated successful closure/sealing of skin and intestinal wounds with minimal tissue damage and healing. Ideally, the swellable MN patch showed the ability to deliver drugs via both diffusion by swelling and enzymatic degradation. Collectively, our developed bio-inspired swellable and tissue adhesive MN patch can be used in diverse practical applications ranging from as a sealant for vascular and gastrointestinal defects to as a transdermal drug delivery vehicle for treatment of skin diseases such as hypertrophic scars and skin cancer.
Biocompatible interpenetrated scaffolds for wound healing processes with bioadhesive properties

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Recent researches are focused on achieving effective wound healing through tissue engineering strategies, however, there are still limitations such as poor integration with the tissue or the traditional invasive methods used for surgical closures. The aim of this project is the preparation of a new mussel-inspired hydrogel system composed of Chitosan (Ch), oxidized Hyaluronic Acid (HAox) and a synthetic catechol conjugate (VHC) obtaining a resorbable biocompatible and bioadhesive interpenetrated network (IPN), able to adhere to body tissues in moist environments activating the wound healing regeneration process. Highly stable IPNs obtained with natural/synthetic polymers and catechol pendant groups are obtained: Ch/HAox (group 1), Ch/HAox/VHC (group 2) and Ch/HAox/VHC/Fe3+ (group 3). Bioadhesion assay measured on porcine skin using the F2258-05 method from the American Society for Testing and Materials, indicates great adhesion strength due to the presence of catechol groups. Also, direct seeding of fibroblasts and mesenchymal stem cells on the processed films reveals that the 3D extracellular matrix-mimicking network, swellability and porous framework of the hydrogels provides an appropriate microenvironment for cell adhesion, migration and proliferation. Furthermore, high antioxidant and antiinflammatory activities coming from the catechol moieties are found in vitro. Lastly, satisfactory in vivo response of the hydrogels in subcutaneous of Albino Wistar male rats is observed at 1-week post-implantation. A mild inflammatory response is observed and the development of small vessels of focal form is more evident for groups 2 and 3, indicating an early inflammatory reparation process. This can be attributed to the polymer bearing the catechol groups which improves the vascularization. Therefore, this catechol containing IPNs possess good bioadhesion, antioxidant and antiinflammatory activities and demonstrate in vivo biocompatibility, which seem to indicate an enormous potential of application as bioadhesive scaffolds for activate wound healing regeneration.

References


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**01-P106** The role of fibrinolysis inhibition in engineered vascular networks derived from endothelial cells and adipose-derived stem cells

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Microvessels generated by co-cultures of endothelial cells with mesenchymal stem cells currently represent one of the most promising approaches in providing oxygen and nutrient supply for tissue engineering. Specifically, co-culture in a fibrin matrix can be exploited as a system for cell and growth factor delivery to assist cells in situ in regenerating tissues. Still, to translate this model into clinics several in vitro parameters including growth medium and scaffold degradation need to be fine-tuned. We recently described the co-culture of adipose-derived stem cells with endothelial cells in fibrin, resulting in capillary formation in vitro as well as their perfusion in vivo. Here, we aimed to further characterise microvascular tube formation in fibrin by determining the role of scaffold degradation, thrombin concentration and culture conditions on vascularisation. We observed that inhibition of cell-mediated fibrin degradation by the commonly used inhibitor aprotinin and tranexamic acid resulted in impaired vascular network formation. Aprotinin had no effect on laminin and collagen type IV deposition or formation of tube-like structures in scaffold-free co-culture, indicating that poor vascularisation of fibrin clots is primarily caused by inhibition of plasminogen-driven fibrinolysis. Co-culture in plasminogen- and factor XIII-depleted fibrin did not result in different vascular network density compared to controls. Furthermore, we demonstrate that thrombin negatively affects vascular network density at high concentrations. However, only transient activation of incorporated endothelial cells by thrombin could be observed, thus excluding a long-term inflammatory response in tissue-engineered micro-capillaries. Finally, we show that vascularisation of fibrin scaffolds in basal medium is undermined because of increased fibrinolytic activity leading to scaffold destabilisation without aprotinin. This suggests that inhibition of scaffold degradation may have beneficial effects when using co-culture in serum- and growth factor-free conditions, thereby fulfilling an important prerequisite for clinical translation of prevascularised tissues. Taken together, our data reveal a critical role of fibrinolysis inhibition in in vitro cell-mediated vascularisation of fibrin scaffolds.

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**01-P107** Synergistic angiogenic effects enabled by combined release of ion and growth factor

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The safe delivery of ions and growth factors that directly promote the angiogenesis is an important paradigm of tissue regeneration. For this purpose, we designed a system for co-delivery of ion and growth factor to be more synergistic to enhance new blood vessel. Mesoporous silica microcarrier was obtained to have mesoporous structure for loading growth factor and to provide abundant source of the silicon ion. Silicon ion was released in therapeutic range (each time point) and up regulated key angiogenic factors such as bFGF, VEGF, and eNOS by blocking PHD-2 in HUVECs and stabilizing hypoxia inducing factor 1α (HIF1α). Moreover, VEGF was selected to deliver in mesoporous structure of the microcarrier for synergizing the angiogenic effect of the silicon ion with sustained release pattern for several weeks. Furthermore, functions of HUVECs, including cell homing, migration and tubular formation, were significantly promoted by the synergistic effects from the VEGF and silicon ion. Through CAM assay, enhanced new blood vessel formation was observed in VEGF/Si group compared with Si and control group. Conclusively, the combined release of silicon ion and VEGF from biomaterials can be a new approach for angiogenesis and tissue repair.

**Keyword:** Silica, Silicon ion, Angiogenesis, Tissue regeneration,

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Introduction: Chorioallantoic membrane (CAM) of chicken embryos have been used as a model to study angiogenesis in ovo for over 20 years. The paper presents an experimental work to predict the degradation model of two bioinks, sodium alginate with bacterial nanocellulose composite and gelatin based bioink, modified with methacryloil substitution groups. Samples of both materials were processed in multi-head bioprinter following crosslinking process with CaCl2 for the sodium alginate and UV light for the gelatine based bioink respectively. Porous and non porous structures of alginate were compared. Tests at 1,4,24 hours and 3,7,10 days were carried out in cell culture media (SBF), comparing mass loss and characterizing the sample. Significant loss of mass was measured in the first hours of the test. Also, porous structures of alginate with nanocellulose by freeze-dry processing were tested under degradation and compared with the ones made by 3D printing. It was observed that, when the hydrogel was completely dried, the nanocellulose fibrils coalesced, forming a nanocellulose network. The degradation model was parametrized under a formula depending on the time and initial mass.

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Methods: Fertile chicken eggs were incubated for 3 days after which, embryos were transferred to shell-less culture system under sterile conditions. A range of commercially available and pre-clinical scaffolds for skin and bone were applied to the developing CAMs at day 6 post ex ovo. The scaffolds tested included but were not limited to Integra®, Matriderm®, demineralized bone matrix (DBM) and polycaprolactone (PCL). Controls were filter discs soaked in VEGF and PBS. From day 9 to day 12, angiogenesis was examined macroscopically. At day 12, embryos were sacrificed ethically and angiogenesis in stereomicroscope images of different scaffolds was quantified using ImageJ software.

Results: There was a clear trend between porosity and the degree of angiogenesis, where more porous scaffolds showed a higher percentage of vascular area relative to the size of the scaffold. This was also dependent on the composition of the scaffold for e.g., a more porous scaffold made of less angiogenic material like PCL was not as angiogenic as a similarly porous scaffold made of fibrin, which is pro-angiogenic. The data suggests that there is a fine balance between structure and composition that can positively or negatively influence angiogenesis.

Conclusion: The data obtained from this study suggests that CAM assays can be used as a proof of concept for biomaterial testing prior to in vivo animal testing. This ex ovo method is safe and inexpensive compared to the currently used methods such as Matrigel assays or ELISAs which are complex and are far from mimicking the in vivo situation. Importantly, in the light of the principles of reducing animals for research (NC3Rs), we share the views of the authors who recently published a review in tissue engineering suggesting that researchers around the globe should adopt this technique for biomaterial testing.

Nano surfaces have improved the clinical success of implants by increasing the rate at which osseointegration is achieved. However, the modulating effect of these complex surfaces during the early healing is not fully understood at the cellular level. Neovascularization is considered an essential prerequisite to osteogenesis as the mesenchymal progenitors of osteogenic cells have a perivascular origin. Thus, our aim is to examine the effect of nanosurfaces on the spatiotemporal pattern of neovascularization in vivo during peri-implant wound healing.

We developed a new preclinical model that integrates a custom-designed murine cranial metallic implant with an optically transparent window chamber compatible with intravital imaging systems. We tracked the neovascularization and osteogenesis in the peri-implant wound site over clinically relevant time scales, from day 3 to 42 post-implantation as a function of implant topography. The morphology of the re-established vasculature was characterized in 3D over time.

The results show that the microvascular density around the nano surface (NT) was significantly higher than the machined surface (MA) at day 7, 11, and 28 post-implantation. 3D analysis of the vascular network revealed that the nano surface contributes to the development of a radially arranged vascular structure with hierarchical branches spatially closer to the surface of the Ti-implant. Further µCT assessment of the bone formation showed contact osteogenesis in the healing volumes around the nano surface. However, the non-modified MA surface only exhibited distance osteogenesis. Thus, nano surfaces not only increased the rate of neovascularization, but also changed the organization, the spatial pattern, and the architecture of the re-established microvasculature that resulted in a change in the mechanism of peri-implant bone healing from distance to contact osteogenesis. The knowledge transferred from the current study helps to design endosseous implants capable of overcoming delayed peri-implant wound healing under diseased conditions such as hyperglycemia through controlling the neovascular rate and morphogenesis.

Pelvic organ prolapse, a disorder in which the muscles of the pelvic floor are weakened over time, affects over a million women each year in the United States [1]. A quarter of these women undergo a reconstructive procedure, increasingly using polypropylene mesh as mechanical reinforcement to the pelvic floor. However, the number of complications such as chronic pain and mesh erosion/exposure in women with vaginal mesh implants were reported at rates as high as 10-20 % [1]. This indicates a limited understanding of the host response to mesh in vaginal tissue and strategies to reduce these complications.

Utilizing a novel surgical technique in New Zealand white rabbits, we implant mesh using the "gold standard" abdominal sacrocolpopexy procedure and evaluate changes in the immunologic response at early (14 days) and tissue remodeling outcomes at late stages (90 and 180 days) of implantation. The procedure begins with an initial hysterectomy followed by securing two 3 x 12 cm² pieces of mesh along both sides of the vaginal wall. The remaining flaps at the top are then secured to a ligament in the sacral/umbilical space, creating the support to the pelvic organs. Upon closing the incision, mesh is implanted in the abdominal muscle. Both of these implantations of mesh allow for the assessment of the immune response in the pelvic area (relevant for prolapse patients) and in the abdominal area (relevant for translation from hernia repair).

The present study also demonstrates the scaleup of a previous methodology for a nanoscale coating [2]. An ideal mesh would provide mechanical support to the pelvic floor while decreasing the inflammatory response and increasing integration with the surrounding native tissue. The results of this study show that implants into vaginal tissues elicited an increased host inflammatory response at 14 days as compared to those in the abdominal wall. However, at chronic time points the inflammatory response in the vagina was reduced as compared to that in the abdominal cavity.

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Hydrogel group. Simvastatin-conjugated gelatin hydrogel could be a new treatment for OA. In addition, expressions of MMP-13, ADAMTS-5 and IL-1β were decreased while COL2 was increased in the simvastatin-conjugated gelatin hydrogel group. The OA progression was delayed by the intraarticular administration of the simvastatin-conjugated gelatin hydrogel in a mouse OA model.

Conclusion:
Mmp-13 was significantly decreased by the treatment with simvastatin depending on concentration and under the stimulation with IL-1β.

Results:
0.1, 1.0 μM) for 24 hrs. Real-time PCR was performed to examine the expression of Col2a1, aggrecan, Adamts-5, Mmp-13.

Methods:
In vitro: Primary mouse epiphyseal chondrocytes were obtained from 6 day-old mice and the first passage cells were cultured in monolayer for immunohistochemistry.

In vivo: OA was induced by destabilizing the medial meniscus of the knee joint in 10-week-old mice. The mice were divided into four groups. (1) 10% Dimethyl sulfoxide (DMSO) group, (2) Drug-free gelatin hydrogel group, (3) Simvastatin solution group, (4) Simvastatin-conjugated gelatin hydrogel group. The OA progression at each time point was evaluated histologically using the Osteoarthritis Research Society International (OARSI) score. The expression of MMP-13, ADAMTS-5, type 2 collagen (COL2) and interleukin (IL)-1β were examined by immunohistochemical analysis. We found higher expression of wound healing markers (fibronectin and tenascin-C) and inflammatory marker (CD18), and more TUNEL-positive cells (apoptotic cells) in the corneas with non-coated PMMA. There was a higher expression of fibroblast marker (Thy-1) and myofibroblast marker (α-smooth muscle actin) in corneas implanted with non-coated PMMA, confirming the slit lamp observation. The results suggested that the corneas elicited a milder response to the HAp coating than to the PMMA. The milder tissue response and the migration of stromal cells near the HAp after 4 weeks were an indication of the improved biointegration of the HAp-coated PMMA cylinders into the host corneas. A further safety and performance study in non-human primate using clinical KPro that is dipcoated with HAp nanoparticles is now warranted.

Reference:

Intraarticular Administration of the Simvastatin-Conjugated Gelatin Hydrogel Attenuates Osteoarthritis Progression in Mice

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Introduction:
Simvastatin, an inhibitor of HMG-CoA reductase, has been widely used for the treatment of hypercholesterolemia. It has been reported that simvastatin suppresses degeneration of human chondrocytes in vitro. The purpose of this study was to examine the effects of intraarticular administration of the simvastatin-conjugated gelatin hydrogel against OA progression in a mouse OA model.

Methods:
C57BL/6J wild-type mice were used in this study. In vivo: OA was induced by destabilizing the medial meniscus of the knee joint in 10-week-old mice. The mice were divided into four groups. (1) 10% Dimethyl sulfoxide (DMSO) group, (2) Drug-free gelatin hydrogel group, (3) Simvastatin solution group, (4) Simvastatin-conjugated gelatin hydrogel group. The OA progression at each time point was evaluated histologically using the Osteoarthritis Research Society International (OARSI) score. The expression of MMP-13, ADAMTS-5, type 2 collagen (COL2) and interleukin (IL)-1β were examined by immunohistochemical analysis. In vitro: Primary mouse epiphyseal chondrocytes were obtained from 6 day-old mice and the first passage cells were cultured in monolayer for 48 hrs. Chondrocytes were first incubated with 0.1 ng/ml IL-1β for 24 hrs and then stimulated with different concentrations of simvastatin (0, 0.1, 1.0 μM) for 24 hrs. Real-time PCR was performed to examine the expression of Col2a1, aggrecan, Adams-5, Mmp-13.

Results:
The OARSI score was significantly decreased in the simvastatin-conjugated gelatin hydrogel-treated group at postoperative 8 weeks compared with that in the other three groups. The immunohistochemical analysis also showed the expression of MMP-13 and ADAMTS-5, IL-1β in chondrocytes were decreased in the simvastatin-conjugated gelatin hydrogel group compared with those in the other three groups at postoperative 8 weeks, while COL2 was increased. In vitro, the expression of Col2a1 and aggrecan mRNA were significantly increased while Mmp-13 was significantly decreased by the treatment with simvastatin depending on concentration and under the stimulation with IL-1β.

Conclusion:
The progression of OA was delayed by the intraarticular administration of the simvastatin-conjugated gelatin hydrogel in a mouse OA model. In addition, expressions of MMP-13, ADAMTS-5 and IL-1β were decreased while COL2 was increased in the simvastatin-conjugated gelatin hydrogel group. Simvastatin-conjugated gelatin hydrogel could be a new treatment for OA.
**01-P115** Synthesis And Characterization Of Injectable In Situ Enzymatically Serum Hydrogel

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Clinically anti-adhesive therapies are mostly based on hyaluronic acid-based membranes, but it is expensive, incomplete coverage, and uncontrollable mechanical property and gelation time for different patient. In this study, we developed a biocompatible, customized and two-stage cross-linked serum hydrogel through different functional group modification. Unlike other one-step-crosslinked hydrogel, this serum hydrogel still show controllable gelation time, mechanical properties, proteolytic degradation, and drug release after gelation by using the 2nd step crosslinking. This hydrogel retained its integrity in vivo for more than 6 months and was eventually degraded due to hydrolysis. The serum hydrogel exhibited little cytotoxicity and hemolysis, and the acute inflammatory response after implanting the hydrogel was acceptable. The soluble chemotherapeutic doxorubicin (DOX) and salicylic acid was encapsulated into the serum hydrogel and the release profile were controllable by crosslinked degrees and methods. We further employed this hydrogel for tumor treatment, demonstrating significantly higher tumor inhibition efficacy than unloaded hydrogel in the in vitro cells. Furthermore, a mouse model of sidewall defect-bowel abrasion was employed, and a significant reduction of post-operative peritoneal adhesion has been found. Our study synthesizes a two-stage cross-linked serum hydrogel that is easy to use and potentially promising for adhesion prevention and drug release for clinical therapy.
**01-P116 Blood prefabrication subcutaneous small animal model for bone substitute materials evaluation**

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Due to the relative big size, large animal bone defect model is usually required for the assessment of bone substitute material particles, resulting in high cost, complicated operation procedure, ethical issue, difficulties in sample analysis, etc. In this study, we proposed a convenient and effective subcutaneous small animal model to address these issues. To mimic bone environment, conventional subcutaneous model requires the addition of osteogenic factors and stem cells, which make it an expensive model with complex experimental procedure. Blood clot has been reported to provide a nutritionally rich microenvironment for bone defect healing process, including platelets, cells trapped in fibrin and a series of cytokines and growth factors. Therefore, we proposed to use the blood prefabrication subcutaneous model for the in vivo osteogenesis assessment, while avoiding additional growth factors and stem cells. CD29+, CD44+, CD90+, and CD45- mesenchymal stem cells could be detected from the blood cells. Blood also endowed porcine hydroxyapatite (PHA) with a microenvironment enriched with osteogenic factors (BMP2/6, Wnt10b, and OSM), osteoclastic activity related factors (CTSK, MMP9 and TRAP), inflammatory cytokines (IL-1ra, IL-1β, IL-18 and TNF), angiogenic factors (PDGF-α) and fibrogenic factors (TGF-β1 and TGF-β3). The optimized subcutaneous model succeeded in testing the PHA’s osteoinductivity, with the results similar to that of the calvarial bone defect, in terms of osteogenesis, osteoclastogenesis and blood vessel formation. These results collectively imply that blood prefabrication subcutaneous small animal model is convenient and effective for the assessment of the osteoinductivity of bone substitute materials.

**01-P117 Effect of PRP treatment on wound healing in radiation induced skin injury**

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**Aim of the study:** Even with modern radiotherapy techniques, approximately 85% of patients will experience a moderate to severe acute skin reactions in exposed areas. Radiation induced skin injury negatively influences cancer control and prognosis. The aim of the study was to evaluate the effect of platelet-rich plasma (PRP) treatment on radiation-induced skin injury in an experimental animal model.

**Materials and Methods:** The umbilical cord blood was used to prepare PRP. Dorsal skin of SKH-1 mice was gently stretched and irradiated using an X-RAD 320 X-ray irradiator. The examination included clinical observations, barrier function examination, and examination of biopsy specimens taken from sacrificed animals.

**Results:** Digital photographs showed the progress in healing of irradiated skin. The wound size in the PRP group was smaller than that of the untreated groups. In particular, the wounds treated with PRP had almost closed at day 28 whereas the untreated had not closed. Transepidermal water loss (TEWL) is a physiological characteristic to measure the efficiency of the skin barrier. The TEWL value in the PRP group was lower than that of the untreated groups. In particular, the TEWL value in the PRP group had decreased almost linear from day 14. Histological analysis revealed larger amounts of type 3 collagen fibers on the wound bed, indicating the enhancing effect of the PRP on granulation. Next, we evaluated the epithelial cell proliferation in irradiated skin tissues to verify the relevance of epithelial regeneration. Keratin 14 and ki-67 were significantly decreased in irradiated group but upregulated in skin tissues from mice with PRP treatment.

**Conclusions:** Under irradiated conditions, the use of PRP enhances epithelial cell proliferation and granulation in regenerating skin, and raises a possibility for using PRP for the management of acute skin reactions by irradiation.
01-P119  Repair of rabbit radial bone defects using bone morphogenetic protein-2 combined with 3D porous silk fibroin/β-tricalcium phosphate hybrid scaffolds

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Our study aimed to investigate the effect of bone morphogenetic protein-2 (BMP-2) bound to silk fibroin and β-tricalcium phosphate (SF/β-TCP) hybrid on the healing of critical-size radial defects in rabbits. A 15-mm critical-size defect was induced at mid-diaphysis in the left radius of 20 New Zealand white rabbits (average age, 3.5 months; weight, 2.5–3.0 kg). The animals were randomized into Group 1 (SF/β-TCP combined with BMP-2), Group 2 (SF/β-TCP alone), and Group 3 (nothing implanted). Radiographs were obtained every 2 weeks and euthanasia was performed after 8 weeks for visual, radiological, micro-computed tomography (micro-CT), and histological studies. Eight weeks after implantation (SF/β-TCP combined with BMP-2), radiographs showed that new bone formed on the surface of the implant and had bridged the defect in Group 1. Micro-CT imaging also confirmed the formation of new bone around the implant, and the newly formed bone was quantified. Histological examination revealed newly formed bone in the implanted area. Meanwhile, there was no formation of new bone in Group 3. Among the groups, most active formation of new bones was found in Group 1, while there was no difference between Group 2 and Group 3. Based on these results, we concluded that BMP-2-SF/β-TCP showed significant improvement in healing of critical-size defects. Therefore, the combination of BMP-2 and SF/β-TCP would be useful in the field of bone tissue engineering.

Acknowledgments

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**01-P120** Relevance of Hyaluronate Molecular Weight for Regenerative Medicine Applications

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The functional design of the tissue engineering scaffolds and drug delivery systems capable to trigger intended cell actions has been the leading topic for regenerative medicine over the last decades. Inspiration and lessons taken from nature has led to creation of biomimetic structures by implementation and combination of precise surface topography and material composition. Such as combination is capable to determine cell fate and set a defined degradation time and kinetics. And more importantly, the biomaterial composition and the degradation products released may serve as information-rich molecule able to run selected cellular processes. Hyaluronic acid possesses such information richness depending strongly upon the molecular weight and is interesting candidate for drug delivery systems and scaffolds formation. Choice of the chain length and concentration can be then the trigger to start desired actions in terms of wound healing and angiogenesis. In this work we explored effects of five HA molecular weights - ranging from 8 kDa to 1500 kDa, and different its concentrations on wound healing and angiogenesis on human dermal fibroblasts (NHDF), keratinocytes (HaCat) and vein endothelial cells (HUVEC). Effects on cell proliferation and migration and collagen production were studied in vitro. MTT metabolic activity tests, scratch-wound healing assay and tube formation assay were performed. Expression of selected relevant genes was studied using RT-PCR. Obtained results give a coherent picture about importance of hyaluronic acid properties chosen for your system of scaffold construction.
**Evaluation of nanoclay gel mediated Bone Morphogenetic Protein delivery in an ovine femoral condyle defect**

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**Introduction**

Bone Morphogenetic Protein (BMP) has shown significant promise in the stimulation of fracture healing and arthrodesis. However, inefficient BMP delivery methods necessitate the use of supra-physiological doses which have resulted in significant side effects including osteolysis and heterotopic ossification. We have previously demonstrated the ability of an injectable nanoclay gel (Laponite) to facilitate BMP2 mediated ectopic bone formation in a murine model at doses 3,000 fold lower than the currently used clinical dose. The aim of this study is to evaluate the ability of nanoclay gel to facilitate BMP2 mediated bone formation in a large animal model, specifically, the ovine femoral condyle model.

**Methods**

Bilateral defects 8mm diameter, 10mm depth were made in medial femoral condyles of sheep. Nanoclay gel with BMP with or without collagen, and current clinical treatment InductOS were placed in defects and assessed in comparison with autograft and blank controls. Endpoints were bone volume formed within the defect assessed using micro CT (Skyscan 1176) and histology at 10 weeks.

**Results**

Autograft mediated significantly greater bone formation within the defect than blank control, thereby validating our model. Nanoclay gel BMP composites spontaneously set at point of implantation and mediated bone formation within the defect with no adverse effects.

**Conclusion**

We have confirmed this ovine femoral condyle defect model is an appropriate method for assessment of bone formation at a scale relevant to clinical practice. Critically, we demonstrate nanoclay gels were efficacious in mediating delivery of BMP without adverse effects, a finding of great significance to optimization of BMP delivery systems for clinical orthopaedic practice.
CONTROLLABLE PREPARATION OF SB-3CT LOADED PLGA MICROCAPSULES FOR TRAUMATIC-BRAIN-INJURY PHARMACO-THERAPY

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This study is to explore controllable preparation of poly (lactic-co-glycolic acid) (PLGA) microcapsules to load 2-[[4-(4-phenoxyphenyl)sulfonyl)methyl]thiirane (SB-3CT) for traumatic brain injury (TBI) pharmacological therapy. Capillary-based microfluidic method was proposed to prepare SB-3CT loaded PLGA microcapsules. Drug loading and release behavior of the corresponding PLGA microcapsules were evaluated and correlated with their degradation profile. The obtained PLGA microcapsules had a golf-featured morphology and high mono-dispersion. Precise control on the size and size distribution of the microcapsules could be achieved by varying the geometry of the capillary device and operation parameters to yield uniform and reproducible PLGA microcapsules in the range of 35-65 μm. A high drug encapsulation efficiency of 99% was obtained. Pharmacological therapy of TBI was tried by local injection of PLGA-SB-3CT suspension in rats at the trauma site after TBI. The protection on brain tissue upon administration was demonstrated by accelerated behavioral recovery (beam balance and beam walk latencies, and spatial memory ability) and reduction in the neuronal cell apoptosis in CA2 and hilus hippocampus as well as the injury cortical region. Hence, PLGA-SB-3CT could serve as a promising pharmacotherapeutic option for TBI treatment.
Polypropylene mesh is commonly used for tissue repair but is associated with complications. Excised mesh-tissue complexes from patients experiencing complications is characterized by abundant pro-inflammatory macrophages. Macrophages, however, are plastic cell types with phenotypes along a spectrum of pro-inflammatory and pro-remodeling/anti-inflammatory extremes. It has been demonstrated that modulation of phenotype during initial stages of healing could prevent chronic inflammation, improving downstream outcomes.

Previous work has shown that an IL-4 eluting coating for polypropylene mesh initially polarizes macrophages to the pro-remodeling/anti-inflammatory phenotype, resulting in mitigation of the foreign body reaction downstream. However, timing and duration of the in-vivo immunomodulatory release of IL-4 (and its effect on macrophage phenotype transition) is important for timely shift to anti-inflammatory phenotypes and eventual resolution of inflammation. To customize the release of IL-4 in a spatial and temporal way, we aim to use fluorescently tagged IL-4 with live-animal in-vivo imaging in animals implanted with coated mesh. Successful visualization of IL-4 release will be important for eventual varying of release profiles in order to correlate coating patterns to downstream integration outcomes.

IL-4 was subjected to fluorescent labeling with AlexaFluor 594. To provide the most relevant in-vivo release profile, it is important that the fluorescently tagged protein maintain bioactivity. IL-4 polarizes macrophages to a pro-remodeling phenotype with increased arginase-1 production; therefore, tagged IL-4 vs untagged IL-4 was supplemented into the media of naïve macrophages. The in-vitro culture assay showed that tagged and untagged IL-4 produced equivalent levels of increased arginase-1 when compared to macrophages that were cultured in media without supplementation, (i.e., fluorescent tag doesn't affect bioactivity). Finally, tagged IL-4 was loaded into a dermatan sulfate-chitosan layer-by-layer coating of polypropylene mesh using previously established protocols and imaged. Confocal imaging showed a uniform signal in the red channel of mesh coated with fluorescently tagged IL-4, indicating incorporation of the tagged protein into the coating. In-vivo implantation of this tagged mesh will allow daily live-animal imaging of the same animal until loss of signal so that release profiles can be manipulated and then correlated to downstream outcomes.

Silk suture has been widely used for surgical field and regarded as a non-resorbable material. As silk suture is mainly composed of silk fibroin protein, it is slowly degraded by proteolysis with the help of macrophage. 4-Hexylresorcinol(4-HR) is a well-known antiseptic. In this study, the biodegradability of 4HR-incorporated silk sutures were compared to that of untreated silk sutures and polyglactin 910 sutures, a commercially available resorbable suture. 4HR-incorporated silk sutures exhibited antimicrobial properties. Matrix metalloproteinase(MMP) can digest a wide spectrum of proteins. 4HR increased MMP-2, -3, and -9 expression in RAW264.7 cells. MMP-2, -3, and -9 were able to digest not only silk fibroin but also silk sutures. Consequently, 59.5% of the 4HR-incorporated silk suture material remained at 11 weeks after grafting, which was similar to that of polyglactin 910 degradation(56.4% remained). The residual amount of bare silk suture material at 11 weeks after grafting was 91.5%. The expression levels of MMP-2, -3, and -9 were high in the 4HR-incorporated silk suture implanted site 12 weeks after implantation. In conclusion, 4HR-treated silk sutures exhibited antimicrobial properties and a similar level of bio-degradation to polyglactin 910 sutures and induced higher expression of MMP-2, -3, and -9 in macrophages.
**01-P128** Extracellular matrix mimetic injectable hydrogel that stabilize recombinant human bone morphogenetic protein-2 and improve bone formation in vivo

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Designing injectable gels that mimic the natural extracellular matrix (ECM) has been of great interest in the field of regenerative medicine.¹ We have previously demonstrated that hyaluronic acid (HA) hydrogel having hydrazine crosslinkages could be used for efficient delivery of recombinant human bone morphogenetic protein-2 (rhBMP-2) and form bone in vivo within 6 weeks when injected below the rat periostium.² Though rhBMP-2 is very potent for inducing bone formation, recently, extensive debate has taken place on the clinical use of rhBMP-2 since several complications in patients has been observed. This is mainly due to supraphysiological dose that is clinically used since the collagen-based BMP-2 carrier is inefficient and does not stabilize rhBMP-2 in vivo.³ We have devised a new strategy to engineer hydrazine crosslinked HA hydrogel such that it differentially interact with rhBMP-2 and provide different release kinetics of the bioactive protein.⁴ In order to understand the binding affinity between HA modification and the heterodimer structure of rhBMP-2, we performed computational analysis by performing molecular docking followed molecular dynamics experiments. The results of the computational analysis clearly indicated that electrostatic and Van der Waals interactions play a predominant role in stabilizing rhBMP-2 and control its release.⁵ To further understand the significance of protein release on bone formation, we performed in vivo bone induction experiments in a rat ectopic model. The in vitro release experiments corroborated very well with the in vivo experiments, which clearly indicate that improving BMP-2 interactions with HA has major impact in stem cell recruitment and bone induction in vivo. Such a biomaterial design strategy could also be easily adapted to deliver other growth factors for different biomedical applications.


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**01-P129** Spatiotemporal release of growth factors by localized hydrogel embedding and chemical decoration for enhanced vascularization and bone regeneration

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Insufficient angiogenic ability and osteoinductivity are the two main problems hindering the application of artificial bone scaffolds in the critical-sized segmental defects repair. Incorporation of growth factors in bone scaffolds is considered as an efficient strategy to accelerate tissues regeneration. However, how to realize high-efficient loading, controllable release and bioactivity preservation is still a challenging topic for material scientists. In this study, 2-N,6-O-sulfated chitosan (26SCS) and gelatin methacryloyl-based hydrogels (GelMA) were jointly utilized to chemically decorate mesoporous bioactive glass (MBG) scaffold to realize a spatiotemporal release of osteogenic factor of BMP-2 and angiogenic factor of VEGF. Cell culture experiments proved that the scaffolds with spatiotemporal release of BMP-2 and VEGF obviously enhance osteogenesis and angiogenesis in vitro. Unilateral hindlimb ischemia model was used to study the in vivo osteogenesis and angiogenesis and results proved that the scaffold with spatiotemporal release of BMP-2 and VEGF significantly promoted vessel formation and accelerated bone healing. Our study possibly provides some guidance for the design of new bone materials with “intelligent” delivery of growth factors.
Heparin has many beneficial properties that have been researched for use in a range of biomedical applications. Of particular importance to this work is heparin’s ability to bind, stabilise and release a range of growth factors (GFs). Our goal was to covalently incorporate heparin in a poly (vinyl alcohol) (PVA) based hydrogel to enable a more tailored delivery of GFs. Specifically, we aimed to create stimuli responsive hydrogels using hydrozone crosslinking. These gels present a mild, in situ crosslinking strategy that allowed us to encapsulate basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF), which are both known to be heparin binding molecules that synergistically enhance angiogenesis.

PVA-aldehyde, PVA-hydrazide and heparin-aldehyde macromers were synthesised using established techniques. Hydrogels were formed at 10 wt% in aqueous solution (1 aldehyde:1 hydrazide) with varying amounts of heparin (0-1 wt%). Both GFs (25 ng/gel) were encapsulated in PVA only and PVA-heparin hydrogels. The addition of heparin into the gels demonstrated a slower and more controlled release profile, as compared to PVA only hydrogels. In order to demonstrate that the released GFs retained their biological activity, a BaF32 cell assay, whose cellular activities are directly related to the presence of both functional active heparin and GFs was undertaken. It was clearly shown that these cells responded in similar way to the heparin and GFs released from the gels as they do to native heparin and GF, demonstrating retained bioactivity of FGF released from the hydrogels. Released bFGF also promoted human umbilical vein endothelial cell (HUVEC) outgrowth over 24 h and proliferation for 3 days. Released VEGF promoted HUVEC migration but did not significantly modulate proliferation. Dual-growth factor release from PVA-heparin hydrogels resulted in a synergistic effect with significantly higher HUVEC outgrowth compared to either GF alone. In conclusion, this work has shown that PVA-heparin hydrogels can be used for GF encapsulation, that encapsulated GFs retain their bioactivity, and we can achieve controlled release of multiple growth factors from these gels. All of this combines to demonstrate the potential of these gels in tissue regeneration applications.
Heparin-modified Type I-Collagen Gel for Controlled Release of Pleiotrophin for vascular medicine

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Post-implantation vascular graft failure is mainly caused by in-graft thrombosis and intimal hyperplasia. A fast endothelialisation has the benefit of reducing these events. Grafts enrichment with pro-endothelialisation molecules has been proposed as an effective solution. Pleiotrophin (PTN) [1], a secreted cytokine known for its role in angiogenesis, was reported to ameliorate endothelial cells (ECS) viability and migration ability if compared with Stromal Derived Factor 1 (SDF-1), a chemokine known for its role in angiogenesis and already used for improving vascular grafts endothelialisation. To better control the release of PTN, specific non-covalent interactions were used to stabilize and immobilize it within a collagen scaffold, through an affinity delivery system specific to Type I collagen gels. Heparin has been widely used in the formulation of affinity delivery systems due to its ability to sequester, stabilize and protect growth factors and cytokine [2]. Therefore, the objective of this work was to develop a controlled release system for PTN. Type I collagen gels were used as scaffold for the release system. To increase the binding of PTN to the gel and to prolong its release over time, heparin have been freely added to the standard gel formulation. Mechanical assessment and immunofluorescence-based collagen fibers quantification were performed to evaluate the effects on the structural and mechanical properties of the gels after the addition of heparin. PTN-specific ELISA assay has been used to analyse the ability of the heparin-modified collagen gels to bind and released PTN over time in a controlled way. The effects of the released PTN on the viability of Ecs has been evaluated by Alamar Blue Cell Viability Assay and hemocompatibility tests have been performed to analyse the effects of the addition of both heparin and PTN on the hemocompatibility properties of the collagen gels. Results confirmed that the addition of heparin to collagen gels does not modify the mechanical properties of the native gels but helps the release of PTN over time. Moreover, the beneficial properties towards Ecs were preserved, conferring at the same time better hemocompatibility to the collagen gels. In conclusion, PTN-heparin-modified collagen gels demonstrated a clear added value in vascular medicine because of the enhanced biological performances.

References:

A bilaminated decellularized scaffold with the paracrine factors of mesenchymal stem cells for wound healing

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Tissue engineering scaffolds can provide protection and support for wound repair, revascularization and tissue reconstruction. The lots of factors secreted from bone marrow mesenchymal stem cells (MSCs) can accelerate the vascularization and tissue regeneration. There is an urgent need for methods or materials to repair the wound quickly. According to characteristics of the skin structure and wound repair mechanism, a bilaminated decellularized scaffold (CDS) was fabricated with two layers in this study, while the collagen layer can promote the revascularization and be used as the growth factors delivery. MSCs paracrine supernatant fluid by culturing with serum or not was obtained, and used the protein array technology to detect the production of growth factors. It was shown that in the normal wounds and diabetic wounds of SD rats, compared with the control group (treatment the wound with saline) and vaseline gauze treated group, after processing, the combined CDS group significantly lower viscera index, wound healing rate was significantly higher, fibroblast growth factor-2-2 (FGF-2-2) secretion and vascular endothelial cells of the wound were richer after 7 and 14 days, and the wound healing had almost been done after 28 days. Our study demonstrates that the CDS combined with paracrine factors of MSCs can better repair skin wound of normal and diabetic models, and provides a new industrialized product selection for wound repair.

Key words: Mesenchymal stem cells, Paracrine secretion, Decellularized material, wound healing
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References:

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Injectable basic fibroblast growth factor (bFGF)-loaded alginate/hyaluronic acid hydrogel for rejuvenation of aged larynx

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As we enter into an aging society around the world, patients with laryngeal atrophy and dysfunction are increasing. Patients with decreased laryngeal function have symptoms of voice change, dysphagia, and aspiration pneumonia, which not only decrease the patient's quality of life, but also affects their lives. Although injection laryngoplasty has been widely performed for dysfunctions of larynx, it cannot recover intrinsic functions of larynx. Thus, we fabricated an injectable alginate/hyaluronic acid hydrogel loaded with bFGF for inducing rejuvenation of aged laryngeal muscle. We found suitable in situ forming bFGF–loaded alginate/hyaluronic acid hydrogel for injection laryngoplasty by adjusting concentration of materials and identified bFGF release profile from the hydrogel. After 1 month and 3 months of injecting the hydrogel into laryngeal muscle of 18-month-old rat, rejuvenation efficacy of the bFGF-loaded hydrogel was evaluated by qPCR, histology, immune-fluorescence staining and functionality analysis. bFGF-loaded hydrogel induced an increase in expression of myogenic regulatory factor-related genes, decrease of interstitial fibrosis, hypertrophy of muscle fiber, proliferation of muscle satellite cells, and angiogenesis. In addition, bFGF-loaded hydrogel led successful vocal-gap-closure in the functionality analysis using high-speed camera. Therefore, bFGF-loaded alginate/hyaluronic acid hydrogel can be a candidate for laryngoplasty with therapeutic effect for the rejuvenation of aged larynx.

Injectable thermosensitive hydrogel for bone tissue engineering

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We prepared a covalently bone morphogenetic protein-2 (BMP2)-immobilized hydrogel that is suitable for osteogenic differentiation of human periodontal ligament stem cells (hPDLCs). BMP2-covalently immobilized on an injectable hydrogel (MC-BMP2) was prepared quantitatively by a click reaction between alkyne groups on BMP2-OpgY and azide groups on MC-N₃. Thermosensitivity of MC-BMP2 was observed around body temperature. In vivo osteogenic differentiation of hPDLCs in the MC-BMP2 hydrogel was confirmed by histological staining and gene expression analysis. Histological staining of hPDLC-loaded MC-BMP2 implants showed evidence of mineralized calcium deposits, whereas hPDLC-loaded MC-Cl or BMP2-OpgY mixed with MC-Cl implants showed no mineral deposits. Additionally, MC-BMP2 induced higher levels of osteogenic gene expression in hPDLCs than in other groups. In conclusion, the injectable in situ-forming MC-BMP2 may be used for noninvasive administration of therapies for bone tissue engineering.
**Fabrication, characterization and biological activity of poly(vinyl alcohol) hydrogel containing chitooligosaccharides conjugated with gallic acid**

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Acne vulgaris is one of the most common skin disease which almost all teenage suffer from. And, *Propionibacterium acnes* (*P. acnes*) plays a key to induce inflammation leading to acne as well as decrease defense system against oxidative stress. So, the antibiotics, such as the macrolides, tetracyclines, azelaic acid and erythromycin, are used to reduce microorganism proliferation and inflammation. However, the antibiotic treatment has side effects including cytotoxicity, allergic and diarrhea. Therefore, resent studies have focused on the development of alternative antimicrobial materials. We synthesized chitooligosaccharide (COS) with gallic acid (GA) by the hydrogen peroxide mediated method and evaluated antioxidant and antimicrobial activity. Then, we fabricated PVA hydrogel with chitooligosaccharide conjugated with gallic acid (GA-COS) for acne treatment. As a result, GA-COS with 5-10 kDa showed the excellent antimicrobial activity and improved antioxidant activity against *P. acnes* compared with COS. In addition, the PVA hydrogel with GA-COS showed the inhibition effect on intracellular ROS generation and antimicrobial activity than others.

**Osteogenesis of lower elastic modulus titanium alloy surface immobilized with growth factor-containing gelatin nanoparticles through a natural cross-linker**

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Ti-24Nb-4Zr-8Sn (Ti2448) alloy is a new developed \(\beta\)-type titanium alloy with good biocompatibility and mechanical properties, especially the low elastic modulus (approximately 42 GPa) close to nature bone (< 40 GPa). The low elastic modulus decreases the risk of stress shielding effect which may lead to the following bone resorption and orthopedic implant loosening. However, Ti2448 surface is still bioinert, which may result in a slow osteogenesis with surrounding bone. In the study, we used a unique surface modification process, combining the physical (sandblasting), chemical (acid etching and alkaline immersion) and biological (growth factor immobilization) treatments, to improve the osteogenesis of Ti2448 alloy. The gelatin nanoparticles (GNPs), containing vascular endothelial growth factor (VEGF; V), fibroblast growth factor-2 (FGF-2; F) and bone morphogenetic protein-2 (BMP-2; B), were immobilized on roughened Ti2448 alloy surface using natural cross-linker genipin (VFB-GNPs/Ti2448). Besides, the surface characteristics and human bone marrow mesenchymal stem cells (hBMSCs) responses were evaluated. According to ISO 10993-5, the cytotoxicity of VFB-GNPs/Ti2448 was evaluated in vitro. The results showed that the noncytotoxic VFB-GNPs could enhance the surface wettability and hBMSCs responses, including cell proliferation and differentiation, of Ti2448 alloy. The proposed surface modification process had great potential for low elastic modulus Ti2448 alloy in orthopedic applications.
Degradation of S53P4 bioactive glass by osteoclasts

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In vivo degradation of S53P4 bioactive glass (BAG) is slow and this hinders the full regeneration of bone [1]. Little is known about the influence of cells on this process. Literature shows attachment of inactive osteoclast-like cells on BAG surfaces and hindering of osteoclastic differentiation by soluble silica [2,3]. The aim of this study was to gain insight into the role of osteoclasts (OCs) in the degradation of BAG in physiological fluid conditions, where precipitation of calcium phosphate (Ca-P) on BAG is expected [4,5].

Our study shows that mononuclear cells could successfully differentiate into OCs in the presence of the dissolution ions of BAG, evidenced by the observation of multinucleated, TRAP expressing cells surrounded by an actin ring, under fluorescence microscopy. No significant differences were observed in cell size and number of nuclei, compared to control samples that were cultured in equal conditions, but in absence of BAG dissolution ions. Using SEM, resorption pits were observed on smooth BAG discs that were pre-soaked in PBS to develop a Ca-P layer prior to cell seeding. EDS could not show a clear silica content in the resorption pits, indicating incomplete resorption of the Ca-P layer. The resorption pits observed on the BAG discs showed a very smooth and uniform surface, in contrast to the rough resorption pits observed on control hydroxyapatite discs. These smooth surfaces suggest that the OC was hindered to resorb further, probably by the silica underneath the Ca-P, as observed previously [2,3]. However, re-growth of Ca-P on the surface during the sample preparation for analysis could not be ruled out.

We observed active OCs on pre-soaked BAG, suggesting that they can play a role in the degradation of the Ca-P surface layers but not on the BAG material itself. Although the role of in vivo degradation of BAG by OCs may be different, hindering by silica might be a reason for OCs to play only a minor role in the in vivo degradation of BAG.

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**01-P141**  In vivo evaluation of a novel bioactive bulking agent for the long-term treatment of stress urinary incontinence

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Stress urinary incontinence (SUI) is involuntary leakage of urine due to normal daily life activities. The primary cause of SUI is the weakness of the pelvic floor muscle complex and/or of the urethral sphincter muscle complex, caused by mechanical trauma during childbirth or age-related hormonal changes. It causes a great deal of distress and embarrassment, as well as significant costs. The use of vaginal slings and injectable bulking agents are two of the common treatment modalities for this condition. They either reposition the urethra and strengthen the pelvic floor muscles or re-establish urethral outlet resistance, both improving closing of the urethra responsible for SUI upon increased intra-abdominal pressure. However, due to risk of infection, irritation, inflammation, and urine retention, the performance of vaginal sling procedures is still under discussion [1]. The use of injectable bulking agents is preferred due to its less invasive nature. Currently, there is no commercially available bulking agent, providing functional tissue regeneration to overcome SUI. In this study, a novel collagen-fibrin based bioactive bulking agent has been tested in a rabbit bladder model. The bioactive fibrin micro-beads were functionalized with a recombinant insulin-like growth factor-1 (α₂PI,α₂MMP-IGF-1) using a droplet microfluidic system. Preliminary results showed that our bioactive bulking agent triggered neo-smooth muscle tissue formation at the injection site 3 months after the surgery. Therefore, this new bioactive bulking agent might offer functional regeneration of urinary tract smooth muscle tissue for long-term treatment of stress urinary incontinence.

Reference:

Acknowledgments:
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Evaluation of ADSC's Ability to Trans-differentiate into Chondrocyte from Gellan Gum Hydrogel Scaffolds with Platelet-rich plasma

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Platelet-rich plasma (PRP) is blood plasma that has been enriched with platelets and it is used as a non-surgery treatment for treating cartilage damage. PRP's platelet contain a number of proteins to promote chondrocyte differentiation and protection such as TGF-β, IGF, PDGF. In this study, PRP were compound with 2% Gellan-gum to make scaffold for chondrocyte regeneration. Incorporation PRP with Gellan-gum, investigate the possibility of trans-differentiation of ADSCs to chondrocyte and examine the effect of PRP ratio on cartilage regeneration. Cells were compound with scaffolds before it were harden. Evidence of cartilage regeneration was found in 2 weeks. PRP-GG scaffolds were analyzed using scanning electron microscope (SEM), compressive strength, MTT assay for cell proliferation, RT-PCR for expression of mRNAs and histological analysis, etc. Rabbit adipose derived stem cells were used In vitro biological compatibility. In results, Gellan-gum loaded 20% PRP have the highest proliferation under the same conditions. Furthermore, at the RT-PCR result, PRP showed the trans-differentiation tendency of ADSCs to chondrocyte. Thus, the Gellan-gum with PRP scaffolds can be used as a substitute for trans-differentiation to chondrocyte.

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Titanium Pins Coated with FGF-2-Apatite Composite Layers for External Fixation

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Fibroblast growth factor (FGF)-2-apatite composite layers were formed by coprecipitaiton of FGF-2 with apatite on titanium screws by immersing the screws in supersaturated calcium phosphate solutions containing FGF-2. The titanium screws coated with the FGF-2-apatite composite layers were implanted percutaneously in rabbit tibia. The coated screws highly prevented bacterial infection at the interface between the screw and skin tissue, and were associated with improved bone formation mechanically stabilizing the screws. In addition, interfacial soft tissue bonded to the FGF-2-apatite composite layer was not a fibrous connective tissue but a tissue having structural similarity to the periodontal membrane around a tooth that is rich in blood vessels and Sharpey's fibers. This periodontal membrane-like tissue could contribute to the prevention of bacterial infection. Then, the rate of bone apposition to screw surfaces was evaluated histomorphometrically. On average, screws coated with FGF-2-apatite composite layers showed a significantly higher bone apposition rate than the uncoated or apatite-coated screws. However, the FGF-2-apatite composite layers have a much more marked effect on reducing the incidence of impaired bone apposition than on enhancing the bone apposition rate. Weibull plot analysis revealed that the risk of impaired bone apposition was >100 times lower for screws coated with the FGF-2-apatite composite layers than for screws free of FGF-2 (uncoated screws plus apatite-coated screws). An initial clinical trial of external fixation treatment using titanium pins coated with the FGF-2-apatite composite layer commenced at the University of Tsukuba Hospital.
**01-P144** Use of low molecular weight fucoidans, heparan-mimetics from brown seaweed, to design bioreactive bone substitute

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Periodontitis or peri-apical pathologies usually lead to teeth loss and alveolar bone crest resorption; a major problem for maxillofacial recovery. In periodontal surgery, autologous bone graft is regarded as the best procedure to resolve bone defect. Unfortunately, use of autologous material is limited in volume and surgical procedures increase operating time, patient pain and risks of donor site morbidity. Thus, clinicians have turned toward purely mineral materials or organo-mineral materials such as human highly purified bone matrix (hpBM). hpBM could be an effective remedy owing to their mechanical properties, their porosity close to human bone and their ability to promote cell adhesion on a preserved collagen network. However, faster bio-integration of freezed bone allograft than hpBM is observed. Indeed, hpBM preparation process maintains its mineral fibrillar collagenous matrix but eliminates most of other bone matrix proteins and its capability to regulate growth factors bioavailability. Low Molecular Weight (LMW) fucoidans are sulfated polysaccharides from brown seaweed which are heparan-mimetics, promoting and protecting growth factors. Their polyanionic nature allows electrostatic interaction with polycationic collagen bundles. So the use of fucoidans would be interesting to restore the hpBM capability to trap growth factors. Our previous works demonstrated that LMW fucoidans promote colonization of xenogenic hpBM by human osteoblasts1.

In this study, we used human medullar stromal cells (hMSCs), potentially comprising mesenchymal stem cells, cultured in humanized osteogenic media containing human platelet lysate, β glycerophosphate and ascorbic acid-2 phosphate to seed Fucoidan bioactivated hpBM. Our results show that LMW fucoidans promote osteoblastic differentiation of hMSCs as shown by a stronger alkaline phosphatase expression and a precocious extracellular matrix mineralization. After 20 and 30 days of culture, scanning electron microscopy shows that Fucoidan bioactivated hpBM were deeper, faster and more importantly settled by MSCs than non bioactivated biomaterials. Our results also show a more important synthesis of osteoid layer at the surface of fucoidan bioactivated hpBM trabeculae. Thus, our results show that the collagen matrix within hpBM could be advantageously used to interact with LMW Fucoidans in order to design a bioreactive biomaterial with faster in situ integration.

Reference:

**01-P145** Cell Response to Zirconia Surface Immobilized with Type I Collagen Using Natural Cross-linker for Dental Implant Applications

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Zirconia (ZrO₂) dental implant has good biocompatibility and corrosion resistance, and its color is similar to the natural tooth. However, ZrO₂ surface still belongs to bioinert, which may not achieve osseointegration rapidly. In order to improve the bioinert character of ZrO₂ surface, suitable surface modification is very necessary. However, very limited information on the surface modification of ZrO₂ is available in the literature. The aim of this study was to enhance human bone marrow mesenchymal stem cells (hMSCs) responses to ZrO₂ through surface biomolecule immobilization. In this study, ZrO₂ surface was roughened using sandblasting (designated ZS group). ZS was immersed in alkaline solution for pretreatment (designated ZSA group). The ZSA was immobilized respectively with the natural cross-linker, procyanidin (designated P group), type I collagen (designated C group), and mixture of procyanidin and type I collagen (designated P/C group), respectively. Results showed that type I collagen was successfully immobilized on ZrO₂ surface. Comparing with C group, P/C group surface presented higher quantity and stability of type I collagen. For cell responses, all the test groups were potentially non-cytotoxic. Among the test groups, P/C group showed the best cell adhesion, proliferation and differentiation of hMSCs. In this study, we provide a simple and novel method to immobilize type I collagen on bioinert ZrO₂ surface for dental implant applications and expect that this surface treatment has great potential in clinical applications.
Herein, we report synthesis and characterization of a novel terpolymeric hydrogel consists of sodium hyaluronate, 2-hydroxyethyl acrylate, and poly(ethylene glycol) diacrylate for bioactive molecules release and bone tissue engineering applications. To accomplish elasticity, well-mannered porous architecture with sufficient gel strength, 2-hydroxyethyl acrylate has been first grafted onto hydroxyl groups of hyaluronate via free radical polymerization using potassium persulphate as initiator, and then crosslinked grafted network using diverse amounts of poly(ethylene glycol) diacrylate. The structure and compositions of the synthesized terpolymer have been verified by different physio-chemical analyses. The terpolymeric gel showed pH-dependent release of bioactive molecules such as DMOG as a bioactive molecule for osteogenesis and angiogenesis, and TCN as an antibiotic at 37° C. The in vitro cell study results ascertained that the prepared gel supported excellent osteoblastic MC3T3 cell adhesion, proliferation, and viability, which are stimulated by interconnected porous structure and 3D network of the gel. The H&E, and MT staining results of the in vitro cell cultured samples confirmed that the native gel itself provides excellent environment for the regeneration of extracellular matrix and collagen, even in absence of any external bioactive molecules after 3 weeks. Finally, the novel biocompatible gel could be employed as matrix for DMOG and TCN delivery, as well as in bone tissue engineering applications.

References:
01-P149  The effects of silk proteins on osteoblast differentiation

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Silk is a natural macromolecule obtained from silkworms and can easily obtain a large amount of pure protein. It is known that silk protein supports effective cell proliferation. In this study, we investigated the effect of silk protein on osteoblast differentiation. Silk proteins obtained from five silkworm varieties were separated into sericin and fibroin, respectively. The silk proteins were treated during stem cell culture or osteoblast differentiation. As a result, the differentiation inducing effect was higher in fibroin than sericin, and in white cocoon than in colored cocoon. Also, the effect on silk protein treatment time was higher during stem cell culture than at the time of differentiation. But considering economical efficiency, it is effective to treat silk proteins at the time of differentiation. We expect that silk proteins could be used as an alternative to expensive osteoblast differentiation inducers.

01-P149  Protective effects of platelet gel on radiation induced salivary gland cell damage

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**Background:** Radiation therapy used in the management of head and neck malignancies causes complications such as severe salivary gland (SG) dysfunction and xerostomia, and it is necessary to have an effective treatment strategy. Platelet gel is defined as autologous platelet derivatives with a platelet concentration higher than baseline. Platelet gel is widely used in different areas of Regenerative Medicine in order to enhance wound healing processes. In this study, we confirmed the effect of platelet gel on SG cell dysfunctions by radiotherapy.

**Materials and Methods:** The platelets were separated from the blood of male SD rats (220±20 g) and activated with collagen (2 ug/ml) to obtain platelet gel. The proliferation rate of human primary SG cells treated with/without 5% platelet gel was checked by CCK8. The amount of PDGF-AB, TGF-β in platelet gel, the superoxide dismutase (SOD) and amylase activity of cell supernatant were determined by ELISA. Histologic examinations and TUNEL (Terminal Deoxynucleotidyl Transferase Biotin-dUDP nick and labeling) assay of cell block were performed.

**Results:** The proliferation of SG cells was significantly reduced by irradiation at 2 and 7 Gy. In addition, the proliferation rate of SG cells treated with 5% platelet gel after irradiation was significantly higher than that of untreated SG cells. The SOD activity in platelet gel treated SG cells was higher than in untreated SG cells. As a result of immuno-histochemistry, the expressions of pP21 and 8-OHdG were reduced in the platelet gel treated SG cells compared with the untreated SG cells. In addition, the number of apoptotic cells was markedly decreased in platelet gel treated SG cells, compared to the untreated SG cells.

**Conclusion:** Treatment with platelet gel right after radiation therapy could be used protective agent against radiation induced SG dysfunction.
Breast cancer is one of the most common cancers affecting women’s life often resulting to partial or full mastectomy. Elective breast augmentation is an option for patients looking to restore their emotional well-being, but complications related to foreign body reaction causing capsular contraction, rejection and rupture persist due to the inert nature of traditional implants. Breast tissue is composed of a large volume of highly vascularized fat (37%) which presents constraints from a regenerative perspective. Current clinical treatment options include harvesting autologous fat from a secondary site in the form of a free vascularized flap or injectable fat are available options for a select patient population which is extremely invasive resulting in donor site morbidity or necrosis. Therefore, researchers have been motivated to employ tissue engineering strategies in an effort to regenerate breast tissue. In this study, a patient specific biodegradable implant was developed to support autologous fat tissue within a porous structure while degrading and actively providing volume for mature tissue development. Based on our previous work related to delayed fat injection, the composite scaffold provides a structural bed of capillary formation prior to fat injection while maintaining structural integrity for long-term tissue remodelling and regeneration. Through the combination of multi-material 3D printing and finite element modelling topology optimization, a new shell-based scaffold design exhibiting tunable mechanical properties as well as degradation rate is addressed. A series of medical-grade monofilaments were evaluated and a design library of scaffold topologies were characterized via finite element modelling to optimize the external and internal scaffold geometry. In addition, a channel structure was designed, optimized, and embedded into the scaffold to serving as a network for even distribution of injected fat.
Graphene Oxide/Chitosan Scaffold As An Electrically Conductive Intermediary Applied In Cardiac Tissue Engineering

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Abstract—Myocardial tissue engineering was considered to be the most expectant therapy for the treatment of myocardial diseases. Superior scaffold not only has the potential to provide growth support for cardiomyocytes but also is beneficial for promoting the transmission of electrical signals between cardiomyocytes, forming a continuous electrical conduction system. Here, we prepared conductive scaffolds with porous structure and electrical conductivity composed of chitosan (CS) blending with graphene oxide (GO) for cardiac tissue engineering. We found that the swelling, porosity, and conductive properties of GO/CS scaffolds could be modulated via adjusting the ratio of graphene oxide to chitosan in our study. More importantly, GO/CS scaffolds conductivity ($\approx 0.7 \times 10^{-5}$ S·cm$^{-1}$) fell in the range of reported conductivities for native myocardium tissue and had swelling ratio ranging from 23.20 to 27.38 (1000%). Furthermore, we assessed their biological activity by seeding H9C2 cells in GO/CS scaffolds. Our data showed that these GO/CS scaffolds exhibited good cell viability, promoted cell attachment and extracellular matrix formation, upregulated expression of the cardiac specific gene and protein involved in muscle conduction of electrical signals (inconnexin-43). Overall, it is concluded that the GO/CS scaffolds promoted the properties of cardiac tissue constructs, demonstrated an ideal scaffold for cardiac tissue engineering.

Cell-Seeded Synthetic Scaffold for Esophageal Regeneration

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Esophageal diseases may require resection of the damaged portion. Current standard of care requires the replacement of the esophagus with stomach or the intestine. Such procedures have high rates of mortality and morbidity and highly affect the quality of life of patients. The use of alternative conduits is needed. A tissue engineering approach that allows for the regeneration of esophageal tissues would have significant clinical application. In this study, we describe a bioengineered construct that is comprised of a synthetic scaffold laden with autologous cells that can be surgically implanted to guide regeneration of the esophagus. The tubular synthetic scaffold was created with electrospun polycarbonate-based polyurethane. This was designed to provide a microenvironment conducive to cellular proliferation, with special attention given to the morphological properties, microstructure characteristics, and surface chemistry. In our preclinical model, autologous adipose-derived mesenchymal stem cells were isolated, expanded, and seeded on the scaffold. The 6 cm scaffold was implanted in big animal models in place of a 5 cm circumferential resection of the esophagus. Functional, biochemical and histological techniques tracked host tissue growth and stability. The electrospun scaffolds were compromised of smooth, randomly oriented fibers. The mechanical analysis results ensured that the scaffolds can withstand the physical forces applied during surgery and esophageal remodeling. In vitro, the construct dependably carried metabolically active cells that released bioactive molecules supportive of surgical repair and restoration of esophageal function. In vivo trials resulted in tissue growth that were observed to reconstitute the esophagus with a high degree of continuity and integrity after circumferential full thickness surgical resection. Progressive esophageal regeneration, mediated by a cellulosized scaffold, was observed over the course of this study, as a result from these animals showed gradual structural regeneration of endogenous esophageal tissue layers, including squamous esophageal mucosa, submucosa, and blood vessel formation. The results demonstrate the feasibility of this approach to facilitate the regeneration of full thickness circumferential defects after esophageal resection as would be clinically required for esophageal malignancy.
Biofunctionalization of mechanoinducing scaffolds with multi-layered cell sheets for the advanced bone tissue engineering

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Currently, there are an enormous variety of methods and biomaterials used for fabrication of bone-tissue substitutes. However, optimization of their properties aiming to improve functional and structural restoration of a damaged tissue is still an urgent task. In this study we aimed to produce the hybrid tissue-engineering constructs able to reproduce the phenotype and biomechanics of bone tissue in its native state. The constructs were made with biocompatible, biodegradable, mechanoinducing scaffolds and multi-layered cell sheets. The scaffolds were produced from hyaluronic acid-coated polylactide microparticles using surface-selective laser sintering method. Hyaluronic acid coating enabled wetting of the particles with water that, consequently, served as the photosensitizer. Finally, the scaffolds were reinforced by the treatment with Irgacure 2959 photoinitiator followed by UV irradiation. Resulting scaffolds were thoroughly characterized in terms of structural and mechanical properties, biocompatibility and mechanoinduction using a set of cutting-edge materials testing techniques. It was shown that surface mechanics of the scaffolds dramatically varies depending on the temperature, presumably, due to thermolabile properties of the scaffold polymer which promote swelling of the surface due to water absorption. The scaffolds had porous structure and rough surface providing successful adhesion and expansion of bone marrow mesenchymal stromal cells both from single-cell suspension and cell sheets. Additionally, it induced calcium deposition by the cells associated with osteogenic differentiation. Grafting the mechanoinducing scaffolds with a functional multi-layered biointerface is an innovative and highly promising approach to facilitate host tissue-implant integration.

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Hydrogel mediated relationship between local inflammation and osteogenic capability

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INTRODUCTION

In literature, it is widely reported that during the bone healing process a local inflammation is functional to the tissue repair via the stimulation of angiogenesis, cellular differentiation and remodeling phenomena. In case of a major bone injury which requires a surgery procedure and a bone graft in order to fill the fracture’s gap, the inflammation plays a triple role: i) it is essential to promote the bone’s repair; ii) it becomes necessary to avoid compromising surgery-derived infections and iii) if not regulated, could lead to graft rejection with consequent serious complications. Due to these reasons, the research is developing increasingly better biomaterials that do not harm the host and are as much as possible biocompatible and bio-absorbable, but something more could be done. The implementation of anti-inflammatory drug, loaded into the biomaterial, is proving to be a good option to modulate the in situ inflammatory response, granting a balance between the two pro-inflammatory-needed situations and the anti-inflammation-needed implant rejection avoidance. Within this concept, the present study developed a biomimeralized composite hydrogel, based on chemically-modified hyaluronic acid and in situ synthesized hydroxyapatite, loaded with Diclofenac Sodium (DS), a well-known anti-inflammatory drug. The aim is to modulate the inflammatory response while maintaining the osteogenic capacity of cells, also enhanced by material properties. The composite hydrogels were loaded with the DS and then seeded with mRAW macrophages in order to observe the effects of drug on pro and anti-inflammatory markers, cells viability and morphology. Then, for mMC3T3 osteoblasts the conditioned media (media + cytokines produced by macrophages) was used to evaluate the effect of released cytokines on the expression of specific markers involved in osteogenesis (OPN, RUNX, ALP, OCN) by RT-PCR. A correlation between the DS’ effects on the inflammation-related molecules and the retaining of the osteoblasts’ osteogenic capability was successfully demonstrated.

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**01-P156**  
**Ovine Tendon Collagen Type I: A Rapid Treatment of Full Thickness Skin Loss**

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Full-thickness skin wound is a common skin complication affecting millions of people worldwide. Delay in treatment resulted loss of skin function and integrity that could lead to the chronic wounds or even death. This study aimed to develop a rapid wound treatment modality using ovine tendon collagen type I (OTC-I) bio-scaffold with or without non-cultured skin cells. Genipin (GNP) and carbodiimide (EDC) were used to crosslink OTC-I scaffold to improve its mechanical strength. The physicochemical, biomechanical, biodegradation, biocompatibility and immunogenicity properties of OTC-I scaffolds were investigated. The efficacy of this treatment approach was evaluated in an in vivo skin wound model. The results revealed that GNP cross-linked OTC-I scaffold (OTC-I_GNP) demonstrated better physicochemical and mechanical properties compared to EDC cross-linked OTC-I scaffold (OTC-I_EDC) and non-crosslink OTC-I scaffold (OTC-I_NC). OTC-I_GNP and OTC-I_NC unraveled no cells toxicity as it promoted higher cell attachment and proliferation of both primary human epidermal keratinocytes (HEK) and human dermal fibroblasts (HDF) compared to OTC-I_EDC. Both OTC-I_GNP and OTC-I_NC exhibited an ability as a template to form bilayer-like skin structure in vitro. No sign of immunogenic response was detected in vitro and in vivo among all OTC-I bioscaffolds. Finally, implantation of OTC-I_NC and OTC-I_GNP scaffolds with non-cultured skin cells demonstrated enhanced healing with superior skin maturity and microstructure features, resembling native skin than other treatment groups. Thus, aforementioned findings suggested that both OTC-I scaffolds could be a potential candidate for the rapid treatment of full-thickness skin loss.

**01-P157**  
**Tough Tissue-adhesive Hydrogel with Enhanced Tissue Integration and In-situ Forming Capability for Osteochondral Defect Repair**

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Articular cartilage repair still remains a major challenge in today’s clinical practice due to its poor self-healing capacity. Therefore, it is essential to develop a promising strategy to achieve a one-step cartilage repair for patients suffering from joint diseases and traumas. Inspired by the complex composition and microscopic architecture of native articular cartilage, an injectable hydrogel with tough mechanical property and strong tissue adhesiveness is developed. All hydrogel material compositions are natural polymers or their derivatives resemble the normal cartilage extracellular matrix (ECM). This hydrogel showed superior tunable mechanical properties (mechanical strength ≈ 270 kPa, compressibility ≈ 70%) and rapid recovery ability. More remarkably, it exhibited strong adhesiveness and enhanced integration with tissues. Further studies demonstrated that the hydrogel had good biocompatibility and superior performance in cartilage defect regeneration. Thus, the presented hydrogel is a promising biomaterial for clinical cartilage regeneration and other biomedical applications.
**New designed scaffold based on human umbilical cord extracellular matrix and hyaluronic acid for applications in tissue engineering and regenerative medicine**

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Worldwide, a large number of surgical procedures to replace or repair tissues damaged by disease or trauma are performed every day. The emerging field of tissue engineering aims to develop new possibilities for regeneration of such tissues. This strategy often combines use of cells and suitable biomaterials that serve as matrix for their cultivation.

For this purpose, extracellular matrix (ECM) derived from native human tissues has been recently considered for clinical use and been extensively studied. The advantages of ECM include their full biocompatibility and biodegradability, importantly, this material contains endogenous bioactive molecules that support cell adhesion, growth and proper function leading to production and growth of the new tissue. ECM scaffolds can be derived from a variety of tissues (e.g. skin, bones, articular cartilages, skeletal muscles, blood vessels, heart valves, neural tissue, urinary bladder, placenta, umbilical cord etc.) and prepared in different forms including meshes, hydrogels, sheets or powders.

In this study, we focused on development and characterization of newly designed hydrogel scaffold based on human umbilical cord ECM (UBC-ECM) combined for enhancement of hydrogel mechanical properties with hyaluronic acid - specifically tyramine derivative of hyaluronic acid (TYR-HA). TYR-HA can undergo noncytotoxic crosslinking reaction initiated by H₂O₂ and mediated by horseradish peroxidase. Importantly, this combination allows the formation of a hydrogel under physiological conditions in the presence of cells without reducing their viability. Moreover, this polymerization strategy enables injection applications and hydrogel formation directly in the targeted site.

Our results suggest that the novel UBC-ECM / TYR-HA hydrogels provide suitable environment for various type of human mesenchymal stem cells cultivation and proliferation as well as stem cell phenotype and differentiation ability maintenance. Moreover, presence of the covalently crosslinked interpenetrating hyaluronic acid network significantly enhanced rheological and mechanical properties of the material which can be further optimized and tuned up by concentration of crosslinking agents and modification of crosslinking reaction conditions.

Altogether, our results indicate that UBC-ECM / TYR-HA hydrogels represent promising material for applications in various fields of tissue engineering and regeneration medicine.

**Double layered hydrogels with 3D textile scaffold for cartilage repair**

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Articular cartilage has a complex depth-dependent microstructural organization, mechanical properties, and biochemical composition. Damaged cartilage cannot be repaired by itself due to the lack of blood vessels and cells. Hydrogels have been considered as a suitable material for cartilage repairs since they have many advantages over other materials. However, the low compression strength limited its use for cartilage replacement. In this study, double layered hydrogels with 3D textile scaffold have been fabricated to improve the mechanical strength of hydrogel. The top layer of hydrogel has a compressive modulus of 500kPa. The compressive modulus of the bottom layer of hydrogel has been reinforced with the 3D textile scaffold to have a similar level of the compressive modulus of the top layer. In the bottom layer of hydrogel, the differentiation of mesenchymal stem cells into chondrocyte was observed.
Free-directional printing of novel nanocomposite into self-healing support hydrogel for skeletal biofabrication

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Manufacturing technologies for tissue engineering are rapidly evolving. However, cell printing remains limited in the size and functionality of tissue replacements that can be generated due to (i) resolution, (ii) bioinks elevated polymer content and (iii) limited vertical stacking of printed layers. In the current study, we set out to develop a modified alginate, which is amenable to thiol-ene functionalization and crosslinking via exposure to low amounts of UV light. These new alginate formulations are printable at much lower concentrations (<2%), show tunable mechanical properties (from <500 Pa to >5000 Pa), and enhanced cell viability as compared to traditional alginate printing. Furthermore, these gels can be straightforwardly functionalized with thiol containing biomolecules (e.g. peptide mimics, growth factors, MMP cleavage sites), allowing for extensive customization of the chemical and mechanical properties of a printed scaffold. Showing the utility of this material, cell-laden constructs of up to 25 layers can be printed with good cell viability, and inroads to the creation of interpenetrating and gradient (chemical and mechanical) scaffold geometries have shown promising initial results. With simple, straightforward, and reliable chemical functionalization, a modular hydrogel system has been developed with a wide range of potential applications, lowering the barrier to innovation in the 3D printing of soft tissue constructs.

Thiol-ene alginate as a versatile bioink for bioprinting of cell laden constructs

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Cell-laden bioprinting remains one of the most promising techniques to allow fast, reproducible, and accurate creation of 3D organ or tissue like constructs. However, the limited availability, tailorability, reproducibility, and performance of cell-compatible and printable hydrogels, often termed “bioinks,” has significantly hindered progress in this field. Printing of cells encapsulated in synthetically modifiable hydrogels, or hydrogel precursors, provides a realizable printable platform allowing for tunable materials properties, tunable bioactivity, and active or passive degradation. Towards these goals, we have developed a modified alginate, which is amenable to thiol-ene functionalization and crosslinking via exposure to low amounts of UV light. These new alginate formulations are printable at much lower concentrations (<2%), show tunable mechanical properties (from <500 Pa to >5000 Pa), and enhanced cell viability as compared to traditional alginate printing. Furthermore, these gels can be straightforwardly functionalized with thiol containing biomolecules (e.g. peptide mimics, growth factors, MMP cleavage sites), allowing for extensive customization of the chemical and mechanical properties of a printed scaffold. Showing the utility of this material, cell-laden constructs of up to 25 layers can be printed with good cell viability, and inroads to the creation of interpenetrating and gradient (chemical and mechanical) scaffold geometries have shown promising initial results. With simple, straightforward, and reliable chemical functionalization, a modular hydrogel system has been developed with a wide range of potential applications, lowering the barrier to innovation in the 3D printing of soft tissue constructs.
**Engineering nanotopographically-defined eggshell membrane platforms for biomedical applications**

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Eggshell membrane (ESM), one of the agricultural bioresources, is being recognized as emerging engineering platforms in various fields including agriculture and biological engineering. Eggshell membrane is the protein-rich membrane between the eggshell and egg white, having interesting characteristics such as structural, chemical, and physical properties. However, the raw ESM has many limitations, which raised some problems for its wide use as commercial engineering platforms. Here, we present new ESM-based engineering platforms based on nanotechnology. To develop the electrically and nanotopographically-enhanced ESM platforms, we used graphene, one of the carbon-based nanomaterials, that provides unique electrically and nanotopographically-defined cues to the raw ESM. The graphene-ESM hybrid platforms showed hierarchically micro- and nanoscale structures (i.e., microfibers from ESM and nanosheets from graphene). It is also found that the graphene-ESM hybrid platforms could enhance the electrical and mechanical property compared to the raw ESM. Our approach can enable the design and manipulation of ESM-based functional engineering platforms for biomedical applications.

References:

Acknowledgments:
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Photo-click Thiol-ene Gelatin Based Hydrogels as Bioinks for Bioprinting

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Radical chain-growth polymerization, such as methacryloyl chemistry has been widely employed in 3D bioplotting of cell-laden hydrogel constructs1. However, this chemistry is known to be susceptible to oxygen inhibition, where oxygen in a normoxic environment is able to scavenge propagating radicals, resulting in incomplete crosslinking which negatively influences construct shape fidelity. Furthermore, a heterogeneous network is often formed due to the lack of control over the chain propagation process. Therefore, the aim of this study was to develop novel gelatin based bioinks based on step-growth thiol-ene chemistry, which offers more spatial control over the hydrogel network formation, and is potentially more suitable for 3D bioplotting.

Gelatin was conjugated with two types of functional alkene groups: allyl (GelAGE) or norbornene (GelNOR). 10wt% GelAGE and GelNOR hydrogels were photo-polymerised using dithiothreitol (DTT) as the thiolated crosslinker (1/10 mM/mM Ru/SPS, 1:1-1:6 AGESH or NORSH). Photo-irradiation conditions were kept at 3 minutes of visible light (30 mW/cm², 400-450nm)². Sol fraction and compressive modulus of the fabricated hydrogels were evaluated. Human articular chondrocytes (HAC) were incorporated into the bioink and bioplotted at a density of 10x10⁶ cells/ml using a BioScaffolder (SysEng, Germany).

Both AGE and NOR functional groups were successfully conjugated onto gelatin. However, the AGE conjugation protocol was more controllable, where GelAGE macromers ranging from 24-92% degree of modification and 8-100kDa molecular weight were successfully synthesised. GelAGE hydrogels also exhibited tailorable physico-chemical properties, where increasing AGESH ratios also resulted in increased sol fraction and compressive modulus. However, this effect was not observed in GelNOR. 3D bioplotted GelAGE constructs that maintained high print- and shape-fidelity were fabricated, where complex porous scaffold architectures were successfully plotted. The GelAGE bio-ink was also cytocompatible as shown by high cell viability after the 3D bioplotting process.

We demonstrated a new versatile photo-clickable thiol-ene gelatin based bio-ink that is promising for 3D bioplotting of cell-laden constructs with high shape fidelity and viability.

In vitro and in vivo Assessment Of Strontium-substituted Bioactive Glass And Polycaprolactone Composite Scaffolds Produced Via Melt-electrospinning Writing

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Introduction: Polycaprolactone (PCL) is a biocompatible polymer in the tissue engineering field, it is easy to shape and mechanically suitable for bone defects [1]. Strontium-substituted bioactive glass (SrBG) is a bioactive ceramic material that stimulates osteogenic differentiation [2]. Both biomaterials already have some FDA/CE approval for clinical use. By incorporating SrBG particles into PCL bulk material, we have produced PCL/SrBG composites which were fabricated into ordered scaffolds using the versatile technique of melt-electrospinning writing (MEW). These PCL/SrBG composite scaffolds are expected to regenerate bone with the same efficacy as autografts and serve as promising cost-effective bone substitutes for clinical treatment of bone defects.

Method: Both PCL and PCL/SrBG (50 wt% of SrBG, particle size<6 µm) scaffolds were fabricated via MEW. The scaffolds were characterized using SEM and micro-CT. In vitro assessments of these scaffolds were carried out with MC3T3 cells in growth and osteogenic media for 28 days. Corresponding assays were used to assess cell attachment, proliferation and differentiation at predetermined time points. A rat cranial defect model was used to assess the in vivo osteogenic capacity via scaffolds implantation. Micro-CT and histology were used to examine the bone regeneration of the defects after 4 weeks and 12 weeks.

