**01-SY-1** Application of iPSC technology to disease modeling for chondrodysplasia

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Many causes of chondrodysplasia are associated with gene mutations. Accordingly, mouse genetic models have contributed enormously to the understanding of chondrodysplasia. However, differences between mice and humans mean observations from mouse models should be translated with caution when studying the mechanisms driving human chondrodysplasia. Human tissues affected by the disease are therefore sometimes more appropriate for study, but difficult to obtain from patients. However, the advent of induced pluripotent cells (iPSCs) is making it possible to obtain chondrocytes and cartilage from which the pathomechanisms of chondrodysplasia can be recapitulated in culture dishes. iPSCs can be expanded almost infinitely and differentiated into any type of cells. We converted skin fibroblasts from patients with chondrodysplasia into iPSCs and then differentiated the iPSCs toward chondrocytes followed by creation of cartilaginous tissue. We found that cartilaginous tissues derived from hiPSCs generated from patients with FGFR3 chondrodysplasia reproduce the pathology of the diseases and thus offers an iPSC-based disease model.

**01-SY-2** Engineering bone-like nodules from human iPS cells as a research platform for bone biology

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Bone is a dynamic tissue that constantly repeats formation and resorption. The formation of bone-like nodules in vitro recapitulates the former process and is used frequently in bone research. Here we demonstrated this process in time-lapse imaging utilizing a novel method to induce osteogenic differentiation of human induced pluripotent stem cells (hiPSCs) within ten days. Expression profiling and confocal imaging demonstrated the sequential differentiation process, and final products were composed of sheet-like structures of osteoblast-like cells and dendritic osteocyte-like cells underneath the sheet, which created human bone tissues in defects of mice calvarias. The rapid induction was dependent on retinoic acid (RA) signal through RA receptor α (RARα) and RARβ, which simultaneously activated the BMP and WNT signaling pathways. Using patient-derived iPSCs, our method successfully recapitulated the pathological phenotype of a congenital bone disease, osteogenesis imperfecta, which was rescued by the correction of disease-causing mutations and also partially by drug candidates. These results indicate that bone-like nodules induced by our new method will serve as a novel research platform for physiological and pathological bone formation.
Background: Pulmonary arterial hypertension (PAH) is a fatal lung disease with a poor prognosis. It is characterized by pulmonary vascular remodeling that rises pulmonary artery pressure; consequently, the right ventricle (RV) has to adapt to the increasing vascular load. Even though PAH originates in the lungs, patients ultimately die of RV failure. Unfortunately, there is no in vitro model to study the pathophysiology and development of RV failure. We aim to develop a 3D model of RV failure in PAH by incorporating cardiac cells differentiated from patient derived induced pluripotent stem cells (iPSCs) into an established engineered heart tissue (EHT) approach [1] combined with mechanical stress.

Methods: Late-outgrowth endothelial colony forming cells (ECFCs) were isolated from peripheral blood from PAH patients using the Ficoll separation protocol [2], and iPSCs were generated using a lentiviral approach containing the 4 Yamanaka factors [3]. Human iPSCs were differentiated into cardiomyocytes by adding 3 growth factors (BMP-4, activin A and bFGF) [4]. Subsequent steps will include RV afterload induction through applying different levels of silicone posts stiffness in the EHT approach [1].

Results: PAH and control iPSCs, expressing the pluripotent markers NANOG, SSEA4 and OCT4, spontaneously differentiated into the 3 germ layers as evidenced by immunostaining for β 3-tubulin, AFP and CD31. iPSCs differentiated into cardiomyocytes expressed main cardiac markers and spontaneously started beating 8 days after the start of differentiation.

Conclusions: iPSCs were successfully generated from PAH patient blood derived cells. In addition, cardiomyocytes were differentiated from human iPSCs. After incorporating these cardiomyocytes into the EHT set-up, further experimentation will include use of different levels of pressure overload and iPSC-derived cardiomyocytes obtained from patients with different PAH severities. This 3D RV model is expected to demonstrate its value in disease modeling, drug screening, and regenerative therapies for PAH.