Results: Based on the in vitro test results, the PCL/SrBG (50 wt%) scaffolds showed minimum cytotoxicity and significantly higher capacity to stimulate osteoblast differentiation compared to PCL only scaffolds. According to micro-CT and histology results, the PCL/SrBG scaffolds showed an enhanced capacity to stimulate bone defect healing in vivo.

Conclusion: We are the first to produce the composite PCL/SrBG (50 wt%) scaffolds via MEW. PCL/SrBG composite scaffolds are non-cytotoxic and have shown an osteogenic capacity.

3D Inkjet Printing of Ionically Cross-linked Star Block Copolymer Hydrogels

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Three-dimensional (3D) inkjet printing of hydrogels has significant potential for various biomedical applications including biofabrication, considering its high printing resolution compared to robotic dispensing. However, the range of hydrogels applicable to 3D inkjet printing is limited to those composed of materials such as alginate and fibrin. We have, for the first time, investigated 3D inkjet printing of ionically cross-linked star block copolymer hydrogels, for which the precursor polymer has a dendritic polyester (DPE) core, a poly(oligo(ethylene glycol) methyl ether acrylate) (polyOEGA) inner layer, and a poly(acrylic acid) (PAA) outer layer. The star block copolymer solution (8.0 wt%) showed a viscosity of 7-8 mPa s, which is suitable for ejection from inkjet nozzles. This solution formed a homogeneous hydrogel upon addition of free metallic ions, such as the zinc, copper(II), aluminum, and ferric ion, because of the strategic molecular design of the precursor star block copolymer. The dynamic viscoelasticity of the resulting hydrogels was dependent on the ion species used as cross-linkers. The rapid gelation induced by the free metallic ions enabled 3D inkjet printing of the star block copolymer hydrogels, through ejection of the star block copolymer solution and subsequent ionic cross-linking to achieve layer-by-layer deposition of the gelled droplets. Considering that this material potentially allows facile molecular design of the precursor polymer, as well as large-scale synthesis in an affordable manner, it represents a useful platform technology to expand the design flexibility of 3D-inkjet-printable hydrogels.
**Physical properties of bioprinted hydrogel structures based on decellularized porcine lungs for 3D cell culture**

**INTRODUCTION:** Advancing in lung bioengineering requires a better knowledge of cell-matrix crosstalk. Understanding how stem cells fate is modulated by their 3D physical microenvironment is particularly important. Hydrogels based on Lung Extracellular Matrix (L-ECM) are promising scaffolds not only by providing physiological molecular cues to cells, but can be bioprinted in 3D structures and their physical properties can be tuned. However, physical characteristics of L-ECM hydrogels are still poorly characterized.

**AIM:** To study how mechanical properties and oxygen diffusion of bioprinted L-ECM hydrogels can be tuned for 3D Lung Mesenchymal Stem Cells (L-MSCs) culture.

**METHODS:** Porcine lungs were decellularized by a detergent-based procedure [1]. The resulting L-ECM was freeze-dried, cryomilled, and the resulting powder was enzymatically digested by papain at different concentrations from 5 to 20mg/ml. For cell-laden structures, pre-gels were mixed with L-MSCs and 3D bioprinted (RegenHu, 3DDiscovery) by using F-127 as structural and sacrificial hydrogel. Resulting structures were physically crosslinked by adjusting the pH to 7.4, incubating at 37ºC and chemically crosslinked by using 1mM concentration of genipin. Cells were harvested from the hydrogel 3D cultures by digesting the structures with collagenase. Mechanical properties of the resulting structures were measured by applying tensile deformations (Aurora Scientific, 300C-LR) to acellular hydrogel slices. Oxygen diffusion in acellular structures was measured by using a fiber optic sensor (Pyroscience, OXR50).

**RESULTS:** Hydrogel Young’s modulus increased with the lung matrix concentration and chemical crosslinking (in a tunable range of 0.1-10kPa). Oxygen diffusion did the opposite, being more than two-fold lower in the stiffer hydrogels. Recovered L-MSCs, from the bioprinted structures after 3 days of 3D culture, were able to spread and proliferate when reseeded in standard plastic 2D culture plates.

**CONCLUSIONS:** Cell-laden hydrogels based on L-ECM exhibit tunable physical properties and thus they can be used as bioink to build potentially useful 3D scaffolds for lung tissue engineering.

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**Cell Surface Engineering for Controlling Multicellular Aggregate Formation**

**Multicellular aggregates consisting of different cell types has attracted attention in cell-based therapy and drug screening. However, it is difficult to form multicellular aggregates from cells expressing different types of cell adhesion molecules. We have used poly(ethylene glycol) conjugated to both stranded DNA (ssDNA) and phospholipid (ssDNA-PEG-lipid) for inducing cell attachment. Modification with ssDNA-PEG-lipid results in the display of ssDNA on the cell surface. Hybridization with complementary ssDNA allows for attachment of cells to substrates or different cells. Design of DNA sequences also enables us to induce cell attachment to substrate in a spatially controlled manner [1] and programmed cellular assembly and disassembly [2].**

In this study, we designed PEG-lipid derivatives to induce natively occurring cell-cell interaction by modification of cell surface with recombinant proteins. We synthesized PEG-lipids carrying metal-chelated nitrolotriacetic acid and benzylguanine, specific ligands for His-tag and SNAP-tag incorporated in recombinant proteins. Both PEG-lipids allowed for modification of tagged enhanced green fluorescent protein (EGFP) on cell surface through specific tag-ligand binding. We next tested cell-cell adhesion induced by cell surface modification with E-cadherin, a membrane protein mediating cell-cell adhesion, in order to control structure of multicellular aggregate. Multicellular aggregates of native two epithelial cell lines (HeLa and MCF-7) exhibited core-shell like structure due to lack of E-cadherin expression on HeLa cells. The modification of HeLa with E-cadherin altered the multicellular aggregates from core-shell to intermixed structure. This result suggests that the structure of multicellular aggregates is modulated by E-cadherin modification. The combination of recombinant proteins and ligand-carrying PEG-lipids provides the potential methodology to control cell-cell interactions.

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Bone is the most transplanted tissue in humans with about 1 million procedures annually in Europe. Autologous bone graft is the gold standard in bone regeneration but requires a second surgery, is limited in quantity, and often associated with complications. Bone tissue engineering using calcium phosphate scaffolds and human bone marrow mesenchymal stem cells (hBMSC) is an alternative to autologous bone grafting. This study aims to regenerate bone tissue by using 3D scaffolds loaded with hBMSC, and to measure the mechanical strength of constructs after implantation in ectopic sites of nude mice.

Calcium phosphate scaffolds were made by mixing alphaTCP powder with pluronic and 3D printing different shapes, porosities and compositions. Cylinders of 6 x 6 mm, having rectangular or honey comb porosity patterns were printed and steam sterilized at 121°C. hBMSC from different donors were expanded in αMEM and 5% platelet lysate. 4 x 10⁶ hBMSC were seeded on each scaffold and implanted for 8 weeks in subcutis of nude mice (n=12/group). Ectopic bone formation was characterized by using microcomputed tomography (Skyscan 1076) and decalcified histology by Masson Trichrome staining. Compressive strengths of the 3D printed scaffolds implanted in nude mice were measured and compared to those of femoral bone. 3D printing permitted the fabrication of calcium phosphate scaffolds with different pore structures and sizes. hBMSC attached, proliferated and differentiated into osteoblasts on the 3D printed calcium phosphate scaffolds in vitro. In vivo, abundant bone formation was only observed inside the 3D scaffolds having pores of 250 μm, while little bone was found in scaffolds with pores of 650 μm. When h BMSC were combines with scaffolds, bone volume (BV/TV) was significantly higher in 3D scaffolds with smaller pore sizes than with large pore sizes. The formation of bone tissue significantly increased the compressive strength of the 3D printed scaffolds that was almost comparable to mechanical resistance of femoral bone.

This innovative 3D printing technology allows the fabrication of personalized 3D scaffolds fitting the skeleton anatomy for regenerative medicine. This study showed for the first time that there is an optimal porosity for osteoinduction and that the mechanical strength of the 3D constructs is comparable to that of native bone.

Acknowledgments

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Magnetically responsive cells loaded hydrogels are promising matrices for production of constructs with remote controllability and quick-response via low magnetic field in tissue engineering approaches. In this work, we report a simple gelatin based magnetic responsive bioink that can be printed into 3D responsive complex shapes, obtained by adding PEG-caped Iron Oxide nanoparticles (IOPs). The ink is characterized via optical, transmission and scanning electron microscopy, FTIR, rotational and oscillatory rheology. The kinetic of the 3D IOPs self-assembly patterning method via a cheap magnetic device is analyzed. Scanning electron microscopy evidences an anisotropic organization of the IOPs in the GelMA when magnetically assembled, while FTIR shows preservation of the GelMA matrix. Rheological characterization indicates a shear thinning behavior of the ink with and without IOPs, and no modification of the UV triggered gelation time. Metabolic activity of hMSCs at the surfaces is not affected by the substrates, indicating a good ink cytocompatibility. When C2C12 cells are embedded in GelMA/IOPs, the IOPs improve viability (UV-protective effect). This magnetically responsive ink is used for printing 3D bio-inspired complex shapes, useful for soft-robotics and cells mechano-stimulation for the maturation of TE constructs.

Biofabrication techniques can be used to build-up cell-containing hydrogels in a spatial tissue-imitating 3D arrangement. However, the development of suitable hydrogel carrier fluids for the biofabrication process still remains a challenge due to the contradicting requirements of three-dimensional printability and optimal cytocompatibility. Blending of hydrogels has been reported as a strategy to provide tailored materials which aim to address both requirements. We hypothesized that hydrogel blends of agarose and type I collagen are suitable materials for the manufacture of vascularized tracheobronchial models enabling 3D printing and simultaneously supporting the formation of a capillary-like network within the gel.

Agarose-collagen blends were characterized mechanically, rheologically, and cell biologically. The stiffness and relaxation of the hydrogels were measured in compression and tensile tests. The gelling temperature and time, the viscosity of the precursor solutions, and the shear moduli of the resulting gels were quantified using a rotational rheometer. The 3D printability of the hydrogel blends was evaluated using a custom-made drop-on-demand printing system. The viability and morphology of human mesenchymal stem cells (hMSC) and human umbilical vein endothelial cells (HUVEC) were assessed in different combinations of agarose and collagen. Maintenance of chondrogenic marker expressions was confirmed using RNA-sequencing; thus, these findings suggest that the incorporation of Si+ ions into bioactive TCP scaffolds is a possible strategy for developing bi-lineage scaffolds that improve osteochondral defect repair.
01-P174 Microfluidic bioprinting of cell-laden microfibres

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Introduction: A bioprinter, which is capable of depositing cell fibers in a layer-by-layer fashion, is conceived and constructed. Cell fiber is a thin (approximately 100 µm), long (greater than a meter) and finely handleable cellular construct in which the intrinsic cellular morphologies and functions are reconstituted. This work takes advantage of the cell fiber technology, by printing cell fibers, rapid fabrication of macroscopic, dense and porous tissue constructs could be achieved.

Methods: To construct the bioprinter, several components of it was originally designed, including the microfluidic printhead for the generation of core/shell hydrogel microfibers on-a-fly, and the syringe-vacuum substrate for the smooth and feasible deposition of the microfibers. Based on the microfluidic printhead and the syringe-vacuum substrate, commercially available motor stage and syringe pump will be adopted and installed. With all its components ready, the bioprinter for cell fiber printing will be finally assembled and tested. Using the bioprinter, optimized set of parameters for stable printing of core/shell hydrogel microfibers will be established. Then, we will demonstrate the printing of cell fibers using HepG2 cells, and investigate the printed constructs in details, regarding its dimensional parameters and the ability to form cell fibers upon culture.

Results: The bioprinter is able to print cell fibers with printing speed > 15 mm/s, shell diameter ~300 µm, core diameter ~ 100 µm.

The cell fiber printer is able to print macroscopic (thickness >1.5 mm) sized, dense and porous tissue constructs. The constructs are able to be maturated after printing using rotary culture method within 4-5 days. After maturation of the cell fibers, alginate shell can be subsequently removed. H&E staining results reveal that the cell fiber inside the printed construct has high density and high viability, showing tissue-like morphology. In addition, the albumin secretion function is also validated using ELISA test, showing enhanced albumin productivity compared to 2D cultured cells.

Conclusions: 

Enhancing Bone Repair by Incorporation of Innovative Nanoparticles Containing Osteogenic Factors in Gelatin Methacryloyl Hydrogel Scaffolds

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Development of simple strategies to bone repair for an increasing aging population remains a significant clinical goal. Biofabrication and tissue engineering offer efficacious and cost-effective approaches to stimulate tissue repair with application of hydrogels that support differentiation of human stem cells through the delivery of specific osteogenic factors. However, to date, many factors are often delivered exogenously, with limited spatio-temporal control resulting in overstimulation of surrounding tissue and significant treatment costs. The objectives of this study were to develop a strategy to deliver osteogenic factors, including ascorbic acid-2-phosphate (Vitamin C) and 1,25-OH₂-Vitamin D₃, encapsulated in poly-lactic-co-glycolic (PLGA) nanoparticles (NPs) that would efficiently release the factors and stimulate human bone marrow stem cells (HBMSCs) differentiation into osteoblasts in vitro. In addition, this work set out to optimise the support for cells and NPs through the use of gelatin methacryloyl (GelMA) hydrogel based bioink polymerised using visible light at wavelength of 450 nm, to generate a highly compatible bioink for cell printing. Results from rheological studies of the 10% (w/v) GelMA bioink demonstrated the shear-thinning properties of the hydrogel, which on crosslinking using visible light produced rapid gelation and a bioink ideal for extrusion 3D printing. Amplitude sweep test of the GelMA hydrogel showed a stable hydrogel with a storage modulus of 1000 Pa. The hydrogel porosity, determined by SEM, indicated an average mesh size of 210 +/- 90 µm, allowing cell attachment, penetration and proliferation within the GelMA structure. At a seeding density of 1x10⁴ HBMSCs per 10x10x5 mm scaffold, extensive cell proliferation (21-fold increase (p<0.05)) was observed after 28 days. High-performance liquid chromatography demonstrated rapid release of the osteogenic factors from NPs, with 40 µg of Vitamin C per mg of NPs (40% of total release) observed within 24 hours followed by a steady release up to 48% of total amount after 12 days. The current work is centred on the efficacy of dual release of Vitamin C and Vitamin D₃ on HBMSCs. In conclusion, this innovative GelMA and PLGA NPs system provides a new efficacious material platform to address bone formation strategies for a number of orthopaedic challenges, for an ageing population, with the potential to reduce costs therein as well as time to reparation and recovery.
Thiol-ene click conjugation of VEGF peptide to electrospun scaffolds for vascularized bone regeneration

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Vascular endothelial growth factor (VEGF) can promote endothelial cell survival, proliferation and differentiation, finally inducing angiogenesis and promoting bone formation in healing bone tissues. There are many ways to immobilize VEGF on surfaces and scaffolds, such as covalent conjugation, or the use of a crosslinker. However, most chemical reactions are nonspecific and require organic solvents which can compromise VEGF activity or the properties of the scaffolds. Recent advances in the fabrication of functionalizable ‘clickable’ electrospun fibers are reported, which showed potential possibility to solve these problems. Here, we used thiol-ene click chemistry for the conjugation of a VEGF mimetic peptide to the surface of PCL fibrous scaffolds. In this study, cell-encapsulated microgels fabricated by photocrosslinking droplets containing methacrylic gelatin (MGel). The mechanical properties of the microgels could be controlled by the MGel concentrations, while their size could be controlled by varying the flow rates during droplet generation. The viability of macrophages encapsulated in the microgels was well maintained regardless of the physical properties, while their proliferation was dependent on the mechanical properties. More significantly, lipopolysaccharide (LPS) induced M1/M2 differentiation of macrophages was also heavily influenced by the mechanical properties of the microgels. Eventually, these macrophage microtissues were embedded in a hepatocarcinoma tissue constructs as an in vitro tumor model to study the effects of macrophage in different stages of differentiation and mechanical environment on the surrounding cancer cells.
Building a Low-Cost Desktop Bioprinter

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3D Bioprinting applies layer-by-layer based 3D printing technologies to develop precisely designed scaffolds for tissue repair and organ replacement [1]. Extrusion based approach is the most common method implemented by majority of commercial 3D bioprinters mainly due to greater deposition, printing speed, cheaper assembly and operational costs [2]. The technique facilitates extrusion of cylindrical filaments of bioink, employing either a pneumatic, mechanical or solenoid control. The cheapest extrusion bioprinter in the market still costs ~ US $15K. The high cost of bioprinters may restrict many research groups/young investigators from entering bioprinting. A cheaper and more customisable alternative would certainly help faster development of new bioinks and deposition strategies. We have adapted a low-cost (<US$1K) commercial fused deposition modelling (FDM) printer, Ormerod 2 (from RepRapPro) for bioprinting. The Ormerod 2 was modified into a bioprinter by adapting the RichRap design to the Ormerod’s X carriage to print Gelatin methacryloyl (GelMA) hydrogels. The RichRap syringe extruder was chosen as it maximized the torque from the extruder motor by using reduction gears. The main aim was to control the XYZ movements and a syringe extruder that translates a motor’s rotation into linear pressure on a syringe. The functional validity of the assembled printer was assessed with the print fidelity and cell viability. The line width experiment demonstrated that while it is possible to increase the printing speed, the optimized setting of 5mm/sec should be used to avoid the larger variance. The printer was calibrated to print a cylinder of 5mm height and 8mm diameter in each well of a 24 well plate. GelMA (5%) was used as ink base due to its biocompatibility and tuneable mechanical properties whereas Lithium phenyl(2,4,6-trimethylbenzoyl) phosphinate) ([LAP] 0.3%) was added as photo-initiator for post curing under a UV(365nm) light source to increase printed gel stability. The Live/Dead assay showed live WS-1 fibroblast cells in the printed scaffold at least 4 days after printing. The cell proliferation assay using Presto Blue indicated cell growth till a week after printing. These optimization studies lay groundwork for higher-resolution, large scale and faster printing of tissues using our bioprinter.

References:
Rational design and fabrication of soft network composites for soft tissue engineering applications: a numerical model-based approach

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Design strategies inspired by hard biological materials such as nacre, bone and teeth have opened up new avenues in materials science. Thereby, innovative materials outperforming their conventionally engineered counterparts have been developed. However, although there is an increasing need for advanced soft materials to be used in emerging fields such as soft bodied robotics, stretchable electronics and devices, and tissue engineering, only a limited number of studies following the blueprints of nature’s soft materials have been reported.

This research demonstrates a strategy for translating nature’s fibre-reinforcement approach to soft biomaterials. Herein, biodegradable fibrous constructs following the distinct motifs of the reinforcing collagen of soft tissue matrices were 3D printed using melt electrospinning writing technology. The printed networks were then combined with soft hydrogels mimicking the water-saturated proteoglycan matrix of soft tissues. Resulting soft network composites (SNCs) were shown to be flexible yet robust, and exhibit various complex biomechanical behaviours including viscoelasticity, J-shaped non-linearity and anisotropy – hypothetically mimicking biological soft materials. Moreover, our in vitro studies demonstrated that these SNCs can support the viability of mesenchymal stem cells and chondrocytes. Finally, to address the lack of design approaches to tailor and optimize their properties in a non-empirical way, we developed a numerical model-based approach for the rational design of patient-specific SNCs. The approach is based on an in silico design library that allows for the selection of appropriate biomaterial and architecture combinations for the target application, resulting in reduced time and cost.

Together, our data suggests that bioinspired SNCs used in this study in combination with computational models could be employed for the deterministic design of biomaterials to engineer a wide range of soft tissues.

1. bas o, et al. An integrated design, material, and fabrication platform for engineering biomechanically and biologically functional soft tissues. ACS Appl Mater Interfaces, 9, 2017

A Single-Network, Biodegradable Hydrogel with High Elasticity for Bioprinting

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Introduction: Cell printing is highly attractive for their biomedical applications, because of its high precision in cellularized scaffold preparation. Most of the biodegradable hydrogels used in bioprinting are brittle and unstretchable due to lack of flexibility and elasticity, which limits the applications of those cell printed hydrogels. Hence, we designed a biodegradable, highly elastic hydrogel with single-network, which uses visible-light for gelation and greatly simplify the bioprinting process compared to those double or more networks hydrogels. Specifically, a series of biodegradable triblock copolymers of polycaprolactone-poly(ethylene glycol)-polycaprolactone (PCL-PEG-PCL) were synthesized, followed by acrylation. The resultant acrylated triblock copolymers (PEG-PCL-DA) based hydrogels were characterized in mechanical behaviors, cytocompatibility and their feasibility of bioprinting.

Methods and Results: The synthesized PEG-PCL-DA hydrogel can be stretched, compressed, and twisted, and then recovered. Their mechanical properties can be tuned to mechanically match various native soft tissues by altering triblock lengths of the hydrogels and polymer concentrations. The PEG-PCL(24K)-DA-40% hydrogel exhibited good elasticity with small irreversible deformations (~20%) at a maximum strain of 100%. The PEG-PCL-DA hydrogel can support the encapsulated 3T3 fibroblasts growth in vitro. Cell viability of over 83% were observed across three different human cell types in 3D bioprinted PEG-PCL(24K)-DA-10% hydrogel. The hydrogel can be easily printed into complex patterns.

Conclusions: A single-network, biodegradable hydrogel with high elasticity and flexibility for bioprinting was designed. Their mechanical properties is tunable to match with mechanics of various native soft tissues. It also can support high cell survival during bioprinting with complex patterns. These attactive results imply this elastic and bioprintable hydrogel has potential for soft tissue engineering and other biomedical applications.

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References:
A hybrid biomaterial has been developed with a study of its mechanism using alginate as biopolymer and α-TCP as ceramics as a biomaterial for the application in bone tissue engineering and 3D-bioprinting technology. While the film was targeted to be applied in tissue engineering, the gel could be applied to both 3D bioprinting and injectable materials. The analyses showed that the strong mechanical strength and high flexibility of the film were attributed to the ionic and H-bonding interactions between alginate with α-tricalcium phosphate particles and calcium chloride, which is confirmed by various chemical analyses. The in vitro bone cell (MC3T3) culture and cytotoxicity tests by extracts of the films showed excellent biocompatibility, while in vitro cell viability studies inside the hydrogel established its cell adhesive and non-cytotoxic characteristics. The film and hydrogel release model drugs such as bovine serum albumin as a protein drug, tetracycline as an antibiotic and dimethylxalylglycine (DMOG) as an angiogenesis and osteogenesis in a controlled way at pH 7 and 7.4. A certain composition of alginate and α-TCP showed printing ability by 3D-printer. Finally, experiment data showed that the developed biomaterial (Alg-α-TCP) could show a possibility of its applications in bone tissue engineering and 3D-printing technology.

References:
Scaffold materials find important applications in tissue engineering and cell transplantation. Although various techniques have been used to create porous scaffolds for construction of cell-material combinatorial systems, precisional control over the internal nano-/micro-pores in scaffolds are still hard to achieve. Here, an immersion-precipitation phase transformation (IPPT) process was established for fabricating the hollow bead-based scaffolds using polyethersulfone (PES). Hollow beads with the outer diameter in 1.5-4 mm and the wall thickness in about 450 μm were obtained. In particular, the wall of these PES hollow beads contained parallel channels penetrating across whole membrane, with the internal diameter starting from 50 μm near the bead core, gradually decreasing outwards to reach a dense PES skin layer on the bead surface. By controlling the deskinning time in dimethylacetamide, two types of beads, with highly monodisperse pore sizes on the surfaces, 0.401 ± 0.187 μm (bead with nanopores on the surface, BNP) and 9.22 ± 3.83 μm (Bead with micropores on the surface, BMP), were prepared. When loaded with mesenchymal stem cells (MSCs) inside, the cells were found to distribute inside channels and form clusters. Moreover, the MSCs cultured in the hollow bead scaffolds exhibited enhanced anti-inflammatory and pro-angiogenesis gene expression, compared to the MSCs cultured on microplates. Through in vivo noninvasive imaging studies in the murine host, it was found that both BNP and BMP scaffolds significantly extended the residence time of MSCs transplanted in the subcutaneous space in comparison with the directly injected MSCs. Specially, the BNP scaffold supported a higher percentage of cell survival at all time points compared to BMP, and the cellular residence time reached approximately two weeks. Our study shows the IPPT process can generate hollow spherical membranes with oriented channels with precision pore sizes and structures. The hollow beads may be further investigated as cell scaffolding/delivery materials for cell-based therapy.
01-P186  Sinterless 3d-printing Of Porous Bioceramics For Bone Tissue Engineering

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Over the last three decades, additive manufacturing has made substantial progress towards fabrication of tissue engineered scaffolds to replace conventional techniques that are incapable of precisely controlling pore size, interconnectivity and geometry of scaffolds. Calcium phosphates are primary focus for synthetic bone graft substitutes because of their osteoconductivity and ability to bind to living bone. 3D plotting, selective laser sintering and stereolithography have been the most frequent techniques to fabricate custom designed calcium phosphate scaffolds. However, as a commonly used post-processing treatment, the printed scaffolds required to be sintered at high temperatures. This hampers the ability of such techniques to incorporate drugs and growth factors within the ceramic scaffolds to promote osteoinduction. Moreover, sintering may lead to local deformation of scaffolds or phase transformation and conversion of the scaffold material into an undesired phase. In this study, we present a method in which enables to print calcium phosphate scaffolds in a one-step process at room temperature.

01-P187  Development of novel light/ionic double-crosslinkable alginate-based bioink for 3D bioprinting

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3D bioprinting has been regarded as a powerful tool for mimicking natural structure, because it can fabricate demanded hierarchical artificial tissues. Alginate, one of the most frequently used biomaterial for bioprinting, can be crosslinked with calcium ions. However, this ionically crosslinked hydrogel is noticeably impoverished under a physiological, aqueous conditions due to easy loss of calcium ions. Barium or copper ions can increase its stability, however, these also increase cytotoxic effects. In this work, we propose a new visible light activated crosslinkable alginate-based bioink, showing fast and non-cytotoxic gelation. We will present the light/ionic double crosslinked alginate hydrogel with increased mechanical properties and dimensional stability. Further, we printed this alginate-based bioink to cell-laden hydrogel with high accuracy, and cell viability was examined. For the additional biofunctionality, bio-inspired proteins were incorporated. Further development of this novel alginate-based bioink may facilitate not only production of transplantable human tissues but also many other applications, including organic transistors and integrated circuit.
01-P189 The Effect of a Novel Bioplotting Hydroxyapatite/Calcium Carbonate Biomaterial on Rat Bone Regeneration

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A novel method has been developed to fabricate hydroxyapatite/CaCO3 biomaterials by 3D printing technology. The biomaterial was characterized using FTIR, SEM and compressive strength testing. The cytotoxicity, cell viability and osteogenic potential were assessed in vitro using human mesenchymal stem cells (hMSCs) and bovine chondroprogenitors. Tissue reaction to CHACC after implanted juxtaposing to tibia/tibialis, and the effect of CHACC on 3.5 mm diameter femoral intercondylar bone defect regeneration were assessed in vivo in Wistar rat model. The FTIR spectrum showed the peaks at 1036 and 1095 cm\(^{-1}\), 568 and 600 cm\(^{-1}\), 875 and 712 cm\(^{-1}\), 1432 and 1756 cm\(^{-1}\) which proves the main components of CHACC are HA and CaCO3. Viable hMSCs cultured on scaffolds were observed to attach and grow on the biomaterial by SEM. The cell viability was assessed using AlamarBlue assay which showed that the materials were not toxic to hMSCs and bovine chondroprogenitors. No adverse effect was observed after CHACC was implanted between tibia and tibialis where new blood vessels, fibril tissue and bone-like tissue were formed in CHACC porous structure. New bone formation surrounding CHACC implants in femoral bone defects was observed, which is superior to control gelatin sponge implant group where bone defects remained as voids with very limited osteogenesis. In conclusion, CHACC is a novel bone graft substitute with great osteogenic potential for bone regeneration. Further research is warranted before potential clinical application.

Key Words: 3D printing, hydroxyapatite/Calcium carbonate (CHACC), scaffolds, osteogenic

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Additive manufacturing (AM) is an emerging field with high potential in tissue engineering and regenerative medicine applications. Small and complex structures like porous scaffolds or vascular vessels can be patient-customized and rapidly manufactured. However, the optical resolution and build layer thickness of the instrument will determine the minimum pore size and fiber diameter achievable, which plays a crucial role in order to mimic native tissue. Hereby, digital light processing (DLP) has emerged as an interesting technique which allows high resolution with inexpensive equipment that can be implemented into clean room manufacturing environments. DLP is based on photopolymerisable resins that are crosslinked in-situ via UV light, allowing also for cell encapsulation. However, the portfolio of commercially available bio-resins, in particular biodegradable resins is limited and has inhibited full exploitation of this versatile photofabrication technique.

Here, we present a biocompatible and biodegradable ink that is based on a polyester urethane acrylate (PEUAc) which can be manufactured via DLP into 3D scaffolds. The PEUAc were synthesized via ring opening copolymerization of ε-caprolactone (CL) and D,L-lactide (LA) with 75:25 feed ratio at 140 °C in presence of catalytic amounts of stannous octanoate and in different molecular weights (2000, 5000, 8000, 12000, 20000 g/mol). The random copolymers were end-capped with hydroxyethyl acrylate substituted isocyanates which can be easily polymerized during the DLP. These materials as photopolymerised coatings have been tested for cell viability. Live/dead assays and methylene blue staining confirmed a high cell viability after 24h of culture which encourages the design of more complex 3D scaffolds.

Hydrogels have been widely used in tissue engineering as their elements and properties mimic the extracellular matrix (ECM) of natural tissues. For cartilage tissue engineering, the “conventional” environment have been described as having pores of 75-400 µm and porosity of 75-97%. Nanocellulose (NC) is a biocompatible, non-cytotoxic, abundant and sustainable biopolymer proved to be an excellent candidate for cartilage reconstruction by 3D bioprinting, providing an appropriate mechanical and biological environment. Still, no study has provided insight on the structural and mechanical characterization of such crosslinked and sterilized NC-based hydrogels.

Here, the structural and mechanical impact of different crosslinker concentrations (0.1M, 0.5M or 1.0M CaCl₂) and sterilization methods (UV, autoclave and ethanol) on NC-based hydrogels was studied. Pore size was determined using scanning electron microscopy (SEM) images. Mechanical properties (i.e. Young's modulus), swelling and porosity were measured. Bacterial persistence was determined based on optical density and cell viability assessed via live/dead stain.

NC-based hydrogels crosslinked with higher CaCl₂ concentration were stiffer and had a significant 10% decrease in porosity when compared to lower concentrations (~46% for 0.1M). We confirmed the negative correlation between CaCl₂ concentrations and pore size (0.23 µm vs 0.13 µm for 0.1M and 1.0M, respectively) and swelling (1500% vs 600% for 0.1M and 1.0M, respectively). All sterilization methods were effective on decreasing bacterial content (p ≤ 0.01) showing no significant impact on porosity (~40-50%) and no differences in cell viability at day 7. UV had a higher impact on swelling (~100% increase after sterilization, p ≤ 0.0001) when compared to the other sterilization methods (yet all methods led to higher swelling). Autoclave sterilization had the biggest impact on mechanical properties, leading to a decrease of ~30 kPa in Young’s modulus. Both UV and ethanol sterilization led to stiffer NC-based hydrogels (~133 kPa and ~140 kPa, respectively for UV and ethanol vs ~126 kPa for non-stereile). Importantly, the results pin point that the properties of NC-based hydrogels do not fall into the “conventional” ideal chondrogenic environment described in the literature. These findings highlight the importance of fully characterizing biomaterials as the definition of “best environment” for chondrogenesis might be broader than anticipated.
01-P192 A Novel Low-Temperature PCL-Based 3D Printing Resin for Craniofacial Repair

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Craniofacial bone is a particularly difficult tissue to engineer due to the demanding geometric complexity, mechanical stiffness, and cellular infiltration requirements for the biomaterial scaffold. The advent of 3D-printing and custom bioink formulation presents an appealing solution to fabricate technically complex, bone tissue engineering (BTE) scaffolds. Identifying a suitable bioink fulfilling these wide requirements must first be identified. Polycaprolactone (PCL) remains one of the most widely utilized 3D printing materials for BTE, driven by the relative ease of fabrication, non-cytotoxic hydrolytic degradation mechanism, and low material cost. However, while structurally sound constructs can be fabricated, unmodified PCL requires a high extrusion temperature (>70°C) and prevents significant cell infiltration with a uniform surface topology. Therefore, in order to realize complex BTE scaffolds, we have developed a novel biocompatible, low temperature, PCL-based resin.

Results indicate PCL viscosity and printing temperature are effectively lowered by solubilizing PCL in dimethyl sulfoxide (DMSO). Under identical tensile conditions, the novel printed and processed resin has a Young's modulus of 170.4±11.5 MPa (n = 7) compared to unmodified PCL at 263.1±9.7 MPa (n = 10), likely due to increased fiber porosity, conducive for improved in vivo cell infiltration. XTT analysis of L929 fibroblast cells demonstrates, via a one-way ANOVA at the 95% confidence interval, a lack of statistical difference between metabolic activity in the processed resin and a known non-cytotoxic control (high density polyethylene) after 24 hours. Printed objects are processed post-printing to remove the cytotoxic solvent under freeze-drying conditions, yielding a highly textured surface with pore diameters of ~ 10 µm, similar to cortical bone. Compounding has been accomplished with hydroxyapatite and alginate microparticles containing vascular endothelial growth factor, creating a dispersed suspension prior to extrusion. Compounding the resin with encapsulated growth factors creates a sustained elution source of ~ 260 pg/ml/day over 4 weeks within physiologically observed concentrations (~ 150 – 1200 pg/ml blood concentration). By encouraging vascular development, cell infiltration, and remodeling, the degrading bioink should improve implant viability and natural regeneration while maintaining the custom 3D shape and stability.

01-P193 Effect of in vitro Generated Extracellular Matrix on Osteogenic Potential of Additive Manufactured Multiscale Porous Hybrid Scaffolds

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Introduction

The challenge of tissue engineering is to mimic target tissue structural, mechanical and biochemical aspects. Due to variations in the architecture of natural bones and bone defects, additive manufacturing (AM) is an attractive route for custom bone applications by having high controllability on both the inner/outer shape of the scaffolds. Although AM enables to manufacture macroporous scaffolds, current techniques limit the microporosity (MiP). Alternatively, the MiP of materials can be controlled via using emulsion templating by producing polymerized high internal phase emulsion (polyHIPE) which have an interconnected porosity very similar to trabecular bone. On the other side, the biochemical environment of the natural bone is dominated by mineralized ECM containing various biological factors.

Our study aims to combine advantages of AM and emulsion templating to create multiscale porous scaffolds. Additionally, to benefit from complex organization of natural ECM, fast-growing murine MLO-A5 cells were cultured on 3D printed polyHIPE scaffolds (PPHSs) so as to decellularization (dcell). This developed hybrid scaffolds made of pre-generated ECM and polymer were presented for usage of on custom bone grafts.

Experimental

Following synthesis and methacylation of PCL, scaffolds were created via AM of PCL polyHIPE. MLO-A5s were cultured on sterilized PPHS for 4 weeks. In this period, cellular activity, mineral and collagen deposition assays were implemented. Cell penetration depth was assessed from histology images. Microarchitecture of PPHSs and cell attachment were observed under SEM. After dcell, to confirm the efficiency of the process, DNA content was measured. HES-MPs were seeded on both plain and hybrid scaffolds cultured in osteogenic media on the evaluation of metabolic activity, newly formed mineral and collagen deposition. Finally, the ex vivo chick femur defect model was used to explore the potential of the scaffolds on the bone healing process.

Conclusion

Both in vitro cell culture and ex vivo chick femur defect model confirmed that due to its interconnected multiscale porous structure, PPHS successfully supports cell adhesion, proliferation and migration and it is a promising candidate to fulfill the requirements of defect-matching bone grafts. Additionally, our in vitro generated ECM decorated polymer system proposed an applicable approach to improving bioactivity of polymers scaffolds to encourage precursors to differentiate mature bone.
Cell therapies are typically limited by the rapid dispersal of cells on delivery. Scaffolds offer an approach of enhancing therapeutic efficiency and efficacy. This project aimed to combine the bio-intelligent properties of fibrin-based scaffolds with the potential of MSC for activating healing in chronic wounds.

A porous fibrin biomaterial scaffold has been developed using a novel manufacturing process (confidential). This creates a regular, highly porous structure with close pore packing and good interconnectivity. The primary structure is stabilised by glutaraldehyde cross-linking. The resulting scaffold is suited to cell ingress and supporting angiogenesis, and allows fluid exchange between the scaffold and the wound environment.

Scaffold microstructure was visualised using SEM. Analysis of these images gave data on pore size (50-150µm), pore size distribution, scaffold density and dispersion of material during the initial manufacturing stage. Iterative protocol design and development allowed tailoring of these microstructural parameters. Coagulometry (Stago STart Max) was used to optimise formulation.

Despite the open basket-woven structure, scaffolds are readily handleable in both lyophilised and rehydrated forms. Tensile strength, in the range 1-2 MPa, was evaluated using Instron and used as a tool for protocol optimisation.

As the scaffold is designed for in vivo implantation, its biological behaviour is an important consideration. Cytotoxicity (CCK8) was performed to assess the risk of eliciting an adverse response in vivo. This was a useful measure in confirming efficacy of the consumption and removal of excess reagents.

Enzymatic degradation was assessed to optimise material persistence in vivo, allowing for creation of a scaffold suitably robust for implantation that would biodegrade within an appropriate time-frame for wound healing in a chronic wound environment.

The ability to create protein polymeric materials with even, hierarchical nano and micro-structured structure is of significance in development of tissue engineering scaffold materials. This work describes a novel biocompatible fibrin scaffold with optimised microstructure for cell ingress and angiogenesis, excellent handling capabilities in both freeze-dried and rehydrated forms, and biocompatibility as demonstrated by in vitro assays.

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In order to get a completely degradable, controllable and polymer free drug delivery system on degradable metal substrates, the surface composition and morphology of metals were studied recently. The coating technology on the surface of degradable magnesium alloys is relatively mature by various methods. It is usually applied to metal implants to improve biocompatibility, bioactivity and bone conduction properties. In this study, a calcium phosphate (CaP) coating containing clindamycin was formed on the magnesium alloy substrate by biomimetic mineralization, and the release of clindamycin was regulated by the degradation of CaP coating and magnesium alloy substrate. Here we describe the CaP coating on the nanostructure formed on the base of the magnesium alloy. The drug loading and drug release mechanism in the bionic process obtained the pharmacokinetics evaluation and compared with the conventional drug release system. The biodegradable magnesium alloy substrate is immersed in the bionic solution containing clindamycin, and clindamycin is involved in the crystallization process, and is fixed in the newly formed CaP layer. The morphology, composition and formation process of clindamycin containing coating were obtained by scanning electron microscope, energy dispersive spectrometer, X ray diffraction and X ray photoelectron spectroscopy. The rate of clindamycin load and the rate of drug test within different times were measured by HPLC. The results show that the homogeneous CaP is formed on the degradable alloy, and clindamycin can be observed on the surface and the cross section. The characteristics of sustained drug release were studied, and the release of clindamycin inhibited the growth of Staphylococcus aureus. Structural analysis shows that various calcium phosphate phases exist, including three anorthite, slender and orthogonal particles, octacalcium phosphate and hydroxyapatite. The drug release system continues to release drugs within 49 days. The test of antibiotic loading calcium phosphate powder for bacterial growth inhibition in broth and agar showed satisfactory antibacterial properties.
**01-P196 Biphasic Biodegradable Osteochondral Scaffold**

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Three dimensional (3D) scaffolds have many advantageous properties for osteochondral repair, due to its controllable properties such as pore size, porosity and morphology. In this study, biphasic bioactive biodegradable scaffolds have been developed by low temperature 3D printing technology, the upper layer of which is composed of poly lactic-co-glycolic acid (PLGA) for cartilage repair, and the lower one is the composite of poly lactic-co-glycolic acid and tricalcium phosphate (P-T) for subchondral bone repair. The morphologies and porosity of the biphasic scaffolds were observed by scanning electron microscopy (SEM) and micro-CT, respectively. The mechanical properties were tested on an electronic universal mechanical tester. Micro CT data showed that the porosities of PLGA and PT layer were 43% and 48%, respectively. The pore sizes of the macro-pores in PLGA and PT layer were 420-480 µm and 450-550 µm, respectively. SEM images demonstrated the micro pores were about 5-50 µm, as well as the clear boundary between two layers. Mechanical test results showed that the compressive modulus and compressive strength of the PLGA layer were 13.35 MPa and 704.1KPa, and that of the PT layer were 23.71 MPa and 1.173MPa, respectively. Our results demonstrated a bi-layer scaffold was well fabricated with proper porosity and pore size for osteochondral regeneration. The degradation properties are on going and we also try to use it as a delivery system carrying bioactive factors to promote bone and cartilage regeneration.

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**01-P197 Improved 3D printing fidelity of alginate-based hydrogels with carrageenan**

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Recently, 3D bioprinting has been widely used in tissue engineering applications to fabricate scaffolds in various shapes with biocompatible materials. Among numerous bioprinting materials, alginate-based hydrogel is one of the most useful materials because its mechanical properties can be controlled with cross-linking agents such as calcium chloride or calcium sulfate. However, in spite of the mechanical enhancement using diverse cross-linkers, shear modulus of the alginate-based hydrogel is low to form 3-dimensional structure with a high printing resolution as well as a good shape. Also, poor mechanical properties of the materials limit the printability in 3D bioprinting, which restricts its preclinical and clinical applications. Thus, alginate has been combined with other materials to overcome the limitations in 3D bioprinting. Among various biomaterials, carrageenan extracted from red seaweed has been widely used as a gelling agent as well as stabilizing and thickening agent. In addition, carrageenan has biological activities such as antiviral and antiinflammatory effects. Thus, in this study, carrageenan was mixed with 2% alginate solution and its viscosity and mechanical properties were rheologically characterized with multiple concentrations of carrageenan (0, 0.5, 1.0, 1.5%). The acquired rheological data was used to optimize bioprinting parameters such as printing motion speed, pressure intensity, and nozzle size. In the deposition test, the shape fidelity of 3D-bioprinted alginate-based hydrogel with and without carrageenan was compared each other. While the alginate-based hydrogel without carrageenan was collapsed over 10 layers, the alginate/carrageenan composite hydrogel was able to maintain good shape fidelity with the high printing resolution. The experimental results indicate that carrageenan can be a useful biocompatible material to enhance alginate hydrogels, which can expand the applications of alginate hydrogels in tissue engineering and regenerative medicine.
01-P198  Production of cell-laden microcapsule with liquid core in a coaxial electrospray system through enzymatic crosslinking

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Cell-laden hydrogel microcapsules have been investigated for tissue engineering, cell therapy and biopharmaceutical applications. The features required to cell encapsulation technology are that the encapsulation process is mild for cells and the resultant hydrogel does not hinder the living of the encapsulated cells. Here, we propose a method for one-step cell-laden microcapsule by electrospray technique assisted with coaxial device through enzyme-mediated hydrogelation reaction. The cell-laden microcapsule was produced by cross-linking phenolic-substituted alginate (Alg-Ph) through horseradish peroxidase (HRP)-catalyzed reaction by using an electrostatic droplet generation system designed in our lab. Gelatin solution 8% (w/v) containing fibroblast cells as modle at 3×10^6 cells/mL was made to flow from inner a 27-gauge stainless-steel needle at 1.5 mL/h and an outer solution of alginate possessing phenolic moieties (Alg-Ph) 1.5% (w/v) and horseradish peroxidase 30 units/mL was made to flow through a 21-gauge needle at 3 mL/h. Under an open electric field from a voltage generator, concentric drops of two coaxial fluids at the needle tip were broken up into microdrops and sprayed into the gelling bath of PBS buffer containing hydrogen peroxide 1 mM. The cell-laden spherical Alg-Ph vehicles were made hydrogel by the reaction of HRP and hydrogen peroxide as an electron donor. We successfully obtained alginate-based microcapsules with liquid core in the size of 260 µm and 50 µm of gel layer thickness. The produced microcapsules preserved their structural integrity during incubation and the cell viabilities in produced microcapsules were measured to be 87.5±3.3%. The fibroblast cells harvested from microcapsules adhered and proliferated in cell culture dishes in the same way as conventional subculture. The encapsulated cells proliferated and grew in the hollow core and preserved their viability by 90.3±1.9% after 9 days of encapsulation. These results demonstrate the feasibility of cell-laden alginate-based microcapsule production through HRP-mediated reaction in one step by electrospray system assisted with coaxial device. It is expected that this method could provide an advantage in cell encapsulation and its biomedical applications.

Keywords: Alginate derivative, Microcapsule, Enzymatic reaction, Encapsulation


01-P199  Development of a 3D Printable Composite for Maxillofacial Applications

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INTRODUCTION
Following major surgery for removal of a tumour in the head and neck region or trauma, the surgeon is limited with devices available for reconstruction to restore both function and aesthetics. 3D printing offers huge promise in the biomedical field, but is limited by the types of materials that can be 3D printed. In this study printability, biocompatibility, mechanical properties and the effect of addition of calcium phosphate (CaP) fillers to a light curable polymer (polypropylene glycol dimethacrylate) were investigated.

EXPERIMENTAL METHODS
The experimental work initially involved optimisation of rheological behaviour of the polymer for 3D printing. The rate of monomer conversion was determined by exposing 11 mm diameter monomer discs to UV light using Fourier transform infrared spectroscopy (FTIR). Biomedical flexural strength and modulus of the tensile elasticity of the composite discs were tested using a biaxial flexural test. 1:1, 2:1 and 3:1 filler to monomer ratio discs were fabricated and in vitro cell work using MG63 osteosarcoma cells was carried out.

RESULTS AND DISCUSSION
Various factors such as viscosity of the composite, printing speed and the tip size were optimised and 2:1 filler to monomer ratio was found to be the optimum viscosity for 3D printing. Composites containing lower percentage of filler showed a higher monomer conversion rate. The biaxial flexural modulus of the discs indicated a linear relationship with the filler content, where higher CaP ceramics improved the stiffness values. Increasing the filler content also affected cell behaviour.

CONCLUSION
It can be concluded that addition of CaP fillers significantly improve the mechanical properties and biocompatibility of the composites whereas printability and the rate of monomer conversion can be adversely affected. Future work will be focused on characterisation of the 3D printed construct to determine its in vivo biocompatibility and bone integration.
Collagen for Extrusion Bioprinting: A New Bioink for High Fidelity 3D Bioprinting

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Introduction: Extrusion-based bioprinting can produce 3D structures of complex living tissues. Using this approach, microvalve-based 3D bioprinting allows for the rapid and precise dispensing of cell-loaded hydrogel droplets. This technique usually requires individual formulations of hydrogel blends for each application to adapt the printing process and limit shear stresses that can impact cell behavior and viability.

Herein, we demonstrate that the controlled carboxylation of agarose polysaccharide backbone allows obtaining printable and tailorable hydrogel matrices for 3D bioprinting. Comprehensive rheological and mechanical studies revealed that these materials span a range of shear and elastic modulus of natural tissues while exhibiting the same viscosity under printing conditions. As a result, we demonstrate versatile microvalve printing to manufacture 3D structures comprised of defined domains of stiff and soft hydrogels while showing limited death of human mesenchymal stem cells under identical printing conditions.

Furthermore, we will present a new bioink formulation for the bioprinting of carboxylated agarose in fuse-deposition modeling systems. Using a comprehensive analysis of the mechanical properties of these bioinks, a fluid dynamic model was developed, which has allowed us to identify the optimal printing parameters for each formulation and manufacture complex hollow hydrogel objects.
Photocrosslinkable hyperbranched polyglycerol (HPG)-based bioink for 3D printing of soft tissue constructs

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Hyperbranched polyglycerol (HPG) is a branched polymer consisting of ethylene glycol backbone and hydroxyl terminal groups. It is increasingly utilized in biomedical applications due to the facile synthesis, hydrophilicity, and biocompatibility. Herein, the terminal hydroxyl groups are modified with acrylic functional groups to impart radical crosslinking. With the abundance of hydroxyl groups, the degree of acrylate substitution (DS) per HPG molecule can be easily controlled. The resulting acrylic hyperbranched polyglycerol (AHPG) could be used as a crosslinker to fabricate hydrogels with various gel-forming macromers via photoinitiated radical crosslinking reaction. The mechanical properties of the hydrogels could be controlled in a wide range by tuning the DS of AHPG, without the need to change the concentration. The in vitro cell culture studies have also demonstrated the biocompatibility of this AHPG-linked hydrogels. The suitability of this AHPG-linked hydrogel system as a 3D printing bioink to develop scaffold materials for tissue engineering applications was demonstrated by utilizing a DLP (digital light processing)-based 3D printer to generate cell-laden hydrogel constructs with varying size and shape with micron-scale resolution.

Effect of after-treatment on the physical and chemical properties and production condition of silk matrix

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Silk are good natural materials for many biomedical applications involving tissue engineering and implantable devices, because of their body compatibility and wide range of physical structures. Silk matrices are made by forcibly thread off the silkworm onto a flat surface. In this work, we invented the automatic Silk Matrix Making Machine (ASMMM) for silk matrix production. Also, we investigated that optimal condition of silk matrix using silkworm and ASMMM. The weight and thickness of the produced silk matrix were found to be optimal condition when 25℃, 30 larvae and rough paper were used. Silk matrix is expected to be increased as a variety of medical materials. Therefore, post-treatment methods are considered to be necessary. In this study, distilled water, 100% ethanol, enzyme (pancreatin), 10% ammonia water, and soap water were treated on the produced silk matrix. Scanning electron microscope showed that sericin was mostly present in the post-treatment silk matrix, and tensile strength was high when treated with 100% ethanol and 10% ammonia water. In addition, FT-IR measurement showed no difference in amide III band due to beta-structure at 1265 cm⁻¹, and 1235 cm⁻¹, but absorption band was changed only post-treatment with soap water. The crystal structure is similar to that of the control, and when the enzyme, 10% ammonia water and soap water are used, the crystallinity is considered to be enhanced by increasing the peak. It is believe that more efforts should be made to the post-treatment methods of silk matrix. These results are expected to provide basic data for the efficient production of the silk matrix, and it is suggested that the produced silk matrix is useful as a medical material.
Three-dimensional scaffolds provide a suitable environment for tissue regeneration. Scaffolds can also be used for localized drug delivery to deliver the drug to the site of the implants. The 3D printed macroporous microporous biodegradable Polycaprolactone scaffolds for scaffold-based tissue engineering are manufactured by combining fused deposition modelling and salt leaching techniques. The scaffold physicochemical properties are characterized and porosity and morphology of the scaffold is investigated. The microporous scaffolds have higher surface area and show significantly different mechanical properties. Microporosity accelerates the degradation and the interconnectivity of the pores helps for drug delivery and sustain release. Furthermore, porous scaffolds have different surface characterization which effects the blood clotting, cell attachment and tissue regeneration.
**01-P206 Physical Characterisation and Cell Culture Utilising a Set of Plant-Derived Bioprintable Hydrogels**

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We are presently developing biocompatible, printable hydrogels from plant sources toward the development on sustainable alternatives to animal-derived materials. Scaffold material sourced from plants is cheaper, renewable, biodegradable, less immunogenic, and appeals to those patients who may have moral objections to using animal tissue for human therapeutic purposes.

In this paper, we describe the comparative swelling ratio, rheological study—in terms of shear thinning and thixotropy—as well as their mechanical properties of a set of plant-derived hydrogels, including their efficacy as bioinks suitable for 3D biofabrication. We also provide data on cell viability, adherence, proliferation, and spontaneous differentiation of human induced pluripotent stem cells (iPSCs) co-cultured with each plant-based bioink.

**01-P207 Hydrogel-based Electron Paramagnetic Resonance Probes for Long-term Monitoring of Tissue Oxygen Content**

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Ischemic diseases are characterized by low blood flow. Vascularization to restore blood flow represents primary therapeutic goal. Quick and non-invasive measurement of tissue oxygen content is an attractive approach to monitor vascularization process. Among several oxygen detection methods, electron paramagnetic resonance (EPR) stands out due to its non-invasiveness, accuracy, and repeatability. Tetrathiatriarylmethyl (TAM) radical has been widely used as an EPR probe. The major advantage is that it has linear dependence of the peak-to-peak linewidth to oxygen content. However, TAM radical has a short half-life time in vivo. Therefore, it is impossible to use it for long-term oxygen monitoring. To overcome this limitation, a hydrogel-based EPR probe has been synthesized by conjugating TAM radicals to an injectable and biodegradable hydrogel with fast gelation time (within seconds) at 37°C. The peak-to-peak linewidth of hydrogel-based EPR probe was linearly increased with the oxygen content, it also had better stability to oxidoreductants than TAM radical. Moreover, this hydrogel-based EPR probe had a slow degradation rate, allowing the EPR signals to remain stable after 4 weeks of implantation. In summary, the newly developed EPR probe can be used for long-term oxygen measurement in ischemic tissues.

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Stem cell-based therapy has held a great promise in providing desirable solutions for various diseases. However, the main reason for its limited application in clinical trials is the lack of effective long-term cell tracking approaches that are able to promote comprehensive understanding of the fate of transplanted stem cells without impairing their intrinsic properties. Here, we successfully synthesized NaYF₄:Yb³⁺,Er³⁺ upconversion nanoparticle (UCNP)-based fluorescent probes and utilized them to label and track rabbit bone marrow mesenchymal stem cells (rBMSCs) during the osteogenic differentiation in vitro. To improve their biocompatibility and cellular uptake, we modified the UCNPs with the negatively-charged and positively-charged polymers in turn. Cellular uptake and long-term effect of these surface modified UCNPs on stem cell behaviors during the osteogenic differentiation process were systematically evaluated, and no significant difference in cell viability and differentiation capacity in a certain range of nanoparticle concentrations (0~50 µg/mL). Moreover, the surface modified UCNPs at a concentration of 50 µg/mL exhibited the highest biocompatibility and stability, which could well track rBMSCs during the osteogenesis process in vitro. Hence, this study provided the necessary data for the application of these lanthanide-based UCNPs in stem cell labeling and tracking to better understand the mechanism of stem cell fate in tissue engineering, stem cell therapy, etc.

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**01-P210** Visualization of cell apoptosis by utilizing cationized gelatin nanospheres incorporating molecular beacon

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Cell transplantation is one of the promising therapies in regenerative medicine, and several therapeutic effects have been demonstrated for animal models. For the development of cell transplantation, a non-invasive technology to visualize cell biological functions is highly needed. Molecular beacon (MB) is a derivative of stem-loop structured nucleic acid. The quencher and fluorophore are conjugated at both the end sides of MB. In the presence of target messenger RNA (mRNA), the structure of MB is changed to be fluorescent based on the hybridization of target mRNA. Based on this change by the molecular interaction with intracellular mRNA, MB can visualize the cell biological function. To realize this visualization of cell biological function, it is indispensable to create the carrier of MB to allow to internalize into cells. In this study, cationized gelatin nanospheres (cGNS) are used as a carrier of MB. One of the target biological functions is cell apoptosis. MB to detect caspase-3 mRNA of an apoptosis target (casp3 MB) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA of a housekeeping gene (GAP MB) were used. The objective of this study is to design the cGNS system for MB to visualize the cell apoptosis. cGNS incorporating MB (cGNSmb) were prepared at different conditions. The cGNSmb showed a sequence specificity in hybridization and stability against nuclease digestion. After cells cultured with the cGNSmb, camptothecin of an apoptosis inducer was added to the cells. The fluorescent intensity of cGNSGAP MB was constant at any concentration of camptothecin. On the contrary, the fluorescence of cGNSCasp3 MB significantly increased only after the addition of camptothecin. This is because the cGNS permitted MB to effectively internalize into the cell, and react with the intracellular target mRNA. This paper also reports that the intracellular controlled release of MB is promising to prolong the time period of mRNA visualization.

**01-P211** Dual-modal fluorescence/CT imaging for monitoring degradation of PLGA scaffold in vivo by labeling with gold nanoclusters

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Degradable polymers such as poly(lactic-co-glycolic acid) (PLGA) are frequently chosen for tissue engineering applications including bone, cartilage, muscle and skin. The proper degradation rate of scaffolds is the key to the formation and reconstruction of neo-tissue. However, non-invasive monitoring of PLGA scaffolds degradation in vivo is still lacking. In this study, we developed a trackable scaffold by labeling poly lactic-co-glycolic acid (PLGA) with fluorescent gold nanoclusters (AuNCs). The AuNCs possess well fluorescence characteristic as well as X-ray attenuation characteristic. The AuNCs were synthesized, characterized, and their cytotoxicity were evaluated in cultured cells. The particles were incorporated into PLGA solution, then the porous AuNCs labeled PLGA (AuNCs/PLGA) scaffold was fabricated by freeze-drying. The AuNCs/PLGA scaffold can be detected by fluorescence imaging and CT scanning. After the implantation of the scaffolds subcutaneously into the nude mice, degradation of the scaffolds was monitored by fluorescence imaging and CT imaging in five weeks, and the samples were harvested for histological analysis. This new approach provided an accurate and non-invasive way for tracking the degradation of synthetic polymer scaffolds in vivo.
Patient-specific bioactive, antimicrobial PLA-PGA/titanium implants for large jawbone defects after tumour resection

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Jawbone resection is the final surgical treatment for ~5500 patients in EU28 with maxillofacial benign and malignant tumours. The resulting large bone defects lead to scarred, mangled facial appearance and the loss of mastication and speaking function, requiring aesthetic and functional reconstruction as basis for physical and physiologic rehabilitation. Although autologous vascularized bone from fibular or iliac-crest autografts is current gold standard, the portion of transplantable bone is limited and subsequent high-dose anti-cancer chemo-/radiotherapy often results in tissue necrosis.

Consequently, our current research focusses on alternative treatment techniques, separating immediate and long-term reconstruction stages: Immediate reconstruction as Stage (i) targets on drug-eluting, polymer-based spacer implants as substitute to transplanted autografts, which enable local high-dose chemo-/radiotherapy with minimal tissue damage and conserve muscle tension over ~6 months (=on-going R&D of project partners). The final reconstruction as Stage (ii) can then be based on novel patient-specifically manufactured maxillofacial implants, again without autografts (=R&D target of the jawIMPLANT project). The planned neoformation of vascularized bone in such implants within the patient’s own body as “bioreactor” is the safest approach in tissue engineering. Compared to the state-of-the-art (autografts, Ti implants), further targeted USPs of these metal-polymer hybrid implants for the functional (mastication, speaking, etc.) and aesthetic jawbone reconstruction are strongly improved accuracy (dental interocclusion), mechanical strength, antimicrobial protection, low irritation of surrounding tissue and the possibility for CT imaging in oncological re-checks.

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The influence of laser-sintered titanium surface properties on attachment of peri-implantitis-associated pathogens

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Background
Laser sintering is an additive manufacturing process that fuses powdered material in incremental layers. This technology allows the production of complex geometries and so is increasingly used to produce medical implants. However, the impact of this novel manufacturing process on biomaterial surface properties, resultant interactions with biological environments, and microbial colonisation remains unknown.

This study aimed to investigate the influence of titanium (Ti) surface properties on the attachment of bacterial pathogens associated with peri-implantitis (PI), a bacterial-mediated inflammatory disease that can cause dental implant failure.

Methods
Physicochemical characterisation, including Fourier-transform infrared spectroscopy, surface profilometry, contact angle measurements and grain boundary visualisation, was performed on laser-sintered Ti discs.

PI-associated bacteria Fusobacterium nucleatum and Porphyromonas gingivalis were cultured until mid-log phase. Discs were incubated for up to 2h in bacterial suspensions. Attachment and viability were quantified by microbiologic culture counts and image analysis following fluorescence microscopy. Both plain and artificial saliva (AS) pre-treated discs were evaluated to investigate the influence of surface preconditioning on bacterial attachment.

Results
Ti discs presented smooth, hydrophilic surfaces (Ra=0.056µm, contact angle θ=49°).

Attachment to Ti differed between F. nucleatum and P. gingivalis. At 2h, 42% F. nucleatum attached to Ti surfaces with 90% viability, while P. gingivalis showed 14% attachment and 98% viability.

Pre-treatment with AS increased surface hydrophilicity (θ=37°) and modulated bacterial colonisation. F. nucleatum attachment and viability decreased to 17% and 49% respectively, while P. gingivalis attachment increased to 42% and retained high viability (85%).

F. nucleatum formed patterns on the surfaces that correspond to Ti grain boundaries produced during laser-sintering.

Conclusion
This study demonstrates that PI-associated pathogens can colonise implant biomaterial surfaces directly, while AS induced divergent effects on bacterial attachment and viability. These novel findings warrant further exploration. Surface hydrophilicity is important when considering bacterial attachment to biomaterial surfaces. Unique attachment pattern formation observed with F. nucleatum on laser-sintered discs may indicate sensing capacity directing biofilm structure.
**01-P214** Saline Accelerates Oxime Reaction with Aldehyde and Keto Substrates at Physiological pH

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We have discovered a simple and versatile reaction condition for oxime mediated bioconjugation reaction that could be adapted for both aldehyde and keto substrates. We found that saline accelerated the oxime kinetics in a concentration-dependent manner under physiological conditions. The reaction mechanism is validated by computational studies, and the versatility of the reaction is demonstrated by cell-surface labeling experiments. Sialic acid motifs on cell surface proteins were converted to aldehyde and then conjugated with aminoxy labelled FITC. This conjugation was then analyzed by confocal imaging and FACS. Saline offers an efficient and non-toxic catalytic option for performing the bioorthogonal-coupling reaction of biomolecules at the physiological pH. This saline mediated bioconjugation reaction represents the most biofriendly, mild and versatile approach for conjugating sensitive biomolecules and does not require any extensive purification step.

**01-P215** Mechanical and biological performance of Fiber-reinforced Calcium Phosphate Cements for Load-bearing bone regeneration

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Skeletal bone defects represent an increasingly frequent clinical condition. Treatment commonly relies on bone grafting using autologous bone, but the severe drawbacks thereof have instigated the development of a large variety of (synthetic) bone substitute materials.

Calcium phosphate cements (CPCs) are bone substitute materials with advantageous handling and biological properties which can be rendered degradable by incorporating poly(lactic-co-glycolic acid) (PLGA) porogens. However, CPCs lack mechanical strength and toughness, and hence are limited to applications with minimal load-bearing applications. Fiber-reinforcement with polymeric fibers was shown to increase the mechanical strength and fracture toughness of CPCs. Herein, we propose the inclusion of poly(vinyl alcohol) (PVA) fibers to improve the stiffness, toughness and strength of CPCs, without compromising the biological performance. To this end, we determined the mechanical properties of PVA-reinforced CPC and evaluated the biological performance in a rabbit femoral condyle bone defect model.

We used a CPC and CPC/PLGA with or without incorporation of PVA fibers. Each material was tested for mechanical properties using a three-point bending set-up. Next, pre-set specimens of each group were implanted in femoral condyle bone defects of New Zealand white rabbits for 6 and 12 weeks. Histological sections of the region-of-interest were assessed for biocompatibility parameters and newly formed bone and remaining material were quantified.

Fiber-reinforcement with PVA fibers demonstrated a significant improvement of mechanical properties (i.e. work-of-fracture and flexural strength). Biologically, all experimental materials demonstrated an intimate contact with the surrounding bone, without the presence of any fibrotic tissue. Moreover, no signs of acute inflammation, degeneration in tissue morphology or necrosis were observed for any of the experimental materials. New bone formation was significantly higher for CPCs containing PLGA porogens at both 6 and 12 weeks. Interestingly, the addition of PVA fibers to CPCs did not compromise new bone formation, and new bone was formed also near PVA fibers.

We here demonstrate that the incorporation of PVA fibers successfully improves the mechanical properties of CPCs, without compromising the biological properties of these materials. Future studies should focus on the biomechanical performance of PVA-reinforced CPCs using load-bearing pre-clinical models.
New force-controlled bioreactor for real-time viscoelastic measurements in a model of liver fibrosis

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Despite the intrinsic viscoelastic nature of tissue and biomaterials, most of the studies in the literature are focused only on the effect of stiffness on the cells. In addition, these studies are normally based on static parameters (i.e. a time-invariant elastic modulus), while the mechanical properties of natural tissues are subject to a temporal evolution, both in physiological and pathological conditions1. Therefore, the possibility of investigating the mechanical properties of scaffolds and biomaterials during cell culture is of great interest, and several examples can be found in the literature2–4. However, they do not focus on viscoelastic properties, and it is evident that a deeper study of these properties is necessary.

For this reason, a new testing method, the sigma-dot (σ), was developed to allow real-time viscoelastic measurements in the MechanoCultureTR (MCTR) bioreactor (CellScale, Canada). Moreover, the MCTR, which allows the application of a known pressure on the sample while monitoring displacement using hall effect sensors, was re-engineered to allow low force testing of soft tissue constructs. In order to mimic the transition of healthy to fibrotic liver in-vitro, a dynamic model of hepatic fibrosis was developed and its stiffening monitored in the MCTR using the new sigma-dot (σ) testing method.

The method is based on the application of different constant stress rates and on a global fitting of the resultant curves to obtain viscoelastic parameters such as the instantaneous and equilibrium moduli and the relaxation time. Sigma-dot was validated with a standard testing machine (Proline Z005, Zwick/Roell), comparing it with testing methods such as creep and epsilon-dot (ε). Then changes in viscoelastic parameters of liver-derived extracellular matrix gels crosslinked with mTG (microbial transglutaminase) were monitored over time in the modified bioreactor.

Thanks to the integration of the new MCTR and testing method, it was possible to monitor both cell function and time dependent mechanical properties in gels loaded with HepG2 and stiffened with the enzyme.

References

Characterization of mechanoresponsive elements in electrically-stimulated human mammary epithelial cells

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Endogenous electric fields are essential in the physiological processes of all living organisms, namely in controlling cellular functions, such as morphology, gene expression, proliferation, and migration. In order to obtain better control over cellular growth, orientation and differentiation, the application of external electrical stimuli has become increasingly popular. Even though little is known about the physical mechanism that allows a cell to sense an electric field, it seems that it may be through the same pathways involved in mechanotransduction1.

The subcellular localisation and activity of the transcription co-factors YAP and TAZ are tightly regulated by biophysical stimuli2, which indicates that these transcriptional regulators are essential mechanosensors and mechanotransducers. In this work, we applied an external electrical stimulation (ES) on non-malignant mammary epithelial MCF10A cells, provided via a biocompatible, conducting polymer polypyrrole (PPy)3, and studied their biomechanical response. In particular, we investigated how the expression of two YAP/TAZ-regulated genes, CTGF and ANKRDT, was affected. Furthermore, in order to achieve a highly-sensitive characterisation, with temporal and spatial resolution, of how the mammary epithelial cells sense and respond to the applied external electrical stimulation, we employed Atomic Force Microscopy (AFM) and Scanning Ion Conductance Microscopy (SICM). AFM allowed us to directly measure single cell elasticity and morphology changes due to mechanics such as reorganisation of the cytoskeleton, and SICM enable us to acquire non-contact topography images and stiffness maps, by using its capability to measure the ion current through an electrolyte-filled nanopipette.

By synergistically combining SICM, AFM and molecular biology tools, we were able to characterise the mechanical response of living cells upon ES, by measuring intracellular changes and studying gene regulation pathways. This study can potentially shed light on how cells sense and mediate electrical cues, and how these affect cell fate in biomedical applications.

References:
Anterior cruciate ligament (ACL) rupture is the most common injury in contact sports. Due to its poor healing capacity, surgical intervention is often required for restoring the stability of the knee. Nowadays, the gold standard is ligamentoplasty, which consists in the use of bone-patella autograft. Nevertheless, with the expansion of tissue engineering, there are more and more researches focusing on the development of artificial ligaments. The new objective is to develop a biodegradable structure with a controlled degradation rate which can support mechanical strengths during the time of neo-ligament formation and which can act as a scaffold for cell colonization [1,2]. Mechanical behaviour of this synthetic structure and reconstruction of natural tissue by cells are the two key points for such a successful device. Our laboratory works for several decades on the creation of bioactive surfaces using sodium poly(styrene sulfonate) (pNaSS) which can control the cell response[3, 4]. Because the improvement of the biological response does not have negatively impact on the mechanical behaviour of the future implant, especially for an orthopaedic prosthesis, we studied the evolution of these properties on poly(ε-caprolactone) (PCL) fibers bundles at each steps of the grafting process, until sterilization. The results show that PCL bundles have the same visco-elastic comportment than the native ACL. Plus, we demonstrated the possibility to improve the elasticity of the construct with the grafting process, which can be very interesting to avoid distension of the future prosthesis and to restore good knee stabilization.

REFERENCES

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**Engineering tough and stretchable hydrogels**

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Alginate hydrogels, obtained by ionic crosslinking, are versatile materials with a broad range of applications, from biomaterials, mainly for cell encapsulation and drug delivery, to food science. Up to a certain extent, the properties can be tailored by controlling the crosslinking, either by employing different multivalent cations or by different gelation processes, namely internal and external gelation. Solvent treatments may also affect the properties of alginate hydrogels. Yet, up to now, the need for tough hydrogels was met by developing double interpenetrated network hydrogels (IPN), in which the main network is represented by synthetic, non-degradable, polyacrylic acid or polyacrylamide that dominates the mechanical features of the hydrogel, bringing exceptional properties (up to 225 kPa elastic modulus both in tension and compression)¹. In this work, a range of interpenetrated hydrogels with both networks made of alginate, “alginate-in-alginate” IPN, are proposed. They were obtained using a combination of internal and external gelation processes (Alg⁰⁰°⁰) and treatment with a non-solvent, such as ethanol (AlgEtOH). Extensive mechanical analysis was performed using dynamic mechanic analysis in compression and tension mode, as well as rotational and extensional rheology. Additionally, stability assays were conducted in dH₂O at 25 and 37 ± 1°C. The Alg⁰⁰°⁰ hydrogels exhibited lower compressive modulus than AlgEtOH hydrogels (about 50 and 240 KPa, respectively), while in tension Alg⁰⁰°⁰ hydrogels displayed higher tensile modulus than AlgEtOH hydrogels (about 410 and 370 KPa, respectively). The extensional rheology was also performed. The extensional viscosity is within the same order of magnitude for both types of hydrogels at the different extension rates, yet AlgEtOH hydrogels display strain hardening (i.e. at large strain, the stress increases more than proportionally). In this way, tough and stretchable (up to 90% strain) hydrogels were synthesized via a frugal, facile and cheap method. Ongoing work is focused on relating the different mechanical and rheological properties to generate greater insights in the structure and mesh size of hydrogels, which can expand the potential application of these materials in the fields of regenerative medicine and drug delivery.


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**Heparin-Poloxamer and Gellan Gum Composite for Enhanced Injectable Hydrogel**

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Gellan gum(GG) hydrogels are advantageous in tissue engineering due to their ability to provide a similar environment to that of natural extracellular matrix (ECM). Nevertheless, gellan-based hydrogels need to be modified in order to encapsulate living cells, because the gelling point of this temperature-dependent gel is too high(above 42°C). A temperature-sensitive heparin modified poloxamer(HP) polymer decrease GG gelation temperature. In this study, HP-GG hydrogel was fabricated to evaluate mechanical properties of hydrogel and retinal pigment epithelium regeneration. HP polymer was made by chemical reaction and loaded in to crosslinked GG. HP/GG was fabricated by using 1% GG and 0.025wt%, 0.05wt%, 0.1wt% HP was loaded in GG. HP/GG hydrogel were analyzed using SEM, MTT, FTIR, RT-PCR, viscosity and compressive strength. It was shown that HP-GG hydrogel increased physical and biological properties compared to control group.

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**01-P222 Supermolecule-based culture substrates with tethered BMP-2 enhance osteogenic differentiation**

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Polyrotaxane (PRX) is a supermolecule with many cyclic molecules (e.g., α-cyclodextrin (α-CD)) threaded onto an axis polymer (e.g., polyethylene glycol (PEG)) and characterized by modulating its molecular mobility via the threading cyclic molecules along the chain. Previously, we have succeeded in modulating the differentiation of mesenchymal stem cells using PRX-based surfaces. For instance, less mobile PRX surfaces enhance the formation of actin filaments (F-actin) to preferentially induce osteogenic differentiation, whereas highly mobile PRX surfaces preferentially induce adipogenic differentiation accompanied by the inhibition of F-actin formation. Alternatively, we have reported that sulfonated-PRXs can form polyelectrolyte complexes with bone morphogenetic protein-2 (BMP-2), resulting in the acceleration of the osteogenic differentiation of MC3T3-E1 cells as preosteoblasts. In the present study, we prepared sulfonated-PRX based surfaces with BMP-2 for enhancing osteogenic differentiation. The sulfonated-PRX substrates were fabricated by coating sulfopropyl ether-modified PRXs (SPE-PRX) triblock copolymers onto polystyrene surfaces by a drop-casting method. And then, BMP-2 was tethered on the sulfonated-PRX surfaces by electrostatic interactions. For assessing the effect of molecular mobility of the SPE-PRX surfaces, the proliferation of MC3T3-E1 cells was analyzed by microscopic observation. The cell proliferation on less mobile SPE-PRX surfaces with a large number of threaded α-CDs was higher than that on highly mobile SPE-PRX surfaces with a small number of threaded α-CDs. This result indicates that the less mobile SPE-PRX surfaces promote the proliferation of MC3T3-E1 cells and are suitable for inducing osteogenic differentiation. For evaluating osteogenic differentiation, gene expression and mineralization of MC3T3-E1 cells on the surfaces were analyzed by qRT-PCR and alizarin red S staining. MC3T3-E1 cells were cultured on the SPE-PRX surfaces and non-sulfonated PRX surfaces with tethered or added BMP-2. The BMP-2-tethered SPE-PRX surfaces with less mobility induced the highest mineralization and expression levels of the runx2, alp, and ocn genes among all culture conditions. These results indicate that the sulfonated PRX surfaces with BMP-2 strongly induce the osteoblast differentiation by the synergistic effect of the mobility of PRX and tethering of BMP-2.

References:
**01-P225** Perfusion culture system using paper-based bilayer scaffold with porous microfibers for effective myotube formation

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Engineered skeletal muscle tissues for transplantation therapy in regenerative medicine and for in vitro model in drug development are useful and important. Skeletal muscles are formed by fusion of longitudinally adjacent and aligned myoblasts. Therefore, control of the cells orientation is a crucial factor to develop the engineered skeletal muscles. In this study, we used oriented microfiber scaffolds to effectively induce myoblasts alignment and differentiation into myotubes. Poly(caprolactone) (PCL), a biodegradable synthetic polymer, was employed as the microfiber material, and the cellular adhesiveness to PCL microfibers was improved by creating porous structure on the surface. Moreover, various fluidic flows in the body give shear stresses and positively work to develop tissues like vessels. Accordingly, we tested the hypothesis that flow stress also enhances formation of muscle tissue in vitro. For this purpose, we developed a novel bilayer scaffold, in which paper was combined to basal side of the PCL porous microfibers. Medium flow was generated by capillary action of the paper in a self-driven mode. Siphon principle was also utilized to realize continuous flow by bridging two medium chambers in different height with the bilayer scaffold. Porous PCL microfibers were electrospun on a paper with the breath figure method, and approximate single orientation was obtained by collecting with a rotating drum. Mouse skeletal myoblasts (C2C12) were cultured on the bilayer scaffolds in perfusion or static (control) conditions. Growth medium or differentiation-inducing medium were used. Pre-incubation was performed using growth medium in static conditions for 2 days, prior to culture using each medium in each condition for 3 days. The static and perfusion cultures in growth medium proliferated C2C12 cells approximately twice as much as the static culture in differentiation-inducing medium. In addition, aligned myotubes formed in the perfusion cultures occupied approximately two times larger area than those in the static cultures, whichever medium was used. These results suggest that the shear stress by medium flow effectively induced myotube formation, accompanying with enhancement of cell proliferation.
Automated Adherent Cell Elimination by a High-Speed Laser Mediated by a Light-Responsive Polymer

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The purification of different types of cultured cells is critical in various biomedical fields, including basic research, drug development, and cell therapy. Conventionally, fluorescence-activated cell sorting (FACS), affinity beads, gradient centrifugation, and elutriation have been used for cell purification. However, the process of detaching, dissociating, sorting, and reseeding can result in low yield and in altered cell characteristics for adherent cells. We have developed a Laser-induced, Light-responsive- polymer-Activated, Cell Killing (LILACK) system enabling high-speed and on-demand adherent cell sectioning and purification. A visible laser beam, which does not kill cells directly, induces local heat production in only the irradiated area of a light-responsive thin layer. This scheme enables effective cell killing even at very fast beam scanning without damaging neighbouring unirradiated cells. To examine the safety and feasibility of this LILACK system, we examined the effect of the long-term culturing of human induced pluripotent stem cells (hiPSCs) using the developed laser-mediated processing on the light-responsive polymer. After 10 passages through the system, the hiPSCs were characterized. The karyotype of these hiPSCs was maintained in all the cells. These cells expressed self-renewal markers of pluripotent stem cells. When differentiated using embryoid body (EB) formation, these cells differentiated into three germ layers. Additionally, these hiPSCs were free of harmful viruses and mycoplasma. We also developed a label-free cell elimination system based on deep machine-learning imaging analysis. Then, we applied this trained classification algorithm to our laser-mediated cell elimination with only phase-contrast images. After the automated cell elimination, we found that the TRA1-60-positive cell ratio increased after laser irradiation to eliminate the “differentiated” cells classified by this algorithm. These results indicate that in situ label-free cell purification was achieved using our LILACK system combined with imaging analysis based on deep learning. We believe that our methods can be widely used in various biomedical fields, including basic research, drug development, and cell therapy. Since the classification of cell types based on deep-learning methods is advancing rapidly, the importance and functionality of our technology will be further enhanced in the near future.

Novel Human Pluripotent Stem Cell Expansion Polymers: A High-Throughput Micro Array Discovery Campaign

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Human pluripotent stem cells (hPSCs) are able to differentiate into virtually any cell type in the body, potentially providing a new resource for regenerative medicine. However, the use of poorly defined, animal-derived substances as cell attachment surfaces for hPSC expansion is commonplace; this leads to unwanted batch variability, increased chance of contamination and greater regulatory control over the materials used.1–3 Currently, synthetic substrates identified for hPSC expansion have issues associated with their use, often requiring protein pre-conditioning4 which limits their commercial and practical viability. Progress has been made with industrially-viable surfaces developed for a range of culture media,5 however no cost-effective synthetic substrates have yet been shown to support hPSC growth using the most recent, chemically-defined Essential 8™ (EB™) growth medium.5 As with cell attachment surfaces, use of a highly-defined medium such as EB™ reduces batch inconsistencies, facilitating hPSC expansion and research by providing reproducible access to appropriate numbers of cells for biomedical applications.7 Building upon techniques developed in our laboratory,8 we have applied a high-throughput screening approach to materials discovery for hPSC culture in commercially available EB™ medium. Using printed polymer arrays, created from a library of over 280 monomers polymerised both individually and mixed, we have identified the first low-cost polymeric materials suitable for further investigation in pluripotent hPSC expansion. We are currently working towards developing our hPSC culture system for use with existing standard laboratory cultureware, with a view to providing an industrially-viable, defined and scalable solution for hPSC culture.

1. Fox IJ et al, Science 345, 889, 2014
Tissue Engineering Approaches Using Piezoelectric Scaffolds

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Introduction:
Piezoelectric materials are attracting attention from the tissue engineering community[1]. This study demonstrates the efficacy of polypyrrole PVDF piezoelectric films and fibres for bone tissue engineering purposes.

Experimental:
Solution blow spinning is utilised to fabricate aligned fibre mats of PVDF and PVDF/graphene oxide (GO). Scanning electron microscopy (SEM), Fourier-transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC) and X-ray diffraction (XRD) measurements are performed on fibres. Commercial piezoelectric and non-piezoelectric PVDF films along with PVDF and PVDF/GO fibres have been used for cell culture studies. Resazurin reduction assay is utilised for quantification of metabolic activity of cells. A four point bending bioreactor has been fabricated to mechanically deform piezoelectric films to stimulate cells electrically. Modelling and simulation of 4 point bioreactor system is carried out in Abaqus to discuss the limitations of the device.

Results and discussion:
SEM images reveal average fibre diameter of ~1.5 μm. FTIR data is utilised to quantify the amount of β phase(~75% for fibres), DSC shows that the fibres are more than 50% crystalline and XRD qualitatively confirms this. Metabolic activity of MC3T3-E1 cells in static culture(no mechanical stimulation) of PVDF fibres on day 7 is significantly higher than non-piezoelectric films but the piezoelectric PVDF films display significantly higher metabolic activity than PVDF fibres. GO incorporation on PVDF fibres results in higher metabolic activity than fibres which is similar to those of piezo films. Four point bending bioreactor fabricated for mechanical and consequent electrical stimulation of cells on piezo films is validated through modelling, simulation and experiments to deliver up to 5000 με of uniaxial tensile strain.

Conclusions and future work:
Static cell culture experiments reveal that poling PVDF significantly contributes to increased metabolic activity which can be further enhanced by adding fillers and utilising dynamic mechanical stimulation of PVDF scaffolds. A uniaxial tensile strain of 1000 με will be applied to assess the effect of piezoelectricity on cells.

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References:

Enhanced myotube formation on the oriented coaxial core-sheath microfiber scaffold embedding high conductive polymer without applied voltage

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Skeletal muscles cannot sufficiently regenerate when damaged to a large extent by an accident or a disease. Therefore, fabrication of transplatable skeletal muscles in vitro is strongly desired. Skeletal muscles are developed from aligned myotubes, which are formed via fusion of longitudinally adjacent myoblasts. Thus, the myotube formation in vitro is effectively promoted by culturing myoblast along the oriented microfiber scaffolds. Moreover, electric effects associated with conductive scaffolds also positively work to enhance the myotube formation. PEDOT/PSS shows much higher conductivity among the conductive polymers, but cannot be used alone as scaffolds because easily dispersed in water and inferior in cell adhesion. In this study, we developed the oriented coaxial core-sheath microfibers via electrospinning, in which a mixture of cell-adhesive gelatin (GN) and stiff, biocompatible poly(e-caprolactone) (PCL) covered the core PEDOT/PSS fiber. The bilayer structure improved the stability of PEDOT/PSS in an aqueous culture media, and the oriented fiber structure and core PEDOT/PSS positively affected myotube formation.

GN and PCL for the sheath layer were dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol in the ratio of 5/8 (w/v), and the oriented microfibers were electrospun using a rotating drum collector. Cross-linking of GN by exposing the fibers to glutaraldehyde vapor rendered the sheath layer stable in the media. GN/PCL fibers without the core PEDOT/PSS and blended fibers of GN/PCL and PEDOT/PSS without the bilayer structure were also fabricated as control samples. The core-sheath structure was clearly observed by transmission electron microscope, and scanning electron microscope observation indicated average diameters of approximately 1 mm for all the fibers. On the three types of fiber scaffolds in growth medium, mouse myoblasts (C2C12) proliferated equally, and the cell densities were saturated after 5 days. After the saturation in growth medium, C2C12 cells were cultured in differentiation-inducing medium for inducing myotube formation. Longer myotubes were formed on the core-sheath microfiber scaffold than the control samples. These results indicate that the core PEDOT/PSS promoted not cell proliferation but the myotube formation. This positive effect was exclusively observed for PEDOT/PSS but not on similar core-sheath fiber scaffolds with polyaniline or polypyrrole.
**01-P230** Fabrication of long luminal tissues in three-dimensional gel scaffolds with oriented cellulose nanofibers

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Blood vessels are indispensable for fabricating large regenerative tissues to supply oxygen and nutrients throughout the tissues. Small vessels embedded in tissues have luminal structures with one or more layers composed of different cell species. Therefore, precise control of cells’ arrangement and position in a long three-dimensional scaffold to efficiently mature is the effective way for fabricating regenerative vessels. In this study, we developed coaxial bilayer scaffolds of hydrogels (gel fibers) by using microfluidic devices. Our gel fibers had a sheath of alginate gel, which completely covers a core layer of cellulose nanofiber (CNF) gel as a cell-culture substrate.

Throughout the study, we used algicn acid as the sheath layer material, because it rapidly gelaes when complexed with calcium cation. It formed the stable three-dimensional scaffolds and enabled prolonged culture. The core layer as a cell-culture substrate was located at the interior of the sheath layer. In a previous study, we used atelocollagen as substrate for embedding cells, but we got cell aggregation due to its low cell-retention capability. On the other hand, the new CNF gel shows thixotropic nature due to the extremely high aspect ratio of CNF (diameter of several nm, length of several μm). Owing to this useful nature, the CNF can fluidically flow in the pressurized environment within the device, whereas it immediately gelaes when ejected from the outlet. Good cell-retention capability is expected due to this rapid gelation. Bovine vascular endothelial cells (BAECs) and mesenchymal stem cells (MSCs) were embedded in the core gel, and co-cultured to mature into luminal tissues.

The gel fiber scaffolds with tens of centimeters in length was successfully fabricated in optimized conditions. The fiber diameter was approximately 200 μm. BAECs and MSCs were stably retained in the gel fibers over 3 weeks. The cells extended well, adhered to each other, and finally formed long continuous structures. Moreover, it exhibited lumen-like structure after 20 days culture, confirmed by fluorescent staining of the nuclei and actin cytoskeleton. This study indicated that the thixotropic CNF gel fibers are available as an effective scaffold for regenerative vessels. Fabrication of other tissue is currently underway.

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**01-P231** Aging Donor-derived Human Mesenchymal Stem Cells Exhibit Reduced Senescence-associated Activities Following Serial Expansion on a PEG-PCL Copolymer Substrate


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Human mesenchymal stem cells (hMSCs) have been widely studied for therapeutic development in tissue engineering and regenerative medicine. They can be harvested from human donors via tissue biopsies, such as bone marrow aspiration, and cultured to reach clinically relevant cell numbers. However, an unmet issue lies in the fact that the hMSC donors for regenerative therapies are more likely to be of advanced age. Their stem cells are not as potent compared to those of young donors, and continue to lose healthy, stemness-related activities when the hMSCs are serially passaged in tissue culture plates. Here, we have developed a cheap, scalable and effective copolymer film to culture hMSCs obtained from aged human donors over several passages without loss of reactive oxygen species (ROS) handling or differentiation capacity. Assays of cell morphology, reactive oxygen species load, and differentiation potential demonstrate the effectiveness of copolymer culture on reduction in senescence-related activities of aging donor-derived hMSCs that could hinder the therapeutic potential of autologous stem cell therapies.
**01-P232** The sulfation of biomimetic sulfated glycosaminoglycans controls growth factor binding and subsequent cell proliferation and differentiation

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Glycosaminoglycans (GAGs) are major components of the extracellular matrix that possess structural and functional roles in many tissues. The sulfation arrangements of GAGs have been tightly correlated with biological events including morphogenesis, tissue repair and aging. However, it is not clear how such events are affected by sulfation. Elucidating the mode of action of sulfated GAGs will enable the synthesis of biomimetic 2D and 3D substrates that can be used in the treatment of diseases and injuries.

Here, alginate was sulfated at different sulfation degrees and then biotinylated in an end-on fashion. The biotinylated sulfated alginites were then used to modify gold or polystyrene substrates. Binding of growth factors including fibroblast growth factor (FGF), epidermal growth factor (EGF) and nerve growth factor (NGF) to the substrates was assessed quantitatively using quartz crystal microbalance with dissipation monitoring (QCM-D) and ELISA and validated qualitatively with immunostaining. Finally, the morphology and growth of cells including normal and tumour breast epithelial, neuroblastoma and lung cancer cells was evaluated via ImageJ. 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) and 5-bromo-2′-deoxyuridineBrdU.

Sulfated GAG modified substrates with different degrees of sulfation were constructed and assessed using QCM-D. Growth factor binding was found to increase with increased sulfation of GAGs. These findings were confirmed with ELISA and immunostaining. Cell proliferation was typically hindered when sulfated materials were added in solution. However, when neuroblastoma cells were cultured on sulfated substrates, they exhibited more stem cell spheres on the more sulfated substrates.

The ability to prepare sulfated substrates with controlled sulfation levels has strong implications in the biomedical field. It can be used to induce different levels of growth factor binding, increase/inhibit cell proliferation thus having major implications in tissue engineering and cancer therapy.

**01-P233** PVA hydrogel as a substrate for adherent culture of neural stem/progenitor cells

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Introduction: Neural stem/progenitor cells (NSPCs) have the capacity to proliferate and are multipotent, giving rise to neurons, astrocytes, and oligodendrocytes. NSPCs can be amplified in neurosphere suspension cultures. However, the suspension cultures have certain limitations, including the inconvenience of changing the culture medium as well as difficulty of live imaging. In the present study, we prepared a γ crosslinked poly (vinyl alcohol) (PVA) hydrogel as a substrate for adherent NSPC cultures.

Experimental: A PVA solution (3.75, 7.5% and 15.0% (w/v)) were irradiated with various doses (10, 20, or 40 kGy) of γ ray at room temperature (25° C) in the cobalt 60 (60Co) γ ray facility of Osaka Prefecture Univ (OPU). NSPCs were isolated from the fetal brain of an imprinting control region mouse (embryonic day 14) and resuspended in DMEM/F12 medium with EGF (20 ng/ml), bFGF (20 ng/ml), and B27 supplement. Cells (1.0 × 10^5/ml in 10 ml medium) were cultured in dishes at 37° C and 5% (v/v) CO2 to induce neurosphere formation and were expanded by dissociation with trypsin and reseeding every 7 days. All animal experiments were conducted in accordance with institutional guidelines and national standards with approval from the Animal Experiment Committee of OPU. NSPCs after several passages were used for analysis on a PVA gel. Differentiation was determined by evaluating the expression of the markers nestin (progenitors), βIII tubulin (neurons), and glial fibrillar acidic protein and S100 β by immunocytochemistry and quantitative reverse transcriptase PCR.

Results and Discussion: NSPCs adhered to the PVA gel as clusters and grew without differentiating into neurons and glia. The cluster was hemisphere or in a mount like shape on the surface of the PVA gel. The proliferation rate of cells grown on the soft PVA gel (3.75 and 7.5% (w/v) PVA) was approximately 70% of that of neurospheres in suspension. The levels of the marker genes were similar between the two types of culture; although some variability was observed, there were no fold differences in expression. We conclude that γ crosslinked PVA hydrogels can function as a novel scaffold for maintaining adherent culture of NSPCs in an undifferentiated state.

In regenerative medicine, cell separation methods for isolating specific cells from cell mixture suspension is required. Conventional cell separation methods require fluorescent or magnetic antibody for labeling cells. These methods can separate small amounts of cells at once, and labeling substances on cell surfaces can be toxic when cells are transplanted to living body. For overcoming the issue, we developed new cell separation tool for separating targeted cells using thermoresponsive surface modified cell adhesion peptide.

The thermoresponsive polymer poly(N-isopropylacrylamide) (PNIPAAm) was grafted on the glass substrate surface by reversible addition-fragmentation chain transfer (RAFT) polymerization. Subsequently, the cell adhesive peptides Arg-Gly-Asp (RGD) was attached on the terminal group of PNIPAAm. Human umbilical vein endothelial cells (HUVECs) were seeded on the prepared RGD-PNIPAAm grafted surface. Cell adhesion and detachment behavior on the surface was observed at 37 °C and 20 °C, respectively. Cell adhesion on prepared RGD-PNIPAAm surface was observed after incubation at 37 °C. On the RGD-PNIPAAm surface, cells adhered even without fetal bovine serum, and the adhesion was enhanced than that on RGD unmodified surface. By reducing temperature to 20 °C, adhered cells on surface was successfully detached attributed to hydration of modified PNIPAAm on substrates. By modifying the cell adhesion peptide to PNIPAAm on the surface, effective temperature modulated control of cell adhesion and detachment was performed. The intrinsic property of the surface would be useful for selective adhesion of targeted cells, and thermally-modulated cell separation.

Stem cells are attractive reagents for tissue engineering applied on regenerative medicine, translational medicine, and drug discovery. However, the dishes for conventional batch-type cultivation are disposable, and enzymes are used to detached cells from cell culture dishes. In order to reduce the use of disposable dishes and enzymes for batch-type culture processes, we designed to proliferate human embryonic stem cells (hESCs) on the thermoresponsive nanobrush dishes. The thermoresponsive nanobrush surfaces were prepared with coating of thermoresponsive polymers having the low critical solution temperature (LCST) on the cell culture dishes where hESCs can be detached from the thermoresponsive surface by decreasing the temperature of culture medium below LCST. The thermoresponsive nanobrush dishes were prepared by coating of poly(N-isopropylacrylamide-co-butylacrylate) (polyNIPAM-BA) and Pluronic on the cell culture dishes. In this study, hESCs are successfully cultured on the thermoresponsive surface and partially detached from the surfaces by incubating at low temperature (7-8°C) for 30 minutes. The optimal surface composition for hESCs detached by decreasing temperature of the culture medium was investigated. The surface water contact angle measurement and X-ray photoelectron spectroscopy were used to analyze the hydrophilicity and chemical element composition of dishes surface, respectively. This partial detached process system allows cells maintain their pluripotency on the surface and prevents hESCs from enzymatic digestion damages. The continuous harvest of stem cells should simplify the culture process and reduce the equipment requirements for stem cell culture. In the future, we also expect to scale up cell numbers via applying the continuous harvest system on 3D cultivation. These improvements should provide a great benefit to clinical application in regenerative medicine.
Coaxial electrospun microfibers containing valproic acid for neural tissue engineering applications

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Valproic acid (VPA), a known histone deacetylase inhibitor used to treat epilepsy and bipolar disorders, is a promising therapeutic intervention in animal models of spinal cord and peripheral nerve injuries. It has been demonstrated that VPA promotes neurite outgrowth and neuronal survival. Association between VPA and biomaterial scaffolds can be used for local and controlled release of this drug and improve its effects in neural regeneration. In this study, VPA was encapsulated in core-shell microfibers by coaxial electrospinning and its biological potential was analyzed. The core of the fibers consisted of 25 mg/mL valproic acid sodium salt, 10% polyethylene glycol and 2% bovine serum albumin; the shell solution was 18% poly(lactic-co-glycolic acid) in 1,1,1,3,3,3-hexafluoro-2-propanol. The core solution was injected at a flow rate of 0.2 ml/h, the shell solution at 2 ml/h and the applied voltage was 16 to 25 kV. The fiber morphology was analyzed by scanning electron microscopy (SEM) and their diameter calculated using ImageJ software. Verification of the core–shell structure was performed by laser confocal scanning microscopy (CLSM). The biological potential of the biomaterial was evaluated using the rat pheochromocytoma PC-12 cell line by MTT assay; cell morphology was analyzed using SEM and CLSM. The fibers presented a uniform morphology without any beads and a hydrophobic surface, with a contact angle of 129.7° ± 3.0. The average diameter of the fibers was of 2.00 μm ± 0.61. By using fluorescein in the core, it was possible to confirm the presence of the encapsulated fluorescence inside the fibers by CLSM. In vitro, the coaxial fiber scaffold robustly supported attachment of the PC12 cells, as shown by the SEM images. The MTT assay demonstrated that the cells proliferated and maintained viability; for the cells on the scaffolds with VPA, the absorbance was 0.058 ± 0.009 on day 3, similar to the cells seeded on the scaffolds without VPA (control) the absorbance of which was 0.066 ± 0.021 on day 3. Future evaluations using WST-8 cell proliferation assay on cultivation days 3 and 7 will be performed. These results indicate that VPA-PLGA coaxial microfibers have great potential for neural tissue engineering applications.

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Fabrication of long myotube bundles in three-dimensional gel scaffolds with oriented cellulose nanofibers

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Regenerative skeletal muscles for transplantation to damaged tissues and pharmaceutical testing have attracted much attention. Myoblasts fuse and differentiate to oriented myotubes, which are bundled together into the ordered structure of skeletal muscles. Therefore, one of the most effective way for fabricating regenerative skeletal muscles in vitro is to precisely control arrangement and position of myoblasts and mature them in a long three-dimensional scaffold. In this study, we developed bilayer fiber scaffolds, in which a sheath of alginate gel completely covers a core layer of cellulose nanofiber (CNF) gel, by using microfluidic devices, embedded mouse myoblasts (C2C12) in the core CNF gel, and matured them to form long myotube bundles. Conventionally, atelocollagen has been used as substrate for embedding cells, but caused cell aggregation due to its low cell-retention capability. On the other hand, our CNF gel shows thixotropic nature due to the extremely high aspect ratio of CNF (diameter of several nm, length of several μm), resulting in fluidization by the applied pressure for flowing in the microdevice and gelation immediately after ejection from the device. That is, good cell-retention capability in culture is obtained. Moreover, alginic acid used in the sheath rapidly gelates in the presence of divalent cation and forms the stable three-dimensional scaffolds with the capacity for prolonged culture.

We successfully fabricated gel fiber scaffolds with tens of centimeters in length and precisely regulated diameters of the whole fiber and core layer by the flow rates. The embedded C2C12 cells extended in the longitudinal direction of the gel fibers and formed oriented structures more than 2 cm long within 1 day culture. The fluorescent staining of the nuclei and myosin heavy chains in the structures obtained after prolonged culture clearly indicated the fusion and multinucleation of the cells and formation of myotube bundles. We presumed that the orientation of CNFs in the longitudinal direction of the gel fibers induced the myotube formation, based on the result that the high-aspect-ratio CNFs were oriented by flow stress.
Functionalization of Cell Culture Surfaces using Human Hair Keratins and Keratin Associated Proteins

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Human hair proteins have shown great potential in biomedical applications due to their biocompatibility, abundance and potential for autologous strategies. Herein, we explored the development of hair protein-based 2D templates as cell culture substrates. Hair proteins were immobilized on plasma-treated polystyrene substrates via free-radical assisted covalent crosslinking. This coating offered long term stability up to 21 days in cell culture media and high protein retention (76.79 ± 2.69 %) even after harsh solvent washing. This stable interaction was likely a result of the antioxidant behaviour of reduced cysteine residues on hair proteins, which readily react with free-radicals on the plasma-activated surface. Separately, two main components of hair proteins, keratin intermediate filaments proteins (KIFP) and keratin-associated proteins (KAP), were separately extracted from hair. A peelable and clear KIFP thin film was obtained by solvent casting at room temperature without additional crosslinkers or plasticizers. This film was mechanically stable and had tensile strength that was 27 times higher than films made with total human hair proteins with plasticizer. We hypothesize that KAP can be additionally used as an exogenous crosslinker to produce mechanically tuneable KIFP thin films. Further understanding of the mechanisms of interactions between KIFP and KAP could allow the development of novel and versatile hair protein-based 2D templates for biomedical applications.
Neural stimulation/recording electrodes have been widely employed to investigate neural activities, diagnose and treat many diseases. Many strategies are promoted to develop ideal electrode-tissue interface with properties as follows: electrochemical properties including high charge storage capability (CSC), low electrical impedance and high charge injection limit; long-term stability during continuous stimulation; biocompatibility ensuring cells survive and network formation.

In this study, we deposited iridium (Ir) on the polished metal substrate by radio-frequency magnetron sputtering, and activated Ir to form iridium oxide (IrOx) thin film by repetitive potential sweeps in diluted sulfuric acid (H2SO4). After activation, the IrOx presented a porous structure, with increasing CSC value from 2.2 mC/cm2 of Ir to 54.35 mC/cm2. The impedance of IrOx thin film decreased nearly two orders of magnitude at 1 kHz compared to bare Ir. The IrOx thin film also showed good mechanical and electrochemical stability. IrOx improved the adhesion, proliferation of NSCs compared to Ir without exhibiting any cytotoxicity. In addition, we applied electrical stimulation on pheochromocytoma cells (PC12) seeded IrOx. The proliferation of PC12 was not influenced by the electrical stimulation. The direction of neurite growth was aligned to the direction of electrical fields. Also, the electrical stimulation increased the neurite extension and network formation.

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Interaction of gelatin hydrogels with different mechanical properties and macrophages

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Macrophages play an important role in inflammatory reactions. The macrophages functions often modify the subsequent immunological events. Considering the biocompatibility of biomaterials implanted, their in vivo fate greatly depends on the macrophages responses. The objective of this study is to obtain fundamental knowledge on the interaction of biomaterials and macrophages. It is well recognized that the cell behavior is influenced by several biomaterials properties, such as the surface and bulk physicochemical natures. In this study, varied concentrations of gelatin solution were crosslinked by glutaraldehyde to prepare gelatin hydrogels with different stiffness. Compressive tests of hydrogels prepared were performed to determine their elasticity. RAW264.7 cells of a macrophage cell line and mouse bone marrow-derived macrophages were cultured on the gelatin hydrogels to evaluate the attachment and proliferation of cells. In addition, the production profiles of cytokines and nitric oxide (NO) were examined in terms of macrophage polarization, such as pro-inflammatory (M1) and anti-inflammatory (M2) natures. The elasticity of gelatin hydrogels varied by the concentrations of gelatin in hydrogel preparation. Macrophages attached on the gelatin hydrogels, although the number was less than that of the cell culture polystyrene dish. The production of tumor necrosis factor α and NO was suppressed for macrophages cultured on the gelatin hydrogels. We also report the effect of hydrogel properties on the human monocyte derived macrophages.

Development of simple and convenient system for single cell separation

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The construction of method to obtain single cell species is required in various fields. Several systems for cell sorting have existed already. One is flow cytometry methods such as fluorescence and magnetic associated cell sorting which are the most used methods. Moreover, it is suggested that single cell species can be separated by cell size fractionation using membrane system. It is well recognized that the cell behavior is influenced by several biomaterials properties, such as the surface and bulk physicochemical natures. In this study, varied concentrations of gelatin solution were crosslinked by glutaraldehyde to prepare gelatin hydrogels with different stiffness. Compressive tests of hydrogels prepared were performed to determine their elasticity. RAW264.7 cells of a macrophage cell line and mouse bone marrow-derived macrophages were cultured on the gelatin hydrogels to evaluate the attachment and proliferation of cells. In addition, the production profiles of cytokines and nitric oxide (NO) were examined in terms of macrophage polarization, such as pro-inflammatory (M1) and anti-inflammatory (M2) natures. The elasticity of gelatin hydrogels varied by the concentrations of gelatin in hydrogel preparation. Macrophages attached on the gelatin hydrogels, although the number was less than that of the cell culture polystyrene dish. The production of tumor necrosis factor α and NO was suppressed for macrophages cultured on the gelatin hydrogels. We also report the effect of hydrogel properties on the human monocyte derived macrophages.
Gelatin hydrogel microspheres improve the activity of multi-layered cell sheet

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Cell sheet is one of the promising and powerful technologies for cell therapy. As a trial to enhance the biological functions, the multi-layering of cell sheet has been investigated. However, cells in the multi-layered are weaken or often die mainly due to a poor supply of nutrients and oxygen. The objective of this study is to improve the poor nutrients and oxygen supplies to cells by making use of gelatin hydrogel microspheres (GHM). Gelatin is well known as one cytocompatible polymer. Since the GHM have water inside, it is expected that the nutrients and oxygen can go through the water phase of GHM. We examined the cell viability and proteins expression of multi-layered cell sheets incorporating GHM to compare with those of GHM-free ones.

GHM were prepared by a water-oil emulsion method, followed by sieving with size from 20 to 32 µm in diameter, and thermally crosslinking at 140 °C for 72 hr. Mixed fibroblasts and cardiac cells were seeded on a cell culture plate. After 3 days incubation, the cell sheet prepared was piled up 5 times incorporating 500 µg/cm² of medium-swollen GHM inbetween each sheet to prepare the 5 layered cell sheet with GHM. As a control, the same procedure was performed except for the GHM incorporation to obtain 5 layered cell sheet without GHM. Following incubation, the cell viability, ischemic condition, ATP or the extra cellular matrix productions, apoptosis inhibition, and transcript factors expression were evaluated.

The amount of ATP produced was significantly higher for the 5 layered cell sheet with GHM than for that without GHM. High expression of anti-apoptosis factors was observed for the 5 layered cell sheet with GHM. In addition, there was significant difference in the expression level of hypoxia inducible factor, anti-apoptosis factors, and transcription facilitators between the 5 layered cell sheets with and without GHM.

Electrochemical Manipulation of an Epithelial Monolayer Supported by a Biodegradable Polymeric Nanosheet for Cell Transplantation Therapy

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Cell transplantation therapy based on cell sheet engineering is a promising approach for the treatment of patients with difficult-to-treat diseases and impaired physical function. Recently, we investigated the use of biodegradable polymeric nanosheets as cell scaffolds. The cell/nanosheet constructs raise the possibility of transplanting cell monolayers into a narrow space, such as the subretinal space, in the body in a minimally-invasive manner [1]. However, it had been difficult to control the timing of the detachment of the cell/nanosheet constructs from a substrate in conventional methods. Then, we investigated the reductive desorption of a self-assembled monolayer (SAM) of thiol molecules to detach the cell/nanosheet constructs [2].

A poly(lactic-co-glycolic acid) (PLGA) nanosheet was attached by hydrophobic interactions onto the surface of a SAM of L-cysteine coated onto a gold electrode. Retinal pigment epithelial (RPE) cells were cultured on the nanosheet to form a monolayer. An AA-size dry battery was used to apply a negative electrical potential, causing reductive desorption of the SAM from the gold surface. Within one minute of application of the voltage, the cell/nanosheet of several mm in diameter was successfully detached without the loss of cell viability in a gentle stream of the electrolyte solution. The use of a porous electrode shortened the detachment time due to the more efficient permeation of the electrolyte solution to the electrode surface. Cell transplantation following the harvesting process was demonstrated by the local delivery of RPE cell/nanosheet constructs into the subretinal space of rat eyes through a capillary needle. This nanosheet-based approach that allows the on-demand harvesting of cell/nanosheet constructs and their subsequent transplantation in a minimally-invasive manner could play an important role in cell transplantation therapy.

Cell sheets have been widely used to tissue engineering and regenerative medicine as effective approach. In the case of cardiomyocyte sheets transplantation in vivo, more than three layered cardiomyocyte sheets cannot be transplanted at once, attributed to the necrosis caused by insufficient oxygen and nutrient. In this study, vascular endothelial growth factor (VEGF) releasing fiber mat was prepared and used for enhancing vascularization in cardiomyocyte sheets in vivo. Polyvinyl alcohol (PVA) nanofibers were prepared through electrospinning. Then, poly(lactic-co-glycolic acid) (PLGA) nanoparticles containing VEGF were loaded on PVA fiber mats through electrospray deposition. The prepared VEGF releasing fiber mat was transplanted with six layered cardiomyocyte sheets to athymic nude rat. At two weeks after transplantation, the thickness of transplanted cardiomyocyte sheets was evaluated by immunostaining. Cardiomyocyte tissue transplanted with VEGF releasing fiber mat maintained their thickness compared those with VEGF-free fiber mat as control. Also, the transplanted layered-cardiomyocyte sheets included blood vessels with larger diameter. The result indicated that the prepared VEGF releasing fiber mat can maintain thick cardiomyocyte tissue in vivo, because of enhanced vascularization and supplying oxygen and nutrients to cardiomyocyte tissues. The VEGF releasing fiber mat would be useful for enhancing vascularization within transplanted layered cardiomyocyte sheets in vivo.
**Aqueous Solutions of Poly (2-n-propyl-oxazoline): An Easy-to-prepare Coating of Standard Cell Culture Dishes for Cell Sheet Engineering**

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Cell sheet technology is a well-known method based on culture dishes grafted with a thermoresponsive polymer, poly(N-isopropylacrylamide) (PIPAAm). PIPAAm exhibits a lower critical solution temperature (LCST) in water at 32 °C, meaning that above 32 °C, PIPAAm chains are dehydrated, leading to hydrophobicity of the coated dish surfaces. Below 32 °C, the polymer reversibly changes to hydrophilic properties. Cells are able to adhere and proliferate on the surface and, by simply decreasing temperature, whole cell sheets can be obtained.

Besides PIPAAm, other polymers also show LCST behavior in water, e.g., polymers based on poly(2-oxazolines) (POx). POx are known as smart polymers because of their tunable solution properties and biomedical applications, therefore playing an emerging role in the field of life sciences. Depending on the side-chain of the polymer, the LCST in water can be tuned to a physiological range between 20 and 37 °C, which leads to potential applications in cell culture.

In this study, we examined whether a covalent coupling of polymer layers on surfaces is necessary for cell sheet preparation, or whether an easy to apply procedure can be established that can be transferred to standard cell culture well-plates and prepared in each standard biological laboratory. Different dishes were repeatedly covered with 0.1% poly(2-n-propyl-2-oxazoline) (PnPrOx) in water and dried to create a fully covered thermoresponsive surface. Contact angle measurement was performed proving the thermoresponsive characteristic of the coating. Furthermore, different cell types were seeded and cultured until confluency on PnPrOx-covered surfaces. By decreasing the temperature to 16 °C for 1-2 hours depending on the cell type, viable cell sheets detached and could be harvested and used for cell sheet technology approaches. We tested different cell types for their potential to generate cell sheets via this method and analyzed the sheets via histological stainings, proving the functionality of the cell sheet. Also, adhesion forces were measured on individual cells in order to quantify the forces between the cell and the substrate via Fluidic Force Microscopy (FluidFM®).

With this method, we established an easy, user-friendly and low-cost variation of creating thermoresponsive surfaces applicable for cell sheet technology and tissue engineering purposes by simply covering surfaces of different types of dishes with PnPrOx solved in water and drying afterwards.

**In vitro and in vivo evaluation of human conjunctival epithelial sheets on PLGA membrane co-cultured with human tenon’s fibroblasts**

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To evaluate PLGA-based membranes as a substrate with co-culture of human conjunctival epithelial cells (hCjECs) and human tenon’s fibroblasts (hTFs), hCjECs proliferation and differentiation with or without co-culture of human Tenon’s fibroblasts (hTFs) were compared with transwell membrane in vitro, and to determine whether epithelial sheets grown on PLGA membranes can repair injured rabbit corneal epithelium by transplantation for 2 weeks in vivo.

Primary hCjECs were cultured on PLGA or the original PET membrane-based transwell inserts with or without co-culture of hTFs. Cell behaviors such as proliferation and differentiation were compared. For in vivo assessment, the corneas of rabbits were burned, and PLGA-based epithelial sheets were then transplanted for 2 weeks before histological staining was conducted and analyzed to determine the effectiveness of the repair. Primary human epithelial cells on the PLGA membrane showed an increased proliferation when co-cultured with fibroblasts, which was confirmed by CCK-8 analysis, and upregulation of Ki67, with the expression of the epithelial marker CK19. The stratified squamous cell marker MUC1 and conjunctival cell marker MUC5AC were also expressed in the epithelial sheet. The epithelial defect in the burned corneas was decreased in the PLGA-based epithelial sheet treatment group (6.1% ± 1.6% of the area) compared with that in the no-treatment group (30.5% ± 6.3%) 2 weeks after transplantation. We developed a co-culture system using a human feeder cell layer and PLGA membrane-based transwell inserts to create human conjunctival epithelial sheets. This system represents a promising strategy to regenerate corneal epithelium by transplantation.

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01-P251 Preparation of dextran-based polyampholytes for cryopreservation

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Cryopreservation is one of the important techniques to store cells for cell-based tissue engineering and regenerative medicine. Dimethyl sulfoxide (DMSO) has been widely used as a cryoprotective agent. However, DMSO has a cytotoxicity and an influence on the differentiation nature of more than 25 different human stem cell lines. It is, thus, important to develop new cryoprotectants.

As one trial, polyampholytes are reported to demonstrate the cryopreservative properties. It is indicated that polyampholytes stabilize the lipid bilayer of cells, and then enzymatic digestion of cellulose under confluent cell layer enabled cell detachment with minimum cell damage yielding cell sheets. For the surface adhesion of the cellulose, carboxymethyl cellulose (CMC) molecules were conjugated with DOPA, and pre-treated on to the surface of the culture vessels. Then, human mesenchymal stem cells (hMSCs) or limbal stem cells (hLSCs) was cultured and harvested using cellulase containing cell culture medium. Single hMSC showed higher proliferative activity showing aggregated morphology compared with trypsin-treated hMSCs. Also, a hLSC sheet was generated with well-preserved morphology after cellulase assisted cell sheet generation. These results demonstrate that CMC-DOPA coating combined with cellulase enzymatic harvesting is an effective candidate method for harvesting cell sheets.

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We have designed a two-component matrix (SPRPix) including 1) a solid anisotropic scaffold prepared by electrospinning from a mixture of recombinant spider silk proteins – spidroin 1 (rS1/9) and spidroin 2 (rS2/12) — and polycaprolactone (PCL) (rS5-PCL), 2) a "liquid matrix" based on platelet-rich plasma (PRP) and a neural medium containing growth factors. In experiments in vitro, we have studied biocompatibility and applicability of SPRPix for neural tissue engineering. It was shown that encapsulation of human directly reprogrammed neural precursor cells (drNPC-01) in PRP dramatically activated neurogenesis and promoted formation of neural tissue organoids connected by long processes of neurons. drNPC-01, obtained by direct reprogramming of bone marrow-derived mesenchymal cells, were mixed with freshly prepared liquid PRP, with the addition of Ca²⁺ and neuronal differentiation factors, and were placed into the anisotropic complex spidroin scaffold, in which PRP formed a clot. Neural differentiation was activated in drNPC-01 embedded in such a two-component matrix, which was confirmed by the cascade expression of βIII-tubulin and MAP2. drNPC-01 adhered well to the spidroin scaffold, and the processes of differentiating neurons interacted with its microfibrils and oriented parallel to them.

For preclinical study of the SPRPix we have designed a spinal cord injury (SCI) model on nonhuman primates (Macaca mulatta). Using this model we have conducted an experiment with intraspinal implantation of suspended drNPC-01, drNPC-01 with fibrin-based regenerative matrix and drNPC-01 incorporated in the SPRPix. Implantation of the prepared tissue engineered construct containing drNPC-01 into the brain and spinal cord of Macaca mulatta monkeys has shown good biocompatibility with the neural tissue: no astro- and microglial reaction was present around the implanted construct. At the same time, human drNPC-01 within the construct composition grafted effectively, survived during 3 months of observation and differentiated with the formation of MAP2 positive neurons. Monkeys with SCI after implantation of drNPC-01 demonstrated the recovery of paralyzed hindlimb as well as recovery of SSEP and MEP of injured pathways. As a result of the study we may recommend the use of SPRPix scaffold with drNPC-01 as a promising approach for SCI regenerative therapy.

**Tissue Engineered Constructs Based on Neural Precursor Cells, Recombinant Spidroin and PRP for Spinal Cord Injury Treatment (in vitro and in vivo study)**

**Alginates Hydrogels to Encapsulate hiPSC-derived Neurons for Parkinson’s Disease**

**Background:** Over the past decade, cell therapy for CNS injury and disease has looked extremely promising. However, both pre-clinical and clinical evidence shows high rates of cell death after implantation and one cause of this is the host cellular response. The overall aim of this programme of work is to develop an advanced therapeutic for PD which overcomes the challenges of cell survival, focusing on protecting the cells from detrimental host glial cell and immune responses.

**Methods:** GMP-ready human induced pluripotent stem cells (hiPSCs; UK Cell and Gene Therapy Catapult) were expanded and maintained in Essential 8 media, then differentiated into midbrain neural progenitors and characterised by immunocytochemistry (Kirkeby, Nelder, & Parmar, 2013). SH-SY5Y cells (Sigma) were maintained in 1:1 Hams F12/EMEM. 2% alginate solution was made using 2g alginate sodium salt in 102mM calcium chloride solution in the well and incubated for 15 minutes. The hydrogels were then removed and placed in respective cell culture media. Viability was assessed using fluorescence microscopy to detect SYTO-21 and Propidium Iodide. Host cell responses to encapsulated and unencapsulated donor cells were modelled in vitro.

**Results:** hiPSCs were successfully expanded and differentiated to a neural phenotype. A new protocol for encapsulating cells in an alginate hydrogel was developed and optimised using SH-SY5Y cells. Alginate gels were then used to encapsulate hiPSC-derived neurons and the ability of encapsulation to protect cells post transplantation was tested in vitro.

**Conclusion:** A new alginate encapsulation technique using 24-well Thincerts was developed. Encapsulation of hiPSC-derived neurons using alginate has the potential to protect therapeutic cells from detrimental host cell responses. Future work will further investigate the ability of biomaterial encapsulation to improve the long-term survival of these cells.
Improving Functioning of Insulin-secreting Cells by Multicellular Spheroid Formation

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Rapid progress in cell culture technology has enabled us to prepare various cells from stem cells including embryonic stem cells or induced pluripotent stem cells. These achievements have opened the possibility of mass preparation of tissue-specific cells for cells-based therapy. However, low survival rate and insufficient functioning of transplanted cells are the cause of the limited therapeutic effect of cell transplantation. Accordingly, it is eagerly required to develop an optimized method for cell transplantation. In this study, we attempted to construct multicellular spheroids of insulin-secreting cells to improve the function of the cells. We prepared multicellular spheroids of NIT-1 mouse insulinoma cells by using poly(N-isopropylacrylamide)-coated, polydimethylsiloxane-based microwells. The morphology of NIT-1 spheroids obtained was spherical with a diameter of 200-300 µm. The survival of NIT-1 spheroids transplanted into mice was longer than that of monolayered NIT-1 cells. In addition, transplanted NIT-1 spheroids reduced blood glucose levels of diabetic mice to normal levels faster than monolayered NIT-1 cells did. Then, we attempted to increase the insulin secretion from spheroids by incorporating other cell types into the spheroids. MIN6 mouse pancreatic beta cells were selected as insulin-secreting cells, and multicellular spheroids of MIN6 cells were prepared with or without MAEC mouse aortic vascular endothelial cells or NIH3T3 mouse embryo fibroblast cells. Again, these spheroids had uniformly-sized spherical structures with a diameter of about 300 µm. MIN6/MAEC or MIN6/NIH3T3 spheroids showed significantly increased insulin secretion compared to MIN6 spheroids or monolayered MIN6 cells. These results indicate that the formation of multicellular spheroids containing insulin-secreting cells are useful for improving the functioning of cells and will realize an effective cell-based therapy for diabetes.

References
**01-P256** Magnetic Cell Therapy With Bioresorbable Nanoparticles: an Attractive Advanced Approach In Regenerative Medicine

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Cell therapy is one of the most exciting and promising areas for disease treatment and regenerative medicine. However, the success rate of cell-based therapies, despite their great potential, is limited mainly due to the ineffective delivery and retention of therapeutic cells in the specific organ. Magnetic targeting has emerged as a method to overcome these limitations. So far these attempts have used superparamagnetic iron oxide nanoparticles (SPIONs), only clinically approved metal oxide nanoparticles. Nevertheless the exposure to SPIONs has always been associated with significant toxic effects such as inflammation, apoptosis and generation of ROS.

Our group, by doping hydroxyapatite (HA), the mineral component of bone, with Fe$^{2+}$/Fe$^{3+}$ ions, had obtained novel biocompatible and fully bioresorbable superparamagnetic nanoparticles (FeHA).

This work demonstrates the promising opportunities of FeHA in Mesenchymal Stem Cells (MSCs) labeling. MSCs easily internalized FeHA, after 3h ≈20 pg/cell of iron content (≈10 times lower respect SPIONs) were detected by inductively coupled plasma emission spectrometry (ICP-ES). MSCs became magnetic enough to be guided and retained to specific site by a permanent magnet that usually generates a magnetic field gradient of ≈ 10–50 T/m over a distance of approximately 1 cm. At the same time, MSCs maintained their morphology and cell viability was not negatively affected. Moreover due the well-known osteoinductive feature of HA, magnetic MSCs overexpress genes involved in osteogenic differentiation as demonstrated by RT-PCR. We are also investigating the possibility to combine these above-mentioned results with the contrast ability of FeHA for a real time imaging of the magnetic MSCs in vivo by magnetic resonance imaging.

In conclusion, due to the intrinsic magnetic properties of FeHA, its fast degradation and very low iron content compared to SPIONs, this approach could be simply transferred to different cell types obtaining an attractive advanced approach for several regenerative medicine applications.

References

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**01-P257** Regulation of Proliferation and Functioning of Transplanted Cells by Using Suicide Gene

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Cell transplantation is an attractive therapeutic method for treatment of diseases. This is because single cell transplantation can exhibit high and sustained therapeutic effects compared to conventional drug-based therapy. Furthermore, some pluripotent stem cells such as induced pluripotent stem cells and embryonic stem cells have recently been established, which opens up a possibility of mass production of various tissue-specific cells for clinical application. However, there remain crucial problems of uncontrolled proliferation and functioning of cells after transplantation in cell-based therapy. Any methods to regulate them have hardly been reported. Suicide genes induce apoptosis in cells when specific drugs or compounds are added. The herpes simplex virus thymidine kinase (HSVtk) gene is the most famous and frequently used suicide gene, and HSVtk-expressing cells have been reported to cause apoptosis by ganciclovir (GCV). The safety and effectiveness of the combination of HSVtk gene and GCV were tested and proven in several clinical trials. However, this combination has never been applied to control the proliferation and functioning of transplanted cells in cell-based therapy. In this study, we used HSVtk gene and GCV to regulate cell proliferation and functioning after transplantation of therapeutic cells into mice. We selected MIN6 mouse pancreatic beta cells as model cells, and transfected MIN6 cells with the HSVtk gene to obtain HSVtk gene expressing-MIN6 (MIN6HSVtk) cells. Proliferation of MIN6/HSVtk cells was suppressed by GCV in a concentration-dependent manner; 0.25 µg/mL GCV maintained a constant number of MIN6/HSVtk cells for at least 16 days. Then, we tried to regulate the proliferation and functioning of transplanted cells in mice by GCV administration. MIN6 or MIN6/HSVtk cells were transplanted to streptozotocin-induced diabetic mice. Mice transplanted with MIN6 cells exhibited hypoglycemia irrespective of GCV administration, while normal blood glucose levels (around 150 mg/dL) were maintained in mice transplanted with MIN6/HSVtk cells by a daily administration of 50 mg/kg GCV. These results indicate that the proliferation and functioning of transplanted HSVtk gene-expressing cells can be controlled by GCV administration, and that this method will greatly improve the usefulness and safety of cell-based therapy.
01-P259  A new cell glue for 3D cell structures: Induced cell adhesion using surface modification with cell-penetrating peptide-PEG-lipid

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The ultimate goal of regenerative therapy is the transplantation of functional stem cells-derived tissues and organs to replace those lost as the result of pathology or tissue damage. Since tissues and organs are complicated 3D structures, 3D scaffolds such as decellularized organs and tissues, are required to properly orient living functional cells of different types. 3D scaffolds offer an environment for cell adhesion that differs from that of conventional 2D culture. Therefore, the induction and control of cell attachment, not only to 2D substrate surfaces but also to 3D scaffolds, is of great importance. Here, we propose new type of cell glue made of cell-penetrating peptides (CPP) and PEG-conjugated lipid, which are used for cell-surface modification. PEG-lipid derivatives are incorporated into the lipid bilayer membranes of cells via hydrophobic interactions, and the CPP anchored onto the cell membrane could work as an adhesive domain. In our study, various floating cells, (i.e., T cells, B cells) were used to examine the adhesive efficacy by CPP-PEG-lipid onto material surface as well as PS microfiber-based 3D scaffolds.

Firm cell adhesion with spreading could be induced by cell surface modification with the CPP-PEG-lipid. Cell adhesion was induced by CPPs, but not by any other cationic short peptides we tested. Here, we demonstrated adherence using the floating cell line CCRF-CEM as well as primary human T cells, B cells, erythrocytes, and hepatocytes. The critical factor for attachment was localization of CPPs at the cell membrane by PEG-lipids with PEG>20 kDa. These cationic CPPs on PEG chains were able to interact with substrate surfaces such as polystyrene surfaces (PS), glass surfaces, and PS microfibers that are negatively charged, inducing firm cell adhesion and cell spreading. Also, as opposed to normal cationic peptides that interact strongly with cell membranes, CPPs were less interactive with the cell surfaces because of their cell-penetrating property, making them more available for adhering cells to the substrate surface. With this technique, cells could be easily immobilized onto PS microfibers, an important step in fabricating 3D cell-based structures. Cells immobilized onto 3D PS microfibers were alive, and human hepatocytes showed normal function on the microfibers.

Our novel materials could be useful for fabricating 3D cell-based structures as a cell glue
01-P261
Hyaluronic Acid-based Hydrogel (HA) Containing Mesenchymal Stem Cells (MSCs) for Treating Hypofunction of Salivary Gland (SG) after Radiation Therapy (RT)

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All patients of head and neck cancer suffer from the hypofunction of SG after radiation therapy due to high radiation-susceptible acini which secrete saliva, mucin, and amylase. However, current medicine for the hypofunction (xerostomia) cannot treat the damaged acini. Here, we introduced MSCs to radiation-damaged SG (RDSG) for preventing the progress of the hypofunction. When isolated SG cells from RDSG were co-cultivated with MSCs, the SG cells showed similar expression of mucin and amylase compared to normal SG cells. 10µl HA (2wt%) was employed to enhance the staying duration of MSCs (6.7 x 10⁵ cells) in SG of radiation-treated mice. Regeneration and functional recovery of RDSG were evaluated by histology and SPECT, respectively. After 6 and 12 weeks, MSCs+HA showed 2-fold increase of proliferation compared to MSCs only. Also, MSCs+HA accelerated recovery of intrinsic functions of SG. Thus, the MSCs+HA could be a superior candidate for prevention of damage and regeneration of RDSG.
01-P262 Design of injectable hydrogel system for cell-based therapy

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Fulfillment and advancement of regenerative medicine with cell-based therapy are important for the cure of intractable diseases such as central nervous disorder, cardiac affection and kidney disorder. On the cell-based therapy, it dawns on someone that hydrogel material serve an important role to support the engraftment and to regulate the transplanted cell behavior. We previously developed a functional hydrogel that significantly enhances the survival of cells transplanted into the midbrain striatum [1,2]. Moreover, novel hydrogel system possessing abilities of the protection of grafted cells and the induction of selective differentiation into dopamine neurons was developed for cell transplantation therapy of Parkinson's disease [3].

However, the hydrogel system developed previously has several problems. We used atelocollagen as a base hydrogel. It is often considered that the degradative products of atelocollagen hydrogel might lead to an inflammatory response. Moreover, although our designed hydrogel system can strictly regulate the behavior of transplanted cell, host tissue around the transplanted area cannot be controlled by the present system. If the hydrogel system has the function to control the host tissue, it is strongly expected the function of transplanted cells might be enhanced and tissue regeneration might be facilitated additionally.

Therefore, we aim to develop the novel hydrogel based on hyaluronic acid (HAc) which has the ability of the regulation of transplanted cells and host tissue. In particular, we designed HAc-based hydrogel possessing alpha-helical oligopeptide for cross-linking with coiled-coil interaction. First, HAc was modified with propargyl groups by the condensation between carboxyl group of HAc and amino group of propargylamine. A propargylated-HAc and an oligopeptide having an azido group were reacted with a click reaction. This hydrogel showed the sol-gel transition with shearing stress because alpha-helical oligopeptides can reversibly interact. Moreover, we developed microparticle to selectively release several growth and trophic factors for regulating both the transplanted cells and the host tissue. In this presentation, we will show the results to demonstrate the usability of hyaluronic acid-based hydrogel and microparticle system for dual release of growth and trophic factors.


01-P263 Bioinspired Dendrimeric Nanoassemblies for Deep Tumor Penetration and Multidrug Resistance Reversal

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Chemotherapy resistance remains a great challenge in anticancer treatment due to their intricate mechanisms including complicated physiological barriers (e.g., undesirable blood circulation, inadequate drug influx and poor drug penetration) and multiple cellular factors (e.g., high drug efflux, reduced apoptosis, and elevated anti-apoptosis). We developed tumor-specific stimuli-activated dendrimeric nanoassemblies with metabolic inhibition to overcome drug resistance. The supramolecular nanoparticles hierarchically knocked down the multiple physiological barriers of multidrug resistance, including clinically proven PEGylated corona for optimal drug blood transportation, robust dendritic nanostructures for passive targeting, MMP-dependent tumor microenvironment targeting, tumor-adaptive size/interface for deep tumor penetration and cell internalization, and stimuli-disintegration for intracellular sufficient drug delivery. Meanwhile, tumor hexokinase (HK) was powerfully inhibited to arouse multiple pathways against complicated cellular factors of drug resistance, because HKs catalyze the first committed step of glycolysis metabolism. Furthermore, metabolism-blocking strategy evoked the multiple pathways to reverse the cellular factors of MDR, including ATP depletion, apoptotic enhancement, and anti-apoptotic reduction. The predominant curative effects to MCF-7R tumor supporting our design strongly surmounted both physiological barriers and cellular factors of multidrug resistance.

On the other hand, we developed the virion-like nanoparticles mimicking molecular component, nanostructure, secondary architecture and bioactivity. We comprehensively adopt chemical, physical and biological approaches to disclose the processes and mechanisms on membrane-broken punching for cell penetration, as well as cell-to-cell spread for tissue penetration. Most importantly, the virion-like nanoparticles successfully conquer the obstacles on the treatment of impermeable drug-resistant SKOV3/R tumors with high tumor inhibition in vitro and in vivo. We believe that our strategy is highly valuable for developing advanced dendrimeric nanoassemblies and conquering chemotherapy resistance.

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Controlling the Porous Structure of Alginate Ferrogel for Anti-Cancer Drug Delivery

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Hydrogels have been widely utilized in many drug delivery and tissue engineering applications [1]. Hydrogels responding to external stimuli have been also extensively investigated for controlled drug delivery. Magnetic stimulation can be utilized as a factor of external stimuli, and hydrogel containing materials attracted to the magnetic field is called for ferrogel [2]. In this study, alginate-based ferrogel was fabricated via ionic cross-linking in the presence of SPION (superparamagnetic iron oxide nanoparticle) [3]. Doxorubicin (DOX) was chosen and used for anti-cancer therapy, which can interact with SPION. The release behavior of DOX loaded in ferrogel was delayed due to the charge interaction between SPION and DOX, and then the release rate of the drug was significantly controlled by the magnetic stimulation. However, the deformation degree was limited due to the small pore size of alginate hydrogel. We thus incorporated gelatin microparticles into alginate ferrogel at room temperature and increased the temperature to 37°C to remove the microparticles. Alginate ferrogel excluded the gelatin particles showed substantially increased deformation rate under the magnetic stimulation, and the drug release was much more accelerated compared to alginate ferrogel. Therapeutic efficacy of gelatin particle-loaded alginate ferrogel was also evaluated in a tumor-bearing mouse model. Gelatin particle-loaded alginate ferrogel that releases drug in a controlled manner by the application of the magnetic field can be useful in many biomedical applications.

References
Sequential growth factor release using enzyme-cleavable microcapsules for in vivo cartilage tissue engineering

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Osteoarthritis is a degenerative disease characterised by the deterioration of articular cartilage, which causes stiffness, pain and immobility in millions of people around the world. There has been intense research focus on the restoration of the articular joint using cartilage tissue engineered in vitro by chondrocytes or progenitor cells cultured for several weeks on supporting biomaterials. Nevertheless, these approaches have limitations, such as the amount of cells that can be harvested, donor site morbidity, and the associated costs. Therefore, the aim of this project is to develop an in situ approach for the regeneration of articular cartilage that does not depend on in vitro cell culture or tissue engineering. The system we are developing consists of an implantable polymeric scaffold containing matrix metalloprotease (MMP) cleavable microcapsules that sequentially release growth factors to trigger the recruitment and differentiation of endogenous synovial stem cells. The microcapsules are designed for sequential release of growth factors. First, platelet-derived growth factor will be released from the capsules for the recruitment of endogenous synovial stem cells. Second, transforming growth factor β3 will be released via degradation of the capsules, triggered by cell-secreted proteases, to stimulate chondrogenesis of the recruited stem cells. The microcapsules are assembled by self-polymerisation of dopamine-containing peptides onto a sacrificial template of polystyrene-stabilized calcium carbonate, and characterised using confocal fluorescence microscopy, flow cytometry and zeta potentiometry. Ongoing and future work includes investigation into on-demand release of the two growth factors in the presence of MMP-2/9 (assessed by enzyme-linked immunosorbent assays), and tuning this process so that the sequential growth factor release corresponds with the timescale of in situ cartilage tissue regeneration. We have embedded the microcapsules in polymeric scaffolds and are investigating how growth factor release can be used in the recruitment of synovial stem cells and their differentiation into chondrocytes in vitro. Subsequent in vivo work is planned in an osteoarthritic mouse model. If successful, this approach will exploit the endogenous regeneration capacity of synovial stem cells to avoid the drawbacks related to autologous implantation.

References:
Development of N-acetylglucosamine-bearing polymers that interact with type III intermediate filament proteins for targeting various chronic diseases

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Vimentin, desmin, gial fibrillary acidic protein (GFAP), and peripherin belong to type III intermediate filament family and are expressed in mesenchymal cells, skeletal muscle cells, astrocytes, and peripheral neurons, respectively. These proteins play important roles in the stabilization of cell integrity such as architecture and structure in each tissue. Recent studies indicated that these proteins are highly expressed in lesion sites of various chronic diseases such as fibrosis, cancer, and autoimmune diseases, and most studies focused on pathologic analyses and elucidation of a pathologic mechanism related to high expression of these proteins in chronic diseases. Type III intermediate filament proteins may represent a new target molecule for multiple chronic diseases. Vimentin and desmin possess N-acetyl-D-glucosamine (GlcNAc)-binding properties on cell surfaces. The rod II domain of these proteins is a GlcNAc-binding site, which also exists in GFAP and peripherin. However, the GlcNAc-binding activities and behaviors of these proteins remain unclear. Here, to study whether these proteins can interact with GlcNAc-bearing polymers on cell surface, we developed various well-defined GlcNAc-bearing polymers synthesized by radical polymerization with a reversible addition-fragment chain transfer reagent, and examined the interaction of these well-defined polymers with these proteins. The small GlcNAc-bearing polymers strongly interacted with HeLa cells through vimentin expressed on the cell surface and interacted with vimentin-, desmin-, GFAP-, and peripherin-transfected vimentin-deficient HeLa cells. Surface plasmon resonance analysis revealed that these proteins possess high affinity to GlcNAc-bearing polymers. These results demonstrated that type III intermediate filament proteins possess GlcNAc-binding activities on cell surfaces and the small GlcNAc-bearing polymers are advantage for targeting these proteins. These findings provide important insights into novel cellular functions and physiological significance of type III intermediate filaments and may facilitate the development of new molecular targeting agents against type III intermediate filaments for various chronic diseases.

Injective Dual-Protein NanoComplex for Controlled Salivary Gland Radioprotection and Functional Regeneration in Head and Neck Cancer: A Step Closer towards Clinical Translation and Personalization

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Saliva plays a major role in maintaining oral health. Hyposalivation, due to medications, Sjögren’s or radiotherapy for head and neck tumors, leads to symptomatic dry mouth, difficulty in mastication, swallowing and speech, burning oral sores, acceleration of dental decay and periodontal diseases, thus significantly deteriorating Quality of Life and ability/willingness of patients to survive the battle with cancer.

A nano-sized dual-protein release-controlled delivery system is formulated, characterized and evaluated as an alternative (to commercially-available and dysfunctional radioprotectors; ex: Amifostine I.V.) therapeutic approach/strategy to protect salivary glands from ionizing radiation to the head and neck zone and potentially restore/repair the radiation-induced damage.

Loaded core-shell nanocapsules with the protein(s) were directly administered into the submandibular salivary glands of the experimental groups 24 hours pre-radiation and PBS or single/pure cytokines was injected into the glands, likewise, for the controls. External irradiation at dose with 15 Gy was exposed to head and neck fields of C57BL/6 mice. Salivary flow rates and salivary protein excretion/content were evaluated using an enzyme-linked immunosorbent assay (ELISA) over a 3 months period following treatment. Histopathological evaluation of structures and analysis of apoptosis/proliferation were performed. Timely bio-distribution assays followed.

Experimental animals demonstrated increased salivary flow rates when compared to controls. Protein content was comparable to that of pre-radiation (base-line) level. Histological evaluation revealed that acinar cells showed less vacuoles and nuclear aberrance in experimental group compared to the control group and the amount of mucin stained by alcian blue was larger, in the latter. Protein therapy resulted in less apoptotic activities detected by TUNEL assay and similar proliferative indices as in the control mice. Immunohistochemistry revealed the involvement of the Wnt/β-catenin signaling pathway in radioprotection.

Novel, biocompatible, stable, reproducible and customizable core-shell nanoparticulate layer-by-layer self-assembled delivery system is presented. Findings suggest that the local sequential release of a protein cocktail (in specific dosage and order) into murine salivary gland highly prevents radiation-induced damage via reducing apoptosis. This approach also promotes in situ proliferation of salivary gland cells.
**A Novel Production Method of Gelatin Hydrogel Microsphere as a Sustained Release System of Cisplatin Aiming for Clinical Application**

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**Introduction**

A novel drug-delivery system for prolonged retention was developed for clinical application, gelatin hydrogel microspheres with incorporated cisplatin (GMA-CDDP) was already applied to intraperitoneal chemotherapy against peritoneal carcinomatosis [1]. However, organic solvents and glutaraldehyde as a toxic substance were used in the production process of GM-CDDP. The object of this study was to develop a new production method of GM-CDDP without organic solvents and glutaraldehyde, and to evaluate the potential to sustained release.

**Methods**

In the current study, dehydrothermal crosslinking technique in place of glutaraldehyde was employed. After frozen dehydration for 72 hours, 5wt% gelatin solution was crosslinked by heating (140 °C). In order to obtain 3 types of degree of cross-linking, heating duration was divided in 3 groups (soft cross-linking: 24 hours, moderate: 48 hours, hard: 96 hours). And then, gelatin hydrogel sheet was comminuted to injectable size (100-180 μm) by ultrasonic homogenizer and sieves. After frozen dehydration for 48 hours, cisplatin was added.

The characterization of GM-CDDP of 3 kinds of crosslinked degrees were assessed with in vitro degradability test and release test. Firstly, GM-CDDP were suspended in 1ml of 0.01 mol/l phosphate-buffered saline (PBS), followed by reciprocally shaking at 37°C. The supernatant was pipetted after centrifugation, and immediately after that, the same volume of PBS or PBS containing collagenase D(25μg/ml) after 24 hours was added at each time point. The degradability of GM-CDDP was assessed by quantitating protein in supernatant, and the concentration of released cisplatin was measured from that of platinum on atomic absorption spectrophotometer.

**Results**

In degradability test, gelatin hydrogel microspheres with greater cross-linking showed slower degradation after changing to PBS containing collagenase. % degradability of gelatin at 48 hours was 83% of hard cross-linked gelatin, 92% of moderate, 100% of soft. In release test, initial bursts were observed approximately 30 to 40% in all type of GM-CDDP, and cisplatin was rapidly released with gelatin hydrogel microspheres degradation after adding collagenase.

**Conclusion**

In the current study, we had established a new production method of GM-CDDP for clinical application, and the release rate of cisplatin was controlled by heating duration.

**Reference**


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**Stem Cell-Engineered Nanovesicle Functionalized with Aptamer for Cartilage Tissue Engineering**


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Notch signaling suppresses bone marrow-derived mesenchymal stem cells (BMSC) differentiation toward the chondrocyte, osteoblast, and adipocyte fates, while promoting BMSC proliferation during skeletal development. Here, we hypothesized that notch-antagonistic aptamer can participate chondrogenic differentiation of MSC. Selected notch-antagonistic aptamer in this study induced chondrogenesis of BMSC spheroid without any growth factors. Translocation of notch aptamer to both cytoplasm and nucleus of MSC was also confirmed. Therefore, we can suggest that notch intracellular domain (NICD) by notch-aptamer suppress notch signaling and then induce chondrogenic differentiation of BMSC. In further study, we developed stem cell-engineered nanovesicles functionalized with the notch aptamer (NV/apt) for cartilage tissue engineering. The NV/apt was designed to deliver drugs as receptor-mediated drug delivery system targeting MSC.
**01-P273** Multivalent polyaspartamide crosslinker for engineering cell-responsive hydrogels with degradation behavior and tunable physical properties

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Hydrogels possess favorable physical properties ideally suited for various biotechnology applications. To tailor to specific needs, a number of modification strategies have been employed to tune their properties. Herein, a multifunctional polymeric crosslinker based on polyaspartamide is developed, which allows for the facile adjustment of the type and number of reactive functional groups to fit different reaction schemes and control the mechanical properties of the hydrogels. The amine-based nucleophiles containing desired functional groups are reacted with polysuccinimide to synthesize polyaspartamide crosslinkers. The crosslinking density and the concurrent change in mechanical properties of the resulting hydrogels are controlled in a wide range only with the degree of substitution. This multivalency of the polyaspartamide linkers also allowed for the degradation of hydrogels by the unreacted functional groups involved in the chain lysis. Furthermore, the polyaspartamide crosslinker conjugated with cell-recognition molecules via the same conjugation mechanism (i.e. nucleophilic substitution) render the hydrogels cell responsive without the need of additional processing steps. This versatility of polyaspartamide-based crosslinker is expected to provide an efficient and versatile route to engineer hydrogels with controllable properties for biomedical applications.
**01-P274** Photo-modulated Drug Delivery System for Minimally Invasive Ophthalmic Injections

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Biotechnological advances in recombinant protein and monoclonal antibody technologies has revolutionized the pharmaceutical industries through their biomolecular products. Although they offer higher specificity compared to small molecules, they possess limited stability for long-term storage and controlled release, which lead to aggregation/degradation and loss of their biological activity. The major problem in the treatment of chronic ophthalmic diseases, like age-related macular degeneration (AMD), is the frequency of injections to the back of the eye with biomacromolecular drugs, such as bevacizumab (Avastin®) and ranibizumab (Lucentis®). In order to minimize the negative impacts of this invasive procedure, there is a need for a drug delivery system that serves as a depot to enable sustained release or controlled burst release of these anti-angiogenics.

Our patented protein depot has been developed using FDA approved polymers to ensure its bio-compatibility in contact with human tissues and to exhibit a reversible thermal softening behaviour. Functionalized gold nanoparticles (AuNPs) were embedded directly inside the polymer matrix (hydrogel) as the light-responsive component. Different payloads, ranging from small molecules (hydrophobic or hydrophilic) to various proteins, can be encapsulated directly into the polymer matrix (hydrogel) without chemical modification. The release of the protein from the depot can be designed to have a sustained release profile or/and to respond to visible or near-infrared light. When the drug depot is exposed to light, the polymer inside the depot (fabricated into rods, films, or microparticles) responses thermo-mechanically to let the unmodified protein diffusing from the depot at higher rate.

The release rate of the loaded drug can be tuned on demand by internal (i.e. AuNPs and polymer content) and external parameters (i.e. light intensity) for controlled dosage forms. Biological activity of the photo-released bevacizumab was highly retained as measured by ELISA (human VEGF165 affinity) and vascular endothelial cells proliferation assay. In addition, the formulation did not show in vitro toxicity to ocular cells, and can be implanted via minimally invasive subconjunctival injection through a 30-gauge needle into rabbit eyes. Due to its high versatility, this photo-modulated drug delivery system has a potential to improve the treatment of posterior or anterior ocular diseases.

**01-P275** Anti-inflammation and Wound Healing Efficiency of Thai Silk Fibroin Hydrogels Controlled Releasing Curcumin

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Thai silk fibroin hydrogels were formed by the induction of sodium tetradeyl sulfate, an anionic surfactant which is used in medical products approved by U.S. FDA. The addition of sodium tetradeyl sulfate at various concentrations could accelerate silk fibroin to form gel at 37°C, pH 7.4 within 20-30 min. The mechanisms of gelation were explained by dehydration effect as results of the hydrophobic interaction and hydrogen bonds, leading to the turning of secondary structure of silk fibroin from random coil to β-sheet structure. The electrostatic interaction also played significant role on the gelation rate. The silk fibroin hydrogels were slowly degraded in proteinase enzyme solution at 37°C, pH 5.5 over a 7-day period. The in vitro test with L929 mouse fibroblast cells following ISO10993-5 standard showed that the silk fibroin hydrogels were not toxic (>100% cell viability). The curcumin dissolved in sodium tetradeyl sulfate (0.7 mg/ml) was incorporated into silk fibroin hydrogels. When incubated in proteinase enzyme solution at 37°C, pH 5.5, the silk fibroin hydrogels could sustain the release of curcumin along 2 weeks. The silk fibroin hydrogels incorporating curcumin were applied topically on the full-thickness wound of mice to evaluate anti-inflammation and wound healing efficiency, comparing with the silk fibroin hydrogels without curcumin, medical used fibrin gel, and non-treated wounds. We found that the silk fibroin hydrogels incorporating curcumin significantly reduced the wound size and inflammation particularly within the first 3 days and promoted re-epithelialization after 7 days of treatment. This hydrogel can be further studied in the infected or diabetic wound.

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Collagen Microgels for Regenerative Medicine

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During recent years, microgels have emerged as an effective drug delivery system (DDS), showing advantages such as tuneable size, increased surface area and injectability1. Collagen has been extensively studied as a scaffold for regenerative medicine and drug delivery due to its biocompatibility, non-immunogenicity and degradability2. In this study, we show the use of microfluidic techniques for the automated generation of monodisperse type-I collagen (col-I) microgels and the encapsulation of hollow collagen spheres loaded with glial-derived neurotrophic factor (GDNF) and bone morphogenetic protein 2 (BMP-2), for regenerative therapies in Parkinson’s Disease and bone repair, respectively.

Microgels with different size and stiffness were synthetized in a glass microfluidic device with co-flow configuration. Crosslinking occurred after merging col-I with the PEG-4S crosslinker in the nozzle of a double-chamber capillary within an oil flow. A coiled tube was placed in the outlet in order to increase the residence time of the microparticles, allowing them to gelify inside the microfluidic system. The chemistry, stiffness and degradation of the synthesised col-I microgels were characterized and the viability of Neu-7 astrocytes assessed. Hollow spheres (diameter ~ 200 nm) were prepared by covalently binding collagen on silica templates, which were subsequently removed with hydrofluoric acid. Hollow spheres were loaded with GDNF and BMP-2 by diffusion, seeded with different cell-types and encapsulated in the microgels within the microfluidic device.

We demonstrate that microfluidics is an adequate technique for automatically generating monodisperse collagen microgels and provides a useful tool for the posterior encapsulation of nanospheres and cells. The microgels are non-cytotoxic to cells and foster cell growth at different conditions. Microgels encapsulating hollow spheres showed sustained delivery of different therapeutic factors, in accordance with previous literature.3

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REFERENCES

Iron oxide nanoparticles-incorporated large scale alginate capsules for biomedical applications

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Biocompatible capsules have recently been highlighted as novel delivery platforms of any “materials” to address current problems of living systems such as humans, animals, and plants in academia and industry, especially for biomedical applications. Here, we proposed a new platform based on biocompatible alginate capsules that can control the movements as an active target delivery strategy for various biomedical applications. In this study, we developed large-scale iron oxide nanoparticle-incorporated alginate capsules and investigated the movement performances of capsules under magnetic fields. The capsules described in this work were generated from ionically cross-linked alginate, and various concentrations of iron oxide nanoparticles were inserted into the capsules. It was found that the sizes of large scale alginate capsules could be controlled via various working conditions such as concentrations of alginate solutions and iron oxide nanoparticles. As a proof of concept work, we showed that the iron oxide particles-incorporated large scale alginate capsules could be moved actively by the magnetic fields, which would be a strategy as active target delivery platforms for biomedical engineering.
**Bio-adhesive polymersome for localized and sustained drug delivery at pathological sites with harsh enzymatic and fluidic environment via supramolecular host-guest complexation**

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**Introduction:** Targeted and sustained delivery of drugs to diseased tissues/organisms, where the body fluid exchange and catabolic activity is substantial, is challenging due to the fast cleansing and degradation of the drugs by these harsh environmental factors. We develop a multifunctional and bioadhesive polycaprolactone-β-cyclodextrin (PCL-CD) polymersome for localized and sustained co-delivery of hydrophilic and hydrophobic drug molecules. The PCL-CD polymersome is capable of in situ grafting to the biological tissues via the host-guest complexation between surface CD and native guest groups in tissue matrix both in vitro and in vivo, thereby effectively extending the retention of the loaded cargos in the grafted tissue.

**Methods:** Polycaprolactone (PCL-OH, 5k) was reacted with hydroxyl terminal group (PCL-OH, 5k) and then conjugated with thiolated β-cyclodextrin (βCD-SH) to obtain PCL-βCD. The BSA-FITC and Rhodamine B base were dissolved in distilled water and DMF (H2O:DMF = 2:1, v/v), respectively, and co-loaded into the PCL-βCD polymersome during the self-assembly process. Application of solutions of free fluorescence dye (BSA-FITC) or PCL-CD polymersomes loaded with the same amount of dye to separate halves of the native cartilage results in the similar level of initial fluorescence staining of the tissue that can be visualized under UV exposure (wavelength: 365 nm; intensity: 7 mW/cm2). 50 μl of BSA-FITC-loaded PCL-CD polymersome solution, or 0.05% (wt%) BSA-FITC solution of the same volume was injected into intra-articular space of the operated knees.

**Results:** The Rh of polymersome is 144nm and the Rg/Rh is 1.09 based on the dynamic light scattering. After being washed with the PBS for 5 minutes the half cartilage surface that is coated with the dye-laden PCL-CD polymersome exhibits significantly high fluorescence signal than the control half that is coated with free dye. Even after three days of immersion in PBS, the PCL-CD polymersome coated half cartilage surface still shows more intense fluorescence signal than the control half. The quantification of the fluorescence intensity also shows higher signal from the PCL-CD polymersome treated cartilage surface than the free dye treated control. Real-time monitoring with in vivo confocal microscope reveals more fluorescence signal in the articular cartilage surface of the joints injected with the BSA-FITC-loaded polymersomes than that in the joints received free drug injection.
**01-P280** Facile fabrication of drug-loaded silica nanoparticles using silica forming peptide-fused self-assembled cage protein

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Ferritin (FT) is an iron-storage cage-protein composed of 24 subunits with interior and exterior diameters of 8 and 12 nm, respectively. ApoFT without iron may carry many types of small molecules inside the core of cage. Therefore, FT nanocages have attracted much attention as a nanoreactor or delivery system for small molecules such as a drug. Meanwhile, silica-nanoparticles are in the spotlight as carriers in biomolecular transport because of large surface areas and easy surface functionalization. Here, to enhance the potential of FT as a drug carrier, FT genetically-fused with silaffin R5 peptide at N-terminus (R5FT). The N-terminal R5 peptides are exposed on the surface of the FT cage and precipitate silica on the surface of FT, resulting in silica-enveloped cage protein (SiO$_2$/R5FT). First, doxorubicin (Dox) was loaded into cage interior by re-assembly of R5FT and then Dox-laden R5FT was coated with silica by R5-mediated silica deposition under eco-friendly conditions. The release rate of Dox-loaded in internal cages was retarded by the silica-coated matrix and showed the pH-controlled behavior, in which the release amount was two times faster at pH5 than pH7. R5FT provides a biocompatible manufacturing process for the drug-laden silica nanoparticles over conventional production methods. Therefore, these silica-FT composite particles are advantageous for application in the biological and medical fields.

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**01-P281** Collagen-based core-shell nanofibers for long-term drug release

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For cancer treatment and wound treatment, matrix materials for long-term control of drug release are highly desired. As electrospun nanofibers have a high specific surface area, porosity, and anisotropy, they have good compatibility with living tissues and are expected as one of the matrix materials. Especially biodegradable nanofibers are safe and promising drug-carrier even if they are embedded into the body. However, the controlled release of hydrophilic drug loaded into the nanofibers is much difficult because the high amounts of drugs are released in the early process; that is called as a burst. To solve the problem, in this study, we designed and fabricated core-shell nanofibers, where the drug is incorporated in the core of nanofibers. The rapid release of drug from the core is suppressed by the shell. Here, collagen and polylactic acid (PLA) which are biodegradable are used as core and shell materials, respectively. Collagen has high interaction with peptides and hydrophilic low molecular weight compounds via hydrogen bonds and hydrophobic interactions. The shell of hydrophobic PLA sustains the release of incorporated drug over a long-term period as it degrades. Here, we report the fabrication and the characterization of the drug-incorporated nanofibers, and the evaluation of the drug release profile.
Nanocomposite design of scaffolds for the protein delivery in tissue regeneration

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Delivering proteins with scaffolds is considered important to regenerate damaged tissue. For instance, ultrahigh loading and sustainable delivery are quite useful for clinical application. Herein, we designed 3D scaffolds for this purpose by introducing mesoporous bioactive glass nanoparticles (BGNPs) to microporous polymer scaffold (up to 200% BGNPs/PLA weight). Scaffolds were fabricated by the salt leaching method to provide a microporous structure. Incorporating BGNPs into the scaffold significantly enhanced specific surface area and hydrophilicity which enabled loading of proteins efficiently. Therefore, protein adsorption was higher in BGNPs/PLA scaffold compared with that in PLA group, and especially positive charged proteins were adsorbed 20 times higher than negative charged protein. Moreover, loaded protein was released in a more controlled manner over a few weeks in BGNPs/PLA, however, such protein release was burst (within a week) in pure PLA. Furthermore, fibroblast growth factor 2 (FGF2) was selected as a representative protein to load and deliver for its cell mitosis and tissue repair potential. The FGF2 loaded scaffold showed significantly higher cell proliferation of the rat mesenchymal stem cells (rMSCs) compared with the pure PLA scaffold. Thus, the designed scaffold proved to have ultrahigh and long-term delivery capacity of proteins and thus to be a good candidate for tissue repair graft.
01-P285

Efficient Nucleic Acid Delivery System Using Aptamers for Cancer Targeting Ligands

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Targeted delivery system is efficient to cancer therapy and diagnosis by reducing drug resistance. In this study, we designed various type aptamer-linear nucleic acid structures for efficient targeted delivery system that could deliver anti-cancer drugs and therapeutic nucleic acids like Bcl-2(anti-apoptotic protein) siRNA that blocks target gene expression for cancer cells. The various type aptamer-linear nucleic acid structures could be easily prepared by waston-crick base pairing. We showed multimeric conjugates can much more efficiently deliver nucleic acids than monomer. In addition, multimer conjugates were accumulated in tumor tissue without liver toxicity and non-specific cytokine induction in vivo. Thus, these multimeric aptamer-DNA and DNA-siRNA hybrids could be harnessed as efficient carrier to cancer cells.
Fabrication and characterization of phlorotannins/poly(vinyl alcohol) hydrogel for wound healing application

Geunhyeong Kim, Hyeon-ho Park, Gun-woo Oh, Seok-chun Ko, Hyoung Shin Lee, Won-kyo Jung

Phlorotannins (PH) derived from brown algae have been shown to have biological effects. However, the application of PH in biomedical materials has not been investigated. Here, we investigated the effects of PH on normal human dermal fibroblast (NHDF) proliferation and fabricated a composite hydrogel consisting PH and poly(vinyl alcohol) (PVA) (PVA/PH) by a freezing-thawing method for wound healing applications. Cell proliferation was significantly higher in the PH-treated (0.01 and 0.02%) cells than in non-treated cells. Based on the mechanical properties, the PVA/PH hydrogel had a significantly increased swelling ratio and ultimate strain compared to the PVA hydrogel, but the ultimate tensile strength and tensile modulus were decreased. Additionally, cell attachment and proliferation on the composites were evaluated using NHDFs. The results showed that after 1 and 5 days, cell attachment and proliferation were significantly increased on the PVA/PH hydrogel compared with that on the PVA hydrogel. The findings from this study suggest that the PVA/PH hydrogel may be a candidate biomedical material for wound healing applications.
Introduction and Objectives: Growth factors associated limitations represent a major hurdle to musculoskeletal regeneration. Gene therapy is advantageous over protein delivery and may provide a solution. mRNA-based gene therapy results particularly attractive. The objective of this study was to develop an osteo-inductive transcript-activated matrix (o-TAM) by loading collagen sponges with a chemically modified mRNA (cmRNA) encoding BMP-2.