Acknowledgments:
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References:

Maturation and Disease of Human Neuromuscular Connectivity Revealed through Optogenetics

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A variety of diseases target the neuromuscular junction (NMJ), but are difficult to study in human patients due to the late diagnosis and the lack of functional insights. Here we report the first human, patient-specific tissue-engineered model of the NMJ that combines stem cell technology with tissue engineering, optogenetics, microfabrication and image processing. The combination of custom-made hardware and software allows for repeated, quantitative measurements of NMJ functionality in a user-independent manner. We demonstrate the utility of this model for basic and translational research by characterizing in real time the functional changes during the physiological maturation and disease pathology. This system holds great potential for the study of neuromuscular diseases and drug screening, allowing for the extraction of quantitative functional data from a human, patient-specific system.

Briefly, human skeletal myoblasts were reprogrammed to obtain a donor-specific hiPSC line that was then transduced with a lentiviral construct comprising the optogenetic protein channelrhodopsin-2 (ChR2) fused to the yellow fluorescent protein (YFP) and differentiated into motoneurons. To allow for compartmentalized and three-dimensional culture of both motoneurons and skeletal muscle, we designed a microfluidic platform comprising a muscle chamber, which provides two compliant pillars for muscle attachment, and a motoneuron chamber, which is connected to the muscle chamber via a channel for axon outgrowth. Axons reached the muscle tissue in 5-7 days after the start of the coculture, and NMJs were established after 7-9 days in co-culture. Functionality of the resulting NMJs was evaluated using a custom optical platform that allows for precise and controlled stimulation of the optogenetic motoneurons with blue light. Light-induced muscle contraction was recorded and quantified using image processing, demonstrating functional improvement over the course of 3 weeks following motoneuron seeding. Bungarotoxin and serum from myasthenia gravis (MG) patients were used to model a pathological neuromuscular phenotype. Our system was able to detect and measure disruption of NMJ functionality in these disease models.

This system represents a step forward towards personalized medicine for NMJ diseases, promising a better understanding of clinically relevant genetic and acquired diseases and high-throughput drug testing in all-human donor-specific systems.
**01-SY-5** Stem cell based microphysiological Organ-on-a-Chip systems as in vitro models of human tissue with physiological structure and function

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Drug discovery and development to date has relied on animal models, which are useful, but fail to resemble human physiology. The discovery of human induced pluripotent stem (hiPS) cells has led to the emergence of a new paradigm of drug screening using human patient- and disease-specific organ/tissue-models. One promising approach to generate these models is by combining the hiPS technology with microfluidic devices tailored to create microphysiological environments and recapitulate 3D tissue structure and function. Such microphysiological organ-on-a-chip systems (OoCs) combine human genetic background, in vivo-like tissue structure, physiological functionality, and “vasculature-like” perfusion. Using microfabrication techniques, we have developed multiple OoCs that incorporate complex human 3D tissues and keep them viable and functional over multiple weeks, including a “Retina-on-a-chip”, a “Heart-on-a-chip” and a “White adipose tissue(WAT)-on-a-chip”. The OoCs generally consist of three functional components: organ-specific tissue chambers mimicking in vivo structure and microenvironment of the respective tissues; “vasculature-like” media channels enabling a precise and computationally predictable delivery of soluble compounds (nutrients, drugs, hormones); “endothelial-like” barriers protecting the tissues from shear forces while allowing diffusive transport. The small scale and accessibility for in situ analysis makes our OoCs amenable for both massive parallelization and integration into a high-content-screening approach. The adoption of OoCs in industrial and non-specialized laboratories requires enabling technology that is user-friendly and compatible with automated workflows. We have developed technologies for automated 3D tissue generation as well as flexible plug&play connection of individual OoCs into multi-organ-chips. These technologies paired with the versatility of our OoCs pave the way for applications in drug development, personalized medicine, toxicity screening, and mechanistic research.