Methods: mRNA was modified by performing a nucleotide modification and by introducing sets of long-lived UTR sequences. HEK293 and MC3T3-E1 cells were transfected with all developed cmRNAs to determine the most efficient candidate. Next, selected cmRNA encoding BMP-2 was loaded into collagen sponges with 1.25 µg and 5 µg and subsequently lyophilized to produce the cmRNA o-TAM. Human adipose-derived stem cells (hAMSCs) were transfected by seeding them on the o-TAM. BMP-2 production was evaluated by ELISA. In vitro osteogenesis was followed by qRT-PCT (RunX2, ALP, Collagen-I, Osteocalcin and Osteopontin) and mineralization (alizarin red). Subsequently, in vivo osteogenesis was evaluated in a critical segmental femur defect (5 mm) for up to 8 weeks. 50µg, 25µg, 10µg, or 5µg of BMP-2 cmRNA loaded into sponges were transplanted into the bone defect. Non-coding cmRNA was used, in vitro and in vivo, as control.

Results: Nucleotide modification of the mRNA improved stability. The sequence containing translation initiator of short 5'UTR (TISU) resulted in elevated Metluc and BMP-2 expression in both cell lines up to 72 hours. Thus, TISU BMP-2 cmRNA was used to develop the o-TAM. hAMSCs cultured on BMP-2 o-TAM were able to secrete significant amounts of BMP-2. Furthermore, the expression of osteo-related genes was greatly enhanced as result of the BMP-2 o-TAM transfection. Mineralization of seeded stem cells was also improved. In vivo, bone healing was greatly improved in the BMP-2 o-TAM when compared to the non-coding and empty groups as demonstrated by microCT (BV/TV). A clear dose response has been observed in the treated groups. In the highest dosage group, best bone healing was detected without showing any side effects in rats. Masson trichrome showed areas of new bone formation, while immunohistochemistry for Collagen-I resulted positive in allo-TAM treated groups.

Mitochondria are essential regulators of cellular function and survival because of their vital roles in energy production and apoptosis. Mitochondrial abnormalities or dysfunction can therefore affect the brain, heart and muscle in varying levels of severity, and have been implicated in various well-known diseases such as Alzheimer’s, Parkinson’s and muscular dystrophy. While disease symptoms can be managed with various medications, there are currently no treatments for mitochondrial disease. Although disease symptoms can be alleviated by alleviating these diseases, efficient mitochondrial-targeting strategies are lacking to date. An ideal delivery agent should be able to condense the therapeutic molecule into compact particles for transport, disrupt the endosomal membrane, escape proteasomal degradation, traffic molecules of various size, charge and function to targeted intracellular compartments, and additionally, have reduced cytotoxicity and immunogenicity. The distinct ability of peptides to deliver molecules that are otherwise restricted from crossing cellular/organellar membranes has led us to develop, through a novel design strategy, a versatile carrier for mitochondrial-specific delivery of exogenous DNA. The peptide-based carrier comprises a mitochondrial-targeted peptide corresponding to the partial presequence of cytochrome oxidase subunit IV (Cytcox) fused to a polycationic copolymer of alternating histidine and lysine residues (KH) for its membrane-translocating as well as DNA-binding/condensing functions. An optimized peptide/DNA formulation, identified through qualitative and quantitative studies, fulfills the fundamental prerequisites for mitochondria-specific DNA delivery, successfully transfecting a high proportion (80%) of mitochondria in a human cell line with concomitant biocompatibility. Nuclear magnetic resonance studies confirmed the effectiveness of the peptide design with segregated functions; a helical domain necessary for mitochondrial import and an unstructured region for interaction with DNA involving lysine residues. The results from our study offer useful insights into the rational design of peptide-derived carriers that can be implemented for the delivery of therapeutics in a mitochondrial-based therapy.
**01-P290 Gold Bunch Microsphere, a novel injectable gene carrier for clinical application**

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Gene therapy is the therapeutic delivery of the disease-specific gene into the cells and tissues as a drug to treat disease. This therapy is used for various disease, tissue repair, and engineering. However, this method requires high gene transfer efficiency, and what is needed is a carrier. The carrier must have high efficiency and stability. Therefore, research on this is the key to successful gene therapy. So, we synthesized gold bunch microsphere as a new carrier in this study. A Gold colloid and Catechol-functionalized branched polyethyleneimine (C-bPEI) were combined to form nanoparticles, and gold clusters were synthesized by attaching pEGFP. Each of the gold bunch was packed into poly-(D, L-lactic-co-glycolic acid) (PLGA) to prepare gold bunch microspheres. When the cells are co-incubated with the Gold bunch microspheres, the PLGA degrades and the gold bunch uptake into the cells, and transfection. In this way, gene delivered to cells. In addition, in situ experiments were conducted to synthesize gold bunch microspheres with SOX9 gene, a cartilage-specific transcription factor, for application to stem cells. And the SOX9 gene was also expressed. Therefore, the Gold bunch microspheres can be applied to various fields such as cancer treatment and tissue regeneration depending on the type of gene to be delivered.

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(3) F. Romero-Gavilan et al., Colloids and Surfaces B: Biointerfaces 162 (2018) 316-325

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**01-P291 Accelerating sanitary product market entry. Prediction of Bone Biomaterial in vivo behaviour**

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Years of biomaterials development, raising of tissue engineering and regenerative medicine and a broad cross-talk between basic scientists, clinicians and engineers have built a great consensus on the need to find a short-term way to transfer research to the clinical relevant needs(1). There is still a lot of work to do, even the development of new procedures for the testing of biocompatibility(2). Taking advantage of our expertise in osteogenic coatings for dental implants and based on the well-known fact of the immediate formation of an outermost layer of proteins on implanted materials, a correlation between most abundant proteins and material in vivo response has been established(3). The goal is to be able to predict in vivo biomaterial behaviour in short times with and in vitro methodology(2).

The synthesis of a good number of sol-gel osteogenic coatings doped with different elements, i.e. Sr, Ca, Mg, that participate in the bone regeneration cascade has been performed. Afterwards, in vitro, in vivo and proteomics procedures were carried out for their evaluation. In vivo experimentation was carried out with a rabbit proximal tibia model. Proteomics assay was conducted by incubating the discs with human serum for 3 h. Non-adhered proteins were removed through washes and proteins attached to the surfaces were eluted and evaluated using mass spectrometry (LC/MS/MS). Data were analyzed with Progenesis QI software. A number of 130-180 proteins related to blood coagulation, fibrinolysis, immune response and osteoregeneration were detected in different proportions in each material surface. Comparison between surfaces showed up to 20 proteins predominantly adsorbed in each of them. A thorough combined study of the whole data set revealed a very clear guideline to relate proteomics with biological processes connected with biomaterials implantation. This study demonstrates that proteomics allows determining a group of biomarkers related to bone regeneration and immune response that could constitute in the short run an easy-to-handle test to predict implants in vivo behaviour.

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**01-P292** Development of Phosphate Based Glass for Possible Antimicrobial and Biomaterial Applications

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**Introduction**

Phosphate based glasses that are used in biomaterial and tissue engineering applications have the ability to dissolve completely in aqueous mediums with products that are bio-compatible. These products can be involved directly in tissue engineering application but it also makes them excellent base to incorporate other elements such as copper, silver and strontium for wider application in biomaterial.

**Methods and Experimental**

Phosphate based glass were prepared by melting phosphorous pentoxide, calcium carbonate and sodium dihydrogen phosphate in a platinum crucible at 1100 C and annealing them in a graphite mould. The glass rods prepared were cut into 15 x 2 mm dimensions, their dissolution properties were studied by immersion in dH2O and tap water over period of 0-49 days. 1, 5 and 10 mol% copper or strontium incorporated phosphate glass has also been prepared by addition of copper sulphate or strontium carbonate in the melt. Their dissolution properties were studied by immersion in dH2O on 1, 3 and 7 days. Anion and cation ion chromatography were carried out to study the products released and their amounts. Raman spectroscopy was carried out to understand surface structure. EDX was carried out on the cross-section of the glass to understand the change in the elemental composition due to immersion in aqueous medium. Antimicrobial study is to be carried out to understand the antimicrobial property of the copper-phosphate glass. In-situ study is also to be done with diamond beamline.

**Discussion and Conclusion**

Phosphate glass lost as much as 10.89 % of its mass in dH2O and 1.16 % in tap water. It is expected to dissolve much faster in dH2O due to absence of ions. Phosphate glass is able to release polyphosphates with calcium, sodium and copper cation which shows that they are available for the biomaterial application. EDX study had shown that there is a difference in elemental composition from the surface and bulk of the glass which supports that different elements are released differently in solution. Further antimicrobial test will show how copper incorporated glass can be used to kill microorganisms.

**01-P293** Effect of PLGA based nanoparticles on reproductive system

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Nanoparticles (NPs) have been demonstrated various applications in different fields, including sensing, electronics, and biomedical sciences. Especially NPs have been extensively utilized as therapeutic and diagnostic agents for drug delivery systems and biomedical devices. Recently application of NPs to the reproductive system has been raised. Furthermore, an increasing number of studies have been suggesting that developmental origins of health and disease could come from an early life of the individuals. However, the influence of NPs on the process of development and next generations have not been studied yet.

Here, we demonstrate that poly lactic-co-glycolic acid (PLGA) NPs coated with a cationic polymer of poly (ethyleneimine) (PEI) (PLGA/PEI NP) are non-toxic and biocompatible for pre-implantation embryo development in vitro followed by subsequent fetal development in vivo and healthy live birth without any genetic aberrations. Treatment of PLGA/PEI NP labeled with TRITC, named TRITC nano-tracers (TnT) visualized and traced successful delivery of the NPs into sperms, oocytes and early stage of embryos.

Treatment of TnT at various embryo stages did not affect the development of the pre-implantation embryo to the blastocyst stage with respect to OCT4 expression, ICM/TE ratio, and apoptotic index. Moreover, when TnT treated 2-cell embryos were transferred into recipients, healthy live offspring were born without any distinct morphologic and chromosomal abnormalities.

Furthermore, various phenotypes of the next generation from the mice that exposed to TnT in their early life, clearly demonstrated that TnT does not produce any adverse transgenerational effects on mammalian development. These results show the possibility that TnT could be used to develop a new easy and safety paradigm to introduce exogenous materials into oocytes and embryos without technical difficulties.
**01-P294** Synchrotron-Based µ-XRF and nano-XRF Mapping for Assessing Exposure to Metallic Wear and Corrosion Products in Human Peri-Implant Bone/Bone-Marrow

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The release of metallic wear and corrosion products from orthopedic implants is a long known and still topical problem in joint arthroplasty. Local particle exposure has been correlated to the manifestation of peri-implant osteolysis. In a previous study we have proved exposure to multi-elemental metallic nanoparticles in peri-implant samples including bone marrow (BM). However, simultaneous and spatially-resolved quantification of released metals in peri-implant bone and BM remains elusive. Today, there is no prove that particles originating from implant components accumulate in the adjacent bone and BM. Thus, our aim is the simultaneous multi-elemental exposure assessment by synchrotron-based X-ray fluorescence (XRF) imaging of human peri-implant cancellous bone from patients with artificial joints.

Peri-implant cancellous bone specimens including BM (n=12) were collected during revision surgery of various implant types (knee implants, hip resurfacing implants, stemmed hip implants, hip implants with modular stems). Specimens from non-exposed patients (n=5) were collected during primary implantation of an artificial hip joint and served as controls. The samples were fixed and plastic embedded for sectioning prior to imaging it at the European Synchrotron Radiation Facility (ESRF) beamline ID21 at 30, 3 and 0.5 μm steps and 7.8 keV. Subsequently, regions with high particle exposure were imaged at beamline ID16B to determine particle sizes and elemental composition on the nanoscale at 50 nm step size and 25.6 keV.

The obtained XRF-maps indicated significant exposure to micron- and nano-sized cobalt, chromium, molybdenum, vanadium, titanium, manganese, and iron particles in the BM of peri-implant samples from all implant types. The highest exposure levels were found in samples obtained from patients with hip resurfacing implants and hip implants with modular stems. Nanoparticle exposure was more pronounced in osteolytic regions. Cobalt was the only element which was found integrated into the proximal trabecular bone in a non-particulate state.

Our data demonstrate that metal release from various metallic implants leads to multi-elemental particle exposure in peri-implant bone and BM. We consider ex vivo XRF mapping of human peri-implant samples as an expedient tool to support risk-benefit evaluation in arthroplasty, to guide individual implant choice in the future and for realistic appraisal of wear-related biological effects.

**01-P295** Tailor-Made Conductive Bioinks for 3D Printing of Neural Guidelines

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3D scaffolds for neural tissue engineering (TE) have significant effects on cell development by more realistic mimicking of an actual neural tissue and a higher spatial freedom for cell interaction. A versatile and precise method of 3D printing, which relies on controlled solidification of biocompatible inks, is the most promising method of 3D manufacturing to date. The present work relies on the development of a conductive nanocellulose-based ink for the 3D printing of neural TE guidelines. We provide insight into the factors that help to guide attachment, proliferation and differentiation of neural cells. It is demonstrated that by controlling the properties of the ink constituents, i.e. cellulose nanofibers (CNFs) and carbon nanotubes, it is possible to deposit guidelines with sufficient shape fidelity and electrical conductivity. The confocal microscopy images revealed considerable inclination of neural cells towards following the 3D printed conductive guidelines. Moreover, the results of cell culturing show that the guidelines have positive influence on cells’ attachment, proliferation and differentiation. To our knowledge, this is the first research effort devoted to using cost-effective cellulose 3D printed structures in neural TE, and, apparently, much more are expected to arise in the near future.
**01-P296** Water Soluble Ceria Nanoparticle-Embedded Contact Lens for the Treatment of Eye Diseases Caused by Excessive Reactive Oxygen Species

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Excessive reactive oxygen species (ROS) are considered important components in the occurrence of many eye diseases including cataract, uveitis, retinopathy of prematurity, dry eye disease and age-related macular degeneration. Ceria nanoparticles (CeNPs) have been known as effective ROS scavenging agents by mimicking the activities of superoxide dismutase (SOD) and catalase. For the treatment of eye diseases, liquid eye drops are generally used but the efficacy of the eye drop is low because of ocular barriers including lacrimal drainage losses. There are a few reports using administration of CeNPs via intravitreal or intravenous injections for the treatment of retinal diseases, but these are invasive and limited to the posterior segment of the eye. To overcome these limitations, we propose CeNP-embedded contact lenses (CeNP-CLs). These CeNPs have a water-soluble property and showed the good transparency in the form of polyhydroxyethylmethacrylate-based contact lens. The cell viability was over 90% in the co-culture of human conjunctival cells with CeNP-CLs. CeNP-CLs successfully showed the 
H2O2-scavenging and the SOD-mimetic activities without releasing CeNPs from contact lenses. Despite the use of medium containing high levels of 
H2O2, in vitro cell survival rate was increased in the presence of CeNP-CLs due to CeNP-CL’s ROS scavenging property and good biocompatibility. These results demonstrate the CeNP-CL’s potential of the clinical translation for the treatment of ROS-causing eye diseases without invasive procedures.

**Reference**


**01-P297** Novel Antimicrobial, Hyperelastic, Nanofibrous Polyurethane Meshes for Wound Care

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Wounds and their comorbidities costs billions annually, with bacterial infections preventing healing further. Many antimicrobial agents cause unwanted side effects that can reduce healing, carry risk for antimicrobial resistance and have poor topical delivery systems. This report describes antimicrobial polyurethane electrospun nanofibre meshes for wound healing, with the aim to give an improved antimicrobial release and activity without host cell damage. A cationic antimicrobial (CA) was electrospun with the polyurethane (PU) for a gradual CA release. The name of the antimicrobial used is confidential and has therefore been removed. It was hypothesised that the high surface area and mesh morphology would control the antimicrobial release over time, and that PU would give ideal mechanical properties for a wound dressing.

A CA:PU solution was prepared with CA concentrations of 5%, 15%, 25% and 35% (wt). These were electrospun and the structural and mechanical properties, and activities against cells were characterised. Experiments included tensile mechanical tests, release in PBS, antimicrobial assays, and HaCaT cell toxicity.

The Young’s moduli of PU alone and CA:PU meshes were not significantly different, averaging at 3.95±1.19MPa, an ideal elasticity for wound dressings. Following addition to PBS, meshes displayed a burst release of CA within one hour (67.19±21.65% of total released), followed by a gradual release over 5 days ( ≤ 25% CA:PU samples). All CA:PU meshes had superior antimicrobial activity when compared to control and commercial groups. 5% CA:PU meshes showed no human cell toxicity and were significantly superior to commercial groups tested. However, samples containing ≥15% CA showed increasing levels of toxicity.

Overall, the CA:PU meshes with ≤15% CA displayed encouraging properties for improved wound dressings. An ideal dressing would release the antimicrobial gradually over 2 days to reflect regular dressing changes. Further development will fine-tune the antimicrobial delivery for an optimised material.

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**01-P298** Dual stimuli-responsive PIPAAm-PDMS surfaces for controlling cell attachment and detachment

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Temperature-responsive cell culture surface, poly(N-isopropylacrylamide) grafted tissue culture polystyrene (TCP5) (PIPAAm-TCPS) showed the graft PIPAAm thickness dependency on cell adhesion character. Based on this phenomena, PIPAAm gel grafted polydimethylsiloxane (PIPAAm-PDMS), was newly prepared as new stretchable temperature-responsive cell culture surface, to demonstrate an idea that mechano-stress modulate physical properties of the grafted PIPAAm gel as well as the PIPAAm-PDMS surface. PIPAAm-PDMS surface showed hydrophilic/hydrophobic change with temperature change, while bare PDMS did not. When PIPAAm-PDMS was uniaxially stretched up to 20% of stretching ratio, contact angle of PIPAAm-PDMS surface increased, accompanying with decrease in the thickness of graft PIPAAm layer. AFM images demonstrated that nano-scale of PIPAAm gel aggregation was newly formed after EB irradiation. The uniaxial stretch deformed the nano-scale PIPAAm aggregation, suggesting extension of PIPAAm chains with the stretch. Cell attachment assay showed that stretched PIPAAm-PDMS surface was more cell adhesive than unstretched PIPAAm-PDMS at 37°C. Cells and cell sheet detachment from the stretched PIPAAm-PDMS was also examined by decreasing temperature and/or with uniaxial shrinkage treatment. Low temperature treatment detached cell sheet from the stretched and unstretched PIPAAm-PDMS in 9 minutes, while uniaxial shrinkage alone enabled to detach cell sheet more than 12 hours at 37°C. Dual stimuli, uniaxial shrinkage treatment and subsequent low temperature treatment, allowed for more rapid cells-sheet recovery (6 minutes) than low temperature treatment alone (9 minutes). These results demonstrated the idea; stretching/shrinkage modulated properties of PIPAAm-PDMS surface as well as thickness of graft PIPAAm layer. This new stretchable temperature-responsive cell culture surfaces may allow for efficient fabrication of cell sheet irrespective of various cell species, by using stretch/shrinkage and temperature change.

Reference

**01-P299** Role of polyelectrolyte hydrolysis and electrostatic attraction in Gentamicin release from Layer-by-Layer coated silica nanoparticles

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Layer-by-layer deposition is a versatile techniques that has been employed in numerous industrial applications i.e. biomaterials, drug delivery and electronics to confer peculiar properties to the system. When employed in drug delivery, the active molecule is sandwiched between polyelectrolytes and the release is controlled by the diffusion of the drug through the layers and the possible hydrolysis of the coating (delamination). Poly-beta-amino-esters (PBAE) are a class of hydrolysable polyelectrolytes that have been widely used in DNA delivery and in LbL on medical devices. Their use allowed controlled release of antibiotics and other bioactive compounds from the surface of medical devices without cytotoxic effects for extended periods of time. The general accepted consensus is that the prolonged drug released from LbL coating assembled using PBAE is the results of the polymer hydrolysis; however no attention has been paid to the possible role of the electrostatic attraction between PBAE and the other polyelectrolyte utilised in the LbL assembly in controlling drug release.

In this work, we demonstrated using gentamicin as drug model that the drug release from PBAE containing LbL coating is predominantly controlled by the electrostatic attractions between opposite charged electrolytes as higher level of drug were released at pH7 than at pH5 as the positive charge of PBAE decreases from pH5 to pH7 despite the hydrolysis kinetics been faster in acidic conditions as determined with GPC. Furthermore, when PBAE were replaced with chitosan the release kinetics at pH5 and 7 were comparable as the charge of chitosan is not influenced in this pH range.
**01-P300** Nanocarriers From Plasma Dust Enhance Gene Delivery in Diverse Cell Types

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**Introduction:** While the number of nanocarriers has vastly increased in recent decades, significant issues persist in combining fine control of physical properties, low cytotoxicity and simple functionalization in a single platform. Multifunctional carbon-based nanoparticles (nanoP3), manufactured in a custom-built plasma chamber, are a new platform that achieves multiple functionalities in a single, simple step enabling delivery of multiple molecular cargos into cells. Size, roughness, surface charge and chemical composition were readily controlled, giving rise to a versatile nanoparticle platform [1]. **Methods:** Cytotoxicity assays, such as Alamar Blue, LDH activity assay and Caspase3/7 were performed as per manufacture instructions. Genetic material including siRNA and plasmid were conjugated to nanoP3 (10⁹ particles/mL) in HEPES-NaCl by simple incubation at room temperature for 10 minutes. The unbound cargo was washed three times by centrifugation. Transfection efficiency/protein knockdown were observed via western blot, IVIS imaging, and fluorescent microscopy. **Results:** Unfunctionalized nanoP3 penetrated the cell membrane in all 13 cell types tested: HEK293, MCF7, MCF10A, mFb, hFb, HEPG2, iPSCL2G, hCAECs, HUVECs, hSMCs, AS94, HeLa, and MIN6, without the requirement for penetrating agents. Incubation of nanoP3 for up to 5 days did not affect cell viability or morphology, no increase in both LDH (membrane integrity marker) and Caspase 3/7 (apoptosis) level, with up to 10⁹ particles/mm² in all cells tested. Confocal imaging showed the presence of paclitaxel, IgG-Cy5 and IgG-Cy7 co-localized within the cell membrane, demonstrating a successful co-transportation of cargo into MCF10A, MCF7 and hCAEC cells. To exemplify one possible functional utility of nanoP3, we delivered functional siRNA targeted to luciferase in primary mouse fibroblasts and induced pluripotent stem cells, reducing gene expression 52.64±1.7% and 51.6±3.1% at 72 hours, respectively. NanoP3 functionalised with siRNA targeted to the vascular endothelial growth factor gene (VEGF) in primary HUVECs, impaired the formation of tubules in an established Matrigel assay (56.57±8.87% reduction vs scrambled). Finally, we demonstrated the successful transfection of a GFP plasmid into HeLa and HEK293 cells using only nanoP3. Overall, we demonstrate a cost-effective, high efficiency nanoparticle platform with significant implications for improved intracellular delivery of genetic materials.

**01-P301** Self-assembling Nanopeptide Hydrogel for the Applications in Tissue Engineering and Regenerative Medicine

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Brain injury is a devastating medical condition and represents a major health problem. Tissue and organ reconstruction have been regarded as promising therapeutic strategies. Here, we propose a regenerative methodology focusing on the provision of functionalized nanopeptide scaffolds to facilitate angiogenesis and neurogenesis at the brain injury site. The peptide RADA16-SVVYGLR undergoes self-assembly to construct an interconnected network with intertwining nanofibers, and can be controlled to display various physicochemical properties by the adjustment of microenvironmental factors such as pH and ion concentration. Such scaffolds can support endothelial cells to form tube-like structures and neural stem cells to survive and proliferate. In an in vivo zebrafish brain injury model, sprouting angiogenesis and developmental neurogenesis were achieved, and functional recovery of the severed optic tectum was enhanced in RADA16-SVVYGLR hydrogel-implanted zebrafish. This nanopeptide hydrogel was non-toxic to zebrafish embryos during early developmental stages. This angiogenic self-assembling peptide hydrogel had programmable physical properties, good biocompatibility, and regenerative ability for functional recovery in the injured brain. We suggest that functionalized self-assembling peptides encapsulated with neural stem cells or used alone could be an attractive and effective therapeutic modality for brain injury and diseases (e.g., trauma, stroke, tumor, degenerative neurological disorders, etc.).
Evolution of biomaterials for implants progressively shifted the focus from adequate mechanical strength to improved biocompatibility and absence of toxicity and, finally, to fast tissue integration. Recently, new frontiers and challenges of titanium implants have been addressed with the focus on bioactivity and fighting bacterial infection and biofilm formation. This is closely related to the clinical demand of multifunctional implants able to simultaneously have a number of specific responses with respect to body fluids, cells and pathogenic agents. Nanotechnologies can have a substantial and effective impact in regulating the tissue-implant interface through controlled topography and surface functionalization at the nanoscale and some of them are here explored.

Titanium and titanium alloys surface treated in order to be bioactive (induced precipitation of hydroxyapatite in contact with physiologic fluids) and antibacterial are here characterized and compared. They have in common surface topography at the nanoscale and functionalization with antibacterial metal ions or nanoparticles, but differ in surface reactivity in contact with the body fluids (simulated through SBF-Simulated Body Fluid, albumin and/or hydrogen peroxide complex solutions). The focus is on comparison of their mechanisms of action, kinetics of surface activity and biological response. A bioactive glass belonging to the system SiO$_2$-Na$_2$O-CaO-P$_2$O$_5$-B$_2$O$_3$-Al$_2$O$_3$ is used as reference for comparison with a classic bioactive material; the same glass doped with silver ions by ion exchange in aqueous solutions of silver nitrate was used as reference of antibacterial action through Ag ions release.

Protocols of analysis suitable for evaluating and comparing the mechanisms of bioactivity and antibacterial action (FESEM, Raman, XPS, electrokinetic and electrophoretic zeta potential measurements), kinetics of surface reactivity (release of antibacterial agents, FTIR, cross section observation after soaking in SBF), biocompatibility (corrosion resistance and ion release in complex solutions) and biological response (biofilm formation, osteoblast adhesion and differentiation) of a wide range of inorganic biomaterials have been assessed and are here described and discussed.

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**Low concentration of Silver nanoparticles affects the cellular response of Tumor Necrosis factor**

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Tumor necrosis factor-α (TNFα) is a pro-inflammatory cytokine which is an early marker in cell inflammation; also, it induces apoptosis by different pathways. Silver nanoparticles (AgNPs) are widely known to have anti-inflammatory properties; therefore, it has been used in many products. However, the exact mechanism of its protective effect has not been totally clarified yet. Our study aimed to investigate the effect of low concentration of AgNPs on the cellular response of TNFα and to determine would it increase or decrease the effect? And what is the molecular mechanism of its effect? Lung epithelial cell line (NCH-H292) was exposed to AgNPs (5µg/ml) and/or TNFα (20 ng/ml) for 24 h, the cytotoxic effect of AgNPs has been analyzed showing an increase in the cytotoxic effect by increasing the concentrations of AgNPs. Then, flow cytometric assay has been established; our results showed that the cellular uptake of AgNPs has been significantly increased in presence of TNFα. Moreover, the estimation of apoptosis showed that the AgNPs significantly decreased the apoptotic effect of TNFα. Confocal microscopy was used to localize the tumor necrosis factor receptor 1 (TNFR1) and the results revealed that TNFR1 localized inside the cells with very few receptors scattered on the cell membrane of the cells exposed to both AgNPs and TNFα while the receptors were homogenously distributed on the cell membrane in control cells and cells exposed to TNFα only indicating that AgNPs affect the localization of TNFR1. Accordingly, our study concluded that the low concentration of AgNPs showed an anti-apoptotic effect against the apoptosis induced by TNFα. Also, we provided a new possible mechanism of the anti-apoptotic effect of AgNPs by its effect on the TNFα receptor1.
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**01-P306** Development and characterization of free standing biomembranes combining chitosan and natural-nanoliposomes for tissue engineering applications

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Advances in Tissue Engineering and Regenerative Medicine (TERM) depend increasingly on the availability of specific biomaterials that allow controlling biological systems behaviour [1]. Therefore, many efforts have been devoted in proposing both biologically derived and synthetic materials in contact with biological materials and new tissue engineering strategies that could enhance the regenerative potential of the products.

In this work, chitosan membranes, were elaborated and functionalized by incorporating nanoliposomes based on natural vegetable (soy based) and marine (salmon head) lecithin. The properties of natural nanoliposomes/chitosan blend membranes were evaluated by multi scale techniques such as water contact angle (WCA), water uptake ability, Fourier Transform InfraRed spectroscopy (FT-IR) and Atomic Force Microscopy analysis (AFM). In vitro biocompatibility analysis of chitosan before and after functionalization with nanoliposomes were performed using human mesenchymal stem cells (hMSCs)

This study provide the information about the potential of natural nanoliposomes/chitosan blend membranes to be used as multifunctional biomaterials for supporting cell activity, facilitating regeneration, and guiding tissue repair.

Especially, cytotoxic assays showed that 1) none of the membranes induced any cytotoxic effect and ii) 1mg.ml⁻¹ of salmon nanoliposomes in the chitosan has the highest potential in the development of chitosan-based biomembranes for tissue engineering applications [2].


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**01-P307** New Composite Membranes Polymer-Functionalized Graphene for Haemodialysis

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Polymeric membranes are widely used for various biomedical applications like proteins concentration [1], osseointegration [2] or substitutes as technological solutions for various organs, like artificial lung – oxygenator [3] or artificial kidney – haemodialysis [4]. This work presents the principle for synthesis of a new generation of composite polymeric membranes with functionalized graphene for haemodialysis. Firs, specific proteins, enzymes and vitamins are covalent immobilized on graphene in order to increase to selectivity and specificity for removing targeted compounds for specific medical conditions associated with chronic renal disease. In the second stage of synthesis, functionalized and derivatized graphene are used for obtaining composite polymeric membranes with controlled porosity for haemodialysis. Fully structural and morphological characterization of synthesized materials is presented and hydrodynamic and separation properties. Also, haemotoxicity tests were performed in order to study and prove the non-cytotoxic character of synthesised membranes.

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Acknowledgement: This work was supported by a grant of the Romanian National Authority for Scientific Research and Innovation, CNCS - UEFISCDI, project number PN-III-P1-1.2-PCCDI-2017-0407 – Intelligent materials for medical applications, sub-project - New generation of hemodialysis composite membranes with derivatized graphene.
Collagen hydrogel has been studied extensively as an extracellular matrix (ECM) material in regenerative medicine. However, it has an isotropic structure. In contrast, the native ECM has an anisotropic structure. Collagen hydrogels with anisotropy would be highly desirable because cells are affected by the physical cues from the ECM microenvironment. The electrospinning of collagen dissolved in organic solvents is a widely used method for fabricating anisotropic collagen nanofibers. However, the thus-obtained collagen fibers are water soluble and need to be cross-linked before they can be used as scaffolds for cell culture. In this study, electrospinning using a core-shell nozzle was employed to spin an aqueous acidic solution of collagen and encapsulate it within a shell of polyvinylpyrrolidone (PVP). Subsequently, the core collagen was gelled, and the shell PVP was washed away through a treatment with a basic ethanol solution to obtain anisotropic collagen hydrogel nanofibers. Immunostaining and Fourier transform infrared spectroscopy revealed that the obtained fibers were composed of collagen and that the surface PVP had been removed completely. Circular dichroism measurements confirmed that the fibers exhibited the triple helical structure characteristic of collagen. Further, human umbilical vein endothelial cells cultured on the collagen hydrogel fibers were oriented along the fiber direction. Thus, this method is a suitable one for fabricating fibrous anisotropic collagen hydrogel without chemical and thermal cross-linking and should lead to the design and development of safe medical materials with anisotropy similar to that of the native ECM.

Ti-6Al-4V (Ti64) is one of the widely used implant materials due to its good biocompatibility, mechanical strength and corrosion resistance. However, the high elastic modulus (~110 GPa) of Ti64 (vs. natural bone 1~40 GPa) implant may cause stress shielding effect on the surrounding bone, leading to the bone resorption and the following implant loosening. Therefore, another new potential alternative, Ti-24Nb-4Zr-8Sn (Ti2448) alloy with lower elastic modulus (~42 GPa) close to natural bone, was used in this study. Furthermore, as compared to solid implants, interconnected porous scaffold implant could mimic bone structure, enhance cell ingrowth and reduce stress shielding effect by controlling pore structure. In this study, electron beam melting (EBM) process, a type of three dimensional (3D) printing technique, was used to produce Ti64 and Ti2448 scaffolds with interconnected porous structure. We have applied electrochemical anodization treatments to produce a thick oxide layer with a nanoporous topography on the surface of EBM-produced Ti64 and Ti2448 scaffolds, for the improvement of corrosion resistance and cell responses. The scanning electron microscope images showed that the produced nanoporous oxide layers on the EBM-produced Ti64 and Ti2448 scaffolds had different surface topographies, including hole-, tube- and network-shape pores. All these nanoporous surfaces showed excellent surface hydrophilicity, which are expected to facilitate the adhesion of nanoscaled proteins. The bioactivity, in terms of Ca/P formation ability, and corrosion resistance were also enhanced by the presence of nanoporous oxide layer on the EBM-produced Ti64 and Ti2448 scaffolds. The nanoporous structure produced by electrochemical process on the EBM-produced Ti64 and Ti2448 scaffold surfaces performed as a stimulus to motivate the signaling transduction to mediate the cell responses, including cell adhesion, proliferation and differentiation, of human bone marrow mesenchymal stem cells. The results of this study provide advanced information on the surface modification of EBM-produced Ti64 and Ti2448 scaffolds in orthopaedic implant applications.
01-P310  Detonation nanodiamonds: the effect of size and surface chemistry on protein corona composition and human cell behavior

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Various materials are used for production of different nanoparticles (NP), which provide promising features for biomedical application as a targeting carrier for drugs, genes or imaging agents¹. The surface chemistry, zeta potential, and surface dipoles are important characteristics enable spontaneous and efficient adsorption of molecules from biological environment e.g. proteins. On the other hand, adsorbed molecules may modify NP properties and even cause loss of specificity in their targeting². Thus, attention should be paid to the influence of NP size and surface modification for controlling their biological activity¹.

Detonation nanodiamonds, i.e. DND, are interesting for medical applications thanks to their excellent biocompatibility, chemical and optical properties as well as relatively low-cost synthesis¹. Use of DNDs for drug adsorption, targeting, and release has been successfully exploited for the treatment of tumors³. However, interactions among DNDs and molecules from their environment are still not fully clarified.

In this work, we firstly characterize effect of DNDs with different mean size (2 and 4 nm) and surface chemistry (hydrogen or oxygen groups)⁵ on two types of cells – SAOS-2 and hMSC. Secondly, we studied formation and composition of protein corona on these different types of DNDs, which occurs on particles in contact with biological fluids (e.g. cultivation medium with serum). Our experiments revealed that hydrogenated DNDs cause morphological signs of heavy cellular distress to both tested cell types. We identified proteins binding onto the DNDs by LC-MS/MS. The analysis detected number of proteins common to all types of DNDs but also several unique proteins in dependence on DND size and/or surface modification. The precise knowledge of protein corona composition and identification of NP interactions in biological environment can help in innovation of targeting carriers without loss of specificity.


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01-P311  Study on cell fusion-mediated transdifferentiation via a novel approach

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One of the ultimate goals of regenerative medicine is to substitute damaged cells and/or tissues.

To realize this, the processes of transdifferentiation or reprogramming have been accomplished by cell fusion or iPS cell method.

In this study, we investigated the possibility of transdifferentiation of human cell through cell fusion with mouse cell. Because we aimed to develop a novel way of cell fusion between two different species and to identify the characteristics of fused cells such as transdifferentiation.

Particularly, we used a microfluidic chip which enables to make cell fusion without nucleus mixing because several issues like pluripotency which would be occurred after cytosol exchange are still subject to attractive and undiscovered field for regenerative medicine.

We furthermore aim to investigate reprogramming using embryonic stem and somatic cells such as fibroblasts with this technology. This approach will be useful in a variety of ways to induce direct transdifferentiation or reprogramming and to study the process of those.
**01-P312** Anti-inflammatory drug-eluting implant model system to prevent wear particles induced osteolysis

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Aseptic loosening, as a consequence of an extended inflammatory reaction induced by wear particles, has been classified as one of the most common complications of total joint replacement (TJR). During last 5 years, aseptic loosening was recorded as the reason for revision in more than 46% of TJR single stage procedures. Despite it is high incidence, in the last decade any therapeutic approach has been found to treat or avoid aseptic loosening, leaving revision as only effective treatment for this condition. The local delivery of anti-inflammatory drugs to modulate wear-induced inflammation has been regarded as a potential therapeutic approach to avoid aseptic-loosening. In this context, we developed and characterised an anti-inflammatory drug-eluting implant model system. The model system was obtained by covalently conjugating dexamethasone to carboxyl-functionalised TiO2 particles, obtained by using either, amino or mercapto silane agents. Zeta potential measurements, thermogravimetric analysis (TGA) and drug loading results suggest that dexamethasone was successfully loaded into the carboxyl-functionalised TiO2 particles. Transmission electron microscopy (TEM) images showed that dexamethasone loaded particles maintain their spherical-like shape with a 20-25nm diameter and narrow size distribution. The model-system was then tested for its cytotoxic and anti-inflammatory properties in a LPS-stimulated murine macrophage-like cell line, RAW 264.7. Here we showed that dexamethasone released from carboxyl-functionalised TiO2 particles was able to decrease LPS-induced nitric oxide (NO) production by ~60%, when compared to LPS control-group treatment, at non-cytotoxic concentrations.

**01-P313** Oxygen nanobubbles for the effective delivery of oxygen and drug molecules

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In this study, we prepared nano-sized oxygen bubbles. Nano-sized oxygen bubbles were effectively delivered to cells to help rehabilitate oxygen-deficient cells. It is also demonstrated that oxygen nanobubbles can reverse hypoxic environment of tissues to normal state. Anti-tumor drug molecules loaded onto the surface of nanobubbles were effectively delivered to the cancer cells, and a synergistic increase of the drug effect due to the increase of the local oxygen concentration could be demonstrated. These oxygen nanobubbles are expected to be useful for oxygen and nutrient delivery in three dimensional cell clusters and/or tissues such as organoids.
**Polypropylene Fumarate-Functionalized Graphene Oxide Composite Scaffold for Tissue Engineering**

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Despite that all biocompatible materials used until now for scaffold fabrication, especially for bone reconstruction, fulfilled most of the requirements for an ideal scaffold; there are still few limitations that many times are essential for a good functioning of the new tissue and patient safety (1). For example, using pre-formed implants for bone regeneration involves a complex and high cost surgical technique which sometimes leads to patient discomfort and complications requiring a subsequent surgical procedure for replacement. Moreover, it is difficult to obtain a scaffold which perfectly fills irregular-tissue defects. Furthermore, pre-formed scaffolds usually use toxic chemical cross linkers. In addition, the risk of migration of the implant is higher (2).

The main aim of this study is to perform chemical modification by grafting polypropylene fumarate (PPF) on the graphene oxide surface which will act afterward as compatibilizer between graphene oxide and the polymer matrices and dispersion of functionalized graphene oxide (PPF) into PPF matrix. The functionalized GO are further used to develop injectable, mechanically strong scaffolds for bone tissue engineering applications.

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Acknowledgment

This work was supported by a grant of the Romanian National Authority for Scientific Research and Innovation, CNCS – UEFISCDI, project number PN-III-P1-1.1-PD-2016-0761– Designing new graphene based injectable biomaterials with therapeutic effect and potential applications in bone regeneration.
**01-P317** Stem cell membrane-cloaked gold nanorods for efficient cancer photothermal therapy

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Gold nanorods (AuNRs), which have tunable near-infrared (NIR) absorption and intrinsically high photothermal conversion efficiency, have been actively investigated as photothermal conversion agents for photothermal therapy (PTT). The short blood circulation lifetime and poor in vivo distribution of AuNRs, however, limits their tumor uptake and thus in vivo applications. Here we show that such a limitation can be overcome by cloaking AuNRs with stem cell membranes, with top-down approach.

The surface of nanoparticles coated with cell membrane determines their in vivo behavior. Especially, the stem cell membrane-cloaked nanoparticles can achieve special properties such as serum stability, tumor tropism, long circulation time and immune escaping (avoid RES) owning to membrane antigens and membrane structure.

For more specific affinity to tumor region, chemically engineered synthetic lipid-peptide was incorporated into natural cell membrane. The cloaking of stem cell membranes over AuNR surface does not alter the unique plasmonic property of AuNRs, and the resulting stem cell-membrane coated AuNRs exhibit good colloidal stability.

The resulting particles are thoroughly characterized using SDS-PAGE, western blotting, zeta potential analyzer, TEM, etc. They show special property originated from both stem cell membrane and synthetic lipid-peptide conjugates.

The work presented here provides a new angle on the design of biomimetic hybrid nanoparticles for efficient tumor targeted photothermal therapy.
**Semi-synthetic hydrogel matrices for studying differences in cell behavior in 2D and 3D microenvironment**

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Cells in vivo are arranged in a complex microenvironment consisting mainly of extracellular matrix (ECM) and soluble factors. Researchers have tried to replicate the ECM by utilizing artificially engineered matrices to provide support for cell growth. Our group has developed semi-synthetic matrices consists of polyethylene glycol diacrylate and gelatin methacrylate which will be used to study how cells respond to different micro-environment. The main advantage of these hydrogels is that we can control the stiffness and degradability by varying the composition of the respective polymers. These matrices were characterized for morphology, pore size, stiffness, degradation and swelling ratio. Further, we wanted to study the cellular fate processes on these semi-synthetic matrices. We chose MDA-MB 231 breast cancer cell line as a model system for our studies. We have seen differences between cells cultured on traditional 2D petri plates and those cultured on the surface of or embedded within these hydrogels. We have looked at various aspects such as morphology and migration of cells in these matrices, their gene expression profile related to epithelial to mesenchymal transition, their matrix metallo proteinases (MMPs) secretion profile and cytoskeletal organization. Our results suggest that cellular phenomenon is influenced not just by matrix properties such as stiffness, degradability or cell adhesivity but also depends upon the context in which these are presented to cells. This work has implications in understanding processes involved in cell invasion, cancer metastasis which may subsequently help in developing platforms for drug screening applications.
3D Microfluidic Co-Culture System Mimicking Brain Tissue

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In conventional in vitro culture systems, cells are cultured on mechanically inappropriate 2D flat surface with bulk media supply, which is different from in vivo microenvironment. In brain tissue, neuronal cells are surrounded by soft 3D extracellular matrices (ECMs) interacting with neighboring cells including endothelial cells forming blood vessel by direct contact and secreted signaling molecules. By modulating these microenvironments, the neural stem cell can be controlled to differentiate into neuronal or glial cell types.

To control these microenvironments, we used microfluidic chip system combined with 3D hydrogels, which can give more physiologically-relevant condition compared with 2D platform. Here, we report a brain-mimicking 3D microfluidic co-culture system guiding human neural stem cell (hNSC) differentiation by co-culturing with human endothelial cells (hECs). The precisely designed micro-dimension chip allows highly dense cell seeding, facilitating direct contact to hECs and accumulated paracrine factors guiding hNSC differentiation. We adjusted the mechanical properties and ECM compositions of the hydrogel to the native brain tissue, which could generate sufficient biophysical support while giving an appropriate stem cell niche.

In conclusion, the hNSCs co-cultured with hECs in the 3D microfluidic chip showed enhanced glial differentiation near hECs, which is similar to cellular morphogenesis in a native brain tissue. Our results showed this system could facilitate in vivo-like conditions along with suitable biophysical stimulation, guiding both hNSCs and hECs to form brain mimetic morphology and physiology.

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Bioactive characteristics on bone regeneration with octacalcium phosphate crystals grown with natural polymers

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Octacalcium phosphate (OCP) has been postulated as one of precursors to apatite crystals in bone and tooth (Brown WE et al. 1966). We have found that synthetic OCP enhances additional bone deposition on cortical bone surface and bone regeneration in critical-sized defects in various animal models, if implanted, compared to hydroxyapatite (HA) materials including Ca-deficient HA (i.e. Suzuki O et al. Biomaterials 2006). Furthermore, it was apparent that OCP is capable of enhancing initial stages of osteoblastic differentiation (Anada T et al. Tissue Eng Part A 2008), the late differentiation to osteocytes (Sai Y et al. Acta Biomater 2018) and osteoclast formation from bone marrow cells together with osteoblasts (Takami M et al. Tissue Eng Part A 2009) therefore can be used as a suitable bone substitute material clinically (Kawai T et al. J Tissue Eng Regen Med 2017). Thus, OCP has stimulatory capacities on bone tissue-related cells to accelerate bone formation. However, it is still unclear how the material characteristics in OCP relate to its bioactive property. In the present study, OCP was wet-synthesized in the presence or absence of natural polymers, such as collagen and gelatin molecules in the solutions and analyzed to the effect of the presence of the molecules to regulate the crystal growth of OCP by transmission electron microscopy, scanning electron microscopy, Fourier transform infrared and Raman spectroscopies. Bone regenerative capacity of OCP crystals included in the porous gelatin matrix materials was studied by implanting them in critical-sized rat calvaria defects up to 12 weeks. After decalcification and hematoxylin and eosin staining of the calvaria, the bone formation rate was determined by histomorphometric analysis. Collagen orientation in newly formed bone matrix was measured after picrosirius red staining as an indicator of the bone quality. The results show that (1) the morphology and the crystallinity of OCP were distinct in the type of natural polymers included in the preparation; (2) the inclusion of the co-precipitated OCP crystals in the gelatin matrix enhanced bone regeneration and the collagen orientation compared to the matrix materials only. These results suggest that the crystalline properties of OCP obtained through the precipitation with natural polymers affect the bone regeneration process by this material.
Tissue formation in our body is driven by the spatial and temporal distribution of continuous gradients in mechanical and biochemical signals. Currently available additive manufacturing (AM) technologies are limited to discrete gradients production, resulting in bi/triphasic or multilayered scaffolds, whose functionality relies on a good interface between layers. Here, with a newly developed single-head AM melt extrusion technique, we aim at the creation of scaffolds with continuous gradients in material composition to promote osteogenic differentiation of human mesenchymal stromal cells (hMSCs) in a segmental bone defect.

Graphene-based polymer composites have recently drawn enormous attention for biomedical applications. Varying reduced graphene oxide (rGO) concentration continuously in a single filament while printing, enabled to spatially control the mechanical, electrical and chemical properties of the final gradient scaffold, which have a high impact in osteogenesis. Melt blended PEOT/PBT – rGO composites were prepared to study the effect of rGO in the physico-chemical properties of the composite prior to printing. By increasing the rGO concentration up to 10 wt%, the elastic modulus in compression increased from 125 to 150 MPa. Moreover, the electrical properties of the material were noticeably enhanced, from a non-conductive to tens of MΩ electric resistance. Importantly, higher protein absorption was achieved with higher rGO content.

Notably, cell adhesion and proliferation were significantly enhanced in 3D scaffolds made with composites of only 3 wt% rGO (single concentration, 250 μm filament diameter, 500 μm pore size). In addition, while in control scaffolds alizarin red stain showed matrix mineralization over the scaffolds cross sections after 3 weeks culture in differentiation media, this was observed after only 1 week in 3 wt% rGO scaffolds. The early osteogenesis on these scaffolds was further confirmed with the expression of the osteogenic markers ALP, RUNX2 and Collagen type I. Ultimately, the new AM technology enabled the fabrication of a continuous filament gradient scaffold with varied rGO concentration from 0 to 3 wt%. Compared to similar technologies, continuous gradient scaffolds closely mimicking the in vivo environment could provide hMSCs with the adequate stimulus for bone formation.

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Biomaterial scaffolds for bone marrow mesenchymal cell (BMSC) therapy targeting bone regeneration require macro-porosity for cell and tissue infiltration, and micro- and nano-porosity to improve osteoinduction. This study compares two calcium phosphate (CaP) ceramics sharing the same interconnected macro-porosity but possessing different micro-porosity.

Calcium deficient hydroxyapatite (CDHA) scaffolds were prepared by foaming a liquid phase (1 wt.% Tween 80) and a powder phase (α-tricalcium phosphate (α-TCP) with 2 wt.% hydroxyapatite (HA) and 10 wt.% Pluronic F-127) at a ratio of 0.65 mL/g. β-Tricalcium Phosphate (β-TCP) scaffolds were obtained by sintering CDHA scaffolds at 1100°C. The scaffolds were evaluated in vitro by culturing human BMSC and -tricalcium phosphate (α-TCP) with 2 wt.% hydroxyapatite (HA) and 10 wt.% Pluronic F-127) at a ratio of 0.65 mL/g. β-Tricalcium Phosphate (β-TCP) scaffolds were obtained by sintering CDHA scaffolds at 1100°C. The scaffolds were evaluated in vitro by culturing human BMSC and

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Total porosity of both scaffolds was 82%, with interconnected macro pores with a pore entrance size of 80 mm. CDHA presented additional pores in the nanometric range (between 0.01 and 0.3 mm) and in the micrometric range (2 mm) while β-TCP showed bigger pore sizes (2.5 mm). The microstructure of CDHA consisted of combined crystal structures of needles and plates, while β-TCP displayed the typical sintering necks and polyhedral crystal grains. SSA of CDHA was 17.2 m²/g, while SSA of β-TCP was 0.60 m²/g. CDHA scaffolds showed osteoinductive properties, while β-TCP scaffolds exhibited osteoconductive properties only. BMSC significantly enhanced bone formation. While no difference in the quantity of new bone formed was observed between biomaterials, the distribution was significantly different, whereby CDHA scaffolds contained bone dispersed throughout the entirety of the scaffold, while bone was formed only along the edges of BMSC deposition in the βTCP scaffolds. These results suggest that the novel biomimetic low-temperature CDHA is an interesting biomaterial and clinically relevant for bone regeneration strategies.

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The crystal structure of OspA revealed that this protein has an unusual architecture: it is dumbbell-shaped and contains a three stranded "single-layer" β-sheet in the center. The single-layer β-sheet segment is rich in polar amino acids, and its amino-acid sequence does not follow the canonical, alternating hydrophobic/ hydrophilic pattern usually found in amphipathic b-sheets. We were selected the "single-layer" β-hairpin peptide sequence: KSSTEEKFNEKGE- LSEKKITRA(KSS) [2] in OspA. Basically, β-hairpin and β-strand peptides were formed self-assembling nanofiber structure because of existence of inter molecular interaction. Thus, we discuss the relationship "single-layer" β-hairpin sequence and aggregation (gelation) mechanism.

The "single-layer" β-hairpin peptide was combined with cell adhesive motif derived from cell adhesive protein. Fibronectin has the RGDS sequence from the extracellular matrix protein as a model bioactive ligand. This sequence interacts with cell surface integrin receptors, plays a critical role in cell adhesion, behavior and signaling and is the minimum motif required for cell adhesion on a surface. We designed OspA based "single-layer" β-hairpin peptide: KSSTEEKFNERGDLSEKKITRA (KSSRGD).

Hydrogel was prepared by mixing of peptide solution and PBS solution. Rheological character of hydrogels was measured by rheometer. These peptides were applied for the cell culture plate surface and 3D culture scaffold. The cell adhesion activity was assessed by L929 cell onto KSS and KSSRGDS peptide-immobilized TCP. These "single-layer" β-hairpin peptide peptides have possibility for application of tissue engineering or regenerated medicine.

Porosity is an essential property in the fabrication of scaffold for tissue engineering applications. Pores should be large and interconnected to allow cell growth and cell migration, facilitating the transfer and permeation of solutes as nutrients, metabolites, oxygen, waste, drugs, among others. Water uptake increases the size pore and reduces the mechanical properties of hydrogels, compromising the structural stability of the scaffold. The most common techniques used to create porosity are cryogenic and freeze-drying process, depending on the material used to fabricate the scaffold. The aim of the present investigation was fabricated gelatin-based polymer scaffold using a cryogenic process following a freeze-drying process and chemical treatment based on molecular imprinting technology (MIT). In order to evaluate the relationship between the impacts of scaffold preparation MIT technique in the pore size, we analyzed morphology using confocal microscopy, water uptake, and rheological properties. Hydrogel showed a highly hydrophilic behavior, with a water uptake capacity from 14 times their own mass. Morphology of hydrogel showed high porosity using confocal microscopy (> 100 μm). Along the entire frequency range, G’ modulus higher than G” modulus, indicating a predominantly solid viscoelastic. At lower frequency (0.1 rad/s), G’ modulus and G” modulus showed values of 5600 and 313 Pa, respectively. Based on the results, a gelatin-based hydrogel can be used as scaffold promising for tissue engineering applications.

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References:

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Mesenchymal stem cells (MSCs) are widely used in Tissue Engineering applications due to their ability to differentiate into various cell types such as chondrocytes [1] and neural cells [2]. MSCs fate could be influenced by the interaction between cells and the topography, porosity and mechanical properties of the extracellular environment. [3,4]. Electrospinning is a well-known technique to obtain 3D scaffolds able to mimic the nanoscale structural organization of ECM. It is a versatile technique allowing to use both natural and synthetic polymer and to obtain a fine control over the scaffold geometry [5]. The aim of this work was studied the effect of random and aligned nanofibrous membranes based on synthetic polyurethane on the growth and proliferation of MSCs. Morphological analysis showed homogeneous diameters (~400 nm) for both membrane types. Atomic force microscopy was used to evaluate how soft and hard segments of polyurethane arranged after the electrospinning process and to evaluate the mechanical behavior scaffolds. To determine how the fibre orientation affects the proliferation of MSCs, cells were seeded on to the membranes and cultured for 14 days under normal conditions. Cell viability was assessed by non-destructive resazurin metabolic activity assays. Cell-scaffold interactions were observed using traditional fluorescence and SEM; results showed that cells grew in response to fibre orientation: aligned orientation supported higher proliferation compared to random fiber orientation. Moreover cell differentiation assessment performed by gene expression analysis through digital droplet-PCR demonstrated that MSCs cultured on oriented fibres lost their stemness (CD29 and CD44 decreased) and increased the differentiation potential (collagen increased expression).

In conclusion, electrospun membranes based on synthetic polyurethane are promising substrates to promote growth and differentiation of mesenchymal stem cells, furthermore surface nanoscale fibre properties could be modified in order to enhance the differentiation process.

References:
Introduction: Intervertebral disc degeneration with an associated bulged/herniated disc is a significant cause of low back pain [1]. Current treatments are not able to promote annulus fibrosus (AF) repair which can result in re-herniation [2]. A successful AF repair strategy should provide biomechanical stability to seal AF defects while promoting cell survival and extracellular matrix deposition. The current objective was to design and fabricate a biodegradable shape-memory e-polycaprolactone (PCL)-silk fibroin (SF) anchoring scaffold, which can be delivered minimally invasively, to seal a ruptured AF from within, and simultaneously allow tissue regeneration.

Methods: Photo-crosslinkable polycaprolactone diacrylate was synthesized with either 15 or 95% degree of acrylation (PCL 15 and PCL 95). Ten porous PCL-SF shape-memory scaffolds of each (PCL 15 and PCL 95) were fabricated by a solvent-casting particulate-leaching method. Scaffold morphology was evaluated using SEM. The tensile strength and compressive modulus were determined with a loading rate of 2 mm/min.

Results: No significant differences were observed between the porosity of PCL 15 and PCL 95, and both scaffolds showed a highly interconnected porous cross-section with an average pore size of 278 and 223 µm, respectively. The tensile strength of the PCL 15 and PCL 95 was 2.56 and 4.59 MPa, and the elongation at failure was 61.64 and 43.42%, respectively. The compressive modulus at 30% strain of PCL 15 and PCL 95 scaffolds was 0.76 and 1.30 MPa, respectively.

Discussion: The pore size of our scaffolds were in the range (200-600 µm) that has been shown to increase AF cell attachment and matrix accumulation [3]. The tensile strength of PCL-95 was in the range of native AF tissue measured in the radial direction and comparable to those of other scaffolds studied for AF regeneration [4]. The higher degree of acrylation of PCL 95 provides more functional groups for PCL to react during photo-crosslinking, likely contributing to the higher tensile strength. In future research, the self-fitting behaviour of the shape memory AF repair construct and its biocompatibility will be evaluated to measure cell survival and extracellular matrix deposition as well as its integration with AF tissue.

References:

Introduction: Titanium-ceramic scaffolds enables many novel approaches in modelling, design and fabrication of complex materials with enhanced functionality to improve cell-material interactions. They are mainly used as a mechanical support for cells but is not specifically designed to interact with desired cell populations; yet the initial interaction between cells and the scaffold is very important and will determine the success or failure of the engineered device. Data that would be presented would include its surface characteristics which shows SEM, AFM, XRD and EDX spectrometry which helped identify the surface topography as well as the amount of calcium and cellular presence contained within the scaffold. In addition, data on the mesenchymal stem cell attachment onto the material was also brought to light showing positive attachment through the live and dead stain study. A study concerning the leached out property of the scaffold will also be shown through mass spectroscopy. The preliminary results displayed non-lethal analytes that have been leached out which are comprised of calcium and phosphorous and this can be further metabolised by the cellular environment. In addition, the cytotoxicity tests conducted that show greater support for the metal-ceramic scaffolds when compared to scaffolds comprising of their individual components. This would be compared with titanium-hydroxyapatite which is a contender of this study as this study is to prove that Titanium-Wollastonite would dominate Titanium-Hydroxyapatite in most of its aspects.

Acknowledgement

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**01-P335** Isolation and characterization of nanorod shaped crystalline hydroxyapatite from parrotfish bone

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Hydroxyapatite (HA) has been widely used in bone tissue engineering due to its excellent biocompatibility and osteoconductivity. Parrotfish (Scarus collana) is a member of Scaridae family mainly found around the coral reefs of the western Atlantic Ocean and the Red Sea. The objectives of the study were to i) isolate the HA from the fish bone ii) characterize the physico-chemical properties and iii) compare the osteogenic potential of fish bone derived HA (FHA) with synthetic HA (SHA) using mesenchymal stem cells. The Fourier transform infrared spectroscopy (FT-IR) results suggested that the isolated HA is carbonated, and X-ray diffraction (XRD) results revealed that the HA is coherent with the standard Joint Committee Powder Diffraction Standard Data (JCPDS). The results suggested that the developed FHA were nanorod shaped with a particle size of 40-100 nm. *In vitro* biologic activities suggested enhanced biocompatibility, higher alkaline phosphatase activity and mineralization ability of the fish derived HA compared to synthetic HA. These observations confirm that the HA derived from fish bone is a better alternative to synthetic HA and have the potential for use as a bone scaffold.

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**01-P336** A novel microgrooved collagen scaffold for tendon tissue engineering

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**Introduction**

Tendon tissue engineering seek to create scaffolds that reproduce the biophysical environment of the native tissue. Mechanical stimuli and anisotropic topographies have shown to enhance the expression of tenogenic markers in stem cells in vitro. Few scaffold designs to date allow for the synergistic application of multiple biophysical cues, while being biodegradable. Recently, we developed a simple method to fabricate microgrooved collagen films able to withstand cyclic mechanical forces and to align human bone marrow stromal cells (hBMSCs) in vitro.

**Methods**

Microgrooved collagen scaffolds were produced by solvent casting. Briefly, porcine type I collagen solutions were cross-linked with 4-arm PEG 1mM (4SP) at different pH and temperatures (T°), and poured on top of microgrooved (2x2x2µm) PDMS moulds. Gelation kinetics was analysed by rheometry. Collagen gels were left to dry either in a laminar flow hood or under vacuum. Drying profiles were assessed by sample weight loss. Resulting collagen films were analysed with SEM and AFM.

Alignment of hBMSCs seeded on the films was assessed with DAPI and rhodamine conjugated phalloidin staining.

**Results and discussion**

The cross-linking effect of 4SP on collagen could be conveniently modulated by adjusting pH and T°. In the presence of 4SP, high pH (7.4) and T° (37°C) resulted in short gelation times (<1min), while low pH (4) and T° (21°C) considerably extended the time for gelation (~10min). Samples dried under vacuum showed a much more linear and slow dehydration profile as compared to the flow hood-dried counterparts (3 and 1 day to reach plateau, respectively). Surprisingly, only vacuum-dried low-pH and T° cross-linked samples did reproduce the pattern in a uniform manner as assessed by SEM and AFM. Past gelation it is likely preventing the collagen solutions from infiltrating in the PDMS micrometer topographies. A large number (~80%) of hBMSCs cultured on these films showed to perfectly align along the microgrooves.

**Conclusion**

Solvent casting on PDMS moulds represents a fast, simple and reliable technique to produce micro-sized topographies on collagen scaffolds. Protein and gene expression analysis on hBMSC cultured on static and cyclic loaded films are currently underway to assess the influence of these biophysical cues on cell lineage commitment.

**References**


**Fabrication and characterization of new hybrid gelatin-based hydrogels for developing skeletal muscle constructs**

**01-P337**

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Hydrogels based on gelatin methacrylate (GelMA) are well-known biomaterials for tissue engineering applications.1-3 However, soft GelMA hydrogels show high degradation rates, while stiffer hydrogels compromise the viability of encapsulated cells.4 To reduce the degradation rates independently from the mechanical properties, we fabricated different gelatin-based biomaterials by adding Alginate methacrylate (AlgMA), Carboxymethyl cellulose methacrylate (CMCMA), or Poly (ethylene glycol) diacrylate (PEGDA). Hybrid hydrogels were photocrosslinked in the presence of either lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) or Irgacure 2959,5 as a photoinitiator, using a 3D bioprinter. We studied the impact of these composites on the mechanical properties, swelling ratio, porosity of the hydrogels, and the degradation rates under the effect of collagenase II.

Our results show the feasibility of tailoring hydrogel properties, such as stiffness or porosity varying the composition, the photoinitiator or the UV exposure time. Furthermore, the addition of CMCMA and AlgMA show slower degradation rates under the treatment of collagenase than GelMA hydrogels. Also, we are able to generate micropatterned structures by using a 3D bioprinter, despite combining different materials. Moreover, we studied the viability, proliferation, and myotube differentiation of encapsulated C2C12 cells.

In conclusion, by adding AlgMA or CMCMA in GelMA hydrogels we improved the mechanical stability and we reduced the degradation rate while maintaining its bioadhesive capabilities. So, GelMA-CMCMA and GelMA-AlgMA are promising hybrid biomaterials for tissue engineering applications.


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**Preparation and characterization of hydrogels derived from decellularized urinary bladder matrix (UBM) and small intestinal submucosa (SIS)**

**01-P338**

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[Introduction]

Decellularized tissue is known to have inductive properties and is widely used as scaffolds to reconstruct functional tissue. Extracellular matrix (ECM) hydrogels have been considered to have several advantages including the ability to quickly fill irregularly shaped spaces, the suitable support for host cell infiltration and remodeling, and the inherent bioactivity of native matrix. However, the decellularized tissue was mainly prepared using detergents, and toxicity of detergent residual and tissue damage can be concerned. In this study, we tried to prepare the ECM hydrogels derived from the decellularized urinary bladder matrix (UBM) and small intestinal submucosa (SIS) prepared by high hydrostatic pressure (HHP) method, and investigated the characteristics of both of the ECM hydrogels.

[Methods]

The UBM and SIS were obtained by mechanical removal of the tunica serosa and tunica muscularis externa. The decellularized UBM and SIS were prepared by two different methods: 1) detergent and 2) HHP. Decellularization efficiency was evaluated using hematoxylin-eosin (H&E) staining and DNA quantification. The solubilized ECM was prepared, and the gelation kinetics was determined turbidimetrically as previously described. The structure of ECM hydrogels was characterized by scanning electron microscopy.

[Results&Conclusions]

Complete removal of the cells was confirmed through H&E staining and DNA quantification. The gelation kinetics was much faster the solubilized detergent-ECM than the solubilized HHP-ECM. SEM images demonstrated that the HHP-ECM hydrogel possesses homogenous microfibrils structure compared with detergent-ECM hydrogels. It was suggested that HHP method have relatively less impact on the tissue than detergent method. The HHP-ECM hydrogel could be useful for biomedical applications.

[References]


[Acknowledgments]

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**The synergistic effects from Intra-articular Injection of N-Acetylglucosamine and Hyaluronic acid with Acellular PLGA Scaffolds for Osteochondral Defect Repair in Rabbits**

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**INTRODUCTION:** Repairing damaged articular cartilage is particularly challenging because of the limited self-repair ability of hyaline cartilage. Glucosamine (GlcN) and N-acetylglucosamine (GlcNAc) have used as supplements for the treatment of osteoarthritis for several years with ability in cartilage protection. However, through either oral administration or intravenous injection (IV), the diffusion of GlcN from the circulation into the joint is very inefficient. Besides, Intra-articular injections of Hyaluronic acid (HA) (i.e., viscosupplementation) have approved worldwide for the treatment of pain associated with OA of the knee.

**METHODS:** We investigated the effects from intra-articular injection of GlcNAc and HA combined with acellular PLGA implants in osteochondral defect in rabbits, respectively and synergistically. Twenty-four rabbits were randomized into one of four groups: the scaffold-only group (PLGA) and the scaffold with intra-articular injection of three supplements such as GluNAc, HA and GluNAc with HA (PLGA+G, PLGA+HA, PLGA+G+HA). Macroscopic appearance, histology, which were identified using H&E staining, total collagen and alignment, studied qualitatively using Masson’s trichrome staining, glycosaminoglycan (GAG), identified using Alcian blue staining, and newly formed bone, observed using micro-CT, were evaluated at 4 and 12 weeks after surgery.

**RESULTS SECTION:** The three intra-articular injection groups exhibited similar effects with nearly normal articular surfaces, significantly higher bone volume and trabecular values than the PLGA group at 4 weeks and 12 weeks. Among the three intra-articular injection groups, PLGA+G group exhibited remarkable cartilage regeneration and PLGA+HA group had integrating architecture in bone regeneration; however, the combination of N-acetylglucosamine and hyaluronic acid together with acellular PLGA implantation performing cartilage and bone regeneration simultaneously could significantly promote the full-thickness osteochondral regeneration in rabbit knee joint models.

**DISCUSSION:** In our study, intra-articular injection of these two supplements combined with acellular PLGA scaffold appeared better effects on osteochondral regeneration. The phenomenon from the cartilage regeneration including great gross appearance, hyaline-like cartilage and bone regeneration, well collagen alignment and abundant amount of GAGs expression also observed.

**Influence of Mechanical Strength of Gelatin grafted Poly(D,-L-Lactide) based Three Dimensional Scaffolds on Cell Proliferation**

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Surface and mechanical properties of the biomaterials are determinants of cellular responses. In our previous study, star-shaped poly(D,L-Lactide)-b-gelatin (ss-pLG) was reported for possessing improved cellular adhesion and proliferation. Here, we extended our investigation to establish the cellular compatibility of gelatin-grafted PDLLA with respect to mechanical properties of biological tissues. In this view, linear PDLLA-b-gelatin (l-pLG) was synthesized and tissue-level compatibility of l-pLG and ss-pLG against fibroblasts (L929), myoblasts (C2C12) and preosteoblasts (MG-63) was examined. The cell proliferation of C2C12 was significantly higher within l-pLG scaffolds, whereas L929 showed intensified growth within ss-pLG scaffolds. The difference in cell proliferation may be attributed to the varying mechanical properties of scaffolds; where the strength of l-pLG scaffolds was notably higher than ss-pLG scaffolds, most likely due to the variable levels of gelatin grafting. Therefore, gelatin grafting can be used to modulate mechanical property of the scaffolds and this study reveals the significance of the matrix strength to produce the successful 3D scaffolds for tissue engineering applications.
Silk Fibroin Hydrogels with Different Biodegradation Rates to Improve Cardiac Function after Myocardial Infarction

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Intracardiac hydrogel injection is a promising therapy to suppress the negative remodeling and to regenerate myocardial tissues after myocardial infarction (MI). Both mechanical strength and bioactivity are important hydrogel properties to prevent post-MI remodeling. However, the mechanism of this therapy remains unclear. Here, we focused on hydrogel biodegradation because the balance of the following two events is assumed to influence the remodeling response: structural reinforcement of left ventricular (LV) wall by an injected hydrogel; and replacement of the hydrogel by regenerated tissues. Previously, we revealed the time profile of silk fibroin (SF) hydrogel degradation in vivo. After implantation, a SF hydrogel allows endothelial cell infiltration, followed by macrophage migration into the hydrogel to degrade the SF network. In this study, to change the SF hydrogel biodegradation, we modified the hydrogel with a peptide which stimulates endothelial cell infiltration. This modification resulted in a 1.3-fold increase in the hydrogel degradation speed in vivo, thereby providing slowly (without the modification; LowDeg) and rapidly (with the modification; HighDeg) degradable SF hydrogels. A rat MI model, whose % fractional shortening (%FS) was <25%, was prepared by a permanent ligation of the left anterior descending coronary artery. The model rats received intramyocardial injections of either the LowDeg or HighDeg hydrogels. The injections increased %FS over 25% throughout 12 weeks post-injection for three of five rats in the LowDeg group. In contrast, one of five rats in the HighDeg groups showed the improved cardiac function. Histological evaluations demonstrated that the LV wall of the LowDeg group was thicker than that of the HighDeg group. This study showed the importance of hydrogel biodegradation rate in the hydrogel injection therapy.

Leaves-inspired micro- and nanostructures as functional scaffolds for stem cell and tissue engineering

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The various surfaces in nature have great potential in both the academia and the industry. In particular, the lotus leaves surface exhibit a unique surface consisting of evenly distributed micro and nanoscale structures (i.e., called as “lotus effect” (superhydrophobic property)). For these reasons, many studies were performed about the lotus effect to design and manipulation of engineering platforms or devices. With this consideration, various natural leaves have unique micro and nanoscale structures that can have controlled hydrophilic and hydrophobic properties. Here, we report on the tunable replication of surface topographies of natural leaves of common camellia, Fragrant plantain, and lotus onto thin polymeric films using a capillarity-directed soft lithography technology. To this end, the surfaces of natural leaves were first replicated using polydimethylsiloxane (PDMS) as molds. The poly(ε-caprolactone) (PCL) film was then coated on the glass using spin-coating technique. Finally, the PCL-based unique micro- and nanostructures of natural leaves were generated using PDMS molds. The micro and nanostructure, chemical composition, wettability was characterized using scanning electron microscopy, atomic force microscope, Fourier transform infrared spectroscopy, X-ray diffraction, and contact angle measurement. We also demonstrated that the replicated polymeric surfaces had different hydrophilic and hydrophobic properties according to the mimicking the natural leaf surfaces. Finally, we proposed that leaves-inspired micro- and nanostructures could be used as functional scaffolds for stem cell and tissue engineering. Our new platforms ould be used as a simple, but powerful methodology for design and fabrication of controlled hydrophilic and hydrophobic platforms for biomedical applications.
Effects of Collagen on Physical and Mechanical Properties and Flavonoid Released of Thermosensitive Chitosan/Collagen Hydrogel

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Objectives: The study aimed to fabricate thermosensitive chitosan-collagen hydrogel (ch-col) as control release of flavonoid, Quercetin (QT), and to investigate effects of collagen contents and concentrations of β-Glycerophosphate (bGP) on properties of the hydrogels.

Materials and methods: To fabricate bGP-ch-col hydrogel, chitosan 2% in 0.1M acetic acid was mixed with 1% atelocollagen in different weight ratios: Groups A, chitosan alone (Ch), B, collagen alone (Col), C, 1:1 chitosan-collagen (1:1 Ch-Col) and D, 1:2 chitosan-collagen (1:2 Ch-Col). Sol-gel transition was initiated by 4, 8 and 16% bGP (w/v) and temperature change from 4°C to 37°C. Hydrogels with QT 2mg/ml was incubated in PBS at 37°C. Then, rheological, mechanical and physical properties, microstructure were investigated and total flavonoid content assay was performed to quantify amount of released QT in PBS.

Results: Beta GP-ch-col hydrogels were solidified at 37°C with different setting time. Beta-GP increased pH, decreased setting time and increased mechanical strength of the hydrogels (p<0.05). Collagen contents increased porosity of the hydrogels. Swelling ratios, degradation rates and mechanical strength of 1:1 and 1:2 ch-col hydrogels were not significantly different (p>0.05) but lower than chitosan alone hydrogels (p<0.05). Quercetin was gradually released from bGP-ch-col hydrogels. Amounts of QT released from 1:2 were consistently higher than 1:1 ch-col and lower than chitosan alone hydrogels (p<0.05).

Conclusion: Properties and QT release of bGP-ch-col hydrogel were influenced by concentrations of bGP and collagen ratios. Thermosensitive 1:1 and 1:2 chitosan-collagen hydrogels are potential injectable scaffolds for natural flavonoid delivery and bone tissue engineering.
Tailored Surface Modification of Polycaprolactone Scaffolds for Improved Cell-Substrate Interactions

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Polycaprolactone (PCL) is a biodegradable biomaterial polymer, frequently utilised with 3-dimensional (3D) printing technologies to form scaffolds for tissue engineering. While readily biocompatible, PCL has an inherently hydrophobic nature that impairs its initial interactions with proteins and cells. To overcome this, researchers typically modify the surface of PCL structures by exposing them to a strong base such as sodium hydroxide (NaOH). These treatments vary widely in both concentration and exposure time, despite their prevalence in the literature. Modification using a strong acid such as hydrochloric acid (HCl), has also been very rarely explored, despite its potential utility [1]. Further, protein adsorption would form the initial systemic response in an in vivo environment. Despite this, induced protein adsorption, such as through immersion of a construct in foetal bovine serum (FBS), has been largely underexplored for improving cell response.

In this study, we optimised 3D-printed PCL scaffold surface treatments by screening a wide range of exposures to NaOH and HCl, initially assessing their effects on surface charge (Toluidine Blue), surface morphology (SEM), and mechanical properties (compressive testing). This first screen allowed us to determine a range of chemical exposures that induced a significant change in the surface, but did not compromise the structure of the scaffolds. We found that HCl exposure appeared to increase surface charge with limited morphological changes, while NaOH exposure resulted in dramatic differences to both. We further shortlisted groups from this screening, and then exposed the selected groups to bovine serum albumin (BSA) and FBS immersion to determine their protein binding over time. From this, we made a final selection of groups and evaluated the surfaces using an osteoblast cell culture, assessing cell seeding efficiency (Picogreen), morphology (DAPI/Phalloidin and live/dead staining, SEM), and behaviour (ALP assay, gene expression).

We overall demonstrated that surface modifications of PCL scaffolds may be optimised and performed in a controlled manner, and these modifications result in differences in surface protein adsorption, cell attachment, and cell morphology.

Scaffolds play a role as a temporary framework and an extracellular matrix substitute for cultured cells. They provide cells growth substrate and promote mechanical integrity for the newly formed tissues. Previous studies indicated that there were many limitations when natural or synthetic scaffolds material is applied individually. To overcome this, hybrid scaffolds have been introduced for tissue regeneration by studying cellular interactions with relevant scaffolds. However, this present study only focused on fabrication and characterization of three-dimensional (3D) poly(lactic-co-glycolic acid) (PLGA) incorporated with fibrin (PF), atelocollagen (PA) and both fibrin and atelocollagen (PFA) scaffolds materials. The PLGA (mole ratio 65:35) scaffolds were fabricated using solvent casting and salt leaching method. The PA and PFA were crosslinked using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) and 20mM N-hyroxysuccinimide (NHS). The interaction of incorporated scaffolds materials with PLGA were demonstrated through the notable peaks of amide bonds, as shown by the attenuated total reflectance Fourier transform infrared (ATR-FTIR). Other evaluations included the observation using scanning electron microscopy (SEM), the interconnection of pore structures (porosity), and water uptake capacity (swelling) of the scaffolds. The SEM showed the interconnection between pores in the scaffolds. This is supported by the increased of total porosity in PLGA after the incorporation of fibrin, atelocollagen and both fibrin and atelocollagen. Despite its hydrophobicity, PLGA alone group exhibited the highest percentage of water uptake compared to other hybrid scaffolds namely PF, PA and PFA. Based on the preliminary results, the PLGA based scaffolds may have potential to be used in tissue engineering application.

Keywords: PLGA, fibrin, atelocollagen

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Biodegradable polymeric scaffolds are essential in bone tissue engineering. Chitosan (CS), a natural polysaccharide, is a biocompatible biodegradable polymer widely used in tissue engineering. However, CS is slightly soluble in an aqueous culture medium with poor mechanical properties. On the other hand, poly(l-lactide) (PLLA), a biocompatible biodegradable synthetic polymer has excellent mechanical properties. Grafting a synthetic polymer along the CS backbone is an attractive way to regulate the physical and mechanical properties of CS. In this work, we present the preparation of two CS-g-PLLA graft copolymers, CS-g-PLLA(20) and CS-g-PLLA(50), with 20 wt% and 50 wt% PLLA content, respectively, as well as the evaluation of the morphology, viability, proliferation and osteogenic response of pre-osteoblastic cells on both copolymers.

We have successfully synthesized novel CS-g-PLLA copolymers by grafting end-functionalized PLLA chains onto the hydroxyl groups of CS, varying the PLLA content from 20 to 50 wt%. Cell culture experiments show strong cell attachment, increased proliferation and osteogenic response of pre-osteoblastic cells on the CS-g-PLLA scaffolds.
Mechanism and optimization study of amphiphilic block-copolymer in situ gelations as scaffolds for tissue engineering

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The Objective of this study was to discuss the thermoresponsive / pH-sensitive polypeptide hydrogel properties and the potential of the hydrogel in cell scaffolds. Methoxy-poly (ethylene glycol)-poly (l-alanine) (mPEG-P(Ala)) is a well-known thermoresponsive polypeptide hydrogel. By using mPEG-NH2 and N-carboxy anhydride-alanine (NCA-l-alanine) through ring-opening polymerization. Afterwards, using N-carboxy anhydride β-benzyl l-Aspartate/l-Lysine through ring-opening polymerization and removal of the benzyl protecting groups to form the hydrogel, we have two kinds of hydrogel further, methoxy-poly (ethylene glycol)-poly (l-alanine)-poly (l-Aspartate), mPEG-P(Ala)-P(Asp) and methoxy-poly (ethylene glycol)- poly (l-alanine)-poly (l-Lysine), mPEG-P(Ala)-P(Lys). We synthesized a series of mPEG-P(Ala) diblock copolymers, mPEG-P(Ala)-P(Asp) and mPEG-P(Ala)-P(Lys) triblock copolymers and investigated the hydrophilic / hydrophobic block length effect on the secondary structure influencing the nanostructure of the self-assembled amphiphilic copolymers, the thermosensitivity of the hydrogels in aqueous solution. In this study, we will explore the physicochemical properties of the copolymers in solubility and hydrogel forms were studied in terms of their assembly and transition in response to temperature changes at first. Second, the study will be focused on demonstrating non-toxicity, biocompatibility, and biodegradability. We propose the use of a thermosensitive mPEG-peptide hydrogel as cell scaffold, even more for growth factors delivery. Incorporation and release of molecules will be evaluated in vitro as proof of concept for proteins encapsulation and growth factor in the cell scaffold. Taken together, we expect that the hydrogel will be excellent biocompatibility and low toxicity alone and as a block copolymer. In the meanwhile, thermosensitive mPEG-peptide hydrogels are attractive candidates for proteins/peptide delivery and tissue engineering applications.

Mechanically competent amniotic membrane based composite scaffold for highly aligned tissue fabrication

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The porous structure of electrospun fiber is favourable for constructing aligned engineered tissue substitutes. However, high porosity of electrospun fiber also results in the weaker mechanical strength of the scaffold. In this study, we attempted to produce a mechanically competent human amniotic membrane (HAM) based composite scaffold with aligned topography by fabricating electrospun PLGA fiber (EF) on the membrane. HAM decellularization using thermolysin and sodium hydroxide effectively removed the amniotic epithelium without causing any local tears. Electrospinning of PLGA yielded aligned fiber on HAM but longer deposition time resulted in the loss of alignment, higher fiber diameter and weaker tensile strength of scaffolds when hydrated. Although no enhancement of skeletal muscle cell viability and migration rate was detected on EF-HAM scaffolds, the cells displayed aligned orientation along the fibers compared to control and HAM alone. The analysis of angiogenic factors in conditioned media (CM) derived from muscle cell-seeded scaffolds were performed using multiplex assay. The content of angiogenic factors including angiogenin, CXCL8/IL-8 and VEGF-A secreted by muscle cell-seeded EF-HAM scaffolds were 1.69, 7.53 and 2.25 fold higher, respectively, in control condition, CM from muscle cell-seeded EF-HAM scaffold also induced endothelial cell tube formation on Matrigel confirming the favourable angiogenic properties of our construct. The CM derived from EF-HAM scaffolds induced an increase of 35.1%, 76.3% and 43.0% in the number of junctions, meshes and segments, respectively, compared to control. In summary, EF-HAM composite scaffold design holds promising applications in aligned tissue reconstruction as it is mechanically competent, provides aligned topography to cells and promoting angiogenesis.
Sachiro Kakinoki, Mai Yoshikawa, Satoru Nishioka, Yoshiaki Hirano

Magnesium (Mg) and its alloys have attracted great interest in the application for tissue engineering scaffold and cardiovascular devices due to biodegradable metal. Because the degradation rate of pure Mg is too fast to meet the requirements for medical application, Mg alloys such as an Mg-Y-RE alloy (WE43) are used for cardiovascular stents. However, the toxicity of the additional metals in Mg alloys has not been clarified yet, and pure Mg must be used preferably. On the other hand, Mg and its alloys does not inherent the tissue regenerative capacity. Coating of biodegradable polymers on Mg substrates could potentially delay the degradation rate. For example, Li et al. reported that a polylactic-co-glycolic acid coating successfully alleviates cytotoxicity due to a delay of degradation rate of Mg-6Zn alloy1). But, the biological functionalization of Mg substrate cannot be achieved by polymer coating. We previously reported that peptides containing tyrosine residues are immobilized on metal substrates through the direct oxidation from a hydroxyphenyl group to a quinone2). In this study, the conjugation of chondroitin sulfate (CS) which can enhance osteogenesis and tyramine (Tyrm) with a hydroxyphenyl group was synthesized for the surface modification of Mg substrate.

CS-Tyrm conjugates (CS-Tyrm) was synthesized using amidation reaction with 4-(4, 6-dimethoxy-1, 3, 5-triazin-2-yl)-4-methylmorpholinium chloride (DMT-MM). Briefly, CS and Tyrm were solved in water, and then DMT-MM and N-methylmorpholine were added to allow the amidation for 24 hours. We successfully obtained CS-Tyrm conjugates with different substitution ratios of Tyrm. CS-Tyrm was immobilized on the alkaline-treated pure Mg plate by oxidation reaction resulting that water contact angle was decreased and Tyrm-derived N1s was detected on Mg plate. It is expected that CS-Tyrm has potential abilities as a surface modifier of biodegradable Mg substrates for tissue engineering scaffolds.

References:
Design of Barnacle-mimetic Peptide Enhanced Cell Attachment Activity for Tissue Engineering Scaffold

Yoshiaki Hirano, Daisuke Fujii, Sachiro Kakinoki, Kei Kamino

The barnacles bond two different materials using secretion, which was constructed of multiprotein complex in water. It revealed that amino acids sequence constructed by 20 residues (RRKYSGILGDLIQVAVIRYY: R-Y) plays an important role in retaining secretion strength and forms stable b-sheet structure. R-Y peptide was formed self-assembling nanofiber structure because of existence of inter molecular interaction. These self-assembling peptides were conjugated with cell adhesive motif derived from cell adhesive protein fibronectin. Fibronectin has the RGDS sequence from the extra-cellular matrix protein as a model bioactive ligand. This sequence interacts with cell surface integrin receptors, plays a critical role in cell adhesion, behavior and signaling and is the minimum motif required for cell adhesion on a surface.

We designed 3 types barnacles based self-assembling mimetic peptide:
RRKYSGLGDLIQVAVIYGRGD (R-Y-RGDS)
RGDSRRKYSGLGDLIQVAVIRYY (RGDS-R-Y)
RRKYSIRGDLQAVIYGRGD (R-RGDS-Y)

These peptides were synthesized by using Fmoc chemistry. Secondary structure of R-Y and RGDS containing three types R-Y mimetic peptides were measured by CD and FT-IR spectra. Hydrogel was prepared by mixing of 1~3 % peptide solution and Eagle’s medium. Rheological character of hydrogels was measured by rheometer. Internal structure of hydrogel was analyzed by SEM image. Three types R-Y mimetic peptides were applied for the cell culture plate surface and 3D culture scaffold. The cell adhesion activity was assessed by L929 and hMSC cell onto three types peptide-immobilized TCP. These self-assembling peptides have possibility for application of tissue engineering or regenerated medicine.

Preparation and Characterization of 3D Fibrous Cylinder type Scaffolds for Tissue Regeneration

Tae-Hee Kim, Chae-Hwa Kim, Jung-Nam Im

The major challenges in tissue engineering are to design and fabricate a suitable scaffold to provide a three dimensional support for cell migration, attachment, and proliferation. Ideal scaffolds for tissue regeneration should have extracellular matrix (ECM)-like structure such as, interconnecting pores, biodegradability, elasticity, biocompatibility, and efficient cell attachment and growth. To design functional substitutes for damaged tissues and organs, we have developed totally new concept of fibrous scaffolds by modifying existing textile manufacturing process. We have developed novel three-dimensional fibrous poly(lactic-co-glycolic acid)(PLGA) scaffolds by using tubular knitting and draw texturing process. The PLGA biodegradable multifilament draw-textured yarn is inserted inside a tubular knitted fabric made of PLGA. The scaffold is designed to have high flexibility and porosity with aligned microfibrous bulky structure inside the tube having advanced biomimetic structure for cell growth. Our scaffolds was easily fabricated into variety of shapes and sizes, and showed excellent 3-D structure with inter-connecting pores, biodegradability, efficient cell attachment and proliferation. To control biodegradation rate of scaffolds, we also prepared scaffolds by controlling mixing ratios of PLGA and Poly(lactic acid)(PLA) filaments inside the 3D knitted tube. The results suggest that our scaffolds are promising for clinical applications for tissue regeneration in orthopedics, cardiology, and general surgery, as well as cell culture system for cell therapy.
**01-P355** Fabrication of customized and flexible tubes on the basis of 3D printed sacrificial templates

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Recently, three-dimensional (3D) printing technology has received a great deal of attention in customized products, which require arbitrary and adaptive shaping. A representative application in 3D printing of biomaterials is fabrication of tissue engineering scaffold. Despite the extensive utilization of 3D printing, the direct printing of biomaterials has a limitation in terms of flexibility due to the original stiffness of material and limit of processing resolution. Here, we successfully fabricated an ultrathin tubular freeform structure that has a wall thickness of several tens of micrometers and is capable of providing sufficient mechanical flexibility. Such a thin geometry cannot easily be achieved by 3D printing alone; therefore, it was realized through a serial combination of processes that included the 3D printing of a sacrificial template, the dip-coating of the biomaterial, and the removal of the inner template. In addition to the flexibility caused by thin geometry, we suggest a further combinatory method involving 3D printing, dip coating, and salt leaching for fabrication of 3D freeform porous tubes with softness. Owing to the porous morphology and the controlled thickness, the processed tubular constructs had appropriate flexible properties which were comparable to the native soft tissues with the moduli ranging in several MPa. Collectively, these results are expected to be universally applicable in engineering of tubular shape tissues such as vascular, airway, and abdominal organs.

**01-P356** Self-mineralising synthetic injectable hydrogels for bone repair

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Enzymatically cleavable PEG-co-peptide hydrogels are promising injectable scaffolds for bone tissue repair [1]. The relatively inert PEG matrix can be tailored to mimic a mineralised tissue environment by adding calcium phosphate minerals. These provide cellular attachment sites and serve as depots for the release of calcium and phosphate ions. Here, we co-precipitate calcium phosphate minerals simultaneously to gel formation.

Mineralised hydrogels were formed by mixing 8-armed maleimide functionalised PEG macromeres and a synthetic peptide at controlled pH. Phosphate and calcium ions were added to polymer and peptide component, respectively. The mineralisation process was spatiotemporally observed by dark field optical microscopy. pH changes occurring throughout the mineralisation and gelation process were monitored using confocal laser scanning microscopy together with a pair of pH sensitive and insensitive dyes [2]. Phase, morphology and distribution of formed mineral were examined via Raman microspectroscopy and SEM as described previously [3].

The described process yields hydrogels with embedded mineral particles. Initially, precipitation of a disperse mineral phase of low crystallinity throughout the gel network was observed for all tested groups. However, within minutes, these particles were consumed by fast growing brushite crystals at the gel periphery. If citrate was added or calcium ions were partly replaced by zinc, however, no recrystallization was observed. This is likely due to two different mechanisms: citrate adsorbs to the surface of initially formed amorphous particles hindering surface nucleation, while zinc is incorporated into the lattice of the amorphous phase causing lattice distortions and preventing crystal growth. In both cases, the formed particles exhibit high solubility compared to more crystalline phases suggesting easy remodelling and high availability of calcium and phosphate ions. The described system presents a simple one-step setup to yield composite scaffolds with potentially high osteogenicity.

References:

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Micro-dimpled surface atelocollagen enhances the function of primary human hepatocytes

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Primary human hepatocytes (PHHs) are commonly used as the gold standard for drug development. However, maintaining functional PHHs in vitro has been difficult in conventional collagen coat culture. We developed a new scaffold using atelocollagen. The scaffold consists of higher amounts (mg/cm² order) of atelocollagen, and it was exposed to ultraviolet radiation to induce cross-linking and improve stability. Observation using scanning and transmission electron microscopy revealed that the micro-dimpled surface (MDS) scaffold comprised randomly arranged atelocollagen fibrils. Thus, we named this collagen MDS atelocollagen. PHHs cultured on MDS atelocollagen were round with compact cytoplasim, and they exhibited enhanced albumin secretion levels and elevated cytochrome P450 (CYP)3A4 activity. The expression of hepatocyte-related genes such as serum proteins, drug metabolism-related CYPs and nuclear receptors was maintained in cells cultured on MDS atelocollagen but not in those cultured via conventional collagen culture. Moreover, the abnormal gene expression of cell adhesion molecules observed in conventional collagen culture was suppressed on MDS atelocollagen, suggesting behaviour similar to that of in vivo hepatocytes. Therefore, our results suggest that MDS atelocollagen functionally enhances PHHs, conserving the usability of conventional PHHs collagen coat cultures.

References

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**Biocompatibility of Porous Polycaprolactone Scaffolds with Incorporated Hydroxylated Multi-walled Carbon Nanotubes Decorated with Magnetic Nanoparticles**

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Poly(ε-caprolactone) (PCL) is a biocompatible and biodegradable polymer with numerous confirmed applications in tissue engineering and medicine. In our study we prepared porous PCL scaffold using solvent casting/porogen leaching method. The PCL matrix was enriched with nano-composites (NCs) made of hydroxylated multi-walled carbon nanotubes (MWCNTs-OH) and magnetic iron oxide nanoparticles (IONs). Two types of NCs, with mass ratio of MWCNTs-OH to IONs of 1:1 and 1:4, were used. The intended aim of polymer modification was to change physicochemical surface properties of scaffolds to affect the cell adhesion. Moreover, the presence of the IONs introduced the possibility of remotely influence cells through magnetic field. Biocompatibility of free MWCNTs-OH and NCs dispersed in cell cultivation medium at different concentrations (10-1000μg/ml) was estimated before biocompatibility tests of the PCL/NC scaffolds. The in vitro tests of hybrid scaffolds were conducted on materials with various content of NCs ranged between 1-5 wt %. The biocompatibility tests were performed using human osteoblastic cell line SAOS-2. Cell viability was measured using MTS metabolic activity test and additionally evaluated by cell counting from microphotographs. Colonization of the scaffolds was also estimated using confocal images of fluorescently stained cells. Phase contrast life-cell imaging was used to observe the interaction of cells with the MWCNT-OHs and NCs aggregates. The viability of cells cultivated with the NCs dispersed in the medium was clearly dose dependent. At the lowest concentration (10μg/ml), NCs did not affect the cell viability significantly. One order of magnitude higher concentration (100μg/ml) lowered the cell viability to half and the concentration 1000μg/ml of NCs killed almost all cells. The viability of cells cultivated on the hybrid scaffolds was either dose or type of NC dependent. However, the results indicated that the MWCNT-OH component of the NCs was more responsible for the harmful effect. The lowest toxicity exhibited the hybrid scaffold with 1 wt % of NC with MWCNT-OH/ION ratio of 1:4. This scaffold is the most promising for the subsequent studies including electromagnetic stimulation of cells.

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**Curcumin Immobilized Polymeric Scaffolds for the Detection of Radical Molecules**

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In this study, curcumin-immobilized polymeric scaffolds were fabricated, and their implications for the detection of radical molecules were investigated. Two kinds of polymeric scaffolds, based on poly(lactic-co-glycolic) acid (PLGA)/poly(lactic acid) (PLA) and chitosan, were prepared. Morphology and porosity were analyzed by scanning electron microscopy. Curcumin was efficiently incorporated into PLGA/PLA scaffolds via hydrophobic interaction, which resulted in fluorescence intensities in phosphate buffered saline solution for 3 days. These fluorescence signals into PLGA/PLA scaffolds were greatly reduced by incubation with radical molecules. This radical-derived oxidation of curcumin in PLGA/PLA scaffolds could be applied to ROS sensing in vitro.
01-P361 The size of composite strands in TE scaffolds — how important is and what can influence on?

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Tissue scaffolds obtained by Fused Deposition Modeling (FDM) method are made of layers of directionally aligned microfibers, using computer-controlled extrusion and deposition process. The material properties have the majority in creation of mechanical properties and cell-scaffold interaction. However, the architecture of scaffolds is also very important. It is dependent on e.g. fiber diameter, pore size or pattern what influence scaffolds’ properties like strength, stiffness or porosity. These properties play an important role not only in load transfer but also may influence the cells response[1].

In this study we focused on various properties of poly(ε-caprolactone)-(PCL-) and poly(l-lactide-co-caprolactone)-(PLCL-) based composite materials processed to obtain 250, 410 and 1070 µm single filaments. The fillers were hydroxyapatite (HAp) and barium sulfate which increases the osteointegration and radiopaque properties, respectively. Single filaments were used in degradation and characterization experiments, e.g. mass loss and water absorption calculations, microscopic observations, GPC, DSC, tensile test, uCT and cytotoxicity assay.

The obtained results showed strong difference between degradation processes of both materials. PLCL-based composite’s degradation was size dependent whereas PCL-based was not. Difference in materials crystallinity and molecular mass distribution was observed when diameter of filaments was considered. Moreover, there was the difference in mechanical properties depending on diameter in both materials. Microcomputed tomography showed the stability of visualization capabilities during degradation. Cytotoxicity assay confirmed the safety of materials. Moreover, ongoing experiments will show the in vivo response.

It can be assumed that the dimension of fibers is one of the most important architecture parameter in simple FDM scaffolds. It is important not only because of the architecture point of view, as it strongly influences on the pore size in Z-dimension of simple FDM scaffolds, but also from degradation and stability point of view. Moreover, the materials have the contrasting properties which enable the visualization of the devices in X-rays techniques[2].

References:

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01-P362 A New Technique To Retain Biomolecules On The Surface Of Ceramic Scaffolds

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Combining biological growth factors or certain biomolecules with ceramic scaffolds can promote the cell migration into the scaffold as well as the differentiation into the specific cell type, and ultimately increase the formation of new bone. However, currently employed methods experience insufficient local retention and require high amounts of growth factor to exert a biological effect, especially in vivo. In some cases, rapid clearance of growth factors from implantation sites raises concerns over ectopic bone formation, pain and cancer risk. Here we report a unique method, inspired by natural biomineralisation processes, which provides a robust protection of biomolecules by encapsulating them within porous nanoparticles that are grafted to the surface of scaffolds, under physiological conditions.
**01-P364**  The Evaluation on Extracellular Matrix Compositions of Decellularized Meniscus Tissues by The Developed Sonication Treatment for Tissue Engineering Application

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Meniscus tissue plays important roles in the knee joints but unfortunately the injured tissue will have poor healing ability and requires great interventions to overcome this traumatic injury. Tissue engineered scaffolds serve as one of the interventions to regenerate the required tissue to treat early degenerative joint disease. In this study, the fabrication of decellularized meniscus scaffolds was prepared using developed sonication decellularization system with 40 kHz ultrasound in 0.1% SDS solution for 10 hours treatment time. Sonication-treated scaffolds were evaluated by histological analyses, biochemical assays and surface ultrastructure observation. Histological evaluation based on van Gieson staining portrayed complete cellular components removal compared to control group. Picrosirius red staining and Safranin O/Fast green revealed the well preservation of collagen and glycosaminoglycan (GAG) distribution within the treated scaffolds. Biochemical assays for DNA, collagen and GAG showed markedly reduction in the quantification of each component respectively. The collagen arrangement and orientation within the decellularized scaffolds were disrupted significantly which was evaluated through Scanning Electron Microscope. Therefore, the results showed that the prepared bioscaffolds by sonication decellularization system managed to reduce the immunogenicity of scaffold and successfully retained the properties of the extracellular matrix. Thus, it is a suitable decellularization method to be used in tissue engineering applications.

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**Keywords**: meniscus scaffold; decellularization; sonication system; tissue engineering
**01-P365** Synthesized Magnesium-Zinc-Calcium Alloys with Improved Bio-corrosion Behavior for Bone Regeneration Applications

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Biodegradable magnesium (Mg) implants are one of the attractive materials among other conventional non-degradable implants such as titanium, stainless steels and cobalt-chromium alloys due to their well degradation behavior without toxicity effect. The mechanical properties of Mg alloys are close to that of natural bone and therefore by reducing stress-shielding in between Mg alloy and bone tissue can further help bone healing. Beside tuning the degradation rate of the implanted scaffold, to assure providing enough space for new generated bone tissue, the degradation product has to be considered as well when designing in organic implantable alloy. Considering the latter mention factor the aim of the present research was synthesizing a new type of biodegradable Mg alloys comprising zinc (Zn) and calcium (Ca) as alloying elements. While the amount of Zn element was fixed at 2 wt. % changing Ca concentration from zero to 1, 2 and 3 wt. %. The corrosion behavior of Mg-2Zn-xCa (x = 0, 1, 2 and 3 wt. %) alloys was studied by potentiodynamic polarization (PDP) examinations and interestingly Mg-2Zn-1Ca showed better result in comprising of Mg-2Zn-2 and 3 Ca alloys. Scanning electron microscopy (SEM) confirmed that the severe corrosion observed for the pure Mg sample and Mg-2Zn-2 and 3 Ca alloys. Importantly, for heat treated Mg-2Zn-1Ca ternary alloy, the presence of Ca2Mg6Zn3 intermetallic phase improved the corrosion resistance (Ecorr=-1.57 V and icorr=195 μA/cm²).

In order to find the biocompatibility of synthesized Mg alloys, indirect MTT viability test performed according to ISO 10993-5:2009 standard with Adipose derived Mesenchymal Stem Cells (ASCs). The viability results indicate that Mg alloys have no significant toxicity effect on ASCs viability; however the viability increased in Mg-2Zn-1Ca group. Also, direct ASCs culturing on the surface of Mg alloys represented good attachment and proliferation.

New synthesized biodegradable Mg-2Zn alloy containing 1 wt. % Ca, which is the most abundant element in natural bone tissue, showed considerable improvement in term of degradation product in compare to those of other conventional in organic scaffolds. The corrosion behavior of pure Mg was significantly improved by addition only 2 wt. % Zn and 1 wt. % Ca, which besides providing high mechanical properties makes it a suitable candidate for bone regeneration applications.

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**01-P365** Effectiveness of a Combined Therapy which is Decompression Surgery with Platelet-rich Plasma Injections and Wrapped Biodegradable Dermal Substitute for Preventing Recurrence of Carpal Tunnel Syndrome

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Recurrence of carpal tunnel syndrome (CTS) can be seen up to 30 percent after surgical decompression of median nerve at wrist. Currently nerve wrapping and ultrasound guided platelet-rich plasma (PRP) injection techniques are highly recommended for recurrent CTS. There isn’t any study combining these two methods. The aim of this study is to evaluate the effectiveness of the combination therapy. In our study, there were 10 patients under axillary block; after decompressing the median nerve, our procedure was wrapping the nerve with an acellular dermal matrix in order to eliminate perineural fibrosis and fibrin sealant for stabilizing the matrix; and injecting PRP to the circumference of the nerve for nerve tissue regeneration. After a month long recovery from revision surgery, patients had physical examination and imaging tests. All patients had regression of symptoms and had acceptable results in static two-point discrimination test and median nerve conduction studies.
Induced pluripotent stem cells (iPSC) have shown to differentiate to functional cardiomyocytes with high efficiency through temporally controlled inhibition of the GSK3/Wnt signaling pathways. In this study we investigated the ability of temporally controlled release of GSK3/Wnt small molecule inhibitors to drive cardiac differentiation of iPSC without manual intervention. Porous silica particles were loaded with GSK3 inhibitor CHIR99021 or Wnt inhibitor IWP2, and the particles containing IWP2 were coated with 5wt% PLGA 50:50 to delay release by approximately 72 hours. Induced pluripotent stem cells reprogrammed through mRNA transfection were cultured with these particles up to 30 days. HPLC suggests a burst release of CHIR99021 within the first 24 hours and a delayed release of IWP2 after 72 hours. Annexin V/PI staining did not show a significant effect on apoptosis or necrosis rates. Cultured cells upregulated both early (Nkx 2.5, Isl-1) and late (cTnT, MHC, Cx43) cardiac markers, assayed with qRT-PCR, and began spontaneous contraction at 3.0±0.6Hz at 15-21 days after the start of differentiation. Cardiomyocytes had clear sarcomeric striations when stained for β-myosin heavy chain, and showed expression and punctate membrane localization of gap junction protein Connexin43. Calcium and voltage sensitive imaging showed both action potential and calcium transients typical of immature cardiomyocytes. This study showed that the cardiac differentiation of pluripotent stem cells can be directed by porous silica vectors with temporally controlled release of small molecules inhibitors. These results suggest methods for automating and eliminating variability in manual maintenance of inhibitor concentrations in the differentiation of pluripotent stem cells to cardiomyocytes.
Currently, titanium and its alloys are in widespread use for clinical applications due to their excellent biocompatibility. However, titanium implants have a Young’s modulus significantly higher than that of bone. This can cause stress shielding, resulting in bone resorption and dental implant failure. To solve these issues high-performance thermoplastic polymers, such as Polyetheretherketone (PEEK), are increasingly investigated for orthopaedic and dental applications as an alternative to commonly used titanium alloys. PEEK has several advantages, for instance, it possesses excellent biocompatibility and displays low elastic modulus (about 3.5 GPa).

On the other hand, PEEK also has disadvantages, for instance poor osseointegration. To overcome this, a thin osteoconductive coating is required to increase the rate of osseointegration. Surface modification of PEEK by Physical Vapour Deposition (PVD) offers an excellent way of coating inert polymer substrate with metallic and/or ceramic thin films with good adhesion. Since Titanium (Ti) and Titanium Niobium (Ti-Nb) alloys have excellent biocompatible properties, which have been used to advantage. In this study, Ti and Ti-Nb coatings have deposited by magnetron sputtering in argon (Ar) atmosphere on to the bare PEEK substrates.

Physical and chemical characterisations of these coatings were carried out by scanning electron microscopy, energy-dispersive X-ray spectroscopy, X-Ray diffraction, ATR and FT-IR spectroscopy respectively. By employing plasma-assisted PVD, it was possible to produce thin Ti and Ti-Nb coatings on the bare PEEK surface, obtaining uniform coatings with good adhesion and bonding to the peak substrates. The titanium coating produced has a composition of atomic percentage 100 at% pure titanium, and titanium-niobium alloy coatings have a composition of atomic ratio 50 at% titanium and 50 at% niobium. Structural characterization shows that the titanium coating has a stable α-Ti phase while the Ti-Nb coating exhibits a metastable β-Ti phase. In conclusion, Ti and Ti-Nb coatings were successfully deposited on the surface of the bare PEEK substrates without any alteration in the main functional groups of the PEEK chemical structures. Bioactive coated PEEK substrates will result in improved bioactivity of the dental implant, resulting in strong bonding capability between Ti and Ti-Nb coated PEEK substrates and bone structures.

Bone regenerative medicine combines the use of smart biomaterials and stem cells in order to engineer tissues mimicking the physical and functional characteristics of native bone while avoiding all kinds of infections. Bone joint infections (BJI) are an important threat for public health with massive individual (i.e., second surgery for bone resection and/or reconstruction, long-term incapacity for work, partial or total disability) and societal impacts (high inpatient and outpatient costs). In addition, BJIs are difficult to diagnose or to treat because of differences in bacterial behavior: *Staphylococcus aureus* has the capacity to escape immune system and to be internalized by host cells or embedded in a protective matrix called biofilm. In the context of bone reconstruction, biomaterials functionalized with stem cells were developed to induce bone synthesis in aseptic conditions. Interactions between *S. aureus* and stem cells with different origins including dental pulp (DP), bone marrow (BM) and Wharton jelly (WJ) were followed to select the best combination (stem cells/biomaterials) and to reduce the risk of infections. The capacity of *S. aureus* to be internalized by different types of stem cells was firstly monitored. While the rate of internalization of *S. aureus* in WJ and BM derived stem cells was respectively 0.51% (0.25% - 0.79%) and 0.61% (0.20% - 1.47%), it was higher in DP stem cells, with an internalization rate of 1.05% [0.00% - 2.72%] and higher standard deviation values. These differences were also observed when comparing human exfoliated deciduous teeth derived stem cells (internalization rate = 1.43%, [0.22% - 2.7%]) to dental pulp derived stem cells (internalization rate = 0.81%, [0.00% - 2.57%]) suggesting a higher capacity for *S. aureus* to be internalized by deciduous teeth derived stem cells. Furthermore, the quantity of biofilm formed by *S. aureus* before and after the internalization was compared. No significant difference was observed between all conditions, except for the dental pulp derived stem cells, where a significant increase in biofilm formation was observed (x1.7 ± 0.14; p=0.016, Mann-Whitney test). In conclusion, we screened for the first time the impact of tissue-derived stem cells on the virulent behavior of *S. aureus* in view of their potential application in bone regenerative medicine.
Clay nanoparticles are emerging as a new class of biomaterials with exciting opportunities for tissue engineering and regenerative medicine applications. Recent high-impact studies have demonstrated the ability of Laponite clay nanoparticles to self-organise into injectable microenvironments able to host and stimulate stem cell growth and differentiation at physiological doses of growth factors. Furthermore, Laponite nanoparticles are able to promote in vitro osteogenic differentiation of skeletal populations even in the absence of the osteogenic supplement dexamethasone. Despite these interesting osteogenic properties of clay nanoparticles, relatively little attention has been given to the role of fundamental aspects of clay structure/composition in influencing such interactions. Lithium, a Laponite degradation product, is known to modulate the canonical Wnt signaling pathway through the inhibition of GSK3β and stimulates osteogenic differentiation of responsive population.

Various Li⁺-modified Laponite formulations were generated, with Li⁺ content ranging from 0 to 9.9 g/kg. Human bone marrow stromal cells (hBMSCs) were cultured in the presence of Li⁺-modified formulations, as culture media additives, and assessed for cell viability, proliferation and osteogenic differentiation in comparison with controls. Dispersed Laponite nanoparticles demonstrated high cytocompatibility at concentration <1 mg/ml. Both Li⁺-modified and standard Laponite structures followed the same pattern in enhancing the osteogenic differentiation of hBMSCs in a dose dependent manner. The addition of Laponite nanoparticles triggered an upregulation of osteogenic gene expression (RUNX2, ALP, collagen I and osteonectin), an increase in ALP activity and enhanced Ca-P mineral deposition, reaching a 2-fold upregulation in RUNX2 gene expression (****P = <0.0001) and a 100% increase in ALP activity (p <0.05) compared to clay-free control at day 3 in basal media. Upregulation of osteogenic markers was observed independent of Li⁺ content.

In conclusion, Laponite nanoparticle addition to cell culture media enhances the osteogenic differentiation of hBMSCs independent of the Li⁺ modifications tested in the current study. Other clay chemistries such as surface charge, particle size and cation exchange capacity may play a role in modulating the osteogenesis of hBMSCs. Such studies seeking to elucidate the mechanism(s) behind clay-enhanced stem cell osteogenesis are ongoing.
**Customizable platelet lysate based hydrogels for 3D cell culture**

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Hydrogels have been used in many biomedical applications, including drug and cell delivery systems, cell growth platforms and tissue regeneration. Currently, implantable hydrogels for biomedical applications built into synthetic and natural biodegradable materials are a major research focus. However, at present there are many concerns in implantable systems uses, such as immunogenicity, biological safety, biocompatibility, degradation rate, and mechanical stability. There is also a continuous need for developing new compositions capable of forming in situ biocompatible hydrogel structures that offer improved therapeutic outcomes. Platelet-rich plasma (PRP) and human platelet lysates (PL) are attractive sources of growth factors and other molecules involved in the tissue regeneration process. PRP and PL have been mainly explored as a promising non-xenogenic supplement designed for the expansion of human cells that replaces animal derived serum. Recently, PRP/PL based hydrogels have been investigated as cell culture platforms. Despite all the promising results, in most cases this type of materials suffer from poor mechanical properties and also poor stability in vitro. In an attempt to overcome such limitations, we developed PL based hydrogels with tunable mechanical properties. PL were modified by addition of methacryloyl groups (PLMA) that by exposure to UV irradiation form hydrogels. Our results showed that the achieved materials own increased mechanical properties that can be easily adjustable by changing PLMA concentration or PLMA modification degree. Additionally, PLMA hydrogels enabled sustained release of VEGF and fibrinogen for up to 250 hours and were resistant to protease degradation. Human derived stem cells and murine fibroblasts were successfully encapsulated in the hydrogels, exhibiting high cell viability, adhesion and proliferation. These results support the use of a PLMA hydrogel as a scaffold for stem cell culture and growth factor release. The material here developed could have an autologous origin, being adequate to produce customized robust matrices to be used as general platform for 3D cell culture with no risk of cross reactivity, immune reaction or disease transmission.

**Acknowledgments**

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Crosslinker Selection Determines the Properties of GelNOR Hydrogels for Tissue Engineering Applications

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Introduction: Gelatin-Norbornene (GelNOR) hydrogels can be crosslinked using any multi-functional thiol containing molecule via thiol-ene photo-click chemistry. This crosslinking reaction is highly modular, but data is lacking as to the effect of crosslinker selection on the physico-chemical properties and cell response of the resulting hydrogels.

Aim: To determine the effect of crosslinker selection and concentration on crosslinking efficiency, compressive modulus, and behavior of encapsulated cells in resulting GelNOR hydrogels.

Methods: GelNOR gels at 5wt% and 10wt% were photo-crosslinked with di-thiothreitol (DTT), polyethylene glycol (PEG)-4SH, and PEG-8SH at NORSH molar ratios of 1:1, 1:2, 1:3, 1:6, and 1:12 using the visible light initiation system we previously described [1]. The effect of crosslinker selection on sol fraction, Young’s Modulus and behaviour of encapsulated cells was analyzed using mass loss and swelling studies, uniaxial compressive testing, and human foreskin fibroblasts (HFFs) encapsulation assays respectively.

Results: The sol fraction was lowest at both 1:3 and 1:6 NORSH molar ratios, and at constant NORSH ratio sol fraction values were lower in 10wt% hydrogels than 5wt%. The compressive Young’s Modulus followed a trend inverse to soluble fraction and was highly tailorable with crosslinker, with observed values from 3.96 - 278.35kPa. HFFs encapsulated in 5wt% hydrogels crosslinked with DTT took an elongated morphology from day 6 at all NORSH molar ratios tested. HFFs encapsulated in 10wt% gels at 1:6 NORSH maintained a rounded morphology and could not elongate, but those encapsulated in 10wt% gels at 1:3 NORSH were able to spread after 16 days.

Discussion: The observed difference in cellular behavior demonstrates the ability to direct cellular responses through NORSH molar ratio. The system is also very versatile due to the range of achievable mechanical properties, with softer DTT crosslinked gels possibly suitable for use in soft tissues (e.g. neural), and stiffer PEG crosslinked gels suitable for hard tissues (e.g. osteochondral).

Conclusions: This study has demonstrated the described GelNOR system’s highly tuneable physico-chemical properties, wide range of achievable mechanical characteristics, and ability to direct cellular behavior, identifying its potential for use in a wide range of tissue engineering applications.

References:

Characterization of the osteochondral potential of a novel three-layered scaffold using hAdMSCs

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Objective: Healing of osteochondral defects is still a challenge. Especially the cartilage tissue has a poor self-healing capacity. Therefore, we developed a multilayered scaffold for osteochondral regeneration. Our hypothesis is that such layered scaffold could introduce a directed stem cell differentiation into osteo- or chondrogenesis by mimicking the biological and mechanical properties of the distinct tissues as well as the interface.

Materials and Methods: The scaffold was developed by a layer-by-layer freeze-drying process of chitosan-collagen solutions, partly supplemented with octacalcium phosphate in the bony part. The cartilage part showed a gradual increase of collagen content. The scaffold was characterized using FTIR, SEM-EDX, and µCT. The scaffolds were cut into 3mm cylinders and seeded with human adipose-derived mesenchymal stem cells (hAMSCs) at different densities for up to 35 days. Cell viability and proliferation was tested, as well as gene expression for apoptosis/proliferation markers (Bcl-2, Casp-3, Mcm-5, and Cyclin D1), osteogenesis (RunX2, Coll I and III, Osteocalcin, and Osteopontin) and chondrogenesis (Sox-9, Coll II and X, and Aggrecan). Moreover, the bioactivity potential was evaluated by incubating the scaffolds for up to 7 days in simulated body fluid (SBF) and characterization using FTIR, SEM-EDX and µCT.

Results: The scaffolds showed good cell attachment and proliferation as well as a homogenous cell distribution. RunX2, Coll I, Osteocalcin and Osteopontin expression showed that osteogenesis occurred mostly in the upper part of the scaffold. Meanwhile chondrogenesis occurred in the lower part of the scaffold as indicated by Sox-9, Coll II, and aggrecan expression. In the bioactivity experiments the scaffold showed a good layer specific potential to form a hydroxapatite-like matrix.

Conclusion: Our study shows that each layer of the multilayered scaffold presented a specific potential for induction of osteo- or chondrogenesis. Clear tissue-specific matrix deposition and mineralization was detected when scaffolds were cultured for long observation times.
**01-P377 Core-shell Structured Polymeric Particles Obtained Through a Solvent-free Strategy**

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Hollow materials have been employed as encapsulation devices for a plethora of applications including Tissue Engineering. Core – shell structured particles with a liquid core exhibit (i) a more efficient and homogeneous transfer of solutes, (ii) a higher loading capacity provided by their internal ample space, and (iii) a lighter weight when comparing with their cross-linked core counterparts. Most of the available strategies to fabricate polymeric capsules are based on complex and harsh synthesis procedures, eluding the use of coagulating baths, which can ultimately compromise the cargo stability and loading efficiency. Herein, highly repellent substrates, namely superamphiphobic surfaces (SA), were successfully employed to design ready-to-use monosized and spherical polymeric capsules with a (i) hydrogel shell made of methacrylamide chitosan (MACHI) and (ii) a liquefied core, wherein different molecules and objects can be dispersed. MACHI capsules were fabricated by first crosslinking a spherical alginate (ALG) droplet induced by a SA surface upon adding CaCl₂. Afterwards, the ALG core was entrapped within a MACHI droplet and, finally, the MACHI shell was crosslinked by a UV-mediated process and the core removed via ethylenediaminetetraacetic acid (EDTA) action. The developed strategy benefit from its: (i) cost-effective character inasmuch as it is based on a simple setup; (ii) air-interfaced nature enabling a high loading efficiency; (iii) reproducibility as demonstrated by the easy and precise control over the particle size and shape; (iv) versatility as shown by the fabrication of polymeric capsules from a wide range of materials and by the incorporation of distinct components in a single structure; and (v) mild processing conditions as proved by the safe encapsulation of metabolically active cells. Based on all these features, this simple, yet efficient strategy is envisioned to constitute an innovative approach to produce liquid-core polymeric systems to entrap a variety of sensitive molecules including not only cells, but also proteins, genes, and drugs. Owing to the widespread application of polymeric capsules like the produced ones, modifications to the conventional fabrication techniques are likely to have a strong impact and open new prospects for the development of the next generation of engineered polymeric assemblies for both science and technology.

**References:**  

**01-P378 Evaluation of the functionality of osteocytic network in 3D culture**

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Osteocytes reside as three-dimensionally (3D) networked cells in the lacunocanalicular structure of bones. Due to the osteocyte network being deeply embedded within hard bone tissues ex vivo, it has been extremely challenging to access and characterize these cells. So far, evaluation of osteocyte activity and regulation in vitro have mainly been conducted in 2D systems, with only very few studies conducted in a 3D environment. Three-dimensional (3D) cultures are attractive as they better mimic the physiological features of the ECM surrounding the cell. Hydrogels represent an interesting way to create a 3D environment, due to easily tunable chemical, physical and mechanical properties. Then we use a hydrogel and human MSC to process 3D culture. Through ALP staining, real time PCR, IHC, microCT detection, we found that MSC can osteogenic differentiation and formation of mineralization in both groups, and better interconnected network between cells was significantly increased within low stiffness hydrogel, compared to cells within high stiffness matrices at the same cell densities. These results proved our 3D culture system is worked, and can be used to investigate the mimic bone 3D structure.
In vitro evaluation of 3-dimensional poly (lactic acid-co-glycolic acid) hybridized with atelocollagen and fibrin bioscaffold composite for annulus fibrosus regeneration

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Tissue engineering principles provide an alternative platform for the regeneration of the IVD tissue via the use of the biomaterial scaffold. The current study evaluated the 3D culture of the PLGA incorporated with the atelocollagen and fibrin seeded with the annulus cells of intervertebral disc. The evaluation was performed in three consecutive weeks observing and evaluation of the morphology, compression modulus, gene expression, histology, immunohistology, sulphated glycosaminoglycan (SGAG) and DNA content. A comparison was made between the AF seeded onto the PLGA (PLGA-AF), PLGA with atelocollagen (PA-AF), PLGA with fibrin (PF-AF) and PLGA with both atelocollagen and fibrin (PAF-AF). The constructs displayed glistening morphology with high wet weight and cellular viability by week 3. The PAF-AF (0.062±0.008MPa) and PA-AF (0.041±0.007MPa) demonstrated significant compression modulus as compared to all other constructs. The constructs were able to maintain the phenotype of the AF cells with high expression of the collagen type I and low expression of the collagen type II supported by the immunohistochemistry. The expression of the condrogenic markers, sox-9 and aggrecan were elevated with variation of upregulation and downregulation observed within weeks of culture. The histological findings corroborated with the cellular distribution however, weak evidence of the glycosaminoglycan and proteoglycan were detected. Significant quantitative sGAG (0.279±0.117µg/µl) was detected for the PAF-AF constructs but with no significant differences was noted on the DNA content. Overall, the PAF-AF constructs support the cellular proliferation of the AF and need to be evaluated further in animal model.

Keywords: PLGA; atelocollagen; fibrin; annulus fibrosus

Designing multifunctional laminarin microparticles for 3D cell culture

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Living tissues are hierarchically organized three-dimensional (3D) structures composed of multiple types of cells and extracellular matrix (ECM). Thus, effective strategies to engineer constructs that mimic native tissues requires the development of structures with well-defined spatial distributions of different cells embedded in ECM.

Microfabrication technologies have been explored to produce microgels that can be assembled in functional constructs for tissue engineering applications. Here, we propose microfluidics coupled to a source of UV light to produce monodisperse multifunctional laminarin microparticles by photopolymerization. Laminarin is a natural polymer obtained from brown algae with low molecular weight and low viscosity. These properties make this polymer particularly appealing to be used in microfabrication techniques. Addition of methacrylate groups to the hydroxyl-containing groups of laminarin was performed to make it light polymerizable into a hydrogel.¹

In an attempt to enhance cell adhesion and proliferation, the microparticles were loaded with platelet lysates and further conjugated with an adhesive peptide. The modified microparticles were cultured with L929 cells, the results showed superior cellular adhesion on microparticles with encapsulated platelet lysates. Moreover, the functional particles have demonstrated the ability to provide sustained release of growth factors from platelet lysates.

The multifunctional laminarin microparticles provide an effective support for cell attachment and cell expansion, moreover the microparticles tend to aggregate in robust 3D structures. These results showed the potential for using the laminarin microplatforms to rapidly produce large tissue engineered constructs.


Acknowledgments

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**Self-Assembling Peptide-Based Hydrogels for the 3D Culture and Differentiation of Induced Pluripotent Stem Cells into Cardiomyocytes**

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Stem cells reside in distinct and complex microenvironments from which they interpret and integrate biochemical and mechanical cues that determine their fate. (1) To date, efficient in vitro culture of stem cells remains a significant challenge, limiting full exploitation of these cells in Regenerative Medicine. (2) Of particular interest are induced pluripotent stem cells (iPSCs) which provide the potential for designing autologous cell therapies. (3) Currently, attempts are being made to recapitulate the native cellular niche through design of different biomaterials for 3D cell encapsulation. (4) Of popular choice are hydrogels; water-swollen polymeric networks which can be comprised of both natural and/or synthetic fibres. One promising class of hydrogels are the self-assembling peptide based hydrogels (SAPH). In particular the peptide design developed by Zhang’s group (5), based on the alternating pattern of hydrophilic/hydrophobic amino acids, has been the focus of significant attention in the last decade by our and other groups (6-8). These SAPH offer a fully-defined, tuneable scaffold made from naturally occurring amino acids.

In this work we demonstrated that these SAPH support the viability and proliferative capacity of human iPSCs. Within 3 days, these cells progress from loose, disorganised cell clusters to smooth spheroid aggregates, around 250-300 μm in size. When stem cell media is used, these cells retain their pluripotent nature; expressing typical markers of pluripotency (Oct-4, Sox-2, Nanog) with little influence from the bulk mechanical properties of the gel, nor cell passage. When non-specific media or differentiation media is used, the cells lose their pluripotent nature and start to differentiate. The ‘simplicity’ of these hydrogels provided us a platform to investigate the importance of a number of factors on the differentiation pathway of these cells when cultured in 3D, including the role of cell proteins specific to the stem cell microenvironment – laminin. Together, this work highlights the potential of SAPH as 3D matrices for stem cell based-applications and is the first step in elucidating the factors that allow to control and direct the differentiation of iPSCs in 3D.
3D cell cultures via porous scaffolds for dentin regeneration

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INTRODUCTION
Tooth loss is a common result of a variety of oral diseases due to physiological causes, trauma, genetic disorders and aging, and can lead to physical and mental suffering that markedly lower the individual’s quality of life [1]. Tooth is a complex organ that is composed of mineralized tissues and soft connective tissues. Dentin is the most voluminous tissue of the tooth and its formation (dentinogenesis) is a highly regulated process displaying several similarities with osteogenesis.

In this study a low-cost scaffold made by gelatin biomineralized with magnesium-doped hydroxyapatite and blended with chitosan, was developed for hard tissue engineering. We synthesized a dentin-like scaffolds using a controlled freeze-drying process permitting the formation of microstructural channels comparable to dentin tubules and appropriate for cell penetration and matrix deposition.

Mesenchymal stem cells (MSCs) and dental pulp stem cells (DPSCs) were seeded in direct contact with the scaffolds and cultured with medium supplemented with osteogenic factors. Cell viability and cell morphology were analysed up to 14 days of cultured. Moreover gene and protein quantification were investigated.

The scaffolds show an aligned porosity suitable for the colonization of its inner part by the seeded cells. The scaffold had no cytotoxicity, cells morphology was accordant to a non-stress cell condition and a good adhesion to the scaffold. The SEM observations showed the scaffold’s aligned porosity and rugosity, cells were found through almost the entire height of the scaffold. 3D cell culture with MSCs and DPSCs showed the promising properties of the new scaffolds for tooth regeneration. In detail, the chemical composition of the biomineralized gelatin facilitate the cell adhesion, the aligned porosity is suitable for cell colonization and fine cell/material interactions together with mineral component permit the cells differentiation and matrix deposition.

This preliminary study indicate the potential of a low-cost biomineralized gelatin scaffold as a novel tool for 3D cell culture in dental regeneration.

REFERENCE

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Engineering Controlled Release of Therapeutic Cargo from Photodegradable Hydrogel Carriers using Near-Infrared Light and Upconversion Nanoparticles

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Hydrogel carriers are used to deliver therapeutic cargo such as stem cells to damaged tissue, with attractive properties including tunable mechanics and bioactivity, and injectability. Stimuli-responsive hydrogels such as enzymatically degradable [1] and photodegradable [2] hydrogels are engineered to controllably degrade in the presence of a stimulus to locally release therapeutics, and light-mediated degradation offers the unique opportunity to trigger degradation externally and on-demand. Light-cleavable hydrogels incorporating UV-absorbing o-nitrobenzyl groups within hydrogel crosslinks enable hydrogels to be controllably degraded, even in the presence of living cells, using low-energy UV light [2]. UV light has very low tissue penetration and mutagenic concerns, whereas near-infrared (NIR) can penetrate several cm through tissue, and upconversion nanoparticles (UCNPs) provide an opportunity to exploit NIR. UCNPs were used as transducers to convert NIR into UV by applying 980 nm light to release photo-caged siRNA from intracellularly uptaken nanoparticles [3]. To demonstrate UCNP-mediated hydrogel photodegradation by NIR, a new thiol-functionalized, UV-cleavable crosslinker was synthesized and reacted with 8-arm PEG-maleimide and UCNPs, resulting in a crosslinked hydrogel with entrapped UCNPs. To verify photodegradation and demonstrate controlled release, a fluorescent dye was covalently bound to the network as a facile means to quantify extent of degradation and cargo release, revealing that dye was released by NIR light in a dosage-dependent manner. Future experiments will fully characterize release and degradation kinetics, followed by demonstrating the versatility of the hydrogel platform for controlled delivery applications of model biological cargo in vitro, and eventually verified in vivo in a mouse model.

References:

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Tuneable Spheroidal Hydrogel Particles for Cell and Drug Encapsulation

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Natural systems, such as bacteria and phytoplankton, present varying geometry and preferentially adopt geometries other than spherical, highlighting the relevance of this parameter in evolution. Considering this and the need to mimic native tissues, development of new platforms for cell and drug encapsulation with diverse material geometries has been gaining momentum.

Microspheres are encapsulation systems used for tissue engineering, allowing for efficient nutrient diffusion and metabolite removal, which is a key aspect for cell survival within a hydrogel matrix. Yet, increasing particle dimension compromises diffusion, resulting in lower cellular viability within the core. For drug delivery purposes, particle geometry is an impacting factor in opsonisation and targeted therapy. Moreover, of convex shapes, spheres present the lowest surface area to volume ratio, which is an impacting factor in diffusion, whether of nutrients for cell survival, or kinetic profile of drug delivery. Thus, geometry tailoring allows to produce hydrogels adaptable to specific applications.

Aiming to investigate how increasing surface area and varying particle shape could impact drug release and cell viability, a novel method was developed to produce spheroidal hydrogel particles with tuneable volume and geometrical parameters. Droplets of hydrogel precursor were sandwiched between two superamphiphobic surfaces separated by spacers with different height, and photo-crosslinked to maintain the acquired shape after "de-sandwiching". Numerical modelling studies were performed to study the polymeric droplet geometry deformation, which were consistent with experimentally obtained results in mild conditions. Sphericaloid particles were produced using methacrylated chitosan, capable of encapsulating proteins or cells. Likely due to their higher surface area to volume-ratio, spheroids presented an improved viability of encapsulated cells due to an enhanced nutrient diffusion to the core, and lead to a significantly faster drug release rate from the polymer network. Hence, the described method can be used to produce spheroidal particles with tailored thickness that can be applied for both drug delivery and cell encapsulation strategies.

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Development of 3D new tissue for bladder reconstructive surgery

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In this work were evaluated three decellularized scaffolds, bovine pericardial (TPB), porcine pericardial (TPP) and amniotic membrane, the decellularization process used was reversible alkaline swelling (STX). They were recellularized with rabbit urothelial cells to generate an autologous tissue. The new tissues have been made performed to obtain a minimum cell concentration of 1x10^5 cel/ml and was maintained for 15 days. The new tissues were implanted in the bladder with damage of the same small animal models and monitored during 15, 30 and 150 days with ultrasounds and evaluation of immune response. After those times the bladder was removed and the cellular regeneration was evaluated using eosin-hematoxilin stain and Mason’s trichrome stain. Scanning electron microscopy (SEM) was realized at the bladder samples. The results show a decellularization efficiency between 88 and 98%, depending on the extracellular matrix. With the stains was observed that the matrix helped the regeneration of the area and the immune response of the animal, however the scar generated was lower with the amniotic membrane matrix and porcine pericardium. Good cell regeneration and collagen fibers was observed in the SEM imagens.
**01-P388** Multiscale reconstruction of a synthetic biomimetic micro-niche for enhancing and monitoring the differentiation of stem cells

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Stem cells reside in the 3D niche microenvironment, which provides specific cues including cell-matrix interactions and soluble factors that are essential to the maintenance and differentiation of stem cells in vivo. Herein, we demonstrate a general approach for the synthetic reconstruction of 3D biomimetic niche environment of stem cells by the multiscale combination of macroscopic porous hydrogels and nanoscale UCNP-based nanocomplex. The porous biopolymeric hydrogels emulated the spongy bone microstructure and provided the conducive 3D environment for the attachment, proliferation, and biosynthesis of seeded stem cells. The UCNP-based nanocomplex (Pur-UCNP-peptide-FITC), which was stably encapsulated in the porous hydrogels, emulated the repertoire of inductive agents in the bone matrix by sustaining localized and long-term delivery of inductive small molecules in the synthetic niche microenvironment. Furthermore, the nanocomplex also generated biomarker-specific reporting emission that correlated well with the extent and stage of differentiation of the stem cells residing in the synthetic niche, thereby enabling long term tracking of stem cell fate in living culture. Our findings showed that this multiscale synthetic niche not only enhanced the differentiation of seeded stem cells but also enabled long term tracking on the stage of differentiation of living stem cells via a non-contact, non-destructive, and potentially high-throughput manner. To the best of our knowledge, our work is the first demonstration of multiscale combination of functional biomaterials for reconstructing the controlled stem cell niche microenvironment to enhance and track stem cell differentiation. The modular nature of this synthetic niche platform allows easy tuning of various parameters, such as microstructure morphology, scaffold stiffness, inductive molecule, and the biomarker-specific reporting system, to cater to a wide array of fundamental studies on dynamic cellular events over long term.

**01-P389** Piezoelectric scaffolds for tendon repair through electromechanical stimulation: an in vitro and in vivo study

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Introduction
Electromechanical coupling (piezoelectricity) is present in all living beings and provides the basis for sense, thoughts, and mechanisms of tissue regeneration. Piezoelectric properties of musculoskeletal tissue have been recently measured at the molecular, fibrillary and tissue level and are mainly attributed to collagen type I - a fibrous protein abundant in mammals. Understanding the effect of piezoelectricity on biological processes such as cellular differentiation, cell growth, cytoskeleton rearrangement or inflammation is of pivotal importance for regenerative medicine and biomedical applications¹. In this study, we fabricated piezoelectric regenerative scaffolds to assess the role of electromechanical stimulation on tendon regeneration.

Materials and methods
Piezoelectric scaffolds were made of poly (vinylidenefluoride-co-trifluoroethylene) P(VDF-TrFE), a material capable of generating electrical charges under mechanical loading. P(VDF-TrFE) was electrospun at 1ml/hr and 3500 rpm to fabricate a scaffold made of aligned fibres. Nonpiezoelectric scaffold was made of similar chemistry (fluorinated) and electrospun at same conditions. All scaffolds were functionalized with a fibronectin-mimetic polymer coating. The electrical and piezoelectric properties were assessed with an in-housesystem and $d_{33}$ was determined. Primary Human Tenocytes were cultured (8 hours, 5 and 10 days) in static and dynamic conditions. Gene expression (96 genes) was evaluated to uncover the molecular pathway. Finally, a full-size rat Achilles tendon injury model was employed. Functional, structural and molecular analyses were made at 2, 4 and 8 weeks.

Results
P(VDF-TrFE), scaffolds were observed to mimic the fibrous structure of tendon tissue and were capable of producing electrical charges up to 17 pC/N when mechanically loaded.Genes associated with tendon specific markers (ColI/Col III, Sx, and Mxk) and mechanosensitive ion channels such as PIEZO1, TRAAK, and TRPV1 were significantly upregulated. The upregulated genes were validated with individual real-time Q-PCR and bioinformatics revealed a possible regulated function.

Discussion
This study indicates that scaffolds made of PVDF-TrFE can produce electrical charges when mechanically loaded. Moreover, gene analyses showed a positive regulation of tendon specific markers through activation of mechanosensitive voltage-gated genes.

References
Red Blood Cell (RBC) production from hematopoietic stem cells (HSCs) has been a focus in regenerative medicine for a number of years, yet obtaining adequate numbers of HSCs still needs to be addressed. The existing systems generate low in vitro concentrations of HSCs, furthermore the number of HSCs collected from peripheral blood are much lower than can be derived from bone marrow. Clinically, the HSCs can be mobilised into the blood in order to increase the concentration by using molecules like granulocyte colony stimulating factor (G-CSF). However the medication causes few side effects. We have developed a practical, rapid, safe and cost effective approach to increase HSCs yields from normal human adult whole blood by using surface modification strategies on polymer substrates to promote endothelial cell adhesion that will then cause leukocyte adhesion to the endothelial cells. The polyurethane (PU) surfaces were functionalised with chemical groups including hydroxyl, amine and carboxyl. Human umbilical vein endothelial cells (HUVEC) were cultured on the modified materials for 72 hours. Based on flow cytometry results, a significant increase in the proportion of cells expressing ICAM-1 was observed and no significant difference in the proportion of cells expressing VCAM-1 as compared to cells that were cultured on glass coverslips. The results showed that without using exogenous mediators of inflammation or infection, direct contact between endothelial cells and surface-modified PU caused the cells to actively express endothelial adhesion molecules. It is well known that adhesion of leukocytes to endothelial cells occurs through the interaction with adhesion molecules expressed on endothelial cells. In order to confirm the interaction between leukocytes and endothelial cells, the HUVEC-seeded materials were exposed to freshly drawn whole blood and incubated for 1 hour. Our data showed that as the expression of adhesion molecules increased, substantially increasing the numbers of leukocytes captured. This technique results in an enriched population of unstimulated HSCs from human adult blood that will potentially underpin production of red blood cells.

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Sodium alginate is a polysaccharide that is derived from the cell walls of brown algae; it has been widely used as a cell encapsulation material as well as a drug delivery vehicle. The backbone of this natural polymer consists of random or co-block formations of β-D-mannuronic acid and α-L-guluronic acid, the latter of which allow the solution to polymerize in the presence of divalent cations such as Ca2+. Alginate is non-immunogenic and biocompatible, with a low toxicity and highly tailorable mechanical properties.

By limiting access or adjusting the rate of access to the calcium, the alginate is able to crosslink at varying homogeneities and crosslink densities. Controlling the crosslinking can also alter the diffusive properties. Internal crosslinking as such can allow for a higher degree of control, and the properties are dependent on the concentrations of the crosslinking catalyst.

An alginate co-encapsulation set up was created by externally crosslinking 1.2% alginate by dropwise addition into varying concentrations of calcium chloride. Following a rinse step to remove excess calcium, the alginate beads were encased in a layer of 2% alginate, internally crosslinked using varying concentrations of a 1:2 molar ratio of calcium carbonate and glucono-delta-lactone. The alginate beads were incorporated with 0.1 mg/mL of Direct Red 80, and controls were employed to determine diffusion profiles of both internally and externally gelled alginate. Addition of proteins of various molecular weight and charge are to follow. Hypoxia gradients in normoxia-cultured, alginate-encapsulated human nucleus pulposus cells (hNPCs) will be determined by hypoxia probe and confocal microscopy.

Preliminary data suggests that internally crosslinking alginate allows a burst release of BSA (67kDa) due to the homogeneity of crosslinking and α-L-guluronic acid, the latter of which allow the solution to polymerize in the presence of divalent cations such as Ca2+. Alginate is non-immunogenic and biocompatible, with a low toxicity and highly tailorable mechanical properties.

By limiting access or adjusting the rate of access to the calcium, the alginate is able to crosslink at varying homogeneities and crosslink densities. Controlling the crosslinking can also alter the diffusive properties. Internal crosslinking as such can allow for a higher degree of control, and the properties are dependent on the concentrations of the crosslinking catalyst.

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Preliminary data suggests that internally crosslinking alginate allows a burst release of BSA (67kDa) due to the homogeneity of crosslinking through the structure, where externally crosslinking alginate prevents a burst release due to the tight crosslinking on the boundary of the hydrogel. When adding these crosslinking strategies simultaneously, diffusion of particles and dyes such as Direct Red 80 (1373.07 kDa) are limited in a manner dependent on the crosslinking density. The implications of these results are far reaching – by adjusting diffusion of nutrients, a more applicable ex vivo model can be created to mimic the hypoxic and nutrient deprived conditions of the intervertebral disc in both a degenerative and a healthy state.
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Injectable Collagen Matrices with Bio-Instructive Properties for Dentin-Pulp Regeneration

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Dental pulp plays a vital role in tooth development as it harbors progenitor/stem cells (DPSCs) that proliferate and differentiate into dentin-secreting odontoblasts. Dental pulp injury caused by caries or trauma, leads to inflammation, which if left untreated, results in pulp necrosis. Regenerative endodontics aims to engineer a metabolically active and functional dental pulp. It requires blood vessel formation in an extracellular matrix (ECM)-mimicking microenvironment. Like other stem cells, DPSCs are also multipotent and a unique source for both endothelial and odontoblast cells due to their differentiation ability. Current stem cell transplantation strategies lack efficacy because they lack a suitable cellular niche to modulate lineage-specific differentiation. We hypothesize that oligomeric collagen-fibril matrices of precise stiffness can induce DPSCs differentiation into odontoblastic and endothelial lineages. We evaluated the long-term cell survival and differentiation of DPSCs on matrices of varying stiffness to induce endothelial and odontogenic differentiation, respectively. We hypothesized that the lower stiffness collagen (235Pa) would induce differentiation of DPSCs to an endothelial lineage and the higher stiffness collagen (800Pa) would induce differentiation of DPSCs to an odonto/osteogenic lineage. We further hypothesized that incorporation of vascular endothelial growth factor (VEGF) and bone morphogenetic protein (BMP-2) into the gels would enhance the endothelial and osteogenic differentiation ability. Cell spreading was enhanced in the matrices with VEGF. On day 3, cells demonstrated a tubular morphology with profuse spreading in the presence of VEGF matrices. Cells cultured on 800Pa matrices demonstrated slow migration compared to low stiffness matrices. The expression levels of VE-Cadherin were higher compared to vWF and PECAM, and the effect of VEGF incorporation was evident. ALP activity was observed in cells cultured in 800Pa collagen gels even in the control media demonstrating the effect of stiffness on osteogenic differentiation of cells. The presence of BMP-2 increased mineralization levels in the collagen matrices. Collectively, the current study demonstrates that collagen matrices of specific stiffness (235Pa and 800Pa) could induce endothelial and osteogenic differentiation, respectively, and that the effects are enhanced in the presence of growth factors.

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Hardening and biological properties of nanoparticle-based cement

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Many types of bone cements are commercially available in clinical field. So far, a lot effort has been made to overcome shortcomings such as brittleness or uncontrolled degradation by adding polymers or nanoparticles. Here, we reported a new type of bone cement made from nanoparticle itself. The hardening was enabled by mixing nano-sized cement powders, which containing different amount of Ca ions (0, 5, 15, and 25%) within the silica network, with various aqueous solutions. Eight types of nanoparticle groups, classified by the Ca amount and mesopore size (small and large), were investigated for cementation properties. The characteristic of nanoparticles and ionic strength of the aqueous solutions affected the hardening time of the cement. TEM images of hardened cement revealed that the formation of a few particles on the initial nanoparticle surface. Furthermore, detailed mechanism of cementation, as studied by EDS, XRD, FT-IR, XPS and NMR, demonstrated newly formed small particles in amorphous phase composed of Ca, Si and P might network the initial nanoparticles. Cement exhibited higher surface area compared with the conventional tricalcium phosphate cement (TCP). Consequently, nanoparticle based cement showed higher protein loading capacity (especially positive charged proteins) than TCP (~163 times). Ca and Si ions were released from cement over 2 weeks and these released ions elicited excellent cellular responses including osteogenesis and angiogenesis. Moreover, the implantation of cement in calvarial bone defect model proved excellent osteoinductivity as well as osteoconductivity. Overall, the nanoparticle based cement maybe useful for bone regeneration as an alternative of TCP.
Microfilm-inspired high-throughput platform to screen 3D cell-materials interactions

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Based on the concept that microfilms are scaled-down reproductions of documents, we propose a microfilm-inspired apparatus to screen cell-material interactions in which cell-laden hydrogel microfibers are representative of physiologically relevant 3D tissue engineering microenvironments. Our “filmstrip” is a hydrogel microfiber produced with a microfluidic system and with a flow-focusing chip by the pulsatile flow of polymeric solutions in the inner channel in anti-phase with the outer channels. In our system, the still images (frame) are the individual compartments made from the solution of the inner channel in which cells are encapsulated. Each frame is then separated by an acellular part (frame lines). The length of the frames and of the frame lines can be independently controlled by changing the parameters of the process (pressure or flow rate) while the width is defined by the size of the chip (300-400 um). The frames can be constituted by different materials and/or different cell types. Thanks to a microfluidic mixer, the composition of the cellular frames can vary along the length of the fiber to create linear gradients of materials and cells. This allows creating a library of materials blends and correlating the composition of the microenvironment to specific cell responses. The fibers are produced with an oil-free approach that surpass the constraints of the emulsion systems. The “microfilm”, from few centimeters to some meters in length of fiber, placed on a custom-made transparent spool permits an automated characterization of the cell response and the respective correlation to the original composition of the frame, based on its position along the fiber. Therefore, the identification of the best material/composition for the differentiation of human adipose stem cells (hASCs) into the osteogenic lineage and the co-culture with endothelial cells has been achieved. The herein proposed approach allows the fabrication of multiple 3D cell-laden hydrogel-based platforms for the screening of cell-materials interactions and selection of conditions for the development of improved tissue engineering approaches.

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Novel Alginate Hydrogels: Two Modes of Degradation and Dual Crosslinking for Local Patterning of Biophysical and Biochemical Properties

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Introduction:
Biomaterials with tunable degradation kinetics can be used to mimic the dynamic microenvironment that cells are exposed to and thereby recapitulate the reciprocal interplay between the ECM and the surrounding cells. We developed an alginate-based material system with two different crosslinking types and two different degradation modes. The first one relies on spontaneous Diels-Alder chemistry of norbornene and tetrazine functional groups, with the potential to impart hydrolytic degradation via oxidation of the polymer backbone. The second one relies on UV-initiated thiol-ene chemistry with the potential to impart enzymatic degradation via incorporation of MMP-sensitive peptide crosslinkers.

Methods & Results:
Initial mechanical properties of both systems were controlled by the amount of norbornene on the polymer backbone and the stoichiometric ratio of norbornene to tetrzaine or the concentration of peptide crosslinker, respectively. Degradation of a subset of materials was assessed by tracking the wet weight and elastic modulus in media or enzyme solutions depending on the material’s susceptibility to hydrolysis or enzymatic attack. Materials were rendered cell-compatible via conjugation of thiol-coupled RGD sequences linked to remaining available norbornene groups. 2D and 3D cell compatibility were verified by quantifying cell number and viability over time.

In vivo degradation of the most promising degradable material was determined by implanting Cy5-coupled hydrogels subcutaneously into the backs of C57/B16 mice and measuring their fluorescence signal over 8 weeks. Additionally, histological stainings were performed at week 8 to evaluate cell infiltration and fibrous capsule formation.

Outlook:
As these crosslinking chemistries are orthogonal, they can be combined, resulting in dual crosslinked materials. Furthermore, because the thiol-ene reaction requires initiation by UV, the precise location and timing of this step can be controlled via photomasks to generate patterns. By taking advantage of this feature, we engineered and confirmed patterns in mechanical properties and biomolecule presentation by nanoindentation surface mapping and visualization of selective cell attachment, respectively. This biomaterial-based strategy allowing control over degradability in addition to biophysical and biochemical patterning could direct cell behavior and support guided tissue regeneration.

Acknowledgements:
This work was funded by the DFG Grant CI203/1-2.
**01-P396** Design of a novel microfeatured poly (glycerol sebacate) methacrylate (PGSM) scaffolds for corneal regeneration

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Limbal epithelial stem cell (LESCs) culture has reached the point of development that allows its adequate use in tissue engineering. However, the survival of the cells after transplantation in host tissue has not been successful. The main challenge is the development of an efficient carrier that delivers cells to specific sites and ensures their survival. Previous biomaterials used in corneal regeneration lack a proper limbal stem cell (niche) microenvironment that can offer physical protection to stem cells. The microenvironment improves cell survival after transplantation and mimics the characteristics of the corneal limbus.

We have designed and produced a novel microfeatured poly(glycerol sebacate) methacrylate (PGSM) implantable outer rings with well-defined niches, whose 3D shape can be modified as required. This is the first report of microfeatured PGSM scaffolds for corneal regeneration. The stem cell pockets are expected to improve healing of the cornea through re-epithelization, increase survival through physical protection, and efficiently deliver stem cells to their destination.

Synthesis of PGSM is carried out by the polycondensation of sebacic acid and glycerol. Methacrylate groups have been added to the PGS molecule to obtain an additional level of control over its strength, degradation, crosslinking density and elongation. These groups also allow UV curing, avoiding polymer degradation in heat-based curing. Two variations PGSM scaffolds were synthesized, each with different methacrylation percentages: 50% methacrylated PGSM high internal phase emulsions (HPIEs), and 30% methacrylated transparent PGSM scaffolds. FTIR analysis confirmed the addition of methacrylate groups, and GPC analysis determined an average molecular weight of 20,000 Da. The scaffold morphology was observed through SEM and confocal light microscopy.

PGSM shows high biocompatibility, tuneable degradation, mechanical and physical surface properties, making it an ideal candidate for soft-tissue applications. Previous results obtained from our research group suggest that these microfeatured PGSM-based scaffolds will improve limbal stem cell survival after implantation in the host tissue.

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**01-P397** Evaluation of Stem Cell Behavior in Different Alginate Hydrogel Consistencies

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Alginate and mesenchymal stem cells (MSCs) are studied today due to their potential applications in regenerative medicine. The aim of this research has been to investigate which alginate and calcium chloride concentrations are suitable for MSCs as a strategy in regenerative medicine. MSCs (400,000/well) were mixed to 1% alginate (w/v) and treated with different concentrations of calcium chloride (0; 25; 50; 75 and 100mM) to reticulate the biomaterial. As control, the cells were cultivated in the tissue culture plate. In a further experiment, the cells were mixed to 0.5% and 1% alginate and 50mM of calcium chloride were added. Finally, the MSCs, in a concentration of 100,000, 200,000 and 400,000 cells per well were added to 1% alginate and treated with 50mM calcium chloride. The experiments were performed with MSCs obtained from human exfoliated deciduous teeth; cell viability was assessed via MTT assay and visualized with fluorescence microscopy after fluorescein diacetate and propidium iodide staining. To evaluate the cytotoxicity, the lactate dehydrogenase enzyme and free calcium was quantified. After seven days there was no significant statistical difference between the scaffolds with 1% alginate crosslinked with 50mM calcium and the 100,000 cells and the control wells. However, in higher concentrations (especially 400,000 cells/well) cell viability was superior in the scaffolds. Furthermore, free calcium concentrations remained constant and LDH dosages corroborated with the data above. After seven days, the average absorbances of the 400,000 cells in 1% alginate scaffolds with 25 and 50mM calcium were superior to the control; however, the experiments have demonstrated that 25mM was not sufficient to completely reticulate the alginate, giving rise to soft gels. Furthermore, the 1% alginate scaffolds showed better results in terms of viability when compared to the control and the 0.5% alginate generated very malleable scaffolds, leading to the loss of a number of cells during the manipulations. These results suggest that alginate provides a three-dimensional microenvironment, which seems to favor survival of big quantities of cells, while the same amount of cells cultivated in the tissue culture plate may not have the same conditions. The 1% alginate and 50mM calcium chloride demonstrated the best results in mimicking, the characteristics of a natural extracellular matrix.

Financial support: MCTI, FINEP, CNPq and Stem Cell Research Institute.
**01-P398  Application of carbon nanotubes/silver (CNTs/Ag) – carboxymethylcellulose (CMC) films for differentiating embryo-derived stem cells into neuron-like cells**

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With the development of nanotechnology, carbon nanotubes (CNTs) that have been used industrially can be used for stem cell differentiation. In this study, electrically conductive carbon nanotubes/Ag (CNTs/Ag) with carboxymethyl cellulose (CMC) was developed to neuronal differentiation of embryo-derived stem cells. CNTs/Ag is multifunctional and widely available as nanostructures with electrical conductivity. Although CNTs/Ag was not well dispersed, it can be dispersed by using the viscosity of CMC. We made a conductive films based on the use of sodium CMC to CNTs/Ag in water. Those properties were analyzed by scan electron microscope (SEM), transmission electron microscopy (TEM), energy-dispersive X-ray spectroscopy (EDX), and conductivity measurement. After CNTs/Ag-CMC films were made on cover glass, embryo-derived stem cells (EDSCs) were differentiated. EDSCs could exchange current between cells by CNTs/Ag after an electronic shock. EDSCs grown on CNTs / Ag-CMC films became neuron-like cells and could be identified by western-blot, RT-PCR, and immunofluorescence.

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**01-P399  Effect of Bioengineered Three-Dimensional Hyaluronic Acid Hydrogels on the Signaling Pathways during Mesenchymal Stem Cell Chondrogenesis**

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To design a novel stem cell niche for cartilage tissue engineering, many researchers have tried to investigate how cells interpret microenvironmental signals. Yet most of these signaling studies have been performed in 2D environments. Although 2D in vitro studies are useful to explain general principles about the regulation of cellular signaling pathways by biochemical and biophysical cues, they do not recapitulate phenomena in 3D microenvironments. It is not clear whether regulation of TGF-β3/Smad and MAPK signaling pathways during chondrogenesis in 3D microenvironments are comparable with those in 2D environments. In this study, we prepared cytopatible and photocrosslinkable TGF-β3 conjugated hyaluronic acid (HA) hydrogel system, and then compared 2D and 3D TGF-β3/Smad and MAPK signaling pathways in human adipose derived stem cells (hASCs) using tissue culture polystyrene plates for 2D and HA hydrogels for 3D. We found that chondrogenesis in 3D was enhanced compared to 2D and phosphorylation of Smad2 and ERK increased with TGF-β3 treatment and phosphorylation of p38 and JNK increased when hASCs were encapsulated in HA hydrogels. Taken together, these results showed that TGF-β3/Smad and ERK signaling was dependent on the addition of the biochemical cue, TGF-β3, while MAPK signaling of p38 and JNK was dependent on the biophysical cue, dimensionality. Additionally, our results suggest that the interplay of biochemical and biophysical factors on cellular signaling pathways should be considered when designing novel biomaterials for tissue engineering.
A screening platform to discover novel cell-adhesion peptides for 3D cell culture applications

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Cell-adhesion peptides, inspired by natural extracellular matrix (ECM) macromolecules, have had a tremendous impact on the design of 3D cell culture platforms for the mimicry of mammalian tissues. Healthy and diseased tissues exhibit considerable differences in their extracellular composition. New technology offers the ability to 3D print titanium implants with potential to design a broad range of new surface topographies that accelerate osseointegration processes. The aim of this study was to assess the need for nitric acid treatment of a standard 3D printed titanium surface with respect to promoting osteoblast differentiation.