**02-SY-1** Regenerative Rehabilitation: the key to Translating Tissue Engineering and Regenerative Technologies

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This talk will discuss the rationale and practical implications how rehabilitation principles can be an integral part of regenerative medicine therapy concept.
The field of tissue engineering has long integrated fundamental principles of stem cell biology, biomaterials, and mechanical engineering to better design new tissues. As regenerative technologies become more prevalent in the clinic, it is becoming increasingly apparent that the rehabilitation regimen and management of the intervention after the delivery/implantation is just as critical to the success of the implant as the technology itself. This recognition has generated the new integrative field of Regenerative Rehabilitation. This talk will introduce fundamental principles in Regenerative Rehabilitation and the importance of considering rehabilitation for the translation of tissue engineering and regenerative therapies.
**02-SY-4**  
**Muscle-contraction training showed the potential to maximize the efficacy of cell transplantation treatment for Duchenne Muscular Dystrophy (DMD)**

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Duchenne muscular dystrophy (DMD) is one of the most severe forms of muscle disorders. Muscle in DMD patients are extremely fragile and can be damaged even during normal daily activity. The disease is caused by mutations in the DMD gene that results in the loss of dystrophin protein expression. There is little in the way of treatment for the disease and no cure. We have been developing cell therapy for DMD by the muscle stem cells generated from human iPS cells and other progenitor cells. Although reports have shown the effects of cell transplantation therapy in DMD, optimal methods that maximize the efficacy of the transplantation are still needed.

We evaluated the transplantation of human immortalized myogenic progenitor cells (Hu5/KD3) into skeletal muscle of DMD model mice (DMD-null/NSG). Moreover, we are optimizing muscle contraction training programs that enhance the effect of the cell transplantation therapy toward DMD.

These newly developed techniques could be a basis for effective cell therapy towards DMD patients.

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**02-SY-5**  
**Rehabilitative Exercise and Spatially Patterned Bioengineered Scaffolds for Treatment of Volumetric Muscle Loss**

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Traumatic musculoskeletal injury often results in impaired endogenous tissue regeneration and revascularization. Towards this goal, we bioengineered parallel-aligned skeletal muscle constructs that mimic the physiological orientation of native vasculature and muscle tissue, to examine their therapeutic potential in a model of volumetric muscle loss (VML) in conjunction with rehabilitative exercise. Three-dimensional parallel-aligned or randomly-oriented nanofibrillar collagen scaffolds were fabricated and seeded with mouse myoblasts (C2C12) and human microvascular endothelial cells (HMEC-1). When implanted into the ablated murine tibialis anterior muscle, the bioengineered aligned constructs in conjunction with voluntary caged wheel exercise could significantly improve the density of perfused microvessels by >50%, in comparison to treatment of aligned scaffold without exercise. In contrast, implantation of randomly oriented constructs had no improvement in therapeutic revascularization, regardless of exercise activity. Furthermore, the abundance of neuromuscular junctions was 5-fold higher when treated with bioengineered aligned constructs in conjunction with exercise, in comparison to treatment of constructs without exercise. However, de novo myogenesis was not significantly improved by aligned scaffolds, regardless of exercise activity. These findings demonstrate that voluntary exercise improved the regenerative effect of bioengineered aligned constructs by augmenting neurovascularization, and have important implications in the design of engineered biomimetic scaffolds for treatment of traumatic muscle injury.
Naoki Morimoto, Toshihito Mitsui, Atsushi Mahara, Natsuko Kakudo, Tetsuji Yamaoka, Kenji Kusumoto
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Giant congenital melanocytic nevus (GCMN) is defined as a melanocytic nevus that appears at birth and has a diameter of more than 20 cm. GCMN is reported to occur in approximately 1 in 20,000 newborns and has a risk of transformation that usually results in malignant melanoma. The JACE® cultured epidermal autograft (CEA) was approved for use in the treatment of giant congenital melanocytic nevus in 2016 and it was also covered by public healthcare insurance in Japan. It is indicated for CMN that are difficult to treat using conventional treatments. We apply JACE® after curettage of CMN in neonates or dermabrasion in childhood or adults. We have already applied JACE® for more than 10 patients.