This work presents a platform inspired by high-throughput screening techniques used for drug discovery, to identify the characteristics of untested cell-adhesion peptides. We demonstrate that with this approach, we can screen libraries of cell-adhesion peptides in 3D hydrogels and identify new peptides relevant for cell adhesion and migration. These results are expected to promote the use of a broader spectrum of cell-adhesion peptides, which in turn could lead to improved cell culture models, implants and wound dressings.
Pre-clinical Evaluation of Novel Mucoadhesive Bilayer Patches for Local Delivery of Clobetasol-17-Propionate to the Oral Mucosa

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Oral lichen planus (OLP) and recurrent aphthous stomatitis (RAS) are chronic inflammatory conditions often characterised by erosive and/or painful oral lesions that have a considerable impact on quality of life. Current treatment often necessitates the use of steroids in the form of mouthwashes, creams or ointments but these are often ineffective due to inadequate drug contact times with the lesion. Here we evaluate the performance of novel bilayer mucoadhesive patches for uni-directional delivery of the steroid Clobetasol-17-propionate to the oral mucosa.

Electrospun polymeric patches with an impermeable backing layer and mucoadhesive drug delivery layer were produced and characterised for their physical properties in the laboratory. The drug release profile, drug penetration and cytotoxicity of the system in delivering Clobetasol-17-propionate was evaluated using ex-vivo porcine oral mucosa and tissue engineered human oral mucosa. The ability of the system to deliver Clobetasol-17-propionate effectively into the oral mucosa, and local and systemic drug safety, was then confirmed in in vivo mini-pig studies before evaluation of residence time and acceptability of the drug delivery system in a human volunteer study.

Clobetasol-17-propionate incorporated into the patches was released in a sustained manner in both tissue-engineered oral mucosa and ex vivo porcine mucosa. Clobetasol-17-propionate-loaded patches were further evaluated for residence time and drug release in an in-vivo animal model and demonstrated prolonged adhesion and drug release at therapeutic-relevant doses and time points without local or systemic toxicity. Human studies confirmed long adhesion (residence) times and high levels of patient acceptability for use of the oral adhesive patches in the treatment of oral mucosal disease.

In summary, these data show that electrospun patches are adherent to mucosal tissue without causing tissue damage, and can successfully be loaded with and release Clobetasol-17-propionate and other clinically active drugs into the oral mucosa in a sustained therapeutic manner. These patches hold great promise for improving the treatment of OLP, RAS and other immunoinflammatory oral diseases and are ready to enter phase 3 clinical trials.

Current Challenges and Future Opportunities for Biomaterials In Tissue Engineering and Regenerative Medicine

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Currently design and development of novel biomaterials has reached a “steady state” plateau. Pertinent developments have been either incremental or “refinements” of existing biomaterials (e.g., those approved by federal regulatory agencies). Recent successful developments incorporated information from novel, seminal, and pertinent advances in the biological/physiological/medical fields to biomaterials design, synthesis and formulation. Continuation of such trends are inevitable because the biomedical scientific frontiers are untapped sources of inspiration leading to novel biomaterial-related developments. Such approaches require close attention to advances at the tissue, organ, molecular, cellular and gene levels, keeping track of clinical needs and developments in the frontiers of medicine, as well as creative translation and application of the latest developments in the aforementioned biomedical fields to biomaterial designs and formulations pertinent to applications in tissue engineering and regenerative medicine.

How can the biomaterials field meet these challenges and benefit from the new scientific advances and opportunities? Pertinent approaches include (but are not limited to) the following: (1) investigation, development, and application of novel approaches to biomaterial design, synthesis, and formulation; (2) expanding the training of the next generations of biomaterialists to include a broader immersion in the basic biomedical/physiological sciences; (3) interdisciplinary collaborations among scientists, clinicians, and engineers to bring together knowledge from medicine, cell biology, molecular biochemistry, physiology, biomedical engineering and other developing biomedical fields and thus provide inspiration and novel ideas for future biomaterial-related applications.

Undoubtedly, the biomaterials field is presently in the crossroads but still has the potential for future developments, success, and contributions.
Tissue engineered biphasic silk fibroin scaffolds improve tendon-to-bone healing in a patellar tendon model

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Tissue engineering is an attractive strategy for tendon-to-bone interface repair. The structure and extracellular matrix composition of the interface are complex and allow for a gradual mechanical stress transfer between tendons and bone. Thus, scaffolds mimicking the structural features of the native interface may be able to support functional tissue regeneration. In this study, we fabricated 2 different ways, biphasic silk fibroin scaffolds designed to mimic the gradient in collagen molecule alignment present at the interface. The scaffolds had two different pore alignments: anisotropic at the tendon side and isotropic at the bone side. Total porosity ranged from 50 to 80% and the majority of pores (80-90%) were <100-300 µm. Young's modulus varied from 689 to 1322 kPa depending on the type of construct. In addition, human adipose-derived mesenchymal stem cells were cultured on the scaffolds to evaluate the effect of pore morphology on cell proliferation and gene expression. Biphasic scaffolds supported cell attachment and influenced cytoskeleton organization depending on pore alignment. In addition, the gene expression of tendon, enthesis and cartilage markers significantly changed depending on pore alignment in each region of the scaffolds.

Subsequently, we administered these scaffolds in a patellar tendon enthesis defect model in the rat. As controls, collagen scaffolds and empty defects were used. The animals (n=12 per group) were observed for 4 and 12 weeks by X-ray, µCT, histology, biomechanics and qRT-PCR. No failure in any of the scaffold groups occurred. The empty groups and the collagen control showed ossifications in the tendon region, whereas the biphasic scaffolds did not. Histology and qPCR indeed confirmed tendon regeneration in the tendon part of the biphasic scaffolds. Furthermore, biomechanic testing showed superior results of the biphasic scaffolds over the control groups. The mechanical parameters were close to those of native patellar tendon enthesis.

In conclusion, the biphasic scaffolds fabricated in this study show promising features for tendon-to-bone tissue engineering.

An Antibiotic-Eluting Scaffold with a Microbially Induced Dual-Release Mechanism for the Treatment of Osteomyelitis

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Introduction

Osteomyelitis (OM) is a progressive, inflammatory infection of bone caused by infectious microorganisms, predominately Staphylococcus Aureus (S. Aureus). The standard treatment for chronic OM, which involves surgical debridement of the infected bone and systemic antibiotic administration, often fails to fully eradicate the chronic and, furthermore, contains no component to promote regeneration of the bone. Therefore, there is an unmet clinical need for a therapy that combines a local delivery of antibiotic to aid bacterial clearance with a biomaterial capable of promoting bone regeneration. The objective of this study was to fabricate a biodegradable scaffold capable of delivering an antibiotic in a dual-release (DR) manner, with a burst-release (BR) to eliminate bacteria and a controlled-release (CR) to prevent reoccurrence of infection. We hypothesised that this DR scaffold, when implanted, would have the capacity to treat infection and regenerate bone in a murine model of OM.

Methods

Vancomycin was incorporated into separate BR and CR collagen/hydroxyapatite scaffolds (*formulations not disclosed due to IP restrictions), and the release kinetics of the two scaffolds were determined. The separate scaffolds were then combined into a single DR scaffold using a layering technique [1] and the bactericidal capacity of this scaffold was tested against S. Aureus in vitro. Uni-cortical defects were created in the tibiae of C57BL6 mice and inoculated with S. Aureus. After 2 weeks, the infected sites were debrided and either left empty or treated with the DR scaffold.

Results & Discussion

BR scaffolds released ~85% of the incorporated antibiotic in 2 days, whereas, as desired, CR scaffolds released the antibiotic at much slower rate. Interestingly, when microbial collagenase was added to the CR scaffold, a spike in antibiotic release was observed demonstrating that the scaffold is responsive to microbial activity and will elute its antibiotic when most needed. When BR and CR scaffolds were combined into a DR scaffold, this scaffold was found to be bactericidal in vitro. Most importantly, the DR scaffold reduced bacterial levels in vivo, and demonstrated successful bone regeneration indicating that the scaffold may present a novel treatment option for OM.

References


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Material-driven fibronectin nanonetworks rescue collagen IV secretion in mutant cells

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Mutations in the extracellular matrix (ECM) protein collagen IV (col IV) have been implicated in a broad spectrum of vascular diseases, including intracerebral haemorrhaging. Indeed, col IV is a major component of the specialised ECM structures that underlie all endothelial and epithelial cells - the basement membrane (BM) - providing them with structural support and signalling cues. Mutations in the genes COL4A1/COL4A2 cause defects in the BM, via the incorporation of the mutant protein or its absence due to retention and accumulation in the endoplasmic reticulum (ER stress).

In this study, we aim to provide novel insights into the mechanisms of col IV diseases by investigating the behaviour of cells with a COL4A2 mutation, COL4A2G702D, mutant fibroblasts, using defined engineered microenvironments composed of synthetic polymers - poly(methyl acrylate), PMA, and poly(ethyl acrylate), PEA - which are able to organise the assembly of ECM proteins - laminin, col IV and fibronectin (FN) - upon simple adsorption. On PEA, FN was adsorbed in the form of interconnected nanofibrils, and these nanonetworks were revealed to induce increased secretion of col IV when mutant cells were cultured onto them compared to PMA, where adsorbed FN maintained a globular conformation. Increased levels of molecular chaperones and reduced ER area suggested an increased collagen IV folding capacity on PEA. Enhanced formation of focal adhesions was also seen on FN-coated polymers, where ligand density and actin-myosin contractility accounted for the observed increase in cell adhesion strength. Most interestingly, the protein matrix deposited by mutant cells cultured on the FN nanonetworks showed a significantly higher Young modulus (~7.5 to 18 kPa) than on PMA (~4.3 to 11.5 kPa), as measured by atomic force spectroscopy.

Collectively, these findings provide a basis of concept that materials, through changes in the interfacial layer of adsorbed proteins, may be employed to modulate effects of col IV mutations. Understanding the mechanisms through which these surfaces rescue col IV secretion of mutant cells will prove valuable for the development of new therapeutic approaches to address pathologies due to these mutations.

References:

Acknowledgments:
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A human-derived biomaterial for the automated production of 3D skin models

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Biological scaffold materials derived from decellularized extracellular matrix (ECM) have gained increasing interest because of their ability to mimic the natural environment of cells by supporting the adhesion, proliferation and differentiation. The majority of decellularized tissues so far have been derived from animals or isolated from cadavers. However, these sources of decellularized tissues cannot be standardized. All animal-derived products additionally have the potential to cause immunologic reactions. Therefore, ECM derived from cultured human cell sheets could have considerable merit for medical treatment and disease modelling.

Our group has developed a device for an automated production of bilayered tissue models for drug and chemical testing. However, apart from the automation, a standardized biomaterial is essential for a highly reproducible production of 3D tissue models. Herein we present an acellular ECM-based biomaterial derived from human fibroblast tissue sheets. We use the capability of primary fibroblasts to produce their own ECM under defined conditions. The resulting tissue sheets are further decellularized and processed into an injectable biomaterial. Combined with the cell suspensions it can be further polymerized at physiological temperature. The final skin model shares many properties with native human skin and provides high mechanical stability. This approach allows the usage of different cell lines that secret ECM in various quality, providing a different composition of extracellular matrix proteins. Therefore, customized disease models can be produced in a reproducible manner. The herein described technique thus allows a standardized one-step production of bilayered tissue models and is a promising alternative to animal testing.
01-P410  Complex Coacervation of Gelatin Methacryloyl and Alginate Facilitates Toughness and Ductility of Bioactive Double-Network Hydrogels for Functional Cartilage Tissue Engineering

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The use of conventional hydrogels for the engineering of load-bearing tissues is severely limited by their brittleness, lack of energy dissipation, and inadequate toughness, resulting in poor mechanical durability and ultimately implant failure. Here, we developed mechanically tough and bioactive double-network hydrogels based on gelatin methacryloyl (GelMA) and alginate. We demonstrate that GelMA/alginate hydrogel precursor solutions undergo entropically-driven liquid-liquid phase separation resulting from local electrostatic interactions of oppositely charged macromolecules, a phenomenon otherwise known as complex coacervation. GelMA/alginate complex coacervate systems are characterised by the formation of a highly concentrated coacervate phase dispersed as droplets within a dilute phase of macromolecules (equilibrium phase), which result in micro-structured coacervate hydrogels upon sequential crosslinking. Our data suggests that a high level of molecular entanglement in the coacervate phase allows for effective energy dissipation in response to loading. This process, which we attributed to reversible biopolymer unfolding and the unzipping of alginate networks, produced a biomaterial with superior ductility, shape recovery, stiffness and toughness. We demonstrate that GelMA/alginate coacervate hydrogels promote chondrogenesis of expanded human chondrocytes and the stabilization of the hydrogel component and improves the mechanical strength of the whole construct. Concomitantly, specific stains suggested the osteoblastic differentiation of hMSCs. The hydrogel precursor solutions also display shear-thinning behaviour, making them suitable for 3D printing applications. Taken together, these results demonstrate that complex coacervation of GelMA and alginate can be used to generate tough and cell-instructive hydrogels for the ex vivo engineering of cartilage tissues with enhanced mechanical functionality.

01-P411  3D Hybrid Structures for the Pre-Vascularization of Bone Tissue Engineered Constructs

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The pre-vascularization of tissue engineered constructs is one of the key aspects for their improved integration and subsequent tissue regeneration. The most common cell-based approach in the field is the mono-culture of endothelial cells (ECs) or their co-culture with other cell types within 3D structures. Among these structures, hydrogels are the ones that more closely resemble the native extracellular matrix, and have shown to support ECs assembly. However, when constructs with superior mechanical properties are needed, hydrogels cannot properly cover all the desired scaffolding features alone. For improved structural properties, their reinforcement with other structures enhances the stabilization of the hydrogel component and improves the mechanical strength of the whole construct. These may also provide mechanical stimuli and spatial guidance to cells. In this work, 3D hybrid scaffolds were developed by combining fibrous scaffolds of poly(ethylene oxide terephthalate)/poly(butylene-terephthalate) (PEOT/PBT) with biofunctionalized pectin hydrogels. These were used as supporting structures for the co-culture of human mesenchymal stromal cells (hMSCs) with human umbilical vein endothelial cells (hUVECs). 3D PEOT/PBT fibrous scaffolds were prepared by spatially depositing polymer filaments obtained by wet-spinning using additive manufacturing, as previously described. Peptide-modified pectin hydrogels were obtained by internal gelation via ionic crosslinking. Both cell types were able to establish intercellular networks within the hybrid structures, with hUVECs being able to establish networks that remained stable for up to 28 days of culture. The alignment and spatial arrangement of the hMSCs around the hUVECs networks suggested a mural cell-like behavior. When hydrogels or fibrous scaffolds were used alone, such 3D organization could not be observed. This indicates the synergistic influence of both structures of the hybrid system on the cellular behavior. Concomitantly, specific stains suggested the osteoblastic differentiation of hMSCs. The in vivo inoculation capacity of the developed hybrid system is currently being evaluated.

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FUNDING
In dentistry, membranes are used for periodontal reconstruction in the process known as guided tissue regeneration (GTR). Over the past decade, resorbable collagen-based membranes have become the standard of care for guided bone regeneration procedures in dental surgeries. In this work a novel acellular small intestine submucosa (ASIS) was designed and fabricated via alkyl polyglucoside (APG) decellularization treatment. The APG-treated ASIS consist of a decellularized tissue matrix layer containing matrix fiber in nanometer scale. In the study, APG-treated ASIS was prepared and evaluated the microstructure, composition, mechanical strength and its function as a barrier membrane. The SEM observation showed the gradient porous changes from top to down and from loose to dense in APG-treated ASIS. Multi-layers gradient structure could be observed at 45-degree tilt. The hydroxyproline content of ASIS membrane was 12.8 ± 0.5% comparing to the purified collagen, 11.2 ± 0.8%, as negative control. The cytotoxicity data showed the cell viability (L929) of ASIS polar and additional vehicle extract were 96.51 ± 9.52% and 99.31 ± 9.66%, respectively, comparing to which of negative control was 100 ± 12.93%. The mechanical strength is generally accepted to be greater than about 200 gf. The testing results of suture pull-out strength of ASIS is 275.58 ± 14.46 gf higher than the suggested strength for clinical suture (>200 gf). In the implantation study, the ASIS membrane maintained its integrity in 4 weeks and resorbable in vivo between 4 and 12 weeks, might suggest the ASIS qualified for being a candidate for the dental membrane. This study suggests that APG-treated ASIS could be a good alternative dental membrane in the application of periodontal surgery.

In cardiac tissue engineering using biomaterials with combination of stem cell offers a new therapeutic option for repairing infarcted heart. We found that human heart valve derived scaffold (hHVS) may provide a cardiac microenvironment for stem cells. Upon anchoring onto the hHVS, post infarct murine BM CD117+ cells exhibited an increased capacity for proliferation and cardiomyogenic differentiation. When used to patch infarcted heart of myocardial infarction, either implantation of the hHVS alone or CD117+ cell seeded hHVS significantly improved cardiac function, and reduced infarct size. In addition, we hypothesized cardiac nature protein (NP), mainly including elastin and collagen, in hybrid PCL electrospun nanofibrous scaffold could be effective as cardiac mimicking scaffold. BM CD117+ seeded on 80% NP/PCL exhibited increased capacity for proliferation and cardiomyogenic differentiation. When used to patch infarcted heart of myocardial infarction, either implantation of the hHVS alone or CD117+ cell seeded hHVS significantly improved cardiac function, and reduced infarct size.

Identifying the optimal effective subpopulation in bone marrow stem cell is required for cell-based therapy for myocardial infarction (MI) repairing. A protective role of AT2R has been shown in tissue repair and regeneration in heart. In our study, we found that the CD117+AT2R+ subset is superior to the CD117+AT2R-subset in improving cardiomyocyte protection and migration capacity in vitro. Additionally, intravenous transplantation of CD117+AT2R+ BMWNC resulted in smaller infarct size and lower levels of inflammatory reactions in heart tissue, leading to a higher whole heart function improvement. In conclusion, the CD117+AT2R+BMWNC subpopulation exerts a protective effect against MI and may show therapeutic possibilities.
Osteoinductive extracellular matrix coated graphene oxide-collagen composite scaffolds for bone regeneration

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Graphene and graphene derivatives, especially graphene oxide(GO) are receiving more and more interests in bone tissue engineering. However, there were worries of biocompatibility and nanocarbon toxicity of GO composite materials. Here, we have investigated a biomimetic composite scaffold by assembling Osteoinductive extracellular matrix(OIECM) derived from BMSCs to GO-Collagen(GO-COL) based 3D scaffold. The morphology, mechanical properties, biocompatibility in vitro and osteogenic performance in vivo were examined. Results revealed that the OIECM contains calcium nodules, fibronectin, Collagen I and BMP-2. The enhanced gene expression also suggest that OIECM could induced the osteogenic potential of BMSCs. Furthermore, OIECM-GO-COL composite scaffold significantly improved the cell viability on the scaffold while it still retained the mechanical properties of GO-COL scaffold. Finally, the OIECM-GO-COL composite scaffold shows excellent reparative effects in rat calvarial defects model through synergistic effects of GO and OIECM. This study demonstrated a new way for constructing biomimetic bone substitutes for better biocompatibility in tissue engineering and regenerative medicine.
01-P416 The transition of extracellular matrix and alpha-gal antigen of rat lung scaffold reseeded by human vascular and adipogenic stromal cells

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Regenerated organs are expected to solve the problem of donor-organ shortage in transplantation medicine. One approach for lung regeneration is to decellularize the organ and reseed it with selected cells. The advantage of the procedure is recognized to reduce immunogenicity because all cells can be replaced by autologous cells theoretically. However, we have little knowledge about extracellular matrix (ECM) damage during the decellularization process and about ECM reconstruction during the organ regeneration process. Objective: The purpose of our research is to evaluate ECM damage and reconstruction in decellularized and recellularized rat lung including xeno-antigen (alpha-gal epitope: Galalpha1-3Galbeta1(3)4GlcNAc-R) removal. Methods: The rat lungs were perfused with sodium dodecyl sulfate (SDS) and Triton-X100 via pulmonary artery, then decellularized scaffold was reseeded with rat or human endothelial cells and adiposed mesenchymal stem cells (ASC). The extracellular matrix and alpha-gal antigen were evaluated with immunohistochemistry, western-blotting assay and glycosaminoglycan assay. Results: Alcian blue staining revealed proteoglycan reproduction was increased by ASC addition to RLMVEC-recellularized rat lung. GAG assay revealed that glycosaminoglycan was decreased in decellularized lung, and increased in recellularized lung especially in ASC added group. Alpha-gal protein expression was immunohistochemically undetectable in decellularized lung tissue. In western blotting analysis, the bands of alpha-gal protein were disappeared after recellularization by human cells. Conclusions: The character of replaced ECM might depend on the species and the type of recellularized cells. Therefore, alpha-gal protein will be eliminated after long time culture using human cells.

01-P417 Nature healing-inspired collagen-binding mussel protein-based hydrogel for scarless wound regeneration

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Skin scarring after deep dermal injuries is a major clinical problem due to the current therapies limited to established scars with poor understanding of healing mechanisms. From investigation of aberrations within the extracellular matrix involved in pathophyslogic scarring, it was revealed that one of the main factors responsible for impaired healing is abnormal collagen reorganization. Here, inspired by the fundamental roles of decorin, a collagen-targeting proteoglycan, in collagen remodeling, we created a scar-preventive collagen-targeting glue consisting of a newly designed collagen-binding mussel adhesive protein and a specific glycosaminoglycan. The collagen-targeting glue specifically bound to type I collagen in a dose-dependent manner and regulated the rate and the degree of fibrillogenesis. In a rat skin excisional model, the collagen-targeting glue successfully accelerated initial wound regeneration as defined by effective reepithelialization, neovascularization, and rapid collagen synthesis. Moreover, the improved dermal collagen architecture was demonstrated by uniform size of collagen fibrils, their regular packing, and a restoration of healthy tissue component. Collectively, our natural healing-inspired collagen-targeting glue may be a promising therapeutic option for improving the healing rate with high-quality and effective scar inhibition.
A Novel Injectable Bone Allogeneic Substitute for Maxillo-facial Skeleton Regenerative Medicine

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In the maxillo-facial area, sequels of traumatisms, diseases or surgery, often lead to bone defects that fail to self-repair. Whereas the gold standard of bone reconstruction remains the autologous bone graft (ABG), it exhibits some drawbacks (low amount of available tissue, complication at the donor site and lengthening of the surgical time). To overcome these limitations, synthetic bone materials and allo- or xenografts are used. Despite their clinical success, these bone substitutes still remain far from having the osteogenic capacity of ABG. In this context, this work aims at developing a new injectable allogeneic bone substitute for maxillo-facial reconstruction.

An injectable allogeneic bone substitute (AlloBS, WO2015162372) has been produced from sifted decellularized cortico-spongious powders (CSP, 0.5mm in diameter) of crushed human femoral heads. After being partially demineralized, the CSP is heated to obtain AlloBS, an injectable scaffold composed of particles consisting in a mineralized core surrounded by demineralized bone matrix, engulfed in a collagen I gelatin. To assess the in vivo ability of AlloBS to support bone repair, we used a guided bone regeneration model in syngeneic Lewis 1A rat calvaria. Briefly, two defects per rat (5mm in diameter, n=6 per condition) were filled with AlloBS or CSP. In a second set of experiment we performed a comparative study with calvarial defects filled with AlloBS or synthetic bone substitute (biphasic calcium phosphate (BCP) granules, 0.5-1mm), associated or not with syngeneic total bone marrow (TBM).

After 7 weeks, the percentage of mineral volume (MV) related to total volume (TV) was measured by µCT and histological analyses were conducted using a Movat’s pentachrome staining. Quantitative analysis by µCT revealed a 1.7 (AlloBS), 1.8 (CSP) fold increase in MV/TV compared with empty defects for the first set of experiments, and 1.9 (BCP), 2.1 (BCP+TBM), 1.7 (AlloBS), 2.4 (AlloBS+TBM) in the second set of experiment. Histological analyses confirmed the presence of a mineralized tissue, exhibiting osteoid and a collagen-rich matrix in all the tested conditions. These data show that AlloBS is a promising candidate for maxillo-facial bone reconstruction with substantial osteogenic capacity as well as injectability and ease of manipulation. To assess whether AlloBS may be a relevant clinical alternative to ABG, further experiments in larger animal models of maxillo-facial bone defects are under consideration.

Electrospun patches containing anti-fungal agents inhibit Candida albicans

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Introduction

Oral candidiasis affects many immunocompromised patients and is difficult to target topically due to rapid drug loss from saliva washing that occurs for creams, adhesive tablets and mouthwashes. Additionally, many candida strains are becoming increasingly resistant to current anti-fungal drugs. Hence, exploring alternative therapies, such as medium-chain fatty acids with known anti-fungal properties, is of great importance. Electrospinning is a versatile manufacturing method which can be used to create a mucoadhesive therapeutic delivery device. This project aimed to develop an electrospun polymer patch for sustained therapeutic delivery of fatty acids to inhibit growth of Candida albicans, the main causative organism of candidiasis.

Methods

The anti-fungal ability of fatty acids (C5:0 to C12:0) were tested on azole-resistant and sensitive C. albicans strains. Tests include the agar disc diffusion method to measure inhibition of C. albicans growth around fatty acid-impregnated filter discs, as well as the inhibition of an electrospun PCL patches containing fatty acids using an XTT assay. The fatty acids were electrospun into a poly (ε-caprolactone) (PCL) (10 w/w%) mats. Electrospun patches were punched from the spun mats and the previously mentioned tests repeated for these patches.

Results

In their yeast form both azole-resistant and sensitive C. albicans strains were significantly inhibited by ocatnoic acid (C8:0) on filter discs and nonanoic acid (C9:0) when incorporated in electrospun patches. For biofilm C. albicans, the electrospun PCL patches containing C9:0 to C12:0 showed significant inhibition, with lauric acid (C12:0) showing the best inhibition consistently.

Conclusion

Fatty acids (C7:0 to C12:0) successfully inhibited growth of both azole-resistant and sensitive strains of C. albicans, both in its yeast and biofilm states. Furthermore, the electrospun patch was able to act as a delivery device for the release of fatty acid as a therapeutic agent to inhibit C. albicans.

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Breast reconstruction surgery recently has been increasing after mastectomy of breast cancer patients. However, PDMS implants, which are commonly used, have a problem of capsular contracture due to immune reaction in vivo, hence synthetic polymer filler for breast reconstruction are increasing. The synthetic products generally produce long-term results because they have lower degradation time in vivo than naturally-derived filler, and they are also categorized more further by permanent or semi-permanent substances. Injectable liquid silicone, poly (methyl methacrylate) and polyimide are examples of such a product respectively. But they are associated with a high incidence of side effects.

This paper 2-hydroxethylmethacrylate (HEMA) based-polymer hydrogel was synthesized by redox polymerization to be used as biocompatible material. As redox polymerization proceeded, the solution was generated heat, resulting in a gel. Notably, the hydrogel swelling properties and confirmed the hydrogel has modulus similar to the modulus of standard dermal filler. To evaluate cytotoxicity of hydrogel samples, human Fibroblast and human ADSCs were isolated from human adipose tissue and incubated with hydrogel eluate for 72h and 120h. The result was confirmed that cells were viable. Our findings offers new approach to the synthesis of polymeric filler to be used in soft tissue reconstruction application, such as in breast reconstructive surgery. We developed synthetic filling materials for breast reconstruction by using HEMA based polymer hydrogel. The HEMA based polymer hydrogel filler is non-degradable, and therefore can retain breast shape permanently. The polymer hydrogel synthesized by Redox polymerization and confirmed chemical structure of copolymer by conducting FT-IR and NMR. By controlling the polymer hydrogel ratio, viscosity and degradability of the filler can be specified in vivo. By cytotoxicity assay and live&dead images, we confirmed that the HEMA based polymer hydrogel is safety and biocompatibility materials to cell. To the best of our knowledge, we suggest HEMA based polymer hydrogel filler has stable structures in vivo and biocompatibility for large scale permanent injectable filler for breast reconstruction.

Peripheral nerve injuries (PNI) are associated with poor neuron regeneration capacity, which can potentially be improved through pharmaceutical therapies. Currently no drug treatments are routinely used clinically to improve recovery, despite identified drug candidates demonstrating positive effects on nerve regeneration, including ibuprofen [1]. In past decades, local controlled-release drug delivery systems have become more attractive because of the drawbacks in conventional drug treatments [2]. Local delivery could potentially improve clinical outcomes due to the increased range of doses that can be given and the reduction in side effects. In the case of a nerve transection injury where a biomaterial conduit would be used to bridge a gap then the material could be developed to also release a drug, providing a multiaxial therapy combining structural support and local drug treatment. Many biocompatible materials such as ethylene-vinyl acetate (EVA) are used for an extensive range of clinical applications involving long-term drug release.

This study aimed to characterise and test drug release from various biomaterials in order to obtain an optimal material for implantation and sustained drug delivery for PNI. Ibuprofen-loaded biomaterial formulations were tested using UV-vis spectroscopy to determine drug release profiles in vitro. EVA tubular conduits displayed an optimal drug release profile and so were implanted into a rat sciatic nerve transection model as a cuff around a primary repair.

The results indicated that manipulation of material geometry and drug loading improved the sustained released of ibuprofen from EVA. Furthermore, the in vivo model indicated that EVA was suitable for implantation at the injury site and ibuprofen released from this material caused a significant increase in neuronal growth in comparison to an unloaded EVA tube conduit. In conclusion, this study showed for the first time that local delivery of ibuprofen improves nerve repair and provides the basis for future development of drug-loaded biomaterials suitable for clinical translation to treat PNI.

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**01-P424**

**A selenium nanoparticle - calcium phosphate coating system on scaffolds for antimicrobial, bone regeneration applications**

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Bacterial infection is a common and serious complication following implantation of a medical device or scaffold. This issue is particularly in orthopaedic surgeries following traumatic injuries, which are often associated with heavy contamination. Antibiotic therapy remains the standard treatment but often requires very high doses due to the protective effects of bacterial biofilms that can form on implanted material surface. In addition, with increases in the number of drug resistant bacteria, advanced antimicrobial therapies are required for better patient outcomes. Here we demonstrated that a coating system comprising nanoparticles of the trace element selenium (Se) and calcium phosphate (CaP) material on polycaprolactone scaffolds which could inhibit growth of *Staphylococcus aureus* and reduce biofilm formation in vitro and promote bone growth in a calvaria defect model in rats. The coating process is based on wet chemistry which has great potential to apply to a wide range of surfaces. In vitro bacterial assays showed significant reduction of planktonic bacteria growth as well as bacterial biofilm formation on the coating with Se nanoparticles and CaP compared to coating with CaP only. In vivo experiments showed a slightly higher (p<0.05) new bone formation on the coating with Se and CaP. This study thus demonstrates that this versatile anti-infective coating could be beneficial to improve treatment outcomes of patients receiving scaffolds or implants for bone tissue.
A Thermogelling Hyaluronic Acid Vaginal Stent to Reduce Postoperative Vaginal Scarring

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Up to 50,000 girls and 213,000 adult women yearly in the United States require vaginal reconstructive surgery. There is a high rate of complication associated with these surgeries, such as vaginal tissue scarring, which occurs in 73% of patients. Sequelae from vaginal fibrosis can be life-long and lead to significant reductions in quality of life. Currently, there is no standard of care to reduce vaginal fibrosis after surgical reconstruction. To manage vaginal scar tissue, patients must perform vaginal dilatation multiple times per week leading to inconvenience, pain, bleeding, and other more serious complications such as vaginal perforation. Advances are being made in the surgical theatre, but techniques and therapeutics to enhance postoperative vaginal wound healing are limited. Patients undergoing vaginal dilatation are concurrently prescribed conjugated equine estrogen cream (CEE) to promote tissue healing and reduce vaginal fibrosis. We have developed a thermogelling norbornene-functionalized hyaluronic acid (NorbHA) vaginal stent that can be used postoperatively. Our dissolvable stent delivers estradiol (E2), interleukin 10 (IL-10), or a combination of both in a temporally staggered release system. The vaginal stents were placed into a murine vaginal injury model for 21 days. Results were analyzed after 3, 5, 10, and 21 days post-injury and comparisons were made between the CEE cream and vaginal stent. Macrophage inhibiting factor 1 (MIF1) and transforming growth factor-β (TGFβ) expression were used as markers of inflammation during vaginal wound healing. Vascular endothelial growth factor (VEGF) expression was used as a marker of angiogenesis. Histology and immunohistochemistry were used to assess wound resolution and estrogen receptor (ER) density. A statistically significant decrease in MIF1 expression was found in the group containing both IL-10 and E2 compared to the CEE cream. Greater expression of TGFβ and VEGF were found in the groups containing E2, or E2 and IL-10 compared to the estrogen cream. ER density increases with E2 delivery, but appears to be dependent on circulating estrogen levels.

Mechanical Strength, Biodegradation, and In Vitro and In Vivo Biocompatibility of Zn-Based Biomaterials for Cardiovascular and Orthopedic Applications

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Zn-based biomaterials have emerged as new types of biodegradable metallic materials with great potential for orthopedic devices, cardiovascular stents, and other medical applications recently. Compared to other degradable metallic biomaterials (Mg- or Fe-based), Zn biomaterials have a more appropriate corrosion rate without hydrogen gas evolution. However, research on Zn materials is still largely missing in the literature. Here, we evaluated the potential of Zn-based biomaterials as medical implants, both in vitro and in vivo, against a typical Mg alloy AZ31 as a comparative benchmark control. We found that the mechanical properties of Zn biomaterials were significantly enhanced (microhardness >70 kg/mm², strength >220 MPa, elongation >15%) after alloying with Sr or Mg, surpassing the minimal design criteria for load-bearing device applications. The corrosion rate of Zn-based biomaterials was about 0.4 mg/cm²/d, significantly slower than that of AZ31. The measured cell viability and proliferation of three different human primary cells fared better for Zn-based biomaterials than AZ31 using both direct and indirect culture methods. Platelet adhesion and activation on Zn-based materials was minimal, significantly less than on AZ31. Hemolysis ratio of red cells (<0.5%) after incubation with Zn-based materials was also well below the ISO standard of 5%. Moreover, Zn-based biomaterials promoted stem cell differentiation to induce the ECM mineralization process. In addition, in vivo animal testing using subcutaneous, bone, and vascular implants revealed that the toxicity and immune response of Zn-based biomaterials were minimal/moderate comparable to that of AZ31. No extensive cell death and foreign body reactions were observed, and they promoted local tissue regeneration. Taken together, Zn-based biomaterials may have a great potential as promising candidates for medical implants.
**01-P427 Preparation of biodegradable hydrogels of in situ gelation for cell transplantation**

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Cell transplantation is one of the promising therapies for regenerative medicine. Generally, the direct injection of cells has been mainly carried out. However, the poor in vivo retention of cells and their low functions remain unresolved. Therefore, it is necessary to create the technology and methodology for an enhanced cell retention and function in vivo. Several injectable hydrogels have been reported. In most researches, chemical crosslinking is used to form hydrogels. Since the hydrogel formed does not degrade synchronizing with the cells proliferation, the cells transplanted do not always survive nor function efficiently in the body. As one trial to tackle the issue, biodegradable hydrogels of in situ gelation need to be designed. In this study, biodegradable hydrogels of in situ gelation, are created by making use of reversible “metal-polymer interaction” which has a reversible property of crosslinking. As the polymer, biodegradable gelatin with a high affinity for cells, was selected. Simple mixing with iron chloride(III) (FeCl₃) allowed gelatin to form a biodegradable hydrogel. When the gelatin solution in phosphate buffer saline solution(PBS) was mixed with various concentrations of FeCl₃ in PBS solution, the hydrogels were formed although the extent enhanced with an increase in the FeCl₃ concentration. The resulting hydrogels were degraded to disappear in PBS solution at 37 °C. The hydrogel cytotoxicity depended on the FeCl₃ concentration. Upon mixing with the FeCl₃ /gelatin solution, cells were encapsulated in the hydrogels formed. We also report the in vivo fate of cells after injection of cells-hydrogel mixture.
**01-P429** Multifunctional protein microdevice designed to cope with excess reactive oxygen species

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Micro- and nano-scale intelligent medical devices can revolutionize the prevention and treatment of diseases. Although proteins are promising materials for creating biocompatible devices with biological functions, construction of complicated solid-state architectures using proteins, a vulnerable natural material, remains a key challenge in materials research. In this study, I developed a sophisticated strategy for constructing a multifunctional microdevice for medical applications using multiple proteins; this strategy achieved the retention of function, increased stability, and orientation control of the proteins in the fabricated device [1]. As proof-of-concept, the device, designed to cope with excess reactive oxygen species (ROS) involved in many diseases, was constructed by combining three proteins with different functions. The body of the device was fabricated using albumin and superoxide dismutase (SOD), and the antibody was incorporated into the surface of the device in an orientation-controlled manner. The constructed protein microdevice, which was approximately 100 µm in diameter and of nano-scale thickness, exhibited coordinated activities for coping with ROS, such as capture of the ROS-secreting cells by using an incorporated antibody, followed by the elimination of 70% ROS secreted from the captured cells by the action of SOD in the device. Diapocynin, loaded to the device via the drug-binding ability of albumin, was released from the device, preventing ROS production in the cells. This multifunctional microdevice, constructed from proteins, can have a profound impact on the creation of intelligent protein-based miniature devices used in medical fields.


**01-P430** Development for the macro-encapsulating device for isolation of immune response which is made of Ethylene vinyl alcohol

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For the cell transplantation research and treatment, the method how can control the immune response, is important key technology for engraftment of donor cells. In current study, we have developed the complex device with multi-pore membrane of ethylene vinyl alcohol (EVOH) and hydro gel (hereinafter defined as “EVOH device”).

“EVOH device” can block the attack of immune response cells for the protection of donor cells.

We examined the permeability rate of several proteins (Insulin, Albumin, immunoglobulin G, etc.) by using “EVOH device”. We also examined the foreign body reaction in rat by intraperitoneal and subcutaneous implantation of “EVOH device”. It was indicated that no significant granulation developed around the device 6 moths after implantation.

Furthermore we are evaluating and will report the efficacy of “EVOH device” by in VITRO and in VIVO immune response models.

We expect that “EVOH device” is valuable and unique macro-encapsulation device for cell transplantation, for example the treatment of diabetes, and several hormone deficiencies.
Graphene coated 3-dimensional conductive mat as a neural electrode

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An inflammatory mismatch between the implant and brain tissue is the major reason behind the failure of current commercialised neural electrodes. These electrodes are made of stainless steel, crystalline silicon or metals whose stiffness induces inflammation and the formation of a glial scar. The scar forms a barrier between the neurons and the implanted electrodes that reduces their durability. To solve this problem, we constructed a 3-dimensional electrode with a stiffness modulus that is similar to that of brain tissue, and with topography and electrical properties that can support communication between neurons. A graphene coated 3-dimensional nylon mat with a tuneable electrical property has been fabricated. Graphene is a conductive material, and thus provides the electrical property to the fibrous mat. Mats were coated with graphene using a vacuum filtration technique followed by the reduction using hydroiodic acid. Graphene coated mats were characterised for their electrical properties and suitability as a biomaterial. Primary cortical neurons from rat pups during late pregnancy were cultured on graphene coated nylon mats and function was tested using calcium imaging, live/dead assay and immunostaining. The results showed that graphene coated nylon mats are biocompatible with neurons and provide a platform for the attachment, proliferation and axonal extension of primary neurons. Moreover, in-vivo tests were performed by placing coated mats on the brain, securing them in place. Electrical activity recorded week later in free-moving rats demonstrated the suitability of these conductive mats as electrodes for neural stimulation and recording.

Fabrication of calcium phosphate-loaded carboxymethyl cellulose non-woven sheets for bone regeneration

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Bone tissue engineering is highly anticipated for numerous clinical applications. The scaffold is one of the most important elements in tissue engineering by securing space for tissue formation whilst allowing cells to migrate, adhere and proliferate. At present, many types of synthetic bone replacement grafts are used as void-filler scaffolds to promote bone regeneration in bone defects during orthopedic, spinal, and dental surgeries. According to previous studies, carboxymethylcellulose (CMC) promoted osteogenic differentiation of human bone marrow stromal cells (hBMSCs). Taken together with its high biocompatibility, it is considered that CMC is likely to promote the healing of bones. In this research, we developed a new hybrid CMC nonwoven sheet whose pores are filled with calcium phosphate (CaP) by using alternately soaking process. The potential of CMC/CaP sheets to induce in vitro osteoblast differentiation and in vivo bone regeneration were investigated. In vitro, the CMC/CaP sheet induced osteoblast differentiation of human mesenchymal stromal cells (hMSCs), as shown by calcification and the upregulation of osteoblast marker genes. In absence of CaP, hMSCs on the CMC sheet had enhanced expression of alkaline phosphatase (ALP) only, indicative of early osteoblast differentiation. In vivo, bone regeneration by the CMC/CaP sheet was demonstrated in a mouse calvarial defect model, based on micro-computed tomography (micro-CT), Masson’s trichrome staining, and immunostaining for osteoblast markers. Cells expressing the transcription factor Sp7/Osterix, which is essential for osteoblast differentiation, were detected around the new bone. The combined effect of CMC and CaP enhanced osteoblast differentiation and the CMC/CaP non-woven sheet was found to be an easy-to-handle and flexible scaffold for bone regeneration.
01-P433  Anti-inflammatory and osteogenic effects on PLGA/Inorganic composites for orthopedic applications

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PLGA has been widely used as biomaterials for the pharmaceutical and medical applications. However, the decomposition products of the PLGA are known to form an acidic environment and cause inflammation at the site of implantation. Moreover, it is hoped that Applications of PLGA coated polymer-based for 12 h can improve the mechanical properties of the PLGA material and Mg(OH)₂ significantly improved mechanical strength and showed pH neutralization effect. The superior osteogenic differentiation of normal female human osteoblast (NHOst) cells on the surface of PLGA/β-TCP/Mg(OH)₂ was confirmed by ALP assay, von Kossa and alizarin S stainings, and q-PCR. The cytotoxicity and anti-inflammation were evaluated by CCK-8 assay and ELISA for IL-6 and TNF-α. These results suggest that the use of β-TCP and Mg(OH)₂ as additives in biodegradable polymer-based orthopedic materials has great potential for improving their biocompatibility and biological functionality.

KEYWORDS:
Poly(l-lactide-co-glycolide) (PLGA); beta-tricalcium phosphate (β-TCP); magnesium hydroxide (Mg(OH)₂); osteogenesis; anti-inflammation

01-P434  Bio-devices for Hard Tissue Regeneration

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Hard tissue regeneration is an evolving discipline that applies tissue engineering principles to construct biological devices from natural or synthetic sources. In this particular field, there is a great thrust to develop bioactive devices that enable early discernment of critical bone defects and simultaneously promote bone formation and tissue penetration. In addition, the engineered bio-active devices with favorable biomechanical features assist in restoring the functioning of defective tissues. In this context, the development of biodegradable and bioreabsorbable biological devices with improved mechanical, physical, chemical and structural properties has been beleaguered to modify the cell response and subsequent tissue formation. The adaptation of synthetic glass ceramics, bioceramics as well as the modern tissue engineering technologies allow to maneuver the regenerative biodevice to overcome the biophysical limitations. The design of such a device could be manipulated to modify the surface available for cell attachment as well as to optimize the exposure of attached cells to nutrients. Hence, a bio-device with desirable biological and mechanical properties has been fabricated from bioceramics for application in hard tissue regeneration. The biodegradation kinetics as well as the improved mechanical properties enable the device to function as a scaffold for bone growth. Moreover, the potential of the developed novel device has been tested in animal models in vitro and invivo.
**A novel peritoneal adhesion barrier composed of ultrapure alginites of different molecular weights**

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Post-operative intraperitoneal adhesions are a serious problems in gastroenterological surgery. Especially repeated heptectomy for intrahepatic recurrence causes serious intraperitoneal adhesions, which make the resection difficult due to increased blood loss or injury to neighboring organs. Therefore, although repeated heptectomy is effective in relatively many cases for intrahepatic recurrence, the adhesions frequently make surgeons hesitate to perform heptectomies several times. In addition, current sufficiently commercially-available adhesion barrier materials such as Seprafilm™ or Interceed™ cannot sufficiently prevent the adhesions induced by heptectomy.

Alginate is a well-known polysaccharide which has a good biocompatibility in vivo and gelation properties by calcium ions. It has been reported to prevent adhesions efficiently in several publications but is not used in clinical uses. We successfully established several heptectomy-induced adhesion models using rats. Then, we carefully evaluated an anti-adhesion effect of calcium ion (Ca2+)-crosslinked alginate hydrogels mixed with carboxymethyl cellulose (CMC). Used alginites and CMC were carefully purified and their endotoxin concentrations were quite low. As a result, we firstly found that low molecular weight alginate prevented de novo adhesions, while high molecular weight alginate prevented the adhesion on the resection surface of liver.

Based on this unique property of alginites, we successfully developed a novel Ca2+-crosslinked alginate sponge which has a layered structure of high and low molecular weight alginate. A top layer composed of low molecular weight alginate rapidly dissolved and prevented de novo adhesion, while a bottom layer composed of high molecular weight alginate slowly dissolved was localized on the cut surface of liver to prevent the cut surface adhesion in partial heptectomy model using rats.

**Mesenchymal stem cells response to hybrid functionalized collagen membrane for dental applications**

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Collagen membrane, barrier in maxillofacial bone regeneration, prevents the ingrowth of fibrous tissue into the bone defect impairing bone regeneration. Functionalization of the bone facing membrane with osteoinductive properties could improve bone healing. We report here a hybrid calcium phosphate/biopolymers functionalized collagen membrane as a new strategy for bone regenerative medicine in dental field.

We used a straightforward process based on simultaneous spray coating of interacting species to functionalize collagen membrane with biomimetic calcium phosphate (CaP) coating supplemented with chitosan (CHI) and hyaluronic acid (HA). Physicochemical characterizations of resulting CaP-CHI-HA coating were performed by scanning electron microscopy with a field emission gun (FEG-SEM), high-resolution transmission electron microscopy coupled to electron diffraction (HR-TEM), X-rays photoelectron and infrared (XPS and FTIR) spectroscopies. Biocompatibility of coated membrane was evaluated after 7 days of human mesenchymal stem cells (MSCs) culture through metabolic activity assay (WST-1) and DNA quantification. MSCs morphology was investigated by confocal laser scanning microscopy (CLSM). MSCs pro-healing properties were analyzed through supernatant cytokines and growth factors quantification (ELISA).

FEG-SEM images showed the presence of star-shaped particles wrapped in organic film, confirmed by TEM. Furthermore, HR-TEM, in addition to FTIR spectroscopy and XPS, indicated the presence of non-stoichiometric and poorly crystalline hydroxyapatite. WST-1 and DNA quantification showed a higher proliferation of MSCs on CaP-CHI-HA coating. Cytoskeleton labeling highlighted spread, elongated and aligned MSCs on coated membrane (vs randomly distributed cells on bare membrane). No significant difference was observed in MSCs inflammatory cytokines (IL-6 and IL-8) secretion between bare and coated membrane, whereas osteoprotegerin and angiogenic growth factors (VEGF, bFGF) releases in supernatant were increased in presence of CaP-CHI-HA coating. Thus, CaP-CHI-HA coated membrane provides a suitable environment for MSCs to induce bone healing.

Finally, CaP-CHI-HA coating allowed to reduce significantly Staphylococcus aureus and Pseudomonas aeruginosa adhesion on collagen membrane, conferring anti-adhesive bacteria function to this surface. Therefore, buildup of CaP-CHI-HA on collagen membrane provides an interesting material for maxillofacial bone regeneration.
Minimally-invasive cell transportation method for cell sheet based regenerative medicine

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Transportation of live cells causes oscillation of a complete medium and generate mechanical stress on the cells, thereby deteriorating cell qualities. We developed a gelatin with extremely high gelation temperature (HGG) that is useful as a protective material for live cells. In this study, we explored an optimal protocol for protecting cell sheets with HGG gels for cell transportation.

First, culture media of cell sheets on temperature-responsive cell culture dishes UpCell® (designated here "TR-dishes") were exchanged to buffers containing various concentrations of Ca2+ and Mg2+ ions without the use of gelatin, and then the dishes were left to stand at 30°C until cells caused detachment. Second, the effects of shear moduli of gels on cell sheet qualities were investigated when HGG gels were created on cell sheets as a protective material. Similar experiments were carried out for commercially-available gelatin for biochemical uses (CG). After the removal of gelatin gels at 37°C, the viabilities of cell sheets were determined by trypan blue exclusion tests.

When the cell sheets were left to stand at 30°C in the buffers, the longest duration of cell attachment was obtained at Ca2+ and Mg2+ concentrations of 1.36 mM and 0.81 mM, respectively. The lower concentrations of the ions caused cell detachment in shorter periods. Thus, following cell sheet protection experiments were carried out using the gelatin sols containing Ca2+ and Mg2+ at the optimal concentrations.

The gelation temperature of HGG was determined to be 30°C, much higher than that of CG (26°C). This unique property allowed the protection of cell sheets with HGG gels at temperatures above the detaching temperature of TR-dishes (27°C). In contrast, cell sheets were detached from TR-dishes before CG formed gel.

The cell sheets which were protected with a HGG gel (modulus of 200 Pa; HGG concentration of 3%) maintained the initial cell morphologies and showed survival rates > 90% after the de-protection. Similar results were obtained for the protection experiments using a harder HGG gel (1,300 Pa, 6%). In contrast, the protection with weaker HGG gel (99 Pa, 1%) resulted in much lower cell qualities.

We propose here an optimal protocol for less invasive protection of cell sheets using gelatin gels, in which the existence of Ca2+ and Mg2+ in gelatin sols, high gelation temperature of gelatin, and high moduli of gelatin gels are essential.

Osteogeneic Enhancing Short Peptide with Biodegradable Polymer as a New Bone Hemostatic Material

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[Backgrounds] The beeswax (BONE WAX) has long been used for bone hemostasis. However, BONE WAX is nonresorbable material and may interfere with bone healing through infection and chronic inflammation. We tried to develop a osteogeneic enhancing short peptide with PCL-based biodegradable polymer (PBP) composite wax as a new bone hemostatic material which could archive prompt bone healing. In this study, we demonstrated the effects of PBP wax using a rabbit model.

[Methods] Two bilateral 2-mm circular noncritical-sized defects were made in the tibiae of rabbits. The experimental groups were divided into 4 groups as PBP wax with or without peptide (PBP, pep-PBP) or BONE WAX was applied to the bone defects or no hemostatic material was used as a control (sham). We measured the bleeding volume at the bone defect, and again 2 weeks after the procedure, the mechanical strength of the damaged tibia by three-point testing and callus bone area by micro-computed tomography (CT). After 16 weeks, the pep-PBP group and the BONE WAX group were observed by CT for bone healing.

[Results] The PBP and pep-PBP groups worked at body temperature and those softened could be pressed similarly with BONE WAX on all bleeding surfaces. Hemostasis with PBP and pep-PBP were achieved equally with BONE WAX. The bleeding volume was 0.61g in sham, 0.02 g in BONE WAX, 0.02 g in PBP and 0.02 g in pep-PBP (BONE WAX vs PBP: p=0.99, BONE WAX vs pep-PBP: p=0.99). The mechanical strength of pep-PBP (354.6 ± 28.8 N) after 2 weeks of operation was significantly stronger than that of BONE WAX (203.6 ± 9.6 N, p=0.007). In addition, pep-PBP promoted callus formation excessively compared to other groups. The callus area was significantly correlated with mechanical strength between pep-PBP (38941.2 ± 6788.9 pixels) and BONE WAX (11917.3 ± 2583.0 pixels, r=0.623, p=0.028). After 16 weeks, although BONE WAX had still shown the bone defect, in the pep-PBP group complete bone healing was observed.

[Conclusion] The pep-PBP is an effective hemostatic material as a similar usage of BONE WAX. In addition, pep-PBP did not inhibit bone healing and it may accelerate bone regeneration and the patient’s recovery.
01-P439  Improved Antimicrobial Performance of a Novel Mechanically Blended Biodegradable Polymer Blend
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From superimposing infection upon degenerated tissues to reducing shelf-life of food, pathogenic microorganisms are one of the critical scourges for mankind. Thus, creating antimicrobial property in materials has always been essential in biomedical and food packaging applications. Poly-ε-caprolactone (PCL) is a biodegradable polymer which has been investigated extensively due to its ease of processability as a result of its low melting point and glass transition temperature. In this study, natural antibacterial substances alginic acid (ALG, 20 wt.%) and grapefruit seed extract (GFSE, 20% vol./wt.) were added into PCL matrix using mechanical blending processes to obtain novel antibacterial and biodegradable composite materials, which can be used for various applications like tissue engineering, drug delivery, wound dressing and food packaging. The blend was then compression moulded to fabricate film via compression moulding. Scanning electron microscopy results showed that the film surfaces were smooth and homogeneous. Results from tensile testing revealed that the ALG/GFSE/PCL blend film had higher Young’s Modulus than PCL but was still able to extend at least 3 times of its original length and remained flexible. The antimicrobial activity of the fabricated ALG/GFSE/PCL blend was effective against Pseudomonas aeruginosa (P. aeruginosa). Scanning electron microscopy images showed a reduction in the adherence of P. aeruginosa on ALG/GFSE/PCL film as compared to PCL film. The improved antimicrobial activity and the biodegradable property of the blend indicates the potential of the ALG/GFSE/PCL blend for tissue engineering and food packaging application. [MOE AcRF (Singapore) Grant Number R-265-000-592-114]

01-P440  Preparation of Polyaniline Films in Colloidal Dispersion Mode: Cytocompatibility Study
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The combination of intrinsic electrical conductivity with easy preparation and modification opens the door for the promising application of conducting polymers in engineering of excitable tissues. Although a wide variety of conducting polymers are available, polyaniline (PANI) has emerged as a popular choice due to its attractive properties such as easy and low cost preparation. Moreover, PANI can be prepared by various methods and it is subsequently easily modified in order to change its surface properties. These are crucial, as they predetermine any interaction with biological fluids, proteins and cells. In addition, modifications of these properties are substantial with respect to the possible applications of PANI in tissue engineering or as biosensors. Innovative technique for preparing PANI films with advanced biological properties via in-situ polymerization in colloidal dispersion mode using four stabilizers (poly-N-vinylpyrrolidone; sodium dodecylsulfate; Tween 20 and Pluronic F108) was developed. The surface energy, conductivity, spectroscopic features, and cell compatibility of thin polyaniline films were determined using contact-angle measurement, the van der Pauw method, Fourier-transform infrared spectroscopy, and assay conducted on mouse fibroblasts, respectively. The stabilizers significantly influence not only the surface and electrical properties of the films but also their cell compatibility. Among the stabilizers used, sodium dodecylsulfate has the most positive impact and the best properties in terms of conductivity and cell compatibility were recorded for films incorporating this surfactant. Moreover, these films are non-irritating and display no harmful effects on human skin, which was confirmed by their in-vitro exposure to the 3D-reconstructed human tissue model. It can be concluded, that the preparation of PANI films using a colloidal dispersion mode can lead to a significant improvement in the biological properties of PANI. This significantly enhances the applicability of PANI in tissue engineering and, more generally, in biomedicine. In particular, its use in biosensors capable of conductometric monitoring of ongoing changes on the skin surface should be of particular interest.

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A metal-chelating collagen conjugate as building block of wound healing devices

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One of the key factors delaying wound healing in chronic ulcers is the upregulated level of matrix metalloproteinases (MMPs), which keeps the wound in a persistent inflamed state impairing extracellular matrix (ECM) deposition and remodelling. Collagen materials are widely used as wound healing devices because of the desired compatibility with wound exudate, chemotactic activity and proteolytic degradability. In spite of many collagen dressings commercially available, there are still many limitations on these products, including fast proteolytic degradation, mechanical incompetence and lack of the native collagen triple helix structure. To overcome these challenges, we have designed a chelating collagen conjugate as building block displaying the retained triple helix structure and controlled enzymatic stability. The chelating conjugate contains photo-active compounds and forms a covalent network via UV crosslinking. We have undertaken an \textit{in vitro} evaluation in the presence of activated MMPs up to 14 days, demonstrating the overall enzymatic activity reduction (~30% RFU) and hydrogel degradability (~50% weight loss) compared to a commercial available collagen dressing which completely degraded. The superior proteolytic stability was also confirmed via a 1-month subcutaneous implantation pilot study in rats. Furthermore, a preclinical in vivo study is ongoing using a guided bone regeneration model in rats.
Soft tissue injuries involving tendons and ligaments account for 50% of all annual musculoskeletal injuries reported in the United States, majority of which are associated with suboptimal healing leading to loss of function and patient morbidity. The tissue engineering approach has focused on the use of biomaterials, cells and growth factors to enable functional repair and regeneration. Clinical translational hurdles posed by growth factors have encouraged researchers to explore growth factor alternatives to promote tendon healing. These alternative small molecules have the ability to mimic growth factor action with extended half-life. The present study explores small molecule glucagon like peptide-1 receptor agonist (GLP-1R) known as exendin-4 (Ex-4) as a tenogenic factor. Here in, the study evaluates the possible differentiation of human mesenchymal stem cells (hMSCs) seeded on electrospun polymeric nanofiber scaffolds and treated with Ex-4 continuously. Electrospun nanofiber matrices are chosen to mimic collagen fibers in the tendon and to provide a 3D environment for cells. The hMSCs were treated with different concentration of Ex-4 (10nM, 20nM, 100nM) and their ability to proliferate and differentiate tendon markers were evaluated at different time points. The results were compared to insulin (positive control: 250ng/ml DMEM), IGF-1 (positive control: 250ng/ml DMEM) and vehicle control group. The results showed a progressive growth of hMSCs treated with Ex-4 in concentration of 20nM and 100nM. Therefore, Continuous treatment of hMSCs on electrospun nanofiber matrix with Ex-4 demonstrated the ability of Ex-4 to cue hMSCs toward tendon differentiation.
**01-P445**

**Self-organized nonamer and hexamer peptides as components of potential scaffolds for regenerative medicine**

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Regenerative medicine needs effective scaffolds that can replace the native extracellular matrix (ECM). Such scaffolds should act as temporary equivalents of ECM allowing cell interactions leading finally to acceleration of the recovery process and regeneration of damaged tissues. Achieving all of these properties will be possible by applying natural components based on polypeptides and polysaccharides which should mimic both carbohydrate and proteic components of the matrix. In order to overcome restrictions created mainly by immunological system in the case of application of proteinic materials, the appropriate substitute of this component is formed by self-organization of short peptides. The aim of our study is to characterize biological effects of nonamer and hexamer peptides and check whether the peptides can be used as components of scaffolds for medicine. For this goal we synthesized peptides with following sequences: H-FFF-FFF-OH, H-WWW-WWW-OH, H-WWC-WWC-OH, H-WWC-WWC-WWC-OH, H-YYC-YYC-OH, H-YYC-YYC-YYC-OH (C- cysteine, F- phenylalanine, W- tryptophan, Y- tyrosine), prepared them as layers and observed interactions in *in vitro* cell culture with fibroblasts and macrophages. Based on the data gathered from viability, cytotoxicity and oxidative assays it can be proved that the synthesized materials are not cytotoxic and do not negatively influence the growth of cells. Moreover, homohexamer peptides ((FFF)₂, (WWW)₂) are very effective in accelerating the proliferation and stimulating the activity of fibroblasts and macrophages.

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**01-P446**

**Biocompatibility and oxidative stress induced by modified carbon nanofibres**

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Carbon nanomaterials like graphene, carbon nanotubes and fullerenes have attracted great attention for their vast potential regarding applications in industry (e.g. electrical engineering) and medicine. Although they are the subject of numerous studies, there are still several discrepancies especially in respect to the relation of physico-chemical properties of these nanomaterials with their toxicity. The carbon nanoform, whose biological properties have been tested so far to a bare minimum, are nanofibres. Carbon nanofibres have a series of unique properties, useful especially in the construction of tissue substrates assigned for the regeneration of the bone, cartilage and nervous tissue. In some biomedical application carbon nanofibres could replace nanotubes that are often considered hazardous.

The objective of our study was to evaluate the biological properties of carbon nanofibres (CNF) in comparison to properties of nanotubes (CNT). Several types of nanofibres and nanotubes were added to the cell culture in the form of well dispersed suspension. The potential cytotoxicity of CNF and CNT was studied in L929 fibroblasts cell culture and production of reactive oxygen species was examined in the culture of RAW 264.7 macrophages. The results obtained in the experiment indicated that the biocompatibility of both materials was similar: neither CNF nor CNT showed signs of acute toxicity. The cell viability was depending rather on functionalisation of the materials than on differences between nanofibres and nanotubes. On the other hand, our studies have shown that nanofibres induced significantly increased reactive oxygen species production (ROS). At lower levels, ROS activate signaling pathways advantageous for cells, but at higher levels can damage or kill cells. However, ROS also limits cancer initiation and progression by causing oxidative stress that kills cancer cells.

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Honey Mimetic in situ Forming PEG-based Hydrogel with Antibacterial Properties

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Currently, alternative antimicrobial strategies are under exploration, such as the use of plant based products like honey to combat growing antibiotic resistance. This study aims to mimic the ability of honey to produce antibacterial Reactive Oxygen Species (ROS) in the form of hydrogen peroxide (H2O2) within the hydrogel. This was achieved using two components found in honey: the glucose oxidase enzyme and glucose, which were incorporated into the quick forming hydrogel.

For this study Hyperbranched Polyethylene Glycol Diacrylate (HB PEGDA) was synthesized using Reversible Addition Chain Transfer Fragmentation (RAFT) polymerization. With the mechanism of thiol-ene click chemistry, HB PEGDA is able to form a hydrogel with thiolated Hyaluronic Acid (HA-SH). For a hydrogel with a composition of 5 w/w% HB PEGDA and 1 w/w% HA-SH, an average gelation time of 585±45 seconds was obtained. For the 10 w/w% HBPEGDA and 1 w/w% HA-SH gelation time was 41±4 seconds.

The study characterized the chemical characteristics of the polymer and the hydrogels’ rheological properties, swelling and degradation properties, and enzyme encapsulation properties. The ROS produced with varying glucose and enzyme concentrations at different time points were quantified. Samples were found to contain positive viability with an Alamar blue assay and NIH/3T3 mouse fibroblast cells for cytocompatibility. Given its quick forming property, biocompatibility, and ability to continuously produce antibacterial ROS, this type of hydrogel system makes a promising material for wound dressing applications and should be further characterized and studied.

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LOTUS/CRTAC1B promoted axonal regeneration and functional recovery after spinal cord injury in adult mice

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[Introduction]
Lateral olfactory tract usher substance (LOTUS)/cartilage acidic protein-1B (CRTAC1B) can be found as both membrane and secreted protein that functions as a molecule for neuronal circuit formation. LOTUS binds to Nogo receptor 1 and inhibits all of five axonal growth inhibitors, Nogo, MAG, OMgp, BLys, CSPG. It has been reported that, in LOTUS knockout mice, the motor function recovery after SCI is significantly worse when compared with wild-type mice. The purpose of this study is to evaluate the axonal regeneration and motor function recovery after SCI in LOTUS overexpressed mice.

[Method]
Contusive SCI was induced at the Th10 level in LOTUS-overexpressed mice (LOTUS group; n=20) and wild-type mice (control group; n=16) as reported previously. Hindlimb motor function was evaluated weekly for six weeks using BMS scores; and the DigiGate system and rotarod test was used on the sixth week after SCI. On this sixth week, biotinylated dextran amine (BDA) was injected into the primary motor cortex to trace the corticospinal tract (CST), or fluoro-gold (FG) was injected into the lumbar spinal cord to trace the reticulospinal tract. Two weeks after the injection, electrophysiological analysis using spinal cord-evoked potential was conducted. After the mice were sacrificed, histological analyses were performed.

[Result]
Significant improvements in BMS scores was seen in the LOTUS group compared with that in the control group at one week following SCI and thereafter. DigiGate analysis also revealed a significantly longer stride length in the LOTUS group, and the rotarod test showed significant longer total run time in the LOTUS group. Electrophysiological analysis revealed significantly shorter latency and larger amplitude in the LOTUS group.

Histological analyses revealed that the NF-H, 5-HT and p-GAP43 positive fibers increased significantly at the caudal sites in the LOTUS group compared to the control group. As for the 5-HT positive serotonergic fibers, a major contributor of motor function, a significant increase was seen in the LOTUS group 14 days after SCI and continued to increase up to 56 days. The CST axons increased at the rostral sites in the LOTUS group, but not at the caudal sites of the lesion epicenter in both groups. On the other hand, reticular nucleus neurons retrogradely increased significantly.

[Conclusions]
LOTUS showed beneficial effects for functional recovery in SCI by promoting axonal regeneration and nerve axonal protection.
Spinal cord injury (SCI) leads to devastating neurological deficits that have a strong impact in the physiological, psychological and social behaviour of patients. For these reasons, it is urgent to develop therapeutic strategies that can specifically target this problem.

Various degrees of recovery have been achieved by protecting the neural tissue, by increasing growth promoting cues or by transplanting cells. Our lab demonstrated that the modulation of inflammation using IL4 promoted behavioural and histological improvements in SCI rats (1). Moreover, we also show that a molecular therapy aimed at reducing excitotoxicity promoted motor improvements in paralyzed rats (2). In addition, our lab has been working with Mesenchymal Stem Cells and Olfactory Ensheathing Cells and already demonstrated that the transplantation of these cells alone (3), encapsulated in a functional hydrogel (4, 5) or the use of MSCs secretome (data not published) promoted functional recovery in contused SCI rats and mice. In the TERMIS World Congress 2018 we intend to present the last data from our lab on SCI repair as well as discuss novel integrative approaches that simultaneously tackle several targets of the SCI pathophysiology.

References

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**01-P453** Resveratrol Treatment Downregulates the Expression of Aquaporin-4 and -9 in Diabetic Rats

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Aquaporins (AQP5) are defined as water channel proteins involve in the regulation of water homeostasis by controlling the water flux through the plasma membrane. In the central nervous system, three members of these proteins were dominantly expressed: AQP1, AQP4, and AQP9 (Hirt et al., 2018). Although AQP4 can only transport water, AQP9 was found to be permeable also to glycerol, urea and monocarboxylates (Arciénega et al., 2010). In brain tissue, AQP4 plays central role in neuroinflammation and neurodegenerative diseases (Baudat et al., 2014).

The studies with the diabetic rodents showed that the AQP9 expression in astrocytes and catecholaminergic neurons was negatively regulated by insulin level in the blood, and the high AQP9 expressions were observed in the streptozotocin (STZ) treated animals. (Baudat et al., 2008). In our study, we focused on the AQP-4 and -9 expressions in STZ-treated rats, and the reversal effect of resveratrol on the pathogenesis of the diabetes on brain tissue was shown. The expression of AQP-4 and AQP-9 was upregulated by 5.6 and 46.5 in STZ-diabetic rats. At the same time, BDNF expression reduced by 2.5 times, and tyrosine hydroxylase (TH) expression improved to 13.4 times of control group. After the resveratrol treatment, the normal physiological levels of AQP-4 and -9 were measured. In parallel to AQP-4 and -9, the level of IL-1β and TNFα were decreased in the tissue. Resveratrol helped to reduce to their normal levels, and BDNF expression was increased.

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**01-P455** Comparison of detergent-based decellularization methods for generation of decellularized peripheral nerve allografts: an in vitro and in vivo study

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**Introduction.** Decellularized peripheral nerve allografts (DPNAs) are a promising alternative to nerve autografts for peripheral nerve repair. They provide a rich extracellular matrix (ECM) and a natural nerve architecture to the regenerating microenvironment. Efficient decellularization and preservation of the most important ECM components are crucial for successful regeneration. Therefore, the aim of this study was to evaluate a new decellularization protocol and compare this to two established detergent-based decellularization protocols. **Methods.** Sciatic nerve segments from Wistar rats were decellularized according to one of the following methods: (1) the Sondell method, combining Triton X-100 and sodium deoxycholate; (2) the adapted Hudson method, combining sulfobetaine (SB)-10 and SB-16 and (3) the Roosens method, developed in our lab combining Triton X-100, DNase, RNase and trypsin. Both an in vitro analysis, including histology, biochemistry and a tensile test as well as an in vivo validation in a rat sciatic nerve injury model were performed. **Results.** The in vitro analysis revealed that decellularization with the Sondell method efficiently removed cellular material, but disrupted the endoneurial tubes. The adapted Hudson method did not affect the ECM composition, but did not result in cell-free DPNAs either. The Roosens method obtained the best level of decellularization with good preservation of the ECM. The in vivo study showed that none of the DPNAs performed as good as the autograft after 12 weeks, but the Sondell and Roosens method resulted in better functional recovery than the adapted Hudson method. Moreover, histological analysis revealed that more axonal sprouts reached the distal part in grafts of the Roosens method compared to the other decellularization methods. **Discussion.** The new method developed in our lab combining Triton X-100 with enzyme treatment showed superior results both in vitro and in vivo compared to the Sondell and adapted Hudson method. However, further supplementation with cell therapy could be beneficial to reach the level of regeneration as seen with autografts.

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**01-P456** Directing and promoting neuronal outgrowth and non-neuronal cell migration using phosphate glass fibres embedded in engineered neural tissue for peripheral nerve regeneration

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Advances in biomaterials and tissue engineering have led to the development of Engineered Neural Tissue (EngNT) for peripheral nerve repair [1]. Experiments using EngNT with embedded Schwann cells to repair rat sciatic nerve injuries indicated sub-optimal growth of neurites from the proximal nerve stump into the EngNT [1, 2]. Phosphate glass fibres (PGFs) have been used in hard- and soft-tissue engineering applications [3, 4]. They are biocompatible and biodegradable and have emerged as a potential material to resolve soft-tissue engineering interface issues [5]. The aim of this study was to investigate whether PGFs could improve the interface between the proximal stump of a damaged nerve and EngNT in supporting neurite outgrowth.

Hemisected dorsal root ganglia (DRG) were placed in direct contact with EngNT incorporated with and without PGFs. DRGs attached to constructs were placed vertically into 1.5ml tubes and maintained in a tissue culture incubator (37 °C/5% CO₂) for 72h. Following immunostaining, axonal growth and non-neuronal cell migration into the construct was imaged using an inverted fluorescent microscope. Image analysis was performed using ImageJ.

This study demonstrates that PGFs can be successfully incorporated into EngNT to encourage non-neuronal cell migration and neurite elongation in culture. Incorporating PGFs within EngNT permits non-neuronal cells to travel approximately twice the distance into EngNT. Furthermore, neurites are able to elongate approximately one and a half times the distance into EngNT that was modified with PGFs. Future work involves testing ‘EngNT + PGF’ constructs in vivo to investigate whether ingrowth of neurites from the proximal nerve stump is improved.

**References**

**Extracellular matrix/polydopamine-contained 3D printed nerve conduits for promoting nerve regeneration**

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Abstract:

Autologous nerve graft has good clinical results, but the disadvantages are limited sources, donor site incidence and size matches. As nerve conduits can be used to promote neuronal growth and to guide axonal extension after nerve injury. Therefore, nerve catheter has become the second alternative to autologous nerve transplantation. In this study, we using Digital light processing (DLP) technology in 3D printing to developed a photopolymerizable water-based polyurethane (PU) material. In addition, we mixed polydopamine (PDA) and extracellular matrix (ECM) to enhance neural stem cells in nerve conduit growth and differentiation performance. In result, SEM and immunofluorescence staining images showed good cell compatibility. Furthermore, neurological transection and conduit efficacy 8 weeks after conduit implantation were assessed using a 10 mm sciatic nerve transection rat model. Finally, the electrophysiological test, functional and histological evaluation of the regenerative assessment were performed to compare with the commercial silicon conduit in this study. Thus, our printed PU/PDA/ECM conduit advance reconstructs the microenvironment neural regeneration. This study demonstrates the potential of 3D-printing PU/PDA/ECM nerve conduit for potential clinical applications in the future.
**Bioink Formulations of Hyaluronic Acid Hydrogels for Applications in Nerve Regeneration after Spinal Cord Injury**

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Bioprinting hyaluronic acid (HA) hydrogels empowers researchers to better mimic the native structure of tissues by allowing for spatial control over the general hydrogel structure and distribution of bioactive cues for the regeneration of functional tissues. Bioprinting technologies are capable of depositing different hydrogel biomaterials in complex tissue-like structures; however, many hydrogels precursors are liquid and cannot be bioprinted with high resolution using an extrusion-based bioprinter. While the development of printable biomaterials, or bioinks, has progressed significantly, few bioinks have the necessary physical properties for bioprinting high-resolution, tissue-like structures that promote desirable tissue regeneration. In the current study, we characterized printable formulations of pentenoate-functionalized HA (PHA) hydrogels. HA hydrogels are a promising material for regenerative medicine as HA is present in native tissues (e.g., cartilage, nerve, skin), commercially available, and easily chemically modified to support numerous crosslinking chemistries for hydrogel formation. Specifically, the PHA hydrogels used in these studies leverage the thiol-ene click chemistry and reduce the hydrogel crosslinking time (~2 minutes) compared to other chemical modifications such as methacrylated HA (~10 minutes). To determine printability, the mechanical performance of the precursor PHA hydrogel was evaluated and the print quality via shape fidelity was assessed after printing on a pneumatic-based bioprinter. We found that functionalizing a high molecular weight HA (1.5 MDa) and using a concentration greater than 3 wt% was sufficient to produce a precursor yield stress of at least 390 ± 130 Pa and have high shape fidelity after bioprinting. As a printable and chemically tunable bioink, PHA can be leveraged as a platform for bioprinting spatial patterns of different bioactive signals for further studies in guiding neuronal axon growth and enhancing nerve regeneration after spinal cord injury.
Detecting tumorigenicity following hiPSC-NS/PCs using clinical applicable PET-CT and MRI

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【Introduction】We have previously reported the beneficial effects of transplanting human induced pluripotent neural stem progenitor cells (hiPSC-NS/PCs) into the spinal cord of contusion model rodents. However, transplanting certain hiPSC-NS/PCs, which are known to have tumorigenic properties, resulted in the deterioration of motor function secondary to the oncogenic transformation. We demonstrated that the tumors derived from these “bad clones” consisted of immature undifferentiated human-specific NESTIN positive cells. Current imaging modalities that are available to us in the clinical setting cannot successfully detect these tumorigenic changes. The purpose of this study is to develop a method that allows us to visualize the potential tumorigenic changes using techniques that are readily available such as PET-CT and MRI.

【Method】253G1-NS/PCs (oncogenic clones), 414C2-NS/PCs (benign clones) or phosphate-buttered saline was transplanted into the striata and cervical/thoracic spinal cord of NOD/SCID mice. These cells were cultured and labeled with firefly luciferase genes via lentiviral transduction. Two months after transplantation, gadolinium injected MRI was performed followed by PET with 11C-Methionine (MET) and 18F-TSPO ligand (18F-FEDAC : JNM2010, 51-1301) within 24 hours. The mice were immediately sacrificed and the brain and spinal cord were dissected out for autoradiography (ARG). The correlation between the in vivo imaging data and immunohistochemistry results were evaluated.

【Result】A region of high signal intensity on T2 weighted MRI was identified at the transplanted site of the mice in 253G1 group, but there were no significant findings in the other groups. In vivo dynamic TSPO-ligand PET and 11C-MET PET revealed an increase in tracer uptake at the transplanted site in the 253G1 group which was not found in the other groups. We found that there was a significantly higher binding of 18F-FEDAC at the transplanted site in the 253G1 group using ARG. Immunohistochemistry showed a high level of NESTIN+ cells in the transplanted site, especially in 253G1 group.

【Conclusion】We were successful in detecting the tumor like overgrowth using all three modalities. The detected signal was higher in oncogenic clones compared to the benign clones using PET. In the future, we aim to monitor the dynamics of the transplanted cells using PET to identify any time-dependent metabolic changes.
Ultrasonic-induced neurite outgrowth in primary neurons for axonal regeneration
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[Introduction] Axonal regeneration after spinal cord injury remains a challenge of neuroscience. Neurite outgrowth is one of the essential steps in axonal regeneration. However, central nervous system myelin contains proteins that inhibit neurite outgrowth. Thus, extensive efforts have been conducted to develop strategies that enable injured neurons to re-grow. Low-intensity pulsed ultrasound (LIPUS) enhanced neurite outgrowth in tumor cell lines. Furthermore, LIPUS improved peripheral nerve regeneration in rat. Therefore, LIPUS may have a potential for axonal regeneration after spinal cord injury. Our ultimate goal is to develop LIPUS therapy for spinal cord injury. In this feasibility study, we evaluated whether LIPUS can induce neurite outgrowth in primary neurons.

[Methods] Primary rat [Fisher 344] cortex neurons (Life Technologies) were seeded onto collagen type-I gel in 35 mm culture dish. Neurons were maintained in Neurobasal® Medium (Life Technologies) supplemented with B-27® Supplement (Life Technologies) and GlutaMax™-1 (Life Technologies). After culturing for 3-day, ultrasound was exposed to neurons for 10 min at 37°C. Ultrasound parameters were as follows; 1 MHz center frequency, 10% duty cycle (100 μsec pulse width, 1 kHz pulse repetition frequency), the spatial-average temporal-average ultrasound intensity was 30 mW/cm². Neurons were observed with a phase-contrast microscope in 7 consecutive days. In addition, neurons were stained with calcein-AM and propidium iodine. Both phase-contrast images and fluorescent images were analyzed with Image J® in order to assess the neurite outgrowth.

[Results] The number of calcein-AM positive cells was higher in LIPUS group than in the control group. Calcein-AM positive cells in LIPUS group had more and longer neurites compared with calcein-AM positive cells in the control group.

[Discussion] This preliminary study shows the feasibility of LIPUS for stimulating neurite outgrowth. The effects of single LIPUS treatment were limited in this preliminary study because we used the complete medium for primary neurons. In ongoing study, we intend to illustrate that LIPUS can be useful for stimulating neurite outgrowth in the presence of inhibitors (e.g., Nogo or MAG).

Mechanically induced neuroinflammation to reproduce peri-electrode gliosis macro environment
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Background: Neuroprosthetic electrode implants have been under investigation for decades and have been proven to be safe and efficacious as therapeutic devices for multiple diseases of the central nervous system (CNS) including Parkinson’s and Alzheimer’s disease. Functional neuroelectrode should remain functionally stable in situ to support intimate contact with CNS tissue for long-term recording performance and therapy to achieve optimum therapeutic benefit. However, studies indicate that, in situ, neuronal recording and charge deliverance decrease with time in implanted electrode systems. This loss of function can be attributed in part to an acute inflammatory reaction resulting from the initial mechanical shear stress experienced during the insertion of the neural electrode. This mechanical trauma results in an adverse tissue response characterized by glial scar formation and electrode encapsulation, causing the signal strength to decrease and adjacent neurons to move away from the electrode as a result of the surrounding region of gliosis.

Aim: To develop an inflammatory model using shear flow stress on primary mixed cell population to reproduce gliosis in vitro.

Methods: Using an in-house parallel-plate flow chamber system, ventral mesencephalic mixed primary cells were exposed to different level of pressure-driven fluid flow allowing to apply a defined shear stress (0.6, 0.8, 1, 2 and 4 Pa) for either a 5-minute pulse to reproduce the insertion only or up to several days to reproduce micro-motion movements between the implant and the brain tissue. The cells were then kept in culture for 1 to 10 days before being assessed using markers of neuroinflammation. The morphology and protein expression of neurons and glial cells were quantified by image analysis, ELISA and custom protein antibody microarray were used to detect the level of neuroinflammatory proteins.

Results: Data have shown that the applied shear flow leads to astrocyte reactivity and inflammatory environment. Shear stresses from 0.6 to 4 Pa have all significantly increased the GFAP protein expression and size of astrocyte cell body along with the up-regulation of several neuronal pro-inflammatory markers.

Conclusions: We have developed a novel in vitro model of neuroinflammation using parallel flow shear stress that mimics the insertion of neuro-electrode into the brain. This model will certainly be a precious tool for future researchers developing anti-inflammatory and anti-gliosis molecules.
01-P467  Improvement of renal function after human umbilical cord mesenchymal stem cell treatment on chronic renal failure and thoracic spinal cord entrapment: a case report

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Chronic renal failure is an important clinical problem with significant socioeconomic impact worldwide. Thoracic spinal cord entrapment induced by a metabolic yield deposit in patients with renal failure results in intrusion of nervous tissue and consequently loss of motor and sensory function. Human umbilical cord mesenchymal stem cells are immune naïve and they are able to differentiate into other phenotypes, including the neural lineage. Over the past decade, advances in the field of regenerative medicine allowed development of cell therapies suitable for kidney repair. Mesenchymal stem cell studies in animal models of chronic renal failure have uncovered a unique potential of these cells for improving function and regenerating the damaged kidney. We report a case of a 62-year-old ethnic Indonesian woman previously diagnosed as having thoracic spinal cord entrapment with paraplegic condition and chronic renal failure on hemodialysis. She had diabetes mellitus that affected her kidneys and had chronic renal failure for 2 years, with creatinine level of 11 mg/dl, and no urinating since then. She was treated with human umbilical cord mesenchymal stem cell implantation protocol. This protocol consists of implantation of 16 million human umbilical cord mesenchymal stem cells intrathecally and 16 million human umbilical cord mesenchymal stem cells intravenously. Three weeks after first intrathecal and intravenous implantation she could move her toes and her kidney improved. Her creatinine level decreased to 9 mg/dl. Now after 8 months she can raise her legs and her creatinine level is 2 mg/dl with normal urinating. Human umbilical cord mesenchymal stem cell implantations led to significant improvement for spinal cord entrapment and kidney failure. The major histocompatibility in allogeneic implantation is an important issue to be addressed in the future.
Ischemic stroke is one of the most frequent causes of death and disability globally, with significant clinical and socioeconomic effects. Current treatment strategies are palliative, owing to the lack of efficient therapeutics. Developing novel therapeutic strategies for enhancing the repair of neural structures and the recovery of functions are urgently required. In this work, we aim to employ a stem cell-based approach for treating ischemic stroke. Three-dimensional (3D) aggregates of human mesenchymal stem cells (MSCs), which are capable of secreting neuroprotective agents and inducing therapeutic angiogenesis, are constructed using a methylcellulose hydrogel system. The gene expression profiles, secretion of cytokines and growth factors, and differentiation into neuronal and vascular lineages of the stem cell aggregates are evaluated. Additionally, a neurite outgrowth assay is employed to assess the neuroprotective and neurogenic potential of the developed 3D stem cell aggregates under various stressed culture conditions, such as hypoxia, inflammation, and oxidative stress, which mimic the post-injured microenvironment. Our in vitro results demonstrate that the fabricated 3D stem cell aggregates can induce angiogenesis and secret neuroprotective cytokines under stressed condition. In the following animal study, a mouse model of ischemic stroke that is established surgically will be employed for evaluating the therapeutic efficacy of the fabricated 3D stem cell aggregates. The recovery of functions as well as the detailed molecular mechanisms behind the therapeutic benefits mediated by the 3D stem cell aggregates will be elucidated.
Laminin coated pHEMA-EMTACl hydrogel in the treatment of spinal cord injury

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Spinal cord injury (SCI) is a devastating condition resulting in a disruption of neuronal connections. After injury the spinal cord lesion develops into combination of a pseudocystic cavity, glial and mesenchymal scar resulting in a barrier for tissue repair and axonal regeneration. Building a bridge across the SCI using hydrogels represents one of the therapeutic strategies in experimental SCI repair. We have assessed the use of laminin coated hydrogel based on poly (2-hydroxyethyl methacrylate), PHEMA with dual porosity, seeded with induced pluripotent stem cell derived neural progenitors (IPSC-NPs), in the treatment of chronic SCI. We found in vitro that double porous hydrogel is more suitable for cell proliferation when compared to gels with one type of pores. Larger pores are suitable for cell adhesion and expansion, while smaller pores enable diffusion of nutrients and cannot be occupied by the cells. IPSC-NPs cultured for 3 weeks in hydrogel in vitro were positive for nestin, GFAP and MAP2. These cell-polymer constructs were implanted into rats with balloon compression lesion 5 weeks after lesion induction. Animals were behaviorally tested, and spinal cord tissue was immunohistochemically analyzed 4 months later. The implanted IPSC-NPs survived in the scaffold for the entire experimental period. Host axons, astrocytes and blood vessels grew into the implant and an increased sprouting of host TH⁺ fibers was observed in the lesion vicinity. Despite cavity bridging and robust survival of IPSC-NPs in the hydrogel, no statistically significant improvement of locomotor recovery was observed. The implantation of IPSC-NP-cell polymer construct into the chronic SCI led to the integration of material into the injured spinal cord, reduced cavitation and modest behavioral recovery support with no negative impact on treated animals. However, further co-therapies that will augment the efficacy of neural cell transplant and restore function in chronic SCI, have to be identified. Support: GACR 17-11140S, P304/12/G069, MEYS LO1309

Dynamics of spinal cord injury in vivo using two-photon microscopy

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Spinal cord injury (SCI) is a devastating clinical condition with profound and irreversible impact on patient’s quality of life, with no effective treatments available. Better comprehension of both primary and secondary mechanisms involved in SCI is a critical prerequisite for any development of new therapeutic interventions. However, standard experimental approaches mostly used for studying SCI lack temporal resolution necessary for understanding its inherently dynamic aspects. Therefore, basic successive mechanisms of SCI are still poorly understood. Using repeated intravital two-photon imaging, we studied dynamic reactions of genetically-defined cell types following SCI. We implanted a custom-made imaging chamber, performed laminectomy and covered it with a spinal cord window. Thus, we obtained permanent optical access to the dorsal spinal cord for weeks up to months. Transgenic mouse strains were used to visualize spinal cord neurons and glial cells. One week after the window implantation, we did a laser-induced SCI with transection of axons in the dorsal column. Time-lapse imaging enabled us to repeatedly observe and evaluate the morphological changes after SCI. Individual axons and cells were precisely reidentified and tracked between individual consecutive imaging sessions. We observed heterogeneously asynchronous, spatially intermingled morphological changes and their continuous transitions between diverse states including axonal swelling and dieback, formation of retraction bulbs or spheroids, often with a subsequent fragmentation. In a subset of mice we transplanted GFP-labeled mesenchymal stem cells, tracked the grafted cells in vivo and observed the changes of their morphology. This methodology can be useful for real-time assessment of new therapeutic strategies.

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Mesenchymal stem cells (MSCs) have the ability to differentiate into multiple cell types. Wharton’s jelly-derived MSCs (WJ-MSCs) have early embryonic state retain telomere at highest possible length, which protects them from premature loss of viability. It has been reported that functional recovery of various neurologic disease models such as brain injury. However, the therapeutic effects of human Wharton’s jelly-derived MSCs (WJ-MSCs) in guiding peripheral nerve regeneration remain poorly understood. In this study, to demonstrate the effect of WJ-MSCs on crush injured of the sciatic nerve in the rat. We immediately transplanted the WJ-MSCs into the region of injured sciatic nerve, and then examined the behavior test, differentiation potentiality of transplanted WJ-MSCs into Schwann cells, expression of neurotrophic factors and growth factors, and expression of pro- and anti-inflammatory cytokines. We were assessed by immunofluorescence staining, ELISA at 1, 3, 5, 7 and 56 days after WJ-MSCs transplantation. The results were: (1) a behavior test (Ankle Angle Measurements) showed a significant improvement in the WJ-MSCs transplanted group as compared to none injected group, (2) WJ-MSCs, the expression of growth factor (VEGF) was increased to compared group, (3) WJ-MSCs, the expression of anti-inflammatory cytokines were increased to compared group. We suggest that WJ-MSCs could be effective cell for treating peripheral nerve injury.

Chronic brain stimulation has become a promising physical therapy with the increased efficacy and efficiency in the treatment of neurodegenerative diseases. The application of deep brain electrical stimulation (DBS) accompanied with manganese-enhanced magnetic resonance imaging (MEMRI) allows for unbiased functional anatomy represents a major challenge and goal in understanding the communications between the areas of brain subject to the remedy. Thus, in this study, we developed a new type of conductive nanogel-based neural interface composed of amphiphilic chitosan-modified Poly (3,4-ethylenedioxythiophene) (PMSDT) that can exhibit biomimic structural/mechanical properties and ionic/electrical conductivity comparable to that of Au. More importantly, the PMSDT enables metal-ligand bonding with Mn²⁺ ions, so that the system can release Mn²⁺ ions, but rather than MnCl₂ solution, which can be directly and precisely controlled by electrical stimulation (ES) to achieve real-time high-resolution MEMRI. With the integration of PMSDT nanogel-based coating on the polyimide-based microelectrode arrays, the post-implantation DBS allowed for not only frequency-dependent MR imaging in vivo, but also a small focal imaging in response to the channel site-specific stimulation on the implant. The MR imaging of the implanted brain treated by 5-minute electrical stimulation showed a thalamocortical neuronal pathway after 36h, confirming effective activation of the downstream neuronal circuit following DBS. This study provided a model of MEMRI-functionalized DBS based on functional neural interface engineering and controllable delivery technology, which is expected to be utilized in more detailed exploration on the functional anatomy for the treatment of neurodegenerative diseases.
**01-P474** Analysis of transduction efficiency and tropism of AAV serotypes in chronic spinal cord injury

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Introduction: A combination of several treatment options, including gene therapy, is thought to be necessary for the management of chronic spinal cord injury (SCI). Optimizing the transportation efficacy of these therapeutic genes can potentially improve the performance, and hence the outcome. In this study, we focused on the potential use of adeno associated virus (AAV) vectors as a therapeutic gene transporter. AAV vectors are known to have a safe and long lasting gene expression in vivo, and are widely utilized in gene therapy. It is well known that AAV serotypes, with different capsids, have different cell tropisms. However, a comprehensive study investigating the efficiency of the different serotypes in chronic SCI has never been performed. The aim of this study is to elucidate the character of AAV serotypes in chronic SCI.

Method: We produced AAV serotypes 1, 2, 3, 4, 5, 6, 7, 8, 9, rh10, DJ and DJ/8 with CMV promoter. Firefly luciferase and GFP were used as an in vivo reporter system. First, intraparenchymal injections of each serotype was given to the intact spinal cord (Th10) C57BL6 albino mice and bioluminescence images (BLI) were measured weekly for three weeks. Second, the three most promising serotypes, based on the results of the BLI, were injected into the spinal cord of chronic contusion SCI model C57BL6 mice. BLI were measured and immunohistological evaluation was performed 6 weeks after the injections.

Result: In the first study using intact spinal cords, successful gene expression was observed in all 12 serotypes. Of these, AAV5, AAV6 and AAVrh10 had the highest photon count measurements with BLI three weeks after injection. Therefore, we selected these serotypes for injecting into chronic SCI models (n=4 each). AAVrh10 had the highest photon counts five weeks after injection (p<0.05). Immunohistological analysis was used to identify the efficiency/character of the cell tropism. AAVrh10 transduced neurons and astrocytes more effectively than AAV5 and AAV6. As for oligodendrocytes, effective transduction was seen with AAV6 and AAVrh10 compared to AAV5 (p<0.05). Transduction to microglia/macrophage could not be observed with all three serotypes. A higher infiltration of the injury epicenter was achieved using AAVrh10.

Conclusions: We showed that AAVrh10 was the most effective in transducing the spinal cord of chronic SCI model mice. AAVrh10 could be a good candidate for therapeutic gene transfer and as a tool for research of chronic SCI.

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**01-P475** Enhancing peripheral nerve tissue engineering using gene and cell therapy

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Peripheral nerve injuries occur in up to 5% of trauma patients and are associated with motor, sensory and autonomic impairment. The autologous nerve graft is the current clinical gold standard treatment for extensive nerve damage. However, it is associated with disadvantages and does not result in restoration of function in all patients. Various nerve repair devices which attempt to overcome the limitations of autologous nerve grafts have been produced. An emerging experimental strategy is the use of nerve repair devices that contain genetically modified cells. This study will test the potential ability of collagen engineered neural tissue (EngNT) containing genetically modified cells to enhance peripheral nerve tissue engineering in a rat model. An in vitro proof-of-concept study was performed to deliver luciferase and green fluorescent protein (GFP) genes to rat Schwann cells (SCL4.1/F7) via lentiviral-mediated transduction and to assess cellular viability for the construction of EngNT. The cells were successfully transduced with efficiency dependent on the multiplicity of infection. This was confirmed by fluorescence microscopy, bioluminescence imaging and flow cytometry. The transduced cells expressing both GFP and luciferase marker genes were incorporated into EngNT. Stereoscopic fluorescence microscopy and confocal microscopy confirmed that the transduced cells had distributed evenly throughout the construct. Bioluminescence imaging showed that the Schwann cells were still viable after seeding into the constructs. No fluorescence or bioluminescence was observed in the untransduced cells or in the control constructs seeded with the untransduced cells. Transduced cells were used to construct EngNT and the cell viability in vitro was monitored over a longer 21 day period using bioluminescence imaging. Although cell viability diminishes over time, live cells were still detected at 21 days. The approach has also been applied successfully to clinically relevant sources of therapeutic cells and provides a potential method for delivering genes that increase vascularisation to the EngNT following implantation in vivo. The combination of gene and cell therapy is an emerging experimental strategy which may provide the optimal environment for axonal regeneration following peripheral nerve injury. F.B. is funded by the ENDEAVOUR Scholarships Scheme and the University College London Graduate Research Scheme.
**Remote control of cell signalling using tagged magnetic nanoparticles for neuronal cell differentiation- emerging cell therapies for Parkinsons disease**

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**Introduction**

Signalling pathways such as Wnt and Trek signalling are important controllers of cell fate and regulators of neuronal development. There is therefore great interest in developing signalling modulators for treating neurodegenerative disease. Cell receptors that initiate signalling and neural progenitor differentiation pathways can be targeted using ligand functionalised magnetic nanoparticles (MNP). This allows remote mechano-stimulation of receptors and activation of differentiation pathways or direction of neurite outgrowth. The aim of this research was to investigate the effects of remote activation of the Wnt receptor Frizzled and Trek1 K+ channels on the neuronal differentiation of neuroprogenitor cells.

**Methods**

Target receptor expression was assessed in SH-SY5Y and neural progenitor cells using rtPCR. 250nm MNP were coated with peptides or antibodies allowing MNP tagging to Frizzled/Trek receptors. Remote MNP-receptor complex stimulation was performed in 1h-3h sessions using alternating magnetic field gradients provided by a magnetic force bioreactor. Downstream signalling activity was assessed by monitoring β-catenin mobilisation and TCF/LEF responsive gene expression using a luciferase reporter. Neuronal differentiation marker expression was also determined in vitro and in an ex vivo embryonic rat slice model to assess the effects of remote signalling activation on neuronal differentiation of injected progenitor cells.

**Results**

Basal expression of Frizzled1, 2 and Trek1 was confirmed in SH-SY5Y cells. β-catenin mobilisation and TCF/LEF luciferase reporter activity both increased over 24h in response to MNP and magnetic field stimulation. Short-term expression of stress-response genes NF-kB and COX2 was also observed in response to receptor activation. Expression of dopaminergic markers DAT and TH was augmented in vitro and maintained in ex vivo rat brain slices when neuronal progenitor cells were cultured in differentiation media and treated with MNP and magnetic stimulation.

**Conclusion**

Our results indicate that remote activation of cell signalling pathways using tagged magnetic particles can be used for the control of neuronal precursor cell differentiation. This approach may offer a novel therapeutic strategy for treating neuro-degenerative diseases such as Parkinsons disease.

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**3D Bioprinting Coupled With Electrospinning of Conductive PPy/SF Scaffold for Neural tissue Engineering Applications**

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How to fabricate reasonably nerve grafts with outstanding properties is still a critical issue but remains a challenge for the repair of large nerve defects. Thus, this paper reports on the development of composite scaffold with aligned conduction and nanofibers structure. It was prepared by combing 3D bioprinting followed by electrochemical deposition of polypyrrole (PPy) and electrospinning of silk fibroin (SF) into a combined PPy/SF matrix. PPy-coated aligned scaffolds and combined PPy/SF matrix were characterized by optical microscopy (OM) and Fourier transform-infrared (FTIR). OM showed that the oriented silk was completely coated by PPy while FTIR showed that there are characteristic peak of both PPy and silk. Then the effect of PPy content associated with 3D bioprinting diameter on different performances of the scaffold is assessed. The results showed that the electrical conductivity was in the region of 10⁻⁵–10⁻³ S/cm proportional to the PPy loaded on the silk and inversely proportional to the stability in vitro. In addition, the combined PPy/SF matrices have good biocompatibility, as demonstrated by in vitro cytotoxicity for they maintained higher than 95% of the relative growth rate compared to DMEM control. Furthermore, the biological function was investigated by assessing the adhesion and proliferation of Schwann cells (SCs) on the prepared scaffolds using EDU, immunocytochemistry and SEM tests. These results reveal that the combined PPy/SF matrix would be an effective material in cell culture and nerve tissue engineering, where electrical conduction properties are required. In summary, this work provide the potential use of conductive PPy/SF scaffold as a suitable material for neural tissue engineering.
Neurodegenerative conditions such as stroke, Alzheimer’s and Parkinson’s are debilitating, incurable diseases causing progressive degeneration and/or nerve cell death, resulting in motion or mental acuity deficits and may be associated with age. Today, 16% of Europe’s population is older than 65, a number predicted to equal 25% in 2030. Current yearly estimates for dementia-related care in Europe reach €130 billion, establishing the predominance of age-associated neurodegenerative diseases among Europe’s main medical and societal problems. A major limiting factor in neurodegenerative disease modeling remains the paucity of sophisticated fundamental in vitro studies. Traditionally, neuroscientific research in conventional, monolayer techniques suffers significant drawbacks due to the unrivaled complexity of the human brain as a three-dimensional (3D) structure (Monzel 2017). Specifically, research focused on Alzheimer’s and Parkinson’s diseases has been conducted in animal models lacking naturally occurring pathology. This may well explain the confounding drug failure rate published by the FDA in 2004, citing that 92 percent of drugs passed in preclinical tests, including required animal tests, never make it to the market (Harding 2004). The controversy of animal research exists not only because of ethical concerns but also due to untranslatable results and significant expense, spawning the desire for ethically sourced, economically efficient research techniques. In addition, animal research always includes further unquantifiable variables, for example individual health status, reproducible diet, growth, weight, and inherent genetic discrepancies (Ertl et al. 2009). Conversely, the integration of complex cell biology with microchip technology has the potential to generate new diagnostic tools and disease models to help improve our understanding of the human body, while fulfilling the EU Directive to reduce, replace, and refine animal testing. Currently, our research group is validating a midbrain-on-a-chip capable of evaluating new treatment approaches in a reproducible, cost efficient model precluding animal trials. In this work, we integrate interdigitated electrodes to sense neurotransmitter release, documenting neuron degeneration and offering quantification of regenerative capacities.
To regenerate complex tissues with an oriented extracellular matrix (ECM), such as the spinal cord, injectable hydrogels, which can be applied in a minimally invasive manner, and yet providing structural guidance, are required. While most injectable hydrogels consist of an isotropic network, we recently developed the Anisogel, a tunable, injectable hydrogel system, which allows precise engineering of the construct’s anisotropy in situ after injection. [1] The Anisogel comprises a soft hydrogel surrounding short fibers, which orient in the direction of a low external magnetic field (∼ 50 mT), before complete gelation of the enclosing matrix. The fibers are rendered magnetic by incorporating a low amount of superparamagnetic iron oxide nanoparticles (SPIONs), and cut short using a cryotome device. They are produced via a high-throughput Solvent Assist Spinning (SAS) technique, which enables continuous fiber production with variable diameter (1-50 µm) and, notably, precise surface morphology. Depending on the employed solvent type, microfibers with smooth, grooved or porous surface topography can be fabricated using the SAS method. In addition, the topography properties, such as the pore size and aspect ratio, and the groove width, can be tuned. Therefore, the Anisogel provides control over the anisotropic structure of the 3D microenvironment at two different scales: i) the macroscopic unidirectional architecture resulting from the microscale oriented fibers (diameter ∼ 5-7 µm, length = 100 µm) and ii) the nano-micro anisotropic features, induced by the surface morphology of the fibers. While the unidirectional macroscopic structure of the hydrogel prompts linear nerve growth inside the Anisogels, short fibers with grooved morphology (grooves width ∼ 1-2 µm) led to an increase in neurite length. Importantly, we demonstrate for the first time that the 3D Anisogel supports spontaneous neural signal propagation in the direction of the oriented fibers. The unique properties of this hybrid material provide a versatile platform to investigate the effect of an anisotropic matrix on physiological and pathological processes in vitro and in vivo.


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Nerve Guide Conduits with Intraluminal Guidance Cues for Peripheral Nerve Regeneration

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Introduction
There is a clinical need for improved treatments for peripheral nerve injury to overcome the disadvantages associated with autograft repairs. Nerve guidance conduits (NGCs) can be used as an alternative treatment, however current commercial NGCs have simple designs and are only successful in short distance nerve gaps. Here, conduits were fabricated containing intraluminal guidance cues in the form of either internal microgrooves or aligned microfibres. Both were investigated in vivo to assess the influence of each type of guidance structure on peripheral nerve regeneration.

Methods
Polycaprolactone-methacrylate (PCL-MA) was synthesised and used to fabricate conduits via microstereolithography; plain tubes and tubes containing internal microgrooves were both produced. Aligned PCL microfibres were electrospun and inserted into the plain tubes to act as intraluminal guidance structures. The individual components and combined constructs were analysed via scanning electron microscopy, helium pycnometry and micro-computed tomography.

In vivo implants were carried out in Thy-1-YFP mice to determine the effectiveness of each conduit type in promoting nerve regeneration; the NGCs were used to repair a 3 mm injury of the common fibular nerve and regeneration was assessed via confocal imaging after a 3 week recovery.

Results & Discussion
PCL fibres with diameters ranging from 2–16 µm were electrospun to a high degree of alignment and inserted into the plain PCL-MA tubes. Fibre packing density was quantified to give a percentage fill of the conduit lumen with values ranging between 10-50%. PCL-MA conduits were also produced with aligned microgrooves along the internal wall, with no material occluding the luminal area.

In vivo regeneration through the microgrooved and fibre-filled NGCs was quantitatively assessed via the tracing of fluorescent axons across the injury site and results were compared to empty conduit controls and graft repairs. In the preliminary in vivo trials, axons were seen to traverse the 3 mm nerve gap in each conduit type showing the potential for these intraluminal guidance cues in promoting nerve regeneration.

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Combined Nonwoven-Hydrogel Scaffold for Spinal Cord Tissue Engineering

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High direct and indirect costs of spinal cord injury (SCI) to both individuals (NSCISC, 2013) and health services (Spinal Research, 2015) is apparent, despite low global estimates of traumatic SCI (WHO, 2013). This financial burden is due to the sudden, severe and prolonged impairment to multiple systems within the body. Impairment is caused by the disruption of the anisotropic tissue structure, tightly linked to the function of information transmission, and the spinal cord’s poor regenerative capacity (Silver & Miller, 2004).

With this in mind, an effective scaffold may need to facilitate reconstruction of tissue architecture, and there is also evidence mechanical properties of the tissue differ depending on the scale at which they are investigated (Bartlett, Choi, & Phillips, 2016). A consensus on the most appropriate for spinal cord tissue regeneration has not yet been reached.

A scaffold design process was sought where degree of alignment, wettability and stiffness was easily adjusted. This has been achieved through the combination of a highly aligned nonwoven and a photo-crosslinked collagen hydrogel. Electrospinning of polycaprolactone (PCL) and a self-assembling peptide, P11-8, has yielded a novel highly aligned nonwoven. Alignment of up to 68% of fibres ±10° of 0° and 88% of fibres ±30° of 0° has been achieved, with average fibre diameter of 485 ± 174 nm. Peptide concentration and distribution was confirmed using high-performance liquid chromatography, with concentration ranging from 29.7 µg per cm2 of nonwoven up to 112.4 µg per cm2. Investigation of scaffold mechanical properties at the macro-, micro- and nano-scale has been probed using uniaxial tensile testing, compression testing and atomic force microscopy. PC12 and C6 cell infiltration has been recorded.

Early consideration of material choices already used in medical devices to aid the route to market were taken where appropriate, including the use of PCL in the nonwoven and collagen in the hydrogel. Augmentation of material properties, such as wettability, was achieved using P11-8. This also incorporated a sensitive and quantifiable material into the product which does not add steps to production and is already being produced to good manufacturing practice standards. These considerations are important due to the high cost of taking a treatment through the regulatory framework that surrounds implantable Class III medical devices, with the goal of treating patients cost-effectively.
Using a mouse model of cerebral infarction, we previously demonstrated that injury/ischemia-induced stem cells developed within the post-stroke area. Because they exhibited the traits as neural progenitors, we initially named them injury/ischemia-induced neural stem/progenitor cells (iNSPCs). However, because of their differentiation activities other than neural lineage, we now call these stem cells injury-induced multipotent stem cells (iSCs). We also demonstrated that brain pericytes near cerebral microvessels acquired multipotency following cerebral ischemia in mice and that they could function as iSCs.

Very recently, we demonstrated that brain pericytes can also revert to iSCs in the human brain following cerebral infarction. Here, we introduce the characteristics and future perspective of human iSCs. The Ethics Committee of our college reviewed and approved this study (approval number: 1776). Brain samples were obtained from post-stroke areas in patients requiring both decompressive craniectomy and partial lobectomy for diffuse cerebral infarction. Then, post-stroke human brain tissues were mechanically dissociated and the resulting cell suspensions were incubated under adherent culture conditions. Polymerase chain reaction analysis indicated that putative iSCs did not express astrocytic and endothelial lineage markers, but expressed pericytic and neural crest lineage markers. Immunohistochemistry showed that iSCs differentiated into multiple cells, including neural and mesenchymal lineages, indicating that iSCs share characteristics of neural stem cells and mesenchymal stem cells. In addition, we demonstrated that iSCs have potential to differentiate into electrophysiologically functional neurons. Because human iSCs can differentiate into various cells, including neurons, iSCs have potential to repair the damaged CNS following ischemic stroke. Thus, iSCs may be a promising cell source for patients suffering from cerebral infarction.

For clinical application by iSCs, we now propose two strategies. The first approach is the way to administer bioactive molecules which can contribute to survival, proliferation, migration, and neuronal differentiation of endogenous iSCs. The second option is the one to transplant the cultured autologous iSCs. This method may be applied in stroke patients mainly during subacute or chronic phase, but have advantages for them from a point of view that a number of iSCs can be transplanted repeatedly.
Neurogenesis of Neural Stem/Precursor Cells: Start from Serum Fractions

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Regulating neurogenesis of neural stem/precursor cells (NSPCs) are the important topics in neural tissue engineering field. Biomaterials and extracellular matrix (ECM) are widely used as substrates or scaffolds to regulate the neurogenesis of NSPCs. It has been shown that NSPCs are influenced by a number of matrix characteristics including wettability, surface charges, and so on. However, because of non-anchor features of NSPCs, intrinsic factors in media such as serum also play a vital role for neurogenesis of NSPCs before them attach on matrix. Here, from another point of view — intrinsic solutes of serum in medium, we want to introduce a concept to decipher the effects of serum fraction components on neurogenesis of NSPCs.

By systematical identification of serum components, we successfully found a specific serum fraction with the neurogenesis function that might be used as a novel neuronal differentiation medium. Besides, via combination of the serum fraction-based medium and substrates, it could fabricate a high efficient neuronal differentiation system, suggesting that the system is useful for in vitro drug test of neural cells.

Importantly, we further used the neurogenesis serum fraction as an agent to treat the rats with ischemia brain, which can enhance the neurogenesis of endogenous NSPCs and improve the functional recovery in an experimental model of transient cerebral ischemia. For future applications, we expected our concept, regulation of NSPC neurogenesis by serum fractions, that could be extended to the neural regenerative applications of autologous-serum from patients in neural tissue engineering field.

References

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Neurotrophic Effects of Centella asiatica (L.) Urban on Differentiation of Human Mesenchymal Stem Cells In Vitro

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Introduction
Increasing evidences have shown the potential of various herbs in differentiating human mesenchymal stem cells (hMSCs) to neural lineages. Yet, there is almost no report on such effects for Centella asiatica (L.) Urban, which was used as nervine during prehistorical times. This study was performed to investigate the potential role of raw extract of Centella asiatica (L.), (RECA) on neural differentiation of hWJMSCs in vitro.

Methodology
The cytotoxicity effects of RECA was evaluated via MTT viability assay. Then, RECA at varying concentrations (400, 800, 1200, 1600, 2000 and 2400 µg/ml) was supplemented to hWJMSCs (P3) alone or in combination of neurotrophic factors throughout 9 days of neural induction process. The morphology of induced hWJMSCs was documented and neural markers expression (S100β, p75 NGFR, MBP, GFAP and MOG) was analyzed via immunocytochemistry and quantitative PCR. RECA effects on cell cycle of induced hWJMSCs was evaluated using flow cytometry.

Results
It was found that RECA exerted effects on both proliferation and neural differentiation of hWJMSCs in a dose-dependent manner with IC₅₀ 1875±55.67 µg/ml. The stimulatory effects of RECA on neural differentiation of hWJMSCs was pronounced at 400 and 1200 µg/ml, via the protein expression of the studied markers on the induced cells. Low gene expression level was detected in each of the neural markers and it was insignificantly different across the experimental groups. The normal growth cycle of the hWJMSCs was found not to change upon induction with RECA. All the cells mainly populated in G₀/G₁ phase due to cell growth arrest attributed by cell’s confluency and/or inhibitory effect of RECA.

Conclusion
Although RECA has inhibitory effects on hWJMSCs, it has the ability to differentiate the cells to neural lineages, either it used alone or in combinations of neurotrophic factors.
01-P489 3D in vitro blood capillaries, towards a model of the blood brain barrier

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Drug diffusion across blood capillaries in the human body and brain is a crucial assessment for early selection of drug candidates. Pharmacokinetics evaluation relies on robust and relevant in vitro tissue models. Here, models of capillary networks were developed with a 3D organization in extracellular matrix (ECM). This technology was then optimized to mimic the heavily selective blood brain barrier (BBB).

Two methodologies were developed. First, single human endothelial and fibroblast cells (HUVECs and NHDFs) were coated with fibronectin and porcine type-I gelatin nanofilms, and seeded into cell culture inserts. The alternation of the two cell types layers enhanced the formation of different vascular network structures. The second methodology aimed at increasing the ECM collagen proportion to get closer to 32%, the human body ratio. Collagen sponges were not dissolved but rather homogenized into collagen microfibers which can be directly mixed with the cell suspension and seeded in the inserts. In the BBB model, HUVEC and NHDF were replaced by human immortalized brain endothelial cells (HBMEC), pericytes (HBVPC) and astrocytes (HASTR). Histology, immunofluorescence and electronic microscopy allowed the evaluation of 3D tissue model structure, while permeability and transport were assessed with fluorescent labelled dextran and microspheres.

Both methodologies induced a 3D capillary network formation. Controlled location of endothelial cells was achieved, leading to the formation of open-ended tubulars which permitted the study of the passage of small molecules13. Perfusable capillaries structures were developed on commercial micropatros making them pertinent for high-throughput drug screening. Results from coculture of HUVEC and NHDF were translated to a triculture protocol for HBMEC, HBVPC and HASTR showing promising results. In a next step, validation of the barrier functions and transporter expression will be carried out using human pluripotent stem cells. Finally, implementation in a perfusable device will complete this proposition of a BBB on a chip.


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01-P490  Delivering engineered neural tissue within peripheral nerve repair conduits

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Current surgical treatment for major peripheral nerve injuries relies on the use of nerve autografts. The aim of our research is to use tissue engineering and regenerative medicine technology to develop living replacement nerve tissue that could be used as an alternative to a nerve graft.

Previous work has developed engineered neural tissue¹,² (EngNT), a living replacement endoneurium formed using self-alignment of cells in collagen gels. In order to progress this technology towards clinical translation, the use of human neural stem cells as a potential allogeneic source of therapeutic cells in EngNT is under investigation. In addition, production technology is being developed to facilitate reliable construction of conduits containing EngNT.

EngNT has been generated using CTX cells differentiated towards a glial phenotype. The production of EngNT has been optimised through the development of new equipment that can be scaled up and is amenable to automation and suitable for translational studies. Initial tests involving a rat model of surgical nerve repair indicated that the EngNT implant maintained its overall structure and supported regeneration of host neurons.

REFERENCES:

01-P491  Investigation of a 3D Brain Angiogenesis Model Mimicking CNS Developmental Stages

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[Background & Aims] Neurovascular unit (NVU) is an important concept for the treatment of central nervous system (CNS) and for understanding basic brain functions. Since NVU is composed of neurons, glial cells, blood vessels and extracellular matrix, it is essential to construct brain blood vessels in combination with neural cells for construction of NVU in vitro. It is known that brain angiogenesis, which is the process of new blood vessel formation, is strictly regulated by neural cells during development. Therefore, we aimed to construct a 3D brain angiogenesis model mimicking CNS developmental stages. The purpose of this study is to evaluate the effect of neural cells on brain angiogenesis in 3D culture.

[Methods] Human neural stem cells (NSCs) were cultured to prepare three types of neural cells mimicking CNS developmental stages such as NSC/NPC phase, neuron phase, and astrocyte phase. These neural cells and human brain microvascular endothelial cells (BMECs) were cultured in microfluidic devices which have two serial gel-channels sandwiched by two media-channels. Neural cells were mixed in fibrin gel and injected into one of the two gel-channels while BMECs were cultured in a media-channel. The process of capillary formation was monitored by phase-contrast microscopy.

[Results & Discussion] Firstly, we confirmed differentiation of NSCs and found that three types of neural cells were successfully prepared, which were nestin-positive NSC/NPC-phase cells, Tuj-1/MAP2-positive neuron-phase cells, and GFAP-positive astrocyte-phase cells. Next, these neural cells were cultured with BMECs in a microfluidic device. We found that NSC/NPC-phase cells promoted capillary formation of BMECs compared with BMEC monoculture. By contrast, neuron-phase and astrocyte-phase cells inhibited capillary formation. To further investigate the interaction between BMECs and neural cells, mRNA expression of angiogenesis-related factors was evaluated by quantitative PCR analysis. The results showed that NSC/NPC-phase cells expressed Wnt3a and Wnt7a, while neuron-phase and astrocyte-phase cells expressed Semaphorin3E and Netrin1. These results suggest that angiogenesis was promoted by Wnt3a/7a, and inhibited by Semaphorin3E/Netrin1 in our coculture model. In conclusion, this study demonstrated a coculture model of BMECs and three types of neural cells mimicking CNS developmental stages. This model is useful for evaluating the effect of neural cells on angiogenesis.
Currently, treatment of peripheral nerve injury using autografts is beset by donor site morbidity and current hollow nerve guidance conduits (NGCs) are ineffective over 20 mm (1). To address this, we are now developing the next generation of filled NGCs (2). These NGCs consist of a tubular outer conduit containing a microporous internal matrix that will allow for enhanced Schwann cell migration and axonal regeneration. In an effort to further enhance this matrix, this study will investigate the potential to enhance the regenerative potential of the NGC through the incorporation of extra-cellular matrix (ECM)-derived neuroconductive macromolecules.

In this study, the ECM molecules fibronectin, laminin I and laminin II were incorporated into a previously selected matrix of collagen-chondroitin sulphate. Various combinations and concentrations of these ECM macromolecules were first examined in 2D Schwann cell culture for enhanced proliferation in order to establish optimal parameters. Following this, selected ECM combinations were incorporated into the collagen-chondroitin sulphate slurry and directionally freeze dried under controlled conditions. Effect of ECM incorporation on the porosity and pore alignment was compared against controls using SEM and mechanical testing. Pro-regenerative effects of the 3D ECM scaffolds was assessed using primary rat dorsal root ganglia (DRG) and Schwann cell isolates in culture for at least 14 days.

NGCs were found to be highly porous and uniform throughout. There were clear, significant increases in Schwann cell proliferation depending on ECM combination with the highest levels of proliferation observed when all three ECM macromolecules were present. Further analysis showed additional increases in proliferation when laminin II made up the majority (66%) of the ECM combination. 3D culture of DRGs on the freeze-dried matrices found the most promising evidence of Schwann cell migration (up to 2600μm, day 14) observed in the 66% laminin II group compared to growth of up to 1800μm in control samples.

These findings demonstrate the clear benefit of ECM neuroconductive macromolecule incorporation in NGCs with future work now focusing on progression to in vivo studies.


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Neurons are also exposed to distinguishing physical and chemical environments that can greatly influence their behaviors and functions. In this study, we proposed engineering platforms of laminin-coated matrix nanotopography that generate extracellular physical and chemical cues for neuronal development. Using our platforms, we showed that nanotopographical and biochemical cues could provide suitable environments for neuronal cultures. More importantly, we showed that a laminin-coated matrix nanotopography could control the orientation of neuronal structures as well as accelerate neuronal development through synergistic effects of extracellular nanotopographical and chemical cues. Our study imparts new design principles on the role of nanotopographical and chemical cues in neuronal development for the fabrication of neuroprosthetic scaffolds.
**A preclinical 3D model for testing nerve guidance conduits using high throughput light-sheet microscopy**

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**Introduction**

Clinically used nerve guidance conduits (NGCs) fail to promote peripheral nerve regeneration in >3cm nerve gaps. Internal NGC scaffolds could provide additional guidance to axons and Schwann cells (SCs) to prevent misguidance and enhance nerve regeneration success. Newly developed NGC scaffold designs, which have been in vivo implantation, have compared favourably to hollow conduits and autografts. However, new designs have typically not been directly compared to previous generation NGCs, or to currently used nerve conduits. In this study, a new 3D in vitro testing model for NGCs with internal scaffolds has been developed\(^1\). Aligned polycaprolactone (PCL) microfibers were chosen as internal NGC scaffolds. Different fibre diameters, packing densities and surface modifications were studied in this model.

**Methods**

Aligned PCL fibres were electrospun to 1, 5, 8, 10 and 13 µm diameters. Poly(ethylene glycol) NGCs were fabricated by microstereolithography, fibres were inserted inside NGCs and evaluated. Samples were surface-modified with air plasma. Embryonic chick dorsal root ganglia (DRGs) were placed on top of the fibre-filled conduits. Immunocytochemistry was performed and light-sheet microscopy used to visualise and measure neurite and SC outgrowth from the DRG body towards the end of the 6 mm tube.

**Results**

All DRGs on air-plasma deposited microfibres showed axon and Schwann cell outgrowth. DRGs on non-deposited samples failed to grow out. The minimum outgrowth over 7 days was 2 mm on surface modified 1-13 µm fibres. Fibres of 10 µm performed best with an average axon and SC outgrowth of 2.8 and 3.2 mm respectively. The translation of the model in vivo is currently ongoing.

**Discussion**

This model enabled the direct comparison of different guide designs in one setup and would enable a reduction of downstream nerve injury models. The model identified 10 µm PCL fibres as the best physical guide in NGCs among those tested here and showed the importance of air plasma treatment when using PCL as a material in nerve regeneration. Additionally, DRGs demonstrated their effectiveness for evaluating internal NGC scaffolds as they simulated the proximal nerve stump after nerve injury and presented a source of primary neurons and SCs.

**Acknowledgements**

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**References**


**Fabrication of a Versatile Three-Dimensional Nerve Model**

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Most human tissues require innervation for functionality and homeostasis maintenance. A three-dimensional (3D) in vitro system that integrates a neural component alongside other tissue equivalents (e.g. muscle, pancreas, blood vessels etc.) constitutes an advance in representing the complex milieu of an organ and can be a valuable tool to study biological interactions and test drugs. However, most current organ models either omit or inaccurately depict native innervation. This work shows the development of a 3D platform that recapitulates a nerve microenvironment and allows for the inclusion of other tissues, towards functional innervation. The platform is composed of a polydimethylsiloxane support that houses a cell-seeded aligned nanofibrous scaffold encased in a fibrin hydrogel. Primary Schwann cells (SCs) are seeded in the scaffold, developing overtime an anisotropic band-like structure, reminiscent of Büngner Bands. Within 7 days of culture, the SCs produced nerve extracellular matrix molecules, such as laminin, fibronectin and collagen type IV. Co-cultures of 7 days with either dorsal root ganglion neurons (DRGs) or PC12 cells resulted in extensive and anisotropic growth of neurites, significantly larger and denser than when SCs were absent. Moreover, neurites grew through the volume of the hydrogel, out of the plane provided by the scaffold, thus resulting in a truly 3D tissue structure. When co-culturing these cells for 28 days, myelination was observed, as demonstrated by immunostaining to myelin markers and transmission electron microscopy. In summary, we demonstrate here foundational steps towards a nerve model, which can help in elucidating questions regarding neural biology and act as a platform to explore the impact of tissue innervation on the development and function of other tissues, such as vascular networks.
Human induced pluripotent stem cells (hiPSCs)-based technologies are a promising resource for modeling and regenerating complex tissues, including the central nervous system. Nevertheless, research models still have significant technical limitations such as insufficient maturation, abnormal aggregation and low long-term viability of neurons, which need to be improved to more closely resemble physiological conditions. To address this challenge, we utilize peptide amphiphiles (PAs), a class of biomaterials that have the ability to assemble into high-aspect ratio supramolecular nanofibers capable of morphologically and chemically mimicking the extracellular matrix.

In this study, a PA containing the short bioactive peptide, IKVAV, found in Laminin-1, was used to explore the impact of dynamic properties of supramolecular assemblies on hiPSC-derived neurons maturation. To investigate this effect, three IKVAV PAs containing different intermolecular hydrogen bonding domains were designed. Surface coatings of the three different IKVAV PAs allowed similar neuronal attachment and improved cell survival and cell maturation compared to the commercial full-length laminin. Interestingly, the IKVAV epitope displayed on the weak hydrogen bonding PA nanofiber induced better activation of the laminin signaling pathway observed by higher protein levels of the laminin receptor, β-1 integrin, and the downstream effector integrin linked kinase-1. Consequently, reduced neuronal aggregation, higher branching complexity, and increased electrical activity were achieved on the aforementioned IKVAV-PA after 2 months in vitro.

Overall, the IKVAV PA with weak hydrogen bonding could enhance long-term maturation of neuronal cultures. The reported PA platform may provide a strategy to study various aspects of the human nervous system microenvironment, thereby helping to elucidate new pathological mechanisms and therapeutic strategies for multiple neurological disorders.

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Rett syndrome (RTT) is a X-linked neurodevelopmental disorder that affects 1 in 10,000 female. Over 95% cases of typical Rett syndrome are caused by mutations of the X-linked methyl–CpG-binding protein protein 2 (MeCP2). Different types of mutations in MeCP2 can account for the variability of clinical features in patients with RTT. The C-terminal (CT) truncating mutations in MeCP2 correlate with milder clinical phenotypes. Here, we present a case of atypical form of RTT with small deletion in CT of MeCP2. The patient was diagnosed with RTT at the age of 26, the clinical symptoms include sleep disorders characterized by non-24 hours (~7 days) sleep-awake rhythm, impulsiveness, and dystonia. DNA sequence analysis of the patient demonstrated 5 base deletion (c.1196-1200ΔCCACC,P399QfsX3) was seen within C-terminus of MECP2 gene. The resulting mutated MeCP2 protein retains the critical functional domains of MeCP2 including methyl-CpG binding domain, trans-repressor domain, nuclear localization signal and the majority of WW domain. However, the mutated MeCP2 protein lacks phosphorylation site (S421) triggered by CaMKII and psychostimulants. Upon the phosphorylation of S421 of MeCP2 protein, it trans-locates from nucleus to cytoplasm, resulting in the expression of the target genes. Accordingly, characteristic symptoms of the patient might originate from impaired de-repression of the target genes by MeCP2 initiated by neurotransmitters associated with the dopaminergic signal. The patient was treated with Ghrelin and Aripiprazole. Ghrelin was proved to improve dystonia and Aripiprazole (partial agonist fro dopamine receptor D2) ameliorated her emotional disorders and verbal communication. It is recently reported that dopamine/DRD1 signaling dependent on the Ghrelin receptor, and MeCP2 regulates mTOR signaling. Ghrelin could exert it functions via collecting dopamine and mTOR signaling impaired by MeCP2 deficits. Next, to explore the molecular mechanisms underlying Ghrelin and Aripiprazole treatments, the same MeCP2 mutation seen in the patient was introduced into culture cell line (DLD1 cells) using CRISPR/CAS9 system. Unexpectedly, mutant MeCP2 protein was not expressed in the MeCP2-mutated culture cells (MeCP2Δ1196-1200 DLD1 cells), although MeCP2 mRNA level of the mutant cells was comparable to that of the wild type DLD1 cells. Therefore, it is very important to see the MeCP2 protein expression to discuss the issue of genotype-phenotype correlation.

One out of six children are diagnosed with developmental disorder and the need of the hour is to develop in vitro platforms to screen compounds such as environmental toxicants and pharmaceutical drugs for their potential developmental toxicity. The developing central nervous system is highly susceptible to damage on exposure to toxicants, due to the immature blood-brain barrier. Since biochemical and biomechanical cues play an integral role in regulating cell morphology, movement and metabolism for fetal development, there is a critical need to develop in vitro tests focusing on biochemical and biomechanical outcomes in addition to cytotoxicity outcomes.

Here, we exposed human neural stem cells (NSCs) to a range of concentrations of different classes of compounds (pesticide and pharmaceutical drug) for up to 36 h, and the in vitro cytotoxicity and biomechanical characteristics (using a MFP-3D-BIO AFM) were investigated and correlated. The sub-cellular mechanisms by which toxicity was induced to NSCs by these compounds were identified. Results suggest a concentration-dependent decrease in cell viability and time-concentration dependent compromise in NSC biophysical characteristics. Such decrease in their elasticity suggests damage to actin cytoskeleton of NSCs, possibly leading to apoptosis. These compounds induced changes at single-cell level suggesting need for biomechanical evaluation of cells under toxicant-aberrant conditions.

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Peripheral nerve injury (PNI) is a debilitating condition resulting in chronic or permanent loss of sensory and/or motor function in a localized area of the body. The current clinical gold standard for PNI is the autograft, and while this method has been successful, its drawbacks include donor-site morbidity, limited tissue supply, and a second surgery. An alternative clinical therapy is the decellularized nerve graft. Decellularized grafts maintain some of the native biochemical and biomechanical cues associated with autografts; however, they rely on a limited supply of cadaveric donor tissue, have batch-to-batch variability, and are not easily produced in high quantities. To address these limitations, we propose the use of a recombinantly synthesized, tunable material for use as an engineered nerve guidance conduit (eNGC). These materials mimic key biochemical and biomechanical cues of the native neuronal matrix to enhance neurite outgrowth while being reproducible and scalable for efficient manufacturing.

Leveraging protein engineering, we have developed a series of elastin-like proteins (ELPs) with built-in cell-binding ligands derived from naturally occurring extracellular matrix proteins. This library of ELPs is modular in design and allows for exact control over ligand density. The ELPs can be non-cytotoxically crosslinked to form matrices with a range of stiffness (shear modulus, G' ~ 100 - 20,000 Pa). Importantly, the cell-binding ligand concentration and matrix stiffness are both independently tuned, making ELP matrices amenable to combinatorial studies. We have developed a casting method that allows for the fabrication of a core-shell eNGC made completely from recombinant ELP. Utilizing ELP, we can tune both the outer shell and inner core separately to fulfill different functionalities. Based on in vitro neuronal studies in ELP matrices, the inner core matrix has been designed to be more compliant (shear modulus ~300 Pa) and to contain the fibronectin-derived RGDS cell-binding ligand to promote neurite outgrowth. Conversely, the outer shell has been designed to be stiffer (shear modulus ~15 kPa) and to contain a non-cell-adhesive ELP variant to provide mechanical guidance to the regenerating nerve stump. We have successfully implanted our eNGC into the site of a 15-mm sciatic nerve injury in a rat model. Taken together, our platform represents a fully-synthetic, biomimetic potential alternative to current therapies for peripheral nerve injury.
The development of injectable beta-peptide hydrogels with human amnion epithelial cells (hAECs) for the treatment of ischaemic stroke

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Ischaemic stroke is a leading cause of death and disability worldwide, caused by the sudden blockage of blood supply to the brain that results in neuronal cell death and loss of neurological functions. Current treatments focus on restoring blood flow to the damaged areas but they are only effective within several hours of stroke onset and only aim to stop the progression of the disease. Hence, there is a distinct lack of therapies that are capable of regenerating the stroke infarct to restore lost function and reduce disability. To address this, we propose a biomaterial-based tissue engineering approach that combines the regenerative ability of stem cells with self-assembling peptide hydrogels as a delivery and encapsulation mechanism.

Briefly, we have developed a novel beta-peptide hydrogel containing cell adhesion motifs that displays shear thinning behaviour, rapid self-assembly and recovery. The molecular basis of gelation is the head-to-tail self-assembly of these peptides into nanorods, which then further assemble into nanofibers via hydrophobic interactions of an alkyl chain. Human amnion epithelial cells (hAECs), shown to possess regenerative and anti-inflammatory properties, have been successfully cultured and grown within the gel. This hydrogel-hAECs mixture is able to be injected with high viabilities and gelate in situ to form a biocompatible encapsulating 3D matrix akin to that of brain tissue. Current studies focus on assessing the in vivo responses to the hydrogel and cells using a photothrombotic cortical lesion mouse model of stroke, and their therapeutic outcomes via functional sensorimotor testing. We hypothesise that the gel will be protective of the hAECs against the harsh inflammatory environment within the infarct, leading to greater therapeutic effect and a promising step towards a regenerative treatment for stroke.
01-P505 | Bioinspired Sticky Protein-based Nanofibous Conduit Scaffold for Accelerated Nerve Regeneration

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Treating traumatic nerve injury requires immediate and effective nerve grafting due to limited regeneration capacity of nerve tissue. Currently, autograft is widely used for nerve grafting, but the demand for safe, ready-made, cost effective, and biodegradable polymer-based nerve conduits are gradually increasing. However, conventional synthetic polymer-based nerve conduits have not been able to achieve comparable nerve regeneration prognosis to the autograft due to incomplete functional regeneration. Thus, taking advantage of bioinspired natural polymer and biomimetic topologies are promising strategies for nerve conduit developments. As a bioinspired natural polymer from marine mussel, bioengineered mussel adhesive protein fused with IKVAV peptide (MAP-I) was designed as a conduit material that can accelerate nerve regeneration. Mussel adhesive protein (MAP) can effectively contribute to cell adhesion and proliferation through its exceptional adhesive property and IKVAV peptide takes part in integrin-mediated interaction with neural and Schwann cells on their differentiation and neurite formation. To promote contact guidance of neural and Schwann cells, electrospun aligned nanofiber of MAP-I was fabricated so that the topology of nerve conduit enhances differentiation and guided nerve regeneration. The combination of adhesiveness by MAP, integrin-mediated interaction by IKVAV, and contact guidance by aligned nanofibers were expected to synergistically benefit functional nerve regeneration. Herein, MAP-I aligned nanofibrous conduit was intensively investigated in vitro and in vivo.

01-P504 | Different Brain Tissue Decellularization Procedures Result in Similar Cell Elimination and Extracellular Scaffold Preservation

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BACKGROUND: Whereas research in decellularized scaffolds as platforms for tissue engineering and regenerative medicine has been mainly focused on organs such as lungs, heart or kidney, the data currently available on brain decellularization is scarce. Reducing this knowledge gap is of particular interest given the high prevalence of neuro-degenerative diseases. For instance, to interpret the recent finding that brain tissue in Alzheimer disease is considerably softer that in normal controls (1). AIM: To get insight into brain decellularization, we tested 4 decellularization protocols in terms of remaining cells and extracellular matrix components. METHODS: 9 male Wistar rat brains rats were isolated, cut into 2 mm-thick coronal sections and subsequently decellularized using 4 protocols adapted from previously described procedures for mice and porcine brain decellularization (2,3). Sections from native and decellularized brains were included in OCT and cut into 30 µm slices and were stained with Alcian blue for glycosaminoglycans (GAGs), immunostained for laminin content and labelled with NucBlue® to visualize remaining DNA fragments. Bright field and epifluorescence images were acquired and processed by ImageJ following a blind procedure. Data were analyzed by one-way ANOVA. RESULTS: The four protocols yielded acellular brain scaffolds with approximately 95% reductions in DNA content (p < 0.001) as compared with native tissue, with no significant differences among protocols. GAGs content was virtually unaltered regardless of the decellularization procedure. Laminin content was reduced to values ranging 50-70% when compared to native tissue. CONCLUSION: Similar results were obtained with different variants of brain decellularization process.

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Development of acellular allogeneic nerve grafts for peripheral nerve reconstruction

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Severe peripheral nerve injuries, characterised by nerve gap defects greater than 2 cm, are currently repaired using autografts or synthetic conduits to bridge the gap. However, such techniques have a limited efficacy and functional recovery is seldom achieved. We hypothesised that an acellular nerve graft will support axon regeneration across a defect within a native microenvironment. The aim of the project was to develop a physically compatible, non-immunogenic nerve graft to support axon regeneration.

Human femoral nerves were decellularised using a combination of hypotonic buffers, low concentration sodium dodecyl sulphate (0.1%) and nuclease enzymes, cell removal was confirmed by H&E and DAPI staining and DNA quantification which indicated a reduction in DNA from 346.2 ± 53.8 to 30.5 ± 7.4 ng.mg⁻¹. Histological staining demonstrated acellular nerves to have retained a native architecture and specific ECM components, including collagen type 4, laminin and fibronectin were preserved. Biochemical analyses indicated that collagen and fat content were unaffected by decellularisation, but glycosaminoglycan (GAG) content was significantly reduced from 2.2 ± 0.4 to 0.7 ± 0.3 µg.mg⁻¹.

Acellular nerve segments (~2 cm, n=6) were seeded with primary rat dorsal root ganglia (DRG) neurons. Cell viability was assessed using Live/Dead staining, and neurite extension visualised using immunocytochemistry. Neurons cultured on an acellular nerve scaffold remained viable, and progressive neurite extension was observed.

A decellularised human femoral nerve was developed, and we have demonstrated it to be biocompatible, capable of supporting viable neurons and promoting neurite extension. We hypothesise that the retention of native histioarchitecture and endoneurial structures, which are essential for providing topographic guidance for axon extension, may have supported neurite extension. In addition, acellular nerves retained specific basal lamina components, including collagen type 4 and laminin, which are important for regulating cellular adhesion and growth and promoting Schwann cell-axon interactions, and this may have also facilitated neurite extension in vitro. The presence of GAGs, including Chondroitin sulphate, is thought to inhibit axon regeneration. Therefore, the reduction of GAGs may also be beneficial for promoting neurite growth. The results indicate acellular human nerves may have clinical potential to promote axon regeneration across a defect.
Institute for Neuroscience, Sidney Kimmel Medical College, Thomas Jefferson University, Philadelphia, PA, USA, Toronto, Ontario, Canada, Regenerative Medicine, Tulane University, New Orleans, LA, USA and Technology, University of Coimbra, Coimbra, Portugal, cellular populations (ASCs and OECs) led to significant functional benefits in two distinct models of SCI, highlighting the therapeutic potential of increased EMG signal at the medial portion of the hemidiaphragm. In summary, the combination of functionalized hydrogels with different inflammatory cells and astrocytes. Despite these promising results, cervical SCI has more drastic consequences for the patients, such as respiratory compromise. Moreover, it represents more than half of the SCI cases worldwide. Using a cervical rat model of injury (C2 controls and hydrogel alone-treated rats. A higher number of neurofilament positive cells and less CD11b and GFAP cells was observed, indicating that the combined therapy might have induced axonal preservation/regeneration, associated with decreased infiltration of inflammatory cells and astrocytes. Despite these promising results, cervical SCI has more drastic consequences for the patients, such as respiratory compromise. Moreover, it represents more than half of the SCI cases worldwide. Using a cervical rat model of injury (C2 hemisection), the same combinatorial treatment strategy was evaluated. Five weeks after lesion, spontaneous activity of the affected hemidiaphragm was evaluated through electromyograms (EMGs) of dorsal, medial and ventral portions. All treatments increased significantly the EMG signal at the ventral portion in comparison to the untreated control, but importantly only the combinatorial group presented increased EMG signal at the medial portion of the hemidiaphragm. In summary, the combination of functionalized hydrogels with different cellular populations (ASCs and OECs) led to significant functional benefits in two distinct models of SCI, highlighting the therapeutic potential of this approach.
Development of a new mouse model of short bowel syndrome that may allow for the assessment of therapeutic efficacy of heterotopic transplantation of small intestinal organoids

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Short bowel syndrome (SBS), a malabsorptive state that results mostly from surgical resection of the small intestine, can lead to intestinal failure in its severe form. Although the intestinal organoid transplantation is an emerging area of research, treatment of severe SBS patients with this approach remains challenging. In this study, we first aimed to develop a new mouse model of ileocecal resection (ICR) that shows clinical manifestations as seen in human SBS patients with extensive ICR. We also examined in this study whether transplantation of small intestinal epithelial organoids to the proximal colon is technically feasible in mice.

We found that mice, in which 75% distal small intestine was resected (75% ICR), showed severe clinical signs, such as weight loss and diarrhea, and resulted in higher mortality. Histological examination revealed that mice that underwent 75% ICR showed structural adaptive reactions characterized by villus elongation and crypt deepening. However, CUBN mRNA and its protein product, which play an essential role in vitamin B12 absorption in the ileum, were not compensatory up-regulated in any part of the remnant intestine. The lack of post-operative CUBN induction led to significant decrease in serum vitamin B12 in mice that underwent 75% ICR. Meanwhile, as a result of optimization of the conditions, we developed a new method to strip the epithelium away by perfusion of a chelating agent in a proximal colon segment. In addition, we found that, when small intestinal organoids were derived from EGFP transgenic mice and transplanted, these EGFP⁺ cells were able to engraft such surgically manipulated colons, maintaining their identity of the small intestinal epithelium.

In conclusion, we have shown a new mouse model of massive ICR characterized by severe clinical manifestations. We have also demonstrated that a part of the mouse colonic epithelium can be replaced with the small intestinal one by organoid transplantation. The use of this 75% ICR model has the advantage that functional loss of the distal intestine and its restoration by various therapies could be assessed quantitatively by measuring serum levels of vitamin B12. Thus, the combinatory use of these two mouse models could address the compensatory functions of heterotopically grafted small intestinal organoids in the colon, which will form a basis of heterotopic small intestinal organoid transplantation as a novel option of regenerative medicine for SBS patients.
**01-P513** The prevention of anastomosis leakage by transplantation of autologous adipose-derived stromal cell sheets

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**BACKGROUND:** Although perioperative care and surgical techniques have improved, anastomotic leakage remains a feared and serious complication of intestinal surgery. Adipose-derived stem cells (ASCs) are capable of multiple differentiation pathways, imparting immunomodulatory effects, and secreting factors that are important for wound healing. These characteristics can be exploited to decrease the incidence of anastomotic leakage.

**METHODS:** In order to delay local wound healing at the anastomotic site, we induced ischemia in a portion of porcine small intestine by ligating vessels. Then, we injected mitomycin C into the serosa of the small intestine above the ligated vessels. Anastomotic sites were created by 2 cm incisions made in the opposite mesenteric area. ASCs were isolated from subcutaneous fat of pigs and expanded under a culture condition. ASCs were trypsinized and seeded on temperature-responsive dishes and cultured to form confluent sheets. Three ASC sheets were transplanted onto the serous membrane after suturing. The extent of anastomotic wound healing was evaluated by bursting pressure, hydroxyproline content, and mRNA expression of collagen-1 alpha1 and collagen-3 alpha1.

**RESULTS:** We found that transplantation of ASC sheets increased anastomotic site bursting pressure. Additionally, transplantation of ASC sheets increased the hydroxyproline content of the anastomoses. Furthermore, transplantation of ASC sheets increased mRNA expression of collagen-1 alpha1 and collagen-3 alpha1.

**CONCLUSIONS:** Our findings showed that transplantation of autologous ASC sheets enhanced collagen synthesis and anastomotic strength. Further studies are necessary to identify substances that, in combination with ASC sheets, might enhance collagen synthesis and healing in sites of anastomosis.
**01-P515** Anti-fibrotic efficacy of gelatin sheet containing triamcinolone acetonide

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**[Background]**

There are many situations which fibrosis plays a bad role, and esophageal stricture formation after endoscopic treatment for superficial esophageal cancer is a very challenging complication to treat. Therapeutic efficacy of triamcinolone acetonide (TA) injection into treatment ulcer base is reported, although its effect is limited.

Based on these properties, we hypothesized that local administration of TA with a drug delivery system may affect the TA efficacy. The objective of this study is to examine the therapeutic efficacy of TA released from a gelatin sheet which has been used to confirm its efficacy in tissue engineering.

**[Materials and Methods]**

To prepare a gelatin sheet containing TA (TA sheet), 500mg gelatin was dissolved in 6ml double distilled water and 20mg TA was suspended. These were freeze-dried for 72 hr.

30 mice were prepared, and two skin defects were made on the back. Gelatin-sheet with/without TA was attached to the defect for 15 mice. 15 days after attaching gelatin-sheet, mice were sacrificed and macro findings and histopathology were analyzed. To certain the efficacy of biomaterial, TA sheet versus TA injection experiment on the skin defects have done as well for another 15 mice.

**[Key Results]**

Wound area / original skin defect (%) of TA sheet (26.9±5.5) was significantly larger than that of control sheet (10.7±2.6, p=0.022).

Evaluation for myofibroblast infiltration, α-SMA positive area / whole area(%) of TA sheet (4.65±0.66) was lower than that of control sheet (7.24±0.70 , p=0.023), and

of TA sheet against TA injection was significantly lower as well (5.32±0.45, 7.75±0.71, p=0.016, respectively).

**[Conclusions & Inferences]**

The efficacy of TA sheet to prevent fibrosis have proved through the animal experiments. The TA sheet is promising to clinically treat fibrosis associated with dysfunction of esophageal stricture after endoscopic therapy.
**A novel colon regeneration method: omentum as an in vivo bioreactor**

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**Introduction:** Total or segmental colectomy is the treatment of choice for different diseases including colorectal carcinomas or inflammatory bowel diseases. In this surgical procedure, large intestine is excised from body and an ileal pouch is reconstructed to work as a reservoir and then anastomosed to rectum. Many complications threaten this procedure including pouchitis or even dysplasia.

In search for a naturally derived scaffold, this study uses rats’ decellularized large intestine scaffolds and omentum as a natural bioreactor for their recellularization.

**Materials and methods:** A number of 6 rat colons were decellularized by detergent based method for 24 hours. The decellularized scaffolds underwent DNA quantification test and histological studies to reassure a legitimate acellular scaffold. Then, decellularized tissues were implanted into abdominal cavity wrapped in omentum. Implanted tissues were extracted at 2 months, 3 months, 6 months and 12 months. Tissues were studied by H&E and IHC markers including Anti CK8 for detection of epithelial cells, Vimentin, Desmin and SMA for detection of smooth muscle cells and CD31 and CD34 for detection of hematopoietic stem cells.

**Result:** DNA quantification test revealed a significant decrease in presence of DNA (i.e: cellularity) as compared to native tissue, based on results of spectrophotometric measures. Histological studies showed an intact extracellular structure with less than 5% cellularity. Extracted implants after 2 and 3 months showed high cellularity and angiogenesis with low cellular differentiation.

**Conclusion:** The promising results of this study suggest that due to high vascular density of omentum and its unique regenerative characteristics, it can be quite effective in recellularization of scaffolds, however, there might be need for further studies to evaluate functional capacity of recellularized scaffolds.

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**A 3D printed in vitro small intestine model**

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**Introduction**

Use of animal models in studies of pathogen-host interactions can be reduced by developing in vitro small intestine tissue models. However, current intestinal models often lack physical intestinal 3D features, especially a villi structure that is needed for absorption and a barrier function. As 3D printing enables complex geometries, it is a great technique to fabricate tissue scaffolds with fine 3D features. We developed an intestinal tissue model by 3D printing scaffolds mimicking the villi structure and seeded fibroblasts as confluent sheets around the scaffolds.

To form an epithelium, we cultured epithelial cells on the lumen of the scaffolds. These cell-containing tissue models will be further matured into multilayered tissue constructs in a dynamic bioreactor.

**Results**

Tubular tissue scaffolds were successfully 3D printed by stereolithography to have a lumen side consisting of villi-mimicking spikes. To obtain a multicellular tissue model, epithelial Caco-2 cells were seeded on the lumen side of the scaffolds and human-derived fibroblasts were placed around the scaffolds as cell sheets. The spikes increased the surface area of the lumen and provided the seeded epithelial cells with guidance and mechanical support. Epithelial cells formed a confluent cell layer along the 3D spikes, showing increased enzyme activity, formation of tight junctions, and formation of a microvilli structure, all indicating the differentiation of the cells into enterocyte-like cells. The fibroblast sheets were obtained from a thermostresponsive polyglycidyl ether surface after 24 h of cell culturing. The placement of fibroblast sheets resulted in a high initial density of cells on the scaffold surface, and the later distribution of fibroblasts indicated their proliferation and migration within the scaffolds.

**Conclusions**

Use of SLA enabled high-resolution 3D printing of tubular scaffolds mimicking a native small intestine. Fibroblast sheets were successfully placed around the scaffolds, while epithelial cells were cultured along the lumen side of the scaffolds. To achieve a matured in vitro tissue model, the cell-containing scaffolds will be further cultured in a dynamic bioreactor in physiologically relevant conditions.

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Extracellular matrix-based cell delivery collagen hydrogel system to regulate vascularization for engineering adipose tissue

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Timely tissue vascularization and integration to the patient in engineered tissues play important roles in the success of translation of engineered tissues into clinically relevant therapies. To decrease the time needed to vascularize an engineered adipose tissue, suitable local microenvironments provided by hydrogels to support cell-based formation of three-dimensional perfused and functional vascular networks have been investigated. However, the role of altering architecture, composition and stiffness of biomaterials in functional vascular network formation in vivo remains unclear and keeps controversial. Here, we used injectable collagen hydrogels through physical and chemical crosslinking methods that are capable of independently controlling architecture, concentration and stiffness of collagen hydrogels, and then demonstrated how these parameters control the vascular network formation and further engineer vascularized adipose tissue in vivo. Finally, we successfully engineered the vascularized white adipose tissue construct (~877.6 adipocytes/mm²; 94% of area of a construct) by controlling the cross-linked microstructure of cell-laden hydrogel. These results indicate that the manipulating polymerized microstructure of hydrogel could be used to modulate the extent of vascular network formation and the regenerative capacity of adipose tissue in vivo.
Photo-click Gelatin-Norbornene Hydrogels for Tissue Vascularisation

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Vascularisation is essential to promote viability of the engineered large tissue constructs due to the need of continuous oxygen and nutrient supply. Therefore, biomaterials able to promote formation and maturation of vessels in vitro are highly required. The aim of this study is to evaluate the vasculogenesis potential of a photo-polymerisable thiol-ene gelatin based hydrogel.

Gelatin (10wt%) was reacted with carbic anhydride (20wt%), at 50°C for 24h with pH kept in the range of 7.5 – 8 to produce gelatin-norbornene (gelINOR) macromers. 5wt% GelINOR hydrogels were photo-polymerised (400-450nm, 30mW/cm², 3min, 1/10mM Ru/SPS) with dithiothreitol (DTT). The physico-mechanical properties were characterised with varying DTT concentration. Human umbilical vein endothelial cells (HUVEC) were co-encapsulated within gelINOR hydrogels with two kinds of stromal cells, human mesenchymal stromal cells (MSC) or human dermal foreskin fibroblasts (HFF), at various endothelial to stromal cell ratios. Co-cultures in casted hydrogel disks (Ø5x1mm) were maintained in endothelial growth media for 14 days, following fixation and immunohistochemical evaluation (CD31/F-actin).

GelINOR was successfully synthesised with a 45% degree of modification. Varying Nor:SH (DTT) ratios resulted in tailorable sol fractions and mass swelling ratios. HUVEC was successfully co-encapsulated with MSC or HFF in gelINOR hydrogels, showing high viability (>90%) and a retained HUVEC phenotype over the cell-culture period. Both stromal cell types were able to facilitate the formation and stabilisation of interconnected vessel-like structures.

In conclusion, we have shown that GelINOR hydrogels, with tailorable physico-chemical properties, can be used to promote in vitro vasculogenesis for large tissue engineered constructs.


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Recombinant Spider Silk Functionalized with Antimicrobial Enzymes for Prevention of Implant-Related Infections

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Recombinant spider silk proteins can be produced in bacterial hosts, and purified proteins can assemble into silk in various material formats, such as fibers, thin surface coatings and 3D foams. Such silk scaffolds are considered promising as a biomaterial due to its mechanical properties and cyocompatibility.13 Considering the great need of alternatives to conventional antibiotics, it is attractive to design antimicrobial silk using protein based antimicrobials such as lytic enzymes and antimicrobial peptides. By immobilizing domains with antimicrobial activity onto silk, a lower dose can be used via local application of the active substances, e.g. as silk coatings on orthopedic and dental implant surfaces. We have designed proteins with antimicrobial peptides fused to the recombinant spider silk protein 4RepCT. Moreover, we show that several different bacteriolysins and a biofilm disruptive enzyme (dispersin) can be site-specifically coupled to the N-terminal site of 4RepCT silk proteins by the use of the enzyme sortase. These functionalized silk proteins still possess the ability to assemble into silk fibers while retaining the enzymatic activity. The effects on the viability and adhesion of bacteria on the silk materials are currently being investigated.

References

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Fibrin hydrogels have been used in a variety of tissue engineering applications over the last decade, such as liver, heart, skin, cartilage, bone, muscle, adipose and ocular tissues, as fibrin exhibits excellent biocompatibility, promotes cell attachment, and can degrade in a controllable manner [1]. More recently, fibrin has been used as a component in bioink, the cell laden material used to form 3D structures in bioprinting [2], despite its low mechanical stiffness and low viscosity in solution [1]. To overcome these limitations, previous attempts to 3D bioprint using fibrin have either used the "ink-jet" method [3] or a "mold and fill" strategy [4]. These methods are inherently limited in their ability to produce large constructs with high cell density and complex multicellular structures. However, micro-extrusion bioprinting does not have this limitation [5]. In order to utilise both the advantages of fibrin and micro-extrusion bioprinting, we propose a new method of using fibrin as a bioink component in 3D bioprinting of renal glomerulus. In brief, the surface of patient-derived renal glomerular cells was modified with a surfactant-coated enzymatic crosslinker, and the cells were next seeded into a composite bioink of alginate and pluronic F127 [6]. The bioink was extruded by a system of our own design and the resulting construct was immersed in a solution of fibrinogen and CaCl$_2$ before incubation. Viability 24 hours after printing was 73% ± 6%. This corresponds to a 20% improvement over cells that were bioprinted in a non-enzymatically crosslinked bioink (53% ± 8% p=0.0002). This bioprinting system will be used to build a 3D dimensional model of the renal glomerulus for disease modeling.

**3D Printed Methacrylated Gelatin (GelMA) Hydrogels for Corneal Stroma Engineering**

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Cornea, the avascular tissue located at the front of eye, is crucial for the protection of the eye from environmental factors. Stroma is the thickest section of the cornea, consists of 200-400 lamellae that are organized parallel to cornea surface and orthogonal to adjacent lamellae. This organization is important for the biomechanical and optical properties of the cornea. Use of GelMA for tissue engineered corneal stroma was reported for the first time in the literature by our group. For an enhanced healing, the internal organization of the stroma has to be improved and in this study, GelMA was 3D printed to mimic the microstructure of the stroma.

GelMA was produced from type A porcine skin gelatin and degree of methacrylation was determined by 1H-NMR. 15% GelMA solution (GelMA15) was prepared in the presence of photoinitiator (Irgacure). Two-dimensional (2D) layers were deposited with subsequent layers perpendicular to each other by Bioscaffolder® (SYS+ENG, Germany). Layer thickness (0.14 mm) and the distance between strands (1 mm) were set. To optimize the printing conditions, deposition speed (Fxy, mm/min) and spindle speed (R/S, Dots/mm) were changed. The resultant hydrogels were tested mechanically under compression, in situ degradation for 21 days, water content, and transparency (in the wavelength range 250-700 nm) were determined.

A high degree of methacrylation (ca. 70%) was calculated from NMR data which resulted in crosslinking of the GelMA solution in a short time (5 s with OmniCure, 15 mW/cm², at 365 nm). Stereomicrographs showed that as Fxy increases, fiber diameter decreases and as R/S increases fiber diameter increases. 3D printed hydrogels with smooth fibers and proper design were obtained when R/S was 0.01, 0.02 and 0.03 while Fxy was 200, 200 and 300, respectively (GelMA15-0.01, GelMA15-0.02, and GelMA 15-0.03). Hydrogels retained a significant amount of water in their structures (~90%). Compressive moduli of GelMA15-0.01 and GelMA15-0.02 hydrogels were similar (~11 kPa) and significantly lower than GelMA15-0.02 hydrogels (~18 kPa). Transparency of hydrogels was similar and around 88% which is comparable with that of native cornea. Hydrogels lost only about 8% of their initial weight upon 21 days of incubation in PBS. In conclusion, these results towards in vitro and in vivo studies show that mimicking 3D microstructure of corneal stroma is successful and hydrogels are highly stable and transparent as needed for cornea engineering.

**Digital Light Processing-Based 3D Printing of Gelatin Methacrylamide/N-vinyl-2-pyrrolidone/Hydroxyapatite Composite Scaffolds**

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Precise control over the geometric shape, porosity and internal pore architecture of three-dimensional scaffold is essential for tissue engineering. Digital light processing, one of additive manufacturing techniques, using photosensitive biomaterials is a promising technology for fabricating three-dimensional scaffolds with specific shapes and internal architectures owing to its fine resolution, high fabrication speed and computer-aided design capabilities. In this study, photo-crosslinkable gelatin methacrylamide was prepared by the polymerization of gelatin with methacrylic anhydride. Nanosized hydroxyapatite particles were synthesized by a co-precipitation method in presence of sodium polyglutamate. The nanosized hydroxyapatite particles, N-vinyl-2-pyrrolidone as a co-monomer and VA-086 as a photoinitiator were then mixed with photo-crosslinkable gelatin methacrylamide. Finally, 3D gelatin and gelatin/hydroxyapatite scaffolds were fabricated by digital light processing-based 3D printer. The results demonstrated that the shape of the 3D-printed scaffold was close to that of the design, suggesting its potential in customized scaffold fabrication. The incorporation of hydroxyapatite particles into gelatin scaffolds decreased the pore size and water absorbency of scaffolds.
**01-P526  The artificial trachea fabrication using 3D bioprinting technology and GelMA-HAMA**

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Lately, several types of tracheal scaffolds created by the three-dimensional(3D) printing technique have been reported. In this study, we fabricated artificial trachea using rabbit chondrocytes with Poly(caprolactone) (PCL) and a blended gel consisting of gelatin methacrylation (GelMA) and hyaluronic acid methacrylation (HAMA). GelMA and HAMA were chemically modified to respond to UV light, which were characterized by FT-IR. GelMA and HAMA were blended with various composition ratio and characterized in terms of printability and rheological characteristics to find optimum condition for 3D printing. Rabbit chondrocytes were printed out with the hydrogel and the cell viability was confirmed by CCK-8 and Live/Dead. The partial trachea was fabricated by printed out PCL as a trachea frame and hydrogel as a cell loading material. Cell seeded PCL scaffolds were used as a control. After stabilization of the structure through cultivation for 3 days, they were transplanted into a damaged trachea model for 4 weeks. Harvested tracheas were analyzed in terms of mechanical strength, glycosaminoglycan (GAG) production, histological response related cartilage regeneration. The artificial trachea composed of PCL, hybrid hydrogel of GelMA and HAMA, and chondrocyte showed higher cell viability, GAG production and cartilage-like histological results comparing with 3D printed PCL scaffold. The results suggest a new direction to help regenerate trachea tissue through a novel bio-ink framework technique.

**01-P527  Fabrication of artificial trachea using silk based hydrogel and digital light processing technology**

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Digital light processing (DLP) bioprinter create models with high resolution and with rapid printing speed regardless of the layer’s complexity and area. Although hydrogels have been widely used as 3D bioprinting materials for cell loading, there are only a few reported biomaterials capable for DLP bioprinting. In this study, we modified silk fibroin chemically to respond to the light and applied it to fabricate cell loaded-trachea structures using DLP printer. First model was printed out with human chondrocyte encapsulated silk fibroin for whole trachea and second one as a partial model was printed in two layers with human chondrocyte and human turbinate cells. The printed cell-loaded artificial trachea was incubated for 4 weeks. At each time point a mechanical property (compressive strength), glycosaminoglycan (GAG) synthesis and cartilage related gene expression were analyzed using Universal Testing Machine, DMMB assay and RT-PCR, respectively. Immunohistochemistry was carried out to confirm mucosal epithelial regeneration. GAG production, mechanical strength and gene expression such as collagen type II, SOX-9 and Aggrecan were promoted with time. In the result of IHC, the outer part of the trachea was completely differentiated into cartilage, and the inside was covered with mucosal epithelium. The results demonstrated that DLP system with silk fibroin could be fabricated mechanically and biologically functional trachea. This technique would be able to use to trachea regeneration.
01-P528  Laser-based 3D printing of hydrogels: a versatile approach for accurate 3D cellular models

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The ability to produce three dimensional structures from components that build up human tissue with tunable properties is an exciting outlook of biofabrication. Nonetheless, preserving the freedom of structuring in terms of speed, resolution and design while imitating the complexity of the native tissue architecture and function is challenging. With the currently available 3D printing methods, the realization of high resolution structures with complex architecture is cumbersome. Two photon-polymerization (2PP) is a novel 3D printing approach where the absorption of femtosecond-pulsed laser light leads to localized polymerization of photosensitive materials within the focal volume. 2PP is capable of encapsulating cells inside photosensitive hydrogels at high structural resolution in accordance to computer assisted designs (CAD). Direct cell encapsulation is a powerful tool for fabrication of 3D cell culture models in vitro, providing closer resemblance to the in vivo environment of human tissue compared to classical 2D models. Compared to cell seeding in prefabricated scaffolds, direct encapsulation provides high initial cell loading, uniform cell distribution and directed cell positioning. We report here the development of a novel biocompatible multicomponent system which has the capacity of direct cell encapsulation by printing different cells into various designs while maintaining cell viability and proliferation. The properties of the hydrogel including stiffness, degradation, and swelling can be fine-tuned by changing the concentrations of the components and / or applied energy, creating a versatile platform for in vitro modelling and tissue engineering constructs to study cell response in 3D.

01-P529  Tailoring the mechanical properties of gelatin methacryloyl hydrogels through manipulation of the photocrosslinking conditions

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Photocrosslinkable hydrogels, in particular gelatin methacryloyl (GelMa), are gaining increasing importance in biofabrication and tissue engineering. While GelMa is often described as mechanically ‘tunable’, clear relationships linking the photocrosslinking conditions to reaction rates, and the resulting mechanical properties, have not been described. Meanwhile the conditions employed across the field are disparate, and difficult to compare. In this work, in situ rheological measurements were used to quantify the relative rate of reaction of GelMa hydrogels with respect to light intensity, exposure time and photo-initiator concentration. In addition the UV degradation of the photo-initiator Irgacure 2959 was measured by UV-vis spectroscopy, and used to estimate the rate of free radical production as a function of light exposure. Using these data an expression was derived which predicts the mechanical properties of GelMa hydrogels produced across a wide range of crosslinking conditions. The model was validated through fabrication of mechanical gradients which matched predicted properties. Encapsulated human mesenchymal stem cells showed high viability under the mildest crosslinking conditions, but decreased viability for conditions which generated the highest flux of photoinduced free radical formation. The expressions described may be used to aid rational design of GelMa photocrosslinking strategies, especially in cell encapsulation experiments where minimising the cytotoxic elements in the reaction is a priority.

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**01-P530**  
**Novel printable bioink made of gelatin methacryloyl (GelMA) and type I collagen promotes angiogenesis**

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Three dimensionally (3D) bioprinted organs and tissue substitutes require an effective vascularization to efficiently supply the cells with oxygen and nutrients. Till date, several hydrogel blends have been developed allowing the in vitro formation of capillary-like networks within the gels but comparatively less effort has been made to improve their applicability to 3D bioprinting processes. Therefore, we hypothesized that tailored hydrogel blends of photo-crosslinkable gelatin and type I collagen exhibit favorable 3D drop-on-demand printing characteristics in terms of rheological and mechanical properties and that further capillary-like network formation can be induced by co-culturing human umbilical vein endothelial cells and human mesenchymal stem cells within the proposed blends.

Gelatin was methacrylated (GelMA) at a high degree of functionalization, mixed with cells, type I collagen, and the photoinitiator Irgacure® 2959 and subsequently crosslinked by UV light. After 14 days of incubation cells were immunofluorescently labeled (CD31) and visualized using two-photon laser scanning microscopy. Hydrogels were rheologically characterized and dispensable droplet volumes were measured using a custom-made 3D drop-on-demand bioprinter.

The cell viability remained high in controllable crosslinking conditions both in 2D and 3D. In general, higher UV light exposure and increased Irgacure concentration were associated with a decrease of the cell viabilities. In 3D printable GelMA-collagen hydrogels distinctive capillary-like structures were formed. The characteristic crosslinking time for GelMA in the range of minutes was not significantly altered when GelMA was blended with type I collagen. Moreover, the addition of collagen resulted in enhanced cell spreading, pronounced shear thinning behavior of the hydrogel solution and an increased storage modulus of the crosslinked hydrogel blend.

Thus, we conclude that GelMA-collagen hydrogel blends exhibit favorable biological as well as rheological properties which are beneficial for the manufacturing of 3D bioprinted prevascularized tissue substitutes.

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**01-P531**  
**Fabrication and characterization of the porous duck’s feet collagen sponge for wound healing applications**

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There are several artificial dermis commonly use to cover the wound and promote healing. The major goal of wound management is fast and scarless healing. However, there is no ideal skin substitute, that is effective to accelerate wound healing without scar formation. Artificial dermis substitute also has some drawbacks, such as high cost, insufficient available period and donor pathogen infection. To overcome these problems, we developed duck’s feet collagen (DFC) sponge as artificial dermal substitutes for the treatment of full-thickness skin defects. We measured these DFC sponge’s comparative characteristics and performances with an artificial dermis Colladerm by carried out SEM-EDX analyze, water-binding abilities and porosity test. Biocompatibility test was also performed using CCK-8 cytotoxicity assay. We also evaluated its wound healing effects for a full-thickness skin wound and compared with Colladerm in a rat model. Histological studies were carried via H&E and MT staining. Although the wound healing effect of the DFC sponge was almost similar to that of Colladerm, the DFC sponge did not induce scar formation and wound contracture like Colladerm. We suggest that DFC sponge can be used as an ideal dermal substitute to the treatment of full-thickness skin wound.
In vitro and in vivo evaluation of the duck’s feet collagen sponge for hemostatic applications

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Recently different hemostatic agents have been developed, but most of them are ineffective in severe bleeding and expensive or cause safety concerns. In this study, we fabricated duck’s feet collagen-based porous sponges and investigated its use as a hemostatic agent. We determined the sponge’s physical and biological characteristics and compared with Avitene via scanning electron microscope analysis, water-uptake abilities and porosity test, and cytotoxicity assay. The duck’s feet collagen/silk sponge showed a larger interconnected porous structure compared to others sponges. The duck’s feet collagen/silk sponge also exhibited significantly higher porosity than Avitene. Hemostatic properties of the sponges were evaluated by whole blood clotting and rat femoral artery hemorrhage experiment. The addition of silk to duck’s feet collagen showed better blood clotting ability than Avitene in vitro. However, rat femoral artery hemorrhage test showed a similar hemostatic property between the duck’s feet collagen-based sponges and Avitene. We suggest that duck’s feet collagen-based sponge can be effectively used for hemostatic applications.
Digital light processing (DLP) bioprinter, one of the bioprinting technologies, create models in a layer by layer fashion through photopolymerization with high resolution and high printing speed. This DLP printer allows only photosensitive polymers as bioink, which prohibits the use of many biomaterials. Silk fibroin (SF) has been used for a variety of biomedical applications, however, SF itself has not been used for DLP printing due to absence of crosslinkage sites essential for photopolymerization.

In this study, we developed silk based bioink as an effective bioink for DLP printing by chemical modification of SF (methacrylated SF). We evaluated the degree of methacrylation on SF modified by various amount of methacrylation agents and characterized their physical properties. To make hydrogel, photoinitiator was included into the methacrylated SF solution, which was printed by DLP printer. Mechanical properties by Universal Testing Machine, rheological properties by a rheometer and cell viability and distribution by Live/Dead assay and confocal microscopy, respectively, were analyzed.

Increases in contents of methacrylation agent resulted in higher level of methacrylation on SF. While increases in contents of methacrylation agent increased mechanical and rheological properties, water uptake was decreased. Increase of photoinitiator and methacrylated SF contents advanced the gelation time. Various complex organ structures such as heart, vessel, brain, trachea and ear were mimicked by DLP printers with good strength and cells grew well in the methacrylated SF hydrogel without cytotoxicity.

Based on our laboratory results, we confirmed that methacrylated SF bioink is well-suited for use in the DLP printing process and it could be applied to tissue engineering and the construction of highly complex organ structures.

Millions of platelets, specialized cells that participate in haemostatic and inflammatory functions, are transfused each year worldwide, but their supply is limited. Platelets are produced by megakaryocytes within the bone marrow, the soft, spongy, gelatinous tissue found in the hollow cavities of flat and long bones that support hematopoiesis. The bone marrow structure and extracellular matrix composition, together with soluble factors (e.g. thrombopoietin), are key regulators of thrombopoiesis. Despite this knowledge, the scarcity of clinical cures for life threatening platelet diseases is due to limited insight into the mechanisms that control the developmental process of megakaryocytes and the mechanisms that govern the production of functional platelets within the bone marrow. Silk fibroin, derived from Bombyx mori silkworm cocoons, is a promising biomaterial for bone marrow tissue engineering because of its tunable architecture and mechanical properties, the capacity of incorporating labile compounds without loss of bioactivity and demonstrated ability to support platelet production without premature activation. In this study, we fabricated a custom perfusion chamber to contain a multi-channel lyophilized silk sponge mimicking the vascular network in the bone marrow niche. The perfusion system consisted in an inlet and an outlet and two splitters that allowed funneling flow in each single channel of the silk sponge. Computational fluid dynamic analysis demonstrated that this design permitted confined flow inside the vascular channels. The silk channeled sponge supported efficient platelet release from megakaryocytes. After seeding, the megakaryocytes localized along Stromal cell-Derived Factor 1a functionalized vascular channels in the sponge. Perfusion of the channels allowed the recovery of functional platelets as demonstrated by activation tests upon thrombin stimulation. Further, increasing the number of channels in the silk sponge resulted in a proportional increase in the numbers of platelets recovered, suggesting applicability to scale-up for platelet production. In conclusion, we have developed a scalable system consisting of a multi-channeled silk sponge incorporated in a perfusion chamber that can provide useful technology for reproducing the bone marrow and studying thrombopoiesis ex vivo.
Expression of high molecular weight collagen-like (Gly-Pro-Pro)\textsubscript{n} repetitive protein in \textit{E. coli}

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Tissue engineering scaffold requires biological activities such as cell adhesion, proliferation and differentiation to promote tissue regeneration. Animal-derived extracellular matrix (ECM) proteins such as collagen and gelatin, are widely used for the tissue engineering researches because of their high biological activities. However, it is very difficult to control only a necessary biological activity, because ECM proteins inherit many kinds of biologically-active sequences. In addition, animal-derived ECM proteins have concerns about immunerejection and virus infection. Genetically-engineered artificial proteins are promising xeno-free material. In this study, a high molecular weight collagen-like protein composed of ((Gly-Pro-Pro))\textsubscript{n}Lys-Pro-Pro)\textsubscript{n} (GPP-K) was expressed in \textit{E. coli} for a xeno-free artificial ECM platform.

The \textit{E. coli} competent cell was transformed with the expression plasmid vector encoding GPP-K with His-tags, Glutathione S-Transferase (GST) region and a digestion site of the HRV3C protease (HRV-GPP-K), and cultivated at 30\textdegree C or 37\textdegree C. The expression of HRV-GPP-K was induced with 0.1 mM IPTG at different OD\textsubscript{590} values. After 12 h of protein expression, \textit{E. coli} was harvested by the centrifugation. The \textit{E. coli} pellet was resuspended by buffer solution and frozen at -80\textdegree C. The \textit{E. coli} were disrupted by sonication and insoluble debris was removed by the centrifugation. The supernatant was purified by His-affinity column and HRV3C protease digestion. After dialysis in deionized water, a high-purity GPP-K was successfully obtained.

The condition of HRV-GPP-K expression was successfully optimized (OD\textsubscript{590} = 0.8, expression time: 12 h, expression temperature: 37\textdegree C). Theoretical mass peak at 11.83 kDa was detected by MALDI-TOF/MS. The CD spectrum of GPP-K in the aqueous solution showed a negative band, and a positive band at 220 nm and 225 nm, respectively, indicating that GPP-K forms collagen-like triple helical structure. We succeeded in the expression of GPP-K with collagen-like triple helical structure and are expecting its usability as a xeno-free artificial ECM platform.

Degradation Prediction Model and Stem Cell Growth of Gelatin-PEG Composite Hydrogel

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Gelatin hydrogel has great potential in regenerative medicine. The degradation of gelatin hydrogel is important to regulate in vivo tissue repair process. As a plasticizer, PEG can significantly improve the mechanical property of gelatin hydrogel. However, how preparation parameters affect the degradation rate of gelatin-PEG composite hydrogel is still not clear. In this study, gelatin-PEG composite hydrogel was prepared by the chemical cross-linking reaction using glutaraldehyde (GA). And GA concentration was found to be the significant effect factor for the degradation rate of gelatin-PEG composite hydrogel by means of Plackett-Burman design. Then a mathematical model was built to further investigate the extended effect of preparation parameters (the concentration of gelatin, GA or PEG) on the hydrogel degradation rate using the response surface method (RSM), which was helpful to predict the degradation rate of gelatin-PEG composite hydrogels under different preparation conditions. Moreover, it was found that the addition of PEG could adjust hydrogel degradation more subtly, which might probably have relation with the increasing of the cross-linking degree. In addition, gelatin-PEG composite hydrogel surface well supported the adhesion and growth of human mesenchymal stem cells (MSCs). And PEG was found to increase the compact degree of hydrogel when lower GA concentration was used, which could prevent cell migration into the hydrogel. Therefore, PEG might play an important role in the composite hydrogel, and our results would be useful to optimize the preparation of gelatin hydrogel for tissue engineering. Acknowledgement: This work was supported by the National Natural Science Foundation of China (No.31470944).
**01-P539** Organic nanocoating of titanium surface activates inflammatory and proliferative phase of ossteointegration

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Plant-derived nanoparticles are polysaccharides, mainly represented by Rhamnogalacturonan-I pectins (RGI) and have a great potential to (1) improve bone formation, (2) prevent from inflammation cause by bacterial infection. The objective of the project was to develop a nanocoating at the titanium implants to improve osseointegration in patients with high risk for inflammation, as periodontitis (PD). The inflammatory phase of ossteointegration at titanium surface with RGI nanocoating was evaluated with human primary neutrophils and macrophages by gene expression under activated immune system with bacteria origin (-/+ P.gingivalis(PG)). The environment without bacterial infection was a positive control. The proliferative phase of osseointegration was observed with proliferation, cell metabolic activity and gene expression of primary human fibroblasts in absence and presence of bacterial infection. The osteogenic properties of the RGI nanocoating was evaluated with osteoblast adhesion, proliferation, cell viability, cell cycle, mineralization and Real-Time PCR in different time point -/+ PG bacterial infection. The surfaces with RGI-Is were test group and the surfaces without RGI-Is were control group. RGI-Is nanocoating at the titanium surface compare with uncoated surface down-regulated Il-1, Il-8, TNF-alpha of neutrophil and macrophages infected with PG. The fibroblast proliferation and viability increased at the RGI-Is compared to control. The Col1a, Fn-1, Fgfr-1, Mmp-2 were up-regulated in fibroblast culture -/+ PG on RGI-Is compared to uncoated surface. **TNF-alpha, I-1, I-8 were down-regulated in fibroblast infected with PG on RGI surface compared to uncoated surface.** The osteoblast proliferation, cell viability and mineralized matrix formation was increased on RGI. The RUNX-2, ALPL, OC, COL-1 were up-regulated and RANKL down-regulated in cell culture -/+ PG s on RGI coated surface compare to control. **IL-1, IL-6 and TNF-alpha were down-regulated in osteoblasts culture infected with PG on RGI compared with control.** The results showed that neutrophils, macrophages, fibroblast and osteoblast cultured on RGI nanocoating and infected with PG significantly reduced release of pro-inflammatory cytokines during inflammatory and proliferative phase of osseointegration. Pectin RGI nanocoating is promising candidate for improvement of osseointegration and might play an important role as anti-inflammatory agent in case of compromised patients.
**Chitosan scaffolds by ElectroHydroDynamic techniques**

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ElectroHydroDynamic techniques such as electrospinning and electrospraying are frequently used for scaffold production and drug encapsulation\(^1,2\).

Thanks to an experimental design we have elaborated a method to produce scaffolds based on nanofibres or submicron beads of controlled sizes. Characterization of the scaffolds was led by X-Ray Diffraction (XRD), Fourier Transform InfraRed spectroscopy (FT-IR), water contact angle (WCA), Atomic Force Microscopy (AFM), Scanning Electron Microscopy (SEM) and X-Ray tomography.

The quality of the chitosan as well as solution properties are key parameters for bead-fibre transition, and scaffold morphology.


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**Biopolymer based electrospinning of synthetic polyester for tissue engineering applications**

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The major challenge of tissue engineering domain comprises of mimicking extracellular matrix (ECM) of native tissues close to natural proximity. Electrospinning has emerged as an exceptional alternative to fabricate scaffolds with necessary porosity and ECM analogue architecture. Electrospinning of synthetic polymers blended with biopolymers have showed promising outcome in terms of mechanical strength, biodegradation coupled with biocompatibility, cellular proliferation and adhesion without eliciting much inflammatory responses. In this present study, polycaprolactone (PCL) has been mixed with silk fibroin sponges (SF) in different volume ratios to obtain stable emulsion, and electrospun to obtain a nanofibrous scaffold with porous architecture. The composite nanofibrous scaffolds exhibited comparable mechanical strength, \textit{in-vitro} biodegradation and biocompatibility than pristine PCL electrospun fibers. The \(\beta\)-sheet conformation of silk structure and its inherent RGD sequence imparts sufficient strength and cell proliferation capacity to the scaffolds, making them a promising candidate in the field of tissue engineering.
Viscoelastic response of a scaffold to cell-generated forces influences the in-vitro behavior of the cells. Thus to understand cellular reaction, measurement of matrix local mechanical properties is of great importance. In this study, we have used hyaluronic acid (HA)/collagen (Coll) based cryogel scaffolds for 3D cell culture. We were for the first time able to monitor changes in local viscoelastic properties in direct cell environment continuously using Multiple Particle Tracking (MPT) based micro rheology. In combination with histologic staining, we gained a deeper insight in cell-matrix interactions.

MPT measurements were performed in cell-laden scaffolds by tracking the Brownian motion of inert beads, evenly distributed within the matrix. The resulting trajectories were transformed into mean square displacement traces from which local viscoelastic properties, i.e., storage modulus $G'$ and loss modulus $G''$, were calculated using the generalized Stokes-Einstein relation. As the tracer particles don’t apply any force to cells and were proved to be non-cytotoxic, the MPT method is considered not to affect cell growth. Newly secreted fibers that contribute to matrix elasticity were identified by antibody staining against matrix proteins after 1, 3 and 8 days of cell culture. Main results were that cryogel composition, local distribution of Coll and initial micromechanical properties affected cell behavior. At low HA to Coll ratio, i.e., high Coll content, cells spread faster but proliferation rate was lower than in scaffolds with more HA. Cells adhered preferentially at locations with thick Coll fibers. When Coll was distributed homogeneously, cells adhered and spread more slowly than in networks with heterogeneous Coll distribution. But in latter, the degradation was accelerated. Continuous MPT monitoring showed that after 5 days $G'$ was reduced by 80% of the initial local elastic modulus. Finally, we observed that in gels with low initial local elasticity, 3T3 cells produced higher amounts of fibronectin and additional Coll, compared to cells seeded in more rigid gels.

In summary, the non-invasive characterization of matrix mechanical and heterogeneity properties on microscale is possible by means of MPT. Thus, our study serves as a window to a better understanding of matrix mechanical changes during cell culture on the length scale of single cell size.

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Lack of the donor for corneal graft is the big problem not only in Japan but also globally. With a new technology development, new materials and devices which are utilized as the substitute for cornea are under development. In case of corneal epithelial layer and corneal endothelial layer, cell sheet technology are developed and it is used in clinical cases. On the contrary, reliable substitute for corneal stroma is very few on clinical. In previous studies[1,2,3], we have found that silk fibroin nanofiber nonwoven mat has potential to be a functional scaffold for corneal stroma. However, fibroin nanofiber nonwoven mat was translucent and it was not perfect for using as a corneal material. To overcome this problem, we developed a novel technique to increase the transparency of fibroin nanofiber nonwoven mat. Preparation of fibroin nanofiber mat: fibroin aqueous solution(80mg/ml) and Poly(ethylene oxide)(ave. Mw=900000 g/mol) aqueous solution(5.0ml/ml) was mixed at 4 to 1 [4]. An electrospinning system equipped with a rotating drum collector was used. (The applied voltage:10 kV, the feeding rate: 0.3ml/h, the spinning distance: 180 mm and the 25 G stainless needle). The resulted mat was immersed into 100% Ethanol for the insolubilization and removing PEO. Treatment: The insolubilized mat was immersed into different concentration of calcium chloride(CaCl2) for 30 min, then washed with distilled water. Implantation to rabbit cornea: To evaluate the tissue compatibility, 2 mm diameter of the treated mat was implanted in stroma and the tissue reactions were observed until 12 weeks. Aligned nanofiber mat treated with 160mM CaCl2 for 30 min has excellent transparency. From the SEM observation, structure changes were observed. The gap between fibers became very small, and outer surface of the mat became much more flattened. Even after the treatment, permeability of protein and water was kept same level as the non-treated mat. From the implantation results, the transparency was kept until 12 weeks and any adverse tissue reaction such as edema formation, opacification, and vascular invasion was not observed. Further studies such long term implantation and histological evaluation are required.

Hydroxyapatite negatively influences proliferation of stem cells in sponges made of gelatin and fibroin at early timepoint

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Objective: Scaffolds are an essential part of the Tissue Engineering strategy. They serve as support to the cells to proliferate and differentiate as well as to deposit extracellular matrix and form new tissue. In our study, we aimed to evaluate the influence of hydroxyapatite (HA) supplemented to gelatin/fibroin scaffolds on hAMSCs viability, proliferation and osteogenic differentiation.

Material and methods: Porous scaffolds were produced by a single-step method to obtain sponges, starting from fibroin (FN) / gelatin (GA) (80:20 w/w) water solutions and using low pressure N₂O gas. HA was added to the FN solution, in form of nanopowder, 1:1 in weight before the production of the sponges. Scaffold morphological characterization was performed by SEM and µCT. hAMSCs were seeded on the scaffolds and cell viability (MTT) and proliferation (PicoGreen) was tested. Furthermore, the gene expression regarding apoptosis, proliferation and osteogenesis was evaluated by RT-PCR.

Results and conclusion: Unexpectedly, HA negatively impacted the proliferation of hAMSCs (at early time points). After 7d, there was no significant difference between both scaffolds (i.e. +HA vs -HA). The addition of HA leads to a slightly higher toxicity, but the viability is not influenced by it. Without HA, proliferation related genes are higher expressed than in the scaffold +HA. The expression of osteocalcin and osteopontin was upregulated in both scaffold types. Based on our results, we conclude that supplementation of HA with 1:1 in weight to the FN/GA scaffolds did not show a significant impact on osteogenesis of hAMSCs. Thus, the amount of HA used to supplement this material may need further optimization.

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Wet Spinning and Riboflavin Crosslinking of Collagen Filaments

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Reconstituted collagen is one of the most useful biomaterials for biomedical applications. Collagen in fiber form may be used for the fabrication of tailor-made, fiber-based scaffolds. Fiber-based scaffolds have the benefit of structural and topographical properties that are easily adjustable by textile-technological techniques. A major drawback of collagen fibers is their non-inherent rigidity in the wet state, which is why collagen fibers are typically crosslinked. The aim of this study was to evaluate the possibility of riboflavin as an alternative non-toxic crosslinking agent for the preparation of wet-spun collagen filaments. For this purpose, mechanical and thermal properties as well as the cytocompatibility of wet-spun collagen filaments crosslinked with both riboflavin and the widely used glutaraldehyde were analyzed and compared in the dry and wet state, whereby the wet state is of particular interest for tissue engineering applications. Collagen filaments were produced on a laboratory wet spinning line. The mechanical parameters and the denaturation temperature and enthalpy of collagen filaments were analyzed in both the dry and wet state. It was concluded that the combination of riboflavin and UV light leads to crosslinked collagen filaments having improved mechanical and thermal properties. Furthermore, riboflavin-crosslinked filaments exhibited a higher cytocompatibility for hMSC compared to glutaraldehyde-crosslinked filaments.
An Injectable Scaffold Based on Crosslinked Hyaluronic Acid Gel for Tissue Regeneration

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Injectable scaffolds have great potential in special applications of regenerative medicine. In this study, hyaluronic acid hydrogels (HAGs) were prepared by crosslinking hyaluronic acid (HA) with 1,4-butanediol diglycidyl ether (BDDE). Applications of HAG as an injectable scaffold for regenerating functional tissues were proposed by matching its viscoelastic properties with those of biological tissues. The effect of BDDE concentration on different properties of HAGs was explored. Swelling properties, cross-sectional morphology, and BDDE residues of the resulting gels were investigated. Rheological properties of different HAGs were measured by monitoring their storage modulus (G’) and loss modulus (G”) and compared with those of biological tissues. It was shown that HAGs (BDDE from 0.4 vol% to 1.0 vol%) possessed great water absorbing capability with swelling ratios ranging from 99.7 to 78.9. The higher the concentration of the crosslinker used, the more rigid the resulting hydrogel, subsequently the lower the swelling ratio would be and the higher the G’ and G” values as well. Similar viscoelastic behaviors were found between HAGs and biological tissues, such as epidermis, dermis, articular cartilage and tooth germ. SEM revealed that HAG obtained at 0.4 vol% BDDE had pore diameters ranging from a few microns to around 100 μm with a high degree of interconnectivity. The feasibility of this HAG, as an injectable scaffold, to regenerate cartilage and dentin-pulp complex was then demonstrated using a preliminary subcutaneous microenvironment. The current study could be a reference to account how a crosslinked HA gel should be chosen for specific tissue regeneration.
Hybrid hydrogels for the orthogonal control of skeletal muscle constructs

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In vitro skeletal muscle constructs have been widely engineered by the encapsulation of C2C12 muscle cells in hydrogels made of gelatin or functionalized PEG.1 Also, it has been reported that three-dimensional patterns influence cell viability and the myogenic differentiation, due to the strong dependence on the mechanical and chemical properties of the material.2,3 Here, we combine gelatin with PEGDA, carboxy methyl cellulose (CMC), and alginate for obtaining hybrid hydrogels which allow the orthogonal control of their properties. First, gelatin, CMC and alginate were methacrylated to make them photocrosslinkable. Polymers were combined at different fractions and used as a material to encapsulate C2C12 cells and promote the myotube differentiation. Hybrid hydrogels containing CMC and alginate, showed high water content and swelling, independently of the weight percentage, due to their hydrophilic structure and higher porosity. We also achieved a decoupling of the dependence of mechanical properties from material concentration, obtaining stiffer (Gel-Alg) and softer composites (Gel-CMC). On the other hand, these hydrogels showed high resistance to the degradation capability of collagenase II than gelatin. To study these composite materials, as an engineered skeletal muscle, we fabricated columnar microstructured hydrogels, more than 200 µm in height, with encapsulated C2C12 cells. Even changing mechanical and chemical properties of the material, the addition of CMC and alginate kept the cell viability at high levels, at least until a depth of 100 µm. Furthermore, C2C12 cells proliferated and differentiated into myotubes without affecting the hydrogel structure. Thus, mechanical properties, water content, or chemical composition of these hybrid hydrogels can be tuned without affecting myotube differentiation. These findings reveal promising tools to generate tailored biomaterials in the field of the skeletal muscle tissue engineering. 1. G. Agrawal, A. Aung, and S. Varghese, Lab Chip, 2017, 17, 3447-3461 2. J. Ramon-Azcon, S. Ahadian, R. Obregon, G. Camci-Unal, S. Ostrovidov, V. Hosseini, H. Kaji, K. Ino, H. Shiku, A. Khademhosseini and T. Matsue, Lab Chip, 2012, 12, 2959-2969. 3. A.S. Salimath, A. J. Garcia, J. Tissue. Eng. Regen. Med., 2014, 10, 967-976
01-P550  Functional analysis of heparin-conjugated collagen gel as a scaffold for regenerative medicine

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Heparin has high affinity to a set of proteins which called heparin-binding proteins. Many reports indicated that the heparin could crosslink to a variety of natural polymers to provide controlled release of growth factors, including BMP-2, bFGF and VEGF. We focus on the heparin as a growth factor-immobilization agent, and evaluate effect of heparin-conjugated collagen gel on tissue formation and cell differentiation in vitro.

To check the ability of heparin-conjugated collagen gel, we investigated the tissue vascularization in vitro. In briefly, we focused on a bottom-up approach using endothelial cell-covered spheroids to construct vascularized tissue. To fabricate endothelial cell-covered spheroids, hepatocyte spheroids were coated with heparin-conjugated collagen gel and co-cultured with endothelial cells. Packed spheroids attached to each other forming a large cellular tissue with regular distribution of endothelial cells. By using heparin-conjugated collagen gel and immobilizing some growth factors, the survival and functions of hepatic tissue were up-regulated. It was suggested that the use of heparin-conjugated collagen gel in the process of the formation of hepatocytes-endothelial cells composite tissue contributed to the enhancement of cell survival rate and liver-specific functions of the tissue.

On the other hand, the research on the change of the mechanical properties, which could cause by the cross-linking level of the substrate itself and the concentration of the matrix protein in our study, showed the influence on the state of the cells, not only for hepatocyte but also iPS cells. Therefore, we also evaluated the influence of mechanical properties on cells by using iPS cells.

01-P551  Fabrication, Characterization and Biocompatibility of Photocrosslinked Tyramine-Hyaluronan Hydrogels: Potential Scaffolds for Tissue Engineering

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Hyaluronan derivatives attract considerable attention as precursors for tissue engineering. In this work photo-initiated crosslinking of biocompatible tyramine-hyaluronan (Tyr-HA) using riboflavin as a photo-initiator was studied [1-3]. Tyr-HA readily self-crosslinked into hydrogels under physiological conditions. The rapid crosslinking was monitored by fluorescence spectroscopy indicating short gelation times (in seconds). The tunability of the mechanical and physical properties of the hydrogels was achieved by either altering material composition of a precursor solution or by the process parameters of the photo-crosslinking. The advantageous spatial control of the photo-reaction was demonstrated by shape-controlled fabrication of macro and micro gels using photolithography and laser printing. The biocompatibility of the derivative, hydrogel, photo-initiator, and light towards mouse fibroblasts was examined by the ATP test. 2D and 3D live-dead assays with fibrinogen showed extensive cell adhesion and proliferation over 10 days. Thus, the promising and functional scaffolds for skin engineering were developed.

Human tissues and organs are three-dimensional. In the tissue and organ, 3D cell communications are very important for cell functions via specific gene expression. 3D cell culture, so called spheroid, is an in vitro model of tissue and organ. The conventional spheroid has been "spherical" shape because it is formed by the spontaneous cell-cell interaction with low attachment surface for several days.

We proposed "precision spheroids" that have designer shape and size and are made within about 1 hour using gelatin. We fabricated a giant cylindrical spheroid with over 1 cm in length and 500 µm in width, a fine cylindrical spheroid with 150 µm in width, and a straw like spheroid. By the same method, we also fabricated a designer cell sheet such as square and star shape with less than 100 µm thick. The precision spheroids could be formed using all cell lines tested by us including MDA-MB-231 cells that do not form spontaneous spheroids.

The precision spheroids were applied to two cancer cell migration assays. Firstly, epithelial mesenchymal transition by TGF-beta braked down the precision spheroids made of MDA-MB-231 cells, whereas TGF-beta receptor kinase inhibitor, SB431542, prevented the brake down. Secondly, the straw like spheroids made of MCF7 cells including fully differentiated adipocytes in the tunnel of spheroids were braked down, whereas the spheroids including undifferentiated pre-adipocyte were not. We considered that the migration of cancer cells was dependent of fatty acids from adipocytes because the fatty acid related genes of MCF7 highly expressed.
**01-P555** Development of a Three-dimensional Biocompatible Polymeric Scaffold with Potential to Spheroid Formation Using Colonic Cancer Cells

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Current models for cancer research and drug discovery lack of physiological relevance to in vivo conditions. Three-dimensional (3D) cell cultures have raised as an interesting system to bridge the gap between conventional monolayer cultures and in vivo models, providing a cellular environment more consistent that in vivo.

In this study, we investigated the porosity and interconnectivity properties, and the in vitro biocompatibility and spheroid forming potential of a gelatin-based hydrogel prepared by freeze-drying process following by a chemical treatment based in molecular imprinting technology (MIT). The colon carcinoma cell line HT29-MTX was used as a model.

The use of freeze-drying and MIT generated a 3D structure highly porous and well interconnected. The porosity and 3D structure were carried out by Scanning Electronic Microscopy (SEM) and Confocal microscopy using rhodamine B staining method. The average pore diameter was 100 µm. The resultant hydrogel could be sterilized by steam sterilization. The hydrogels were found to be biocompatible using MTT assay in HT29-MTX cell cultures. In order to evaluate spheroids formation, cell suspensions were injected into the hydrogel and cultured by 5, 10, 20, 30, and 45 days. To evaluate spheroid formation into hydrogel, optical microscopy was employed. The HT29-MTX cells begin to form multicellular aggregates with spheroid-like morphology since the first days of seeding, maintaining an average size (100 µm) but growing in number.

The very encouraging preliminary results from our in vitro studies suggested that gelatin-hydrogel is a potential 3D technology that allowed cell aggregation in a spheroids-like morphology, maintaining long-term viability. Future work will focus on spheroid characterization.

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**Optimizing A Three Dimensional In Vitro Intervertebral Disc Environment For The Co-Culture Of Human Nucleus Pulposus Cells And Mesenchymal Stromal Cells**

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Current clinical trials have attempted the injection of mesenchymal stromal cells (MSCs) into degenerated intervertebral discs (IVDs) to restore height and function. However, retrieval of tissue for intensive characterization of the mechanisms behind any apparent regenerative effect is very limited. In the present research study, a 3D culture system has been developed to act as an ex vivo model for the intervertebral disc that mimics the native environment physically and chemically. Development of a 3D co-culture analogue will help to unravel the cell-cell signaling between MSCs and nucleus pulposus (NP) cells in an in vitro setting.

Alginate constructs were created in two steps; alginate beads were extruded through a needle into a solution of 102mM CaCl₂ and left to crosslink for 10 minutes. The beads were rinsed in PBS and added in a 1:1 volumetric ratio to a solution of alginate, CaCO₃ and Glucono-de-lactone to create 3mm x 3mm cylindrical constructs with a central bead. To optimize culture medium, human NP cells were seeded onto poly-L-lysine coated 96-well plates. Base media (DMEM-lg, 10% FBS, 1% ABAM) was combined with variations of pH, osmolarity and growth factors (L929) to grow within the gels was investigated. Finally, the gels were investigated in the repair of focal corneal defects in rabbit model.

Phototriggered thiol-ene reactions are rapid and facile methods of crosslinking gelatines. They resulted in transparent gel with mechanical properties suitable for soft tissue replacements. Moreover, they could be cured in the presence of cell and maintain high viability. The resulting materials are promising candidates for repair of corneal focal defects.

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Design and characterization of a novel alginate 3D co-culture method for use in cell-cell interaction studies

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Alginate is a highly tailorable biomaterial that has the ability to polymerize in the presence of divalent cations such as Ca2+. By limiting access or adjusting the rate of Ca2+ access, alginate is able to form various geometries before crosslinking dependent on the concentrations of the crosslinking catalyst. In this study, a dual-layered crosslinking strategy involving both internal and external gelation is employed to develop a novel co-culture method for separately encapsulating groups of cells or varying alginate concentrations for drug delivery. Alginate beads at 1.2% and 2% (w/v PBS) concentrations were crosslinked by external ionic gelation using varying needle sizes (26G½, 27G½, 30G½). The alginate solution was extruded through a syringe at a 20 cm working distance into a solution of 10% CaCl2 with a stir speed of 200 RPM. Externally-gelled beads were crosslinked for 10 minutes, followed by 4 x 5 minute PBS soaks to remove excess calcium ions from the bead boundary. Rinsed alginate beads were added in a 1:1 volumetric ratio to uncrosslinked alginate at 1.2% or 2% concentrations. The alginate-bead solution was then internally gelled using a 1:2 molar ratio of CaCO3 and D-(+)-glucono-δ-lactone, creating a dual-layer alginate consisting of an alginate bead surrounded by a secondary alginate layer. Uniform alginate geometries were created by two methods; crosslinking between two glass platens with 3mm spacers or by pulling into a 3mm diameter tube. The alginate beads, cylinders, and combinations of each concentration were soaked in PBS for a period of 28 days during which swelling was analyzed by ImageJ, and retrieval of the beads as well as spontaneous extrusion of the beads from the cylinders was noted.

Initial diameter of the beads was determined to be dependent on the needle gauge. The two methods of uniform geometry creation resulted in similar constructs; however, the tube extrusion technique proved to be far more effective of a full constraint mechanism. The data suggests a combination of 26G½ beads within 1.2% alginate, created by the tube extrusion technique. This combination resulted in a 1:1 volumetric ratio of alginate between the bead and the surrounding cylinder, with minimal spontaneous bead extrusion. Using a combination of crosslinking strategies, the constructs can be tailored toward mechanical and physical properties ideal for the culture of various cell lines.
Hydrolyzed Collagen Promotes Viability Increase in Stem Cells Cultivated in Alginate Hydrogels

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Hydrogels and mesenchymal stem cells (MSCs) are studied due to their potential applications in regenerative medicine. The aim of this research has been to investigate which collagen concentrations in alginate are suitable for MSC cultivation as a strategy in regenerative medicine. MSCs were isolated from human deciduous teeth and cultivated in a concentration of 200,000 cells/well. Cell viability was assessed by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) test and cytotoxicity was analyzed by lactate dehydrogenase (LDH) assay, after three days of cell cultivation. The MSCs were cultivated in a medium with 0, 3.75, 7.5 or 10% (w/v) of hydrolyzed collagen in 1% alginate hydrogels crosslinked with 50mM of CaCl2, for 30 minutes. The results showed that mean of the normalized viable cells was of 100% and the standard error of the mean (SEM) was 7% when the cells were cultivated in 1% alginate without collagen (control); at a concentration of 3.75, 7.5 and 10% hydrolyzed collagen in 1% alginate, cell viability was higher, with values of 129% ± 8 (p=.002), 122% ± 5 (p=.023) and 134% ± 5 (p=.000), respectively. The averages ± SEM concentration of LDH released were 152±8, 142±5, 148±4 and 157±1 U/L at 0, 3.75, 7.5 and 10% collagen, respectively, without significant statistical difference of the LDH leakage between the control and collagen treatment (p>0.05). In conclusion, hydrolyzed collagen associated with alginate hydrogels provided a three-dimensional matrix in which the MSCs were able to increase their viability. The scaffolds did not exhibit cytotoxicity, which also suggests they have pertinent characteristics for future use in tissue engineering applied to regenerative medicine. It can be concluded that these hydrogels could be used in the restoration of morphological or functional functions of tissue, such as cartilage, bone, and skin.

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A 3D Enzymatically Crosslinked Hydrogel Promotes Human Adipose-Derived Stem Cell Spheroids Proliferation and Differentiation

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Human adipose-derived stem cells (hASCs), an abundant source of mesenchymal stem cells (MSCs), not only can differentiate into multiple lineages but also have the potential of transdifferentiation. However, the expression of pluripotency markers which is important for the renewal and differentiation capabilities of hASCs are detected only in the early passages. Accumulating evidence have proved that cells aggregated to form cell spheroids in 3D cell culture, cells mimic the better in vivo microenvironment and could maintain the expression of stemness markers. In this study, gelatin hydrogel crosslinked with microbial transglutaminase (mTG) which is a kind of enzyme that performs high specific activity over a wide range of temperature and pH. In addition, enzymatic crosslinking reaction is milder than physical and chemical methods which may lead to cell death. hASC spheroids formed by seeding cells in the agarose microwells plate, followed by the examination of the pluripotency genes markers expression in spheroids. The properties of gelatin/mTG hydrogel was evaluated including the gelatin time, crosslinking extent, mechanical strength and enzymatic degradation test. To investigate the performance of spheroids in the 3D hydrogel, after the spheroids were encapsulated and cultured in the hydrogel, cell cytotoxicity, proliferation and differentiation potential were assessed.
Innate immune response to engineered tissues is an important determinant of their functional integration. Macrophages play an important role in the initial pro-inflammatory response (M1) and also in the resolution of the immune response (M2) through their phenotypic plasticity. Thus, incorporation of phenotype controlled macrophages into the engineered tissues can provide a microenvironment that is more conducive to healing and integration. To demonstrate the potential benefits of macrophage presence, we encapsulated naïve or activated macrophages in enzymatically crosslinked gelatin hydrogels and demonstrated the effect of the encapsulated cells on incoming monocytes, vascular endothelial cells and fibroblasts in order to mimic the sequence of events during wound healing over a 14 days period. M1 phenotype induction of the encapsulated macrophages resulted in a significant boost (between 2 to 4 fold) of both pro- and anti-inflammatory cytokine secretion (IL-1beta, TNF-alpha, IL-1RA, CCL-18 and IL-10) together with a denser cellular layer on the hydrogels for both fibroblasts and endothelial cells (1). Induction of M2 phenotype by IL-4 supplementation resulted in clustering of macrophages within the hydrogel and decreased pro-inflammatory marker gene expression (CD86, STAT-1, TNF-alpha) (2). M2 phenotype induction of the encapsulated macrophages also significantly altered the attached incoming cell morphology and increased IL1-RA and CCL-18 secretion. Our results demonstrate that phenotype controlled macrophage containing hydrogels can be used to alter the behavior of incoming cells and also the local cytokine microenvironment which can be harnessed to exert control over the integration of engineered tissues.

References:

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Using 3D collagen microfibers tissues to induce and maintain the functionality of both pre- and mature primary adipocytes in long term cultures

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Adipose tissue regeneration is currently a competitive challenge for either cosmetic/pharmaceutical assays and plastic surgery purposes. Conventional in vitro two-dimensional (2D) cell cultures using directly mature adipocytes (AD) showed limited culture time by quickly dedifferentiating [1], while getting sufficiently matured AD by differentiating adipose derived stem cells (ADSC) usually required more than one month [2]. The existing three-dimensional (3D) models accelerated the ADSC adipogenesis, but mature AD still cannot be maintained more than one week in in vitro cultures [3].

In this context, we developed biomimetic 3D-tissues using high density collagen microfibers (until 20-30 wt%, similar to in vivo [4]) from homogenized type I collagen mixed with mature AD or ADSC in 24 well transwells. These 3D-tissues ensured the long term maintenance of unilocular mature AD (fat vesicle diameter of 53.9 ± 7.8 μm at day 0 to 53.1 ± 12.2 μm at day 14) with a viability of 95.6 ± 1.9% at day 14. On the contrary, 2D mature AD showed significantly 4.04 times smaller multiple vesicles. Concerning ADSC, 3D adipogenic genes expression was found at least significantly doubled throughout the differentiation (even 8.3 times higher for GLUT4 at day 21), along with up to 3.7 times bigger fat vesicles observed (9.9 ± 3.6 μm at day 14). Perilipin immunostaining and leptin secretion finally attested the functionality of both adipocytes.

We thus developed an innovative method using collagen microfibers gels to construct 3D adipose tissues similar to in vivo. The obtained long term functional maintenance and the faster adipogenesis make this model relevant for screening assays and reconstructive surgery.

Reference

Tuning Biochemical and Mechanical Properties of 3D Hydrogel Tweak Stem and Cancer Cells Cross-talk

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Stem cells are largely considered as deadly offspring of tumors nowadays, working silently while regulating disease progression and metastasis. Plenty of literatures identifies the cross-talk between the stem and cancer cells¹, but petty hard evidence or models are available exploiting the impact of this cross-talk. Therefore, an attempt is made to uncover the effect of biochemical and mechanical properties on proliferation and spheroid formation ability of osteosarcoma cells in presence of adipose stem cells. Finding a biomimetic biomaterial to culture osteosarcoma cells other than collagen has challenged the researchers. Silk is one of popular choice of biopolymer for bone tissue engineering² and easily be blended with other polymers, such as gellan gum. Blended hydrogels of silk - gellan gum with tunable chemical and mechanical properties are synthesized for co-culturing human osteosarcoma (Saos2) and adipose derived stem cells (ADSCs). The constructs are then screened for spheroid formation. The development of spheroids in selective hydrogel formulation is indicative of regulatory effect of biophysical cues in cancer (spheroid) formation and progression. The heterogeneity lies within the present model (co-culture and blended matrix), highlights the diversity of cancerous niche and is anticipated to evoke further study of screening of cancer chemotherapeutics, more precisely drugs targeted to cancer niche.

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Reference
01-P567  Effects of high molecular weight hyaluronan on immunomodulation

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Background: Hyaluronan (HA) is a glycosaminoglycan composed of alternate units of D-glucuronic acid and N-acetyl-D-glucosamine. HA as an extracellular matrix component shows distinct biological effects depending on its molecular size and target cells in each tissue. Especially, high molecular weight-HA (HMW-HA) is highly expressed not only in some normal tissues but also in immune-resistant tumor tissues, a physiological relationship between HMW-HA and immunomodulation is expected. The objective of this work is to clarify effects of HMW-HA on immunomodulation to apply its knowledge to allogenic cell and tissue grafting.

Methods: Two types of HA (HA200 and HA80, weight-average molecular weight $[M_{w}] = \approx 2.0 \times 10^6$ and $8.0 \times 10^5$ Da, respectively) were used. The effects of HAs on immunomodulation were verified by two kinds of in vitro experimental models using rat bone marrow-derived mesenchymal stem/stromal cells (BMSCs) and human monocytic leukemia cell line THP-1 cells. Primary-cultured BMSCs in DMEM supplemented with 10% FBS were seeded at a density of $5.0 \times 10^4$ cells/cm$^2$ into 24-well culture plate, and stimulated for 24 h with $5.0 \text{ ng/mL TNF-\alpha}$ in the presence or absence of $5.0 \text{ mg/mL of each HA}$ after cell attachment. Immature dendritic cells (DCs) were generated from THP-1 (2.0 $\times 10^5$ cells/mL) in RPMI 1640 medium containing 10% FBS, 5.0 ng/mL IL-4, and 5.0 ng/mL GM-CSF in culture flask. After transferring into 24-well culture plate, cells (4.0 $\times 10^4$ cells/mL) were exposed to each HA (5.0 mg/mL) for 24 h. The gene expression of cytokines and co-stimulating factors in BMSCs and DCs was analyzed by RT-PCR.

Results and Conclusion: BMSCs as well as DCs are considered to be involved in regulation of immune response in local area of tissue. RT-PCR analysis data demonstrated that cells exposed to HA 200 and HA 80 showed quite different behaviors on gene expression. HA200 rather than HA80 induced the IL-10 gene expression related to regulatory T cell (Treg) activation in both TNF-\alpha stimulated BMSCs and THP-1-derived DCs. PDL2 expression suggesting a potential inactivation of naive helper T cell (Th) was also enhanced only in DCs exposed to HA200. Thus, HMW-HA is expected to behave a coordinator of moderation of immune reaction. These findings indicated that HMW-HA might to be able to be utilized as an immunosuppressant in combination with implantation of allogeneic cell and tissue-engineered products.
To create more excellent anti-adhesion materials, we have developed thermally cross-linked gelatin film, using scaffold material for regenerative medicine. We investigated its physical and biological properties, preclinical examination, and further applications, compared with conventional anti-adhesion materials.

At first, we optimized the duration of thermal cross-linking for the gelatin film to show useful biodegradability and anti-adhesion effects. Next, the physical tests such as tensile strength and adhesiveness showed that the gelatin film provide better handling than the conventional film, particularly due to its physical strength. In the biological tests such as anti-adhesive effect using murine adhesion model, influence on fibroblast cell proliferation, and cytotoxicity tests by colony or Live/Dead assays, the gelatin film showed significantly greater anti-adhesion effect than the conventional film without any cytotoxicity. Furthermore, in the preclinical tests using canine adhesion or anastomosis models, the gelatin film also showed superior anti-adhesion effect, involving excellent peritoneal regeneration, and could be used safely for the site of intestinal anastomosis, in comparison with the conventional film.

Additionally, we have developed two type of applications of gelatin film. The former “two-layered gelatin sheet”, which composed of gelatin film and gelatin sponge, showed both more excellent hemostatic and anti-adhesive effects, compared with the conventional materials. The latter “gelatin flakes”, which composed of small fragments of gelatin sponge, could be used in laparoscopic surgery by spraying with excellent anti-adhesive effects.

These results suggest that thermally cross-linked gelatin film and its applications are quite favorable as anti-adhesive materials.
01-P570  Tropical tasar silk protein sericin based matrices for skin tissue regeneration

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Formulating matrices to support and augment the process of regeneration entail appending the fundamentals of an artificial tissue within the scaffolds at an affordable cost. Over the years, naturally obtained silk protein sericin is exploited as cosmetics constituent for its beneficial properties (anti-oxidant, UV B protection and hydrophilicity). The effective and proficient nature of sericin make this water soluble protein a promising biomaterial for tissue engineering and regenerative medicine. It also proves to be advantageous especially in skin repair and restoration due to the above mentioned properties. Here, we fabricate nonmulberry tasar Antheraea mylitta sericin based porous hydrogels (pore size; 57.23–75.22 μm) and nanofibrous matrices (fiber thickness; 80-400 nm) for skin reconstruction. Both kinds of matrices show improved cellular adhesion, migration, proliferation and viability of human dermal fibroblasts and keratinocytes. These matrices don’t induce any significant immune response of inflammatory cytokines and cause minimal hemolysis of human blood. The hydrogels retain the intrinsic antioxidant and antibacterial characteristics. The in vivo study of hydrogels also exhibits minimal inflammation and good biocompatibility of the matrices marking them potential as skin substitute. Cephalexin hydrate loaded sericin based nanofibrous matrices show enhanced healing of wounds with minimal infection in vivo. The histological analysis reveals absolute restoration of the epidermal layer surrounded by dense collagen filled dermis exhibiting the skin appendages. The in vitro and in vivo results validate the prospective wound dressing and skin regeneration ability of this type of sericin based nanofibrous matrices and hydrogels, respectively.

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Disclosure: No conflict of interest amongst authors.
**01-P572 Development of a new anti-adhesion material, thermally cross-linked gelatin film –Investigation of the physical and biological properties**

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In order to create more excellent anti-adhesion materials, we developed a thermally cross-linked gelatin film. The purpose of this study is to reveal the handling properties by examining the physical properties of the film such as the physical strength and the adhesiveness to tissues. Moreover, in order to reveal the anti-adhesion mechanism, we examined the biological properties such as the anti-adhesion effect, the influence on cell proliferation and the cytotoxicity, especially in comparison with the conventional hyaluronic acid and carboxymethylcellulose film (the conventional film).

A tensile test under dry and wet conditions and shearing stress test showed that the gelatin film has significant higher maximum tensile stress and fracture strain than the conventional film. The anti-adhesion effect of the gelatin film was significantly superior to that of the conventional film with excellent peritoneal regeneration. In the cell proliferation test, the number of fibroblast cells on the gelatin film increased at each time point, while no cell proliferation was observed on the conventional film. Furthermore, in the cytotoxicity test using a colony assay and Live/Dead assay, the extract of the gelatin film had no cytotoxicity, while the extract of the conventional film had cytotoxicity considerably.

Therefore, the gelatin film provides better handling than the conventional film, due to better physical strength and ductility of the film. In addition, the gelatin film has a significantly greater anti-adhesion effect than the conventional film without any cytotoxicity.

**References:**


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**01-P573 Photo-crosslinkable, injectable sericin hydrogel as 3D biomimetic extracellular matrix for minimally invasive repair of cartilage**

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Millions of patients worldwide suffer from cartilage injury and age/disease-related cartilage degeneration. However, cartilage, such as articular cartilage, is poor at self-regeneration. Current treatments are often invasive with limited regenerative efficacy. Developing minimal invasive strategies for effective cartilage repair is thus highly desired. Here, we report an injectable, photo-crosslinkable sericin hydrogel as a biomimetic extracellular matrix for minimally invasive repair of cartilage. Sericin was functionalized to generate sericin methacryloyl (SerMA), which formed an in situ photocross-linked hydrogel upon UV light irradiation. Possessing excellent biocompatibility, SerMA hydrogels were adhesive to chondrocytes, and promoted the proliferation of attached chondrocytes even in a nutrition-lacking condition. Additionally, SerMA hydrogels exhibited photoluminescent property allowing real-time monitoring of hydrogels' status. The mechanical properties and degradation rates of SerMA hydrogels were readily tunable by varying methacryloyl modification degrees to meet various repair requirements. Notably, the in vivo implantation of chondrocyte-laden SerMA hydrogels effectively formed artificial cartilages after 8 weeks. Most importantly, the artificial cartilages molecularly resembled native cartilage evidenced by high accumulation of cartilage-specific ECM components and upregulated expression of cartilage-critical genes. Together, these results suggest this type of sericin hydrogel may serve as a promising tissue engineering scaffold for effective, minimally invasive repair of injured cartilage.

**References:**


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SYIS PA-1  A Highly Bioactive Nanofibrous System for on-site Delivery of Endothelial Progenitor Cells with Rapid Angiogenesis Promotes Wound Healing

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Wounds therapy with a rapid healing performance remains a critical clinical challenge. Cell therapy is considered to be a promising approach to improve the efficiency of healing, yet problems such as compromised cell viability and functionality arose due to the inappropriate delivery methods. Therefore, in this study, a nanofibrous scaffold (CPB) composed of collagen, polycaprolactone (PCL) and bioactive glass nanoparticles (BGNs), was constructed for efficient delivery of endothelial progenitor cells (EPCs) and enhancing wound healing. The CPB scaffolds possessed excellent porosity with uniform size and good cytocompatibility. With the stimulation of CPB, the viability and angiogenic ability of EPCs were significantly enhanced through activation of Hif-1α/VEGF/ SDF-1α signaling when compared to CP nanofibers. In vivo, CPB/EPCs constructs significantly enhanced the formation of high density blood vessels, through greatly upregulating the expressions of Hif-1α, VEGF and SDF-1α. Moreover, due to the increased cell homing and fast neovascularization within the wound site, the cell proliferative activity, granulation tissue formation, collagen synthesis and deposition, were greatly promoted by CPB/EPCs constructs, resulting in fast re-epithelialization speed and skin appendages regeneration. Furthermore, CPB/EPCs constructs displayed higher healing efficiency than CP/EPCs graft and CPB alone, suggesting that the viability and angiogenic ability of EPCs activated by CPB scaffold could be maintained after they were delivered to the wound site. Taken all these results together, it is well illustrated that this biocompatible CPB scaffolds can be used as a promising cell delivery system for stem cell transplantation therapies in wound healing.
Reversal of natural chondrocyte aging through modulating cell morphology in a condensation-simulating process

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INTRODUCTION: The mechanism of chondrocyte senescence and function loss due to natural host aging is not clear, and no proven approach exists to reverse their state and restore their regenerative potential. In this study, we hypothesized that natural chondrocyte aging is accompanied by a change of cytoskeletal architecture and morphology, and therefore modulating cell morphology through a condensation-like process will reverse aged chondrocytes back to a phenotypic state comparable to that of young chondrocytes.

METHODS: Human chondrocytes were isolated from articular cartilage harvested from healthy young (<30 years) and old (>60 years) donors with IRB approval. We first characterized the proliferative activity, chondrogenic phenotype, and response to transforming growth factor β3 (TGF-β3) in young and aged chondrocytes. Then, the aged chondrocytes were rejuvenated via suspension culture in agarose, in which cells were forced to adopt a round morphology, mimicking a condensation-like process. The cartilage formation capability of the untreated aged chondrocytes and the “rejuvenated” chondrocytes were assessed by seeding the cells in a photocrosslinked gelatin hydrogel scaffold developed in our laboratory.[1]

RESULTS: Compared to chondrocytes from young donors, aged chondrocytes displayed prominent cytoskeleton, enlarged cell body with a flat morphology, as well as reduced cartilage formation capacity. After morphology modulation by a condensation-like process, aged chondrocytes displayed smaller extension than untreated cells. “Rejuvenated” chondrocytes also displayed enhanced replicative capacity, improved chondrogenic gene expression, and suppressed dedifferentiation. Interestingly, rejuvenated chondrocytes generated better cartilage in 3D photocrosslinked gelatin hydrogel scaffold both in vitro and in vivo. Westernblot results further indicated that this novel morphology modulation process enhanced RhoA and inhibited p-ERK1/2 level, and finally leading to the increased expression of SOX9. In summary, we reported a robust method to reverse chondrocyte aging via 3D modulation of cell morphology, which enhances the regenerative potential of aged chondrocytes, and may allow the application of autologous chondrocyte implantation (ACI) in the aged population.


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Bi-content micro-collagen chip provides contractility-based biomechanical readout for phenotypic drug screening with expanded and profiled targets

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Phenotypic screening regains new momentum in pharmaceutical industry owing to its exceeded success over target-based screenings. Most phenotypic screenings rely on nonspecific biochemical readout regarding cellular viability which hampers the discovery of novel druggable mechanism of actions (MOAs). Here we present Contractility-based bi-Content micro-Collagen Chip (3CChip), which innovates cellular contractility as a biomechanics-related phenotype for drug screening. Bi-content analysis on cell contractility (imaged by iPhone) and viability suggests that the label-free contractility-based analysis exhibits superior sensitivity to compounds targeting contractile elements (e.g. focal adhesion, cytoskeleton), resulting in enlarged target pool for drug assessment. Six typical readout patterns of drug response are summarized according to relative positions of contraction/viability curves and drug targets are profiled into three categories (biomechanical, biochemical and house-keeping) by 3CChip, which would benefit subsequent target identification. The simple-to-use and effective 3CChip offers a robust platform for micro-tissue-based functional screening and may lead to a new era of mechanism-informed phenotypic drug discovery.
The use of nanocarriers for an improved muscle regeneration

Jenny Ann Prange, Michael Duss, Tullio Sulser, Ehud Landau, Daniel Eberli

To exploit the large potential of nano-medicinal techniques, the development and investigation of state-of-the-art drug delivery nanoparticles is required. Differentiated muscles cannot regenerate unless their satellite cells are activated through injury and start developing a new generation of muscle precursor cells (MPCs). These cells are then able to proliferate and build new muscle fibers. However, their use for muscle transplantations remains problematic since cells face a rather not-preferential environment after transplantation.

The use of lipid-based nanoparticles for the delivery of bio-macromolecules has attracted considerable attention due to the current interest in protein-based therapeutics. Cubosomes protect the incorporated therapeutics, which are susceptible to degradation by enzymes, thereby improving their bioavailability and enhance cellular uptake.

One of the first bottlenecks in tissue engineering is the sufficient supply with nutrients and oxygen in the environment where the transplanted cells and tissue should develop.

We aim to improve MPC survival under hypoxic conditions by treating them with cubosomes that are loaded with pyridine-derived peroxide.

The cubosome nanoparticles presented herein were successfully synthesized and loaded with a water-soluble methylated pyridine-derived endoperoxide, that undergoes a retro-Diels-Alder reaction when exposed to aqueous environment releasing oxygen and providing strongly needed substances to the MPCs. Previous experiments showed that cubosomes are able to increase cellular uptake up to 10 fold, rescue disease phenotypes and remain longer in the endo-lysosomal pathway while no impact was seen on cell viability and proliferation.

Cell viability under hypoxic conditions seems improved with cubosome treatment prior to the exposure to hypoxia compared to untreated samples. Analysis of protein expression by WES showed a significant difference in Hif1α expression in cubosome treated samples under hypoxic conditions compared to untreated samples. Interestingly, we observed an increased expression of desmin under hypoxic conditions after cubosome treatment while Pax7 remained unchanged.

The data presented here provides first insights into the use of cubosomes as a delivery system to overcome the limitations of suboptimal conditions after muscle injury. They present a promising approach to support the integration of MPCs into the damaged area for better regeneration.
**SYIS PA-6**  Skeletal muscle fascicle engineering is improved by co-culture with myofibroblasts and TGF-β1

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**Background.** Wound healing in skeletal muscle leads to tissue regeneration or the formation of fibrotic scar tissue and is dependent on the interactions between fibroblasts, myofibroblasts, myogenic cells, and cytokines, such as TGF-β1. Many recapitulations of the in vivo myogenic tissue regeneration environment use simplified in vitro models lacking 3D tissue structure and cellular complexity. The objective of this study was to establish a 3D co-culture model of skeletal muscle fascicles through inclusion of multiple cell types found in the tissue niche and mimicking wound healing paradigms.

**Methods.** Three culture systems were compared: cell monolayers grown on 2D dishes and 3D tissues prepared from self-assembly or collagen 1-based hydrogels. The myogenic impact of TGF-β1 and mono-/co-culture strategies containing human dermal fibroblasts, myofibroblasts, and C2C12 mouse myoblasts was assessed in 2D and 3D. qPCR identified gene expression changes during fibroblast to myofibroblast and myoblast differentiation between culture conditions. Changes to cell phenotype and tissue morphology were characterized via immunostaining for myosin heavy chain, procollagen, and α-smooth muscle actin. Elastic moduli were measured with compression and atomic force microscopy systems, and a slack test was used to quantify differences in tissue architecture and integrity.

**Results.** TGF-β1 improved myogenesis in 3D mono- and co-cultures, but not in 2D. The 3D TGF-β1-treated co-culture demonstrated the highest myogenin and collagen 1 gene expression, indicating that the inclusion of myofibroblasts significantly enhanced myogenesis beyond the capacity of fibroblasts or TGF-β1 in monocultures of myoblasts. These constructs had the greatest tissue stability, integrity, and muscle fiber organization, as demonstrated by their rapid and sustained shortening velocity during slack tests, and the highest Young's modulus of 6.55kPa. Both self-assembled and hydrogel-based tissues yielded the most multinucleated, elongated, and aligned muscle fiber histology. In contrast, the equivalent 2D co-culture with TGF-β1 completely lacked myotube formation through suppression of myogenin.

**Discussion.** These results demonstrate that our 3D co-culture model treated with TGF-β1 more closely mimics myogenesis in vitro than 2D or monoculture systems. Critically, this study highlights the impact of TGF-β1 and myofibroblasts as myogenesis accelerators across multiple tissue engineering platforms.

**SYIS PA-7**  The effect of combined application of calcium polyphosphate granules and bFGF fibrin glue for tendon-bone healing in the tibial tunnel in a rabbit ACL reconstruction model

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**Objective** This study was designed to investigate the effect of combined application of calcium polyphosphate granules and bFGF fibrin glue for tendon-bone healing in a rabbit ACL reconstruction mode.

**Methods** Seventy-two male New Zealand white rabbit were divided into 4 groups (n=18 for each group) to receive bilateral ACL reconstruction using the long digital extensor tendon autograft: Group A (Nothing added into the tendon-bone interface), group B (CPP granules and fibrin glue without bFGF), group C (bFGF 600 mg/ml in fibrin glue), and group D (CPP granules and bFGF 600 mg/ml in fibrin glue). The decalcified histological sections were stained with Hematoxylin-Eosin, Masson and safranin O for histological scoring. Immunohistochemical stain of Col-I, Col-II, VEGF and OCN were done.

**Results** Histological results indicated that the quality of tendon-bone healing in group B and D were better among groups. Immunohistochemical analysis showed high express of VEGF in group C and D, and high express of OCN and Col-I in group B and D. The BMD values were higher in group B and D. Micro-CT showed peri-graft bone mass and microarchitecture in group D were higher than the other groups. The ultimate failure load and stiffness of group D were the highest among the four groups at week 6 after surgery.

**Conclusion** The bFGF, CPP and combined use of them could significantly improve tendon-bone healing in the tibial tunnel and promoted osteogenesis and fiber connection at the tendon-bone interface after ACL reconstruction in the rabbit model.
Tissue engineering strategies showed successful management of bone defects with stem cells and scaffolding materials to develop bone-analogous constructs (1). Several in vitro studies have shown that hydroxyapatite derived from fish bone exhibits enhanced osteogenic potential compared to synthetically derived hydroxyapatite. This study compared the bone regeneration of fish bone derived hydroxyapatite (FHA) alone and in combination with mesenchymal stem cells (MSCs) in the rabbit femoral condyle bone defects. The study was conducted in the femoral condyle of fifteen New Zealand white rabbits. A total of 30 defects (5 mm in diameter, 8 mm in depth) were created, in either side of the condyle of femoral bone. Defects were randomly assigned to 3 groups: (1) FHA+MSCs, (2) FHA, and (3) Unfilled defect (control). The study was conducted in the femoral condyle of fifteen New Zealand white rabbits. A total of 30 defects (5 mm in diameter, 8 mm in depth) were created, in either side of the condyle of femoral bone. Defects were randomly assigned to 3 groups: (1) FHA+MSCs, (2) FHA, and (3) Unfilled defect (control). The bone volume, bone mineral density and the remaining graft particles were assessed using micro CT and histologically after 8 weeks of healing. The values were tabulated and analyzed statistically. The bone healing seen in both defects filled with FHA and FHA+MSCs were compared to the control group. Micro CT showed significantly higher bone volume and bone mineral density in the defects filled with FHA in combination with MSCs (P<0.05). Quantitative analysis of the histologic sections also showed significantly higher bone volume in the defects filled with FHA+MSCs (58.5±5.3) compared to FHA alone (52.2±5.9). Though the remaining graft particle volume showed a reduction in defects filled with MSCs and FHA it was not significant compared to FHA alone. Based on the results it can be concluded that naturally derived hydroxyapatite along with mesenchymal stem cells enhanced the bone regeneration in rabbit femoral condyle.

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References
**SYIS PA-11**

**Tissue engineering of esophagus: omentum-based recellularization technique**

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**Introduction**: Discovering a compatible scaffold for esophageal replacement in cases of esophageal carcinomas, atresia and caustic injuries has been a critical issue for decades and now with the rise of tissue engineering era, there are new hopes for discovering a more efficient scaffold. In the following study we introduce a novel technique for preparation of an in vivo recellularized esophageal scaffold by the aid of omentum.

**Materials and methods**: A number of rats’ esophaguses were decellularized by a perfusion-based protocol to create a naturally derived esophageal acellular scaffold. Scaffolds underwent DNA quantification test to assure that they have been completely decellularized and they would not produce an immunogenic response. Besides, Scaffolds’ biomechanical features were further investigated by tensile stress test.

On the next phase, Acellular scaffolds were planted in a wrap of omentum in abdomen for different periods of time and the biopsies were taken from one, two, three, six, twelve and eighteen months old scaffolds to investigate cell seeding and differentiation. Samples were stained by H&E, Movat Pentachrome and Sirus Red. IHC assays including SMA for detection of smooth muscle cells, CD34 for detection of stem cells, CK-pan and Ck5/6 for detection of epithelial cells were performed as well as biomechanical tests.

**Results**: DNA quantification of acellular scaffolds showed an absorbance of 2.6 ng/mL compared to 66.4 ng/mL in native scaffolds which demonstrates an efficient decellularization method. Explanted grafts showed regeneration of all the major cell types including epithelial cells, smooth muscle cells and stem cells; furthermore, angiogenesis was observed mostly on the outer layers of implanted scaffolds. Although all major cell types were present in the implanted scaffolds, cellular orientation and architecture of esophageal tissue was not established in implants of three months old or less.

**Conclusion**: Our study demonstrated adequate cell seeding of acellular scaffold via omental wrap; suggesting that omentum could be utilized as an efficient bioreactor in production of tissue-engineered esophagus.
Articular cartilage is not easy to recreate, since it is a highly organized tissue with zonal defined characteristics. Most current treatment strategies simply consider cartilage as a uniform piece of tissue, thereby ignoring its complexities for easy fabrication. In this study, we upgraded the bioprinting of articular cartilage substitutes from 3D to 4D to fabricate constructs with synchronized remodeling of the bioink whilst bioprinting using magnetism in a 4D real-time, biomimetic and zonal fashion.

Streptavidin-coated iron nanoparticles were embedded in printable bioinks with varying concentrations of low gelling temperature agarose (0-1 %) and type I collagen (0.1-0.3 %) and gelled in the presence of a magnetic field (2 mT) for preliminary screening of fiber alignment characteristics within the range of bioinks. Collagen fiber alignment was visualized by second-harmonic generation imaging using a two-photon microscope and quantified with a Fast Fourier Transform algorithm to translate the circularity of its distribution. Selected bioinks were loaded with human mesenchymal stem cells and chondrocytes and printed using a custom-designed drop-on-demand bioprinter. The effect of fiber alignment in one or multiple directions mimicking the superficial, middle and deep cartilage layers on the printed construct’s mechanical and biological properties was characterized by unconfined compression tests as well as histological/immunohistochemical (Hematoxylin and eosin, Toluidine Blue, COL II, ACAN) and biochemical/molecular (GAG, COL II, ACAN) analyses.

Pure type I collagen bioinks showed the most effective unidirectional collagen fiber alignment amongst studied samples. Printable bioinks with the highest agarose content exhibited diminished or inexistant fiber alignment, independently of the presence of a magnetic field. Bioinks of 0.5 % agarose with 0.2 % collagen showed the best unidirectional fiber alignment using a magnetic field during the gelation period. Compressive tangent moduli of printed constructs with unidirectionally aligned fibers perpendicular to the applied force were significantly higher than the modulus of samples with random alignment. Biological data showed successful GAG production and chondrogenesis in printed constructs 21 days after incubation with or without fiber alignment.

Automated and biomimetic solutions such as herein presented hold promise for improving the performance of articular cartilage substitutes in orthopedic research.
Conclusions: HL-1 cells were successfully bioprinted within a 2 mg/mL collagen solution and remained viable. Moreover, the cardiac phenotype was preserved, as evidenced by the cardiac markers expression. Further hydrogel formulations, namely fibrin and decellularized extracellular matrix, are being tested to optimize the bioink composition for cardiac tissue engineering applications. The optimal bioprinted cardiac scaffold could be useful for disease modeling, drug screening, and regenerative therapies.
SYIS PA-16  Spatiotemporal control of spiral waves in human cardiac cell models through optogenetics

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Traditional methods for spatiotemporal control of arrhythmias in the heart rely on the use of electrodes. These methods are laborious and technically limited, such that only local pacing algorithms using a handful of electrodes have been studied. Progress in arrhythmia management requires the development of novel spatiotemporal control schemes in biological systems. In pursuit of these goals, a high-throughput platform was developed for optical actuation and sensing of emergent cardiac behaviors through the use of optogenetics.

Channelrhodopsin-2 (ChR2) is a cation channel that depolarizes the cell membrane in response to blue light and anion channelrhodopsin 2 (GtACR2) is an anion channel that hyperpolarizes the cell membrane in response to blue light. Transgenic hiPSCs expressing ChR2 and GtACR2 were created through lentiviral transduction and differentiated into cardiomyocytes. In parallel, a projector was integrated into an optical mapping system for simultaneous patterned illumination and imaging. Patterned illumination of optogenetic cardiomyocyte monolayers successfully perturbed existing conduction patterns.

To characterize the impact of anatomic defects with high precision, we used patterned illumination of the hyperpolarizing GtACR2 protein to generate light-inscribed conduction blocks in cardiac monolayers. We dynamically modulate the light patterns to control spiral wave formation, pinning, migration, and termination as a function of the inscribed functional geometry.

Patient-derived hiPSCs were then used to model LQT8, where an L-type calcium channel mutation prolongs the action potential duration (APD). Spiral waves can be defibrillated by illumination of the spiral core in ChR2-expressing cardiomyocytes. We find that spiral wave arrhythmias are harder to defibrillate as the APD increases, which correlates with the increased incidence of arrhythmias in patients with LQTS, and that illumination of the spiral core is required for defibrillation.

This work demonstrates high-throughput testing of spatiotemporal control schemes for arrhythmia control in disease-specific contexts. We believe that extensions of this approach will lead to novel scientific discoveries regarding the relationships between cardiac tissue geometry, ion channel mutations, and arrhythmia control. These new fundamental and clinical insights point the way towards the development of patient-specific therapeutic strategies.

SYIS PA-17 Inervated and Vascularized Immunocompetent Human Tissue Engineering Skin to Study of Cutaneous Neuroinflammation

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Immune reactions in the skin are initiated by the cutaneous dendritic cells (DCs). The potential sensitizing effect of a compound can be predicted in vitro using human monocytes differentiated into DCs (Mono-DCs). However, these simplistic models remain inaccurate because the activation of cutaneous DCs by sensitizers may be triggered or modulated by microenvironmental interactions with multiple types of non-immune cells [1].

Our goal is to develop an immunocompetent human tissue-engineered skin (TES) that will combine DCs with all structural and functional elements of the skin, i.e. an epidermal barrier laid upon a dermis containing a pseudo-vascularization and nociceptive neurons [2]. Collagen matrix was seeded with fibroblasts and endothelial cells, then with precursors of nerve fibers derived from either human iPSC or murine embryonic DRG. Finally, we introduced Mono-DCs and keratinocytes.

We observed that in situ differentiated neurons grow axons towards the epidermis as usually observed in normal human skin. What’s more, the neurons derive from iPSC, express neuropeptides and calcium channel as normal nociceptive fibers. Moreover, Mono-DCs settled as expected beneath the epidermis and remained sessile to stimulation for several weeks.

The model will be used to predict the irritant potential of chemical compounds, and the impact of nerves on DC activation. Further, the iPSC technology allows us to create a “one patient” TES with all cells from the same donor to start a personal medicine tool.

References:

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