In this treatment, we usually obtained a skin sample about 1cm² in size, then we waited for culture which usually takes 3 weeks. We applied JACE® on partial-thickness skin defects after removal of CMN, and it was fixed using a tie-over dressing. The tie-over dressing was removed about 1 week after grafting. CEA took most completely in all patients and the wounds were epithelized within 2 weeks. However, the major complication of this treatment is re-pigmentation or recurrence of nevus, because it is difficult to remove nevus cells existing in the deeper part of nevus by curettage or dermabrasion. To overcome this issue, we developed a novel treatment to inactivate nevus tissue using high hydrostatic pressurization (HHP) at 200 MPa and reconstruct the skin defect after full-thickness removal of nevus using the nevus tissue itself in combination with CEA. In this treatment, the full thickness nevus of the target is removed and grafted again to the original site after its inactivation using HHP at 200MPa. The inactivated nevus does not have any cellular components however, fibroblasts and capillaries will infiltrate the inactivated nevus within a few weeks and the inactivated nevus will serve as a recipient floor for CEA. We have already started the first-in-man clinical trial and will show some cases in this presentation.
Background and Objective: Non-healing limb ulcers are a major cause of amputation. Recently, we have reported the novelty of serum free ex vivo expansion system called Quantity and Quality Culture System (QQc) using peripheral blood mononuclear cells (PbMNC) as non-invasive and effective new generation cell therapy. This study demonstrates the safety and efficacy of QQ cultured PbMNC on non-healing limb ulcers as a prospective phase I/II clinical trial. Material and Methods: 200ml of peripheral blood was drawn from patients with chronic (>3 months) ischemic foot ulcers in an outpatient basis. Mononuclear cells were isolated and cultured in QQc for one week without passaging or media changes. Under local anesthesia, 2x10^7 cells were injected within 20 cm^2 of the chronic wound and wound healing was monitored by photometrically. The adverse effects were evaluated according to the National Cancer Institute Common Terminology Criteria for Adverse Events. Wound closure, VAS scale, skin perfusion pressure (SPP), TcPo2, laser doppler, thermography and angiography were performed to evaluate efficacy post 2,4,8 and 12 weeks therapy. Results: A total of ten limbs were enrolled. The age ranged from 64 to 74 years old. Eight males and one female. Seven patients had diabetes with renal failure and two with collagen disease as past medical history. Blood sugar levels were controlled for all patients, and HbA1C was below 8.0%. All of the wounds extended into bone or tendon and were located in the digits of the foot. Case one with adverse effect underwent infection from injection site with alternative ulcer which had later healed. There was no death, other serious adverse events, or major amputation seen 12 weeks following transplantation. Increased vascular perfusion with decrease in VAS scale were seen in all patients. Interestingly, SPP significantly increased after therapy. Conclusion: The outcomes of this prospective clinical study indicate the safety and feasibility of MNC-QQc cell therapy in patients with non-healing limb ulcers. This methodology will allow us to transplant highly vasculogenic EPCs from small amount of blood draw. This will be the world’s first non-invasive and effective peripheral blood vascular stem cell therapy for limb salvage.

Methods: Several variations of radiation doses and frequencies were delivered to ASCs (in vitro) and dorsal skin of 7-week-old mice (in vivo), evaluating subsequent changes of the radiated cells and skin. Six months after radiation, cutaneous punch wounds were created to compare wound healing of each dose fractionation protocols. We also tested injection of fat or fat-derived products to treat such radiated wounds. Results: In the single dose of radiation to cultured ASCs, dose-dependent increase in cell death was observed up to a dose of 2 Gy. However, the single dose of 2 to 20 Gy, the rate of cell death was not significantly different. In mice models, 2 Gy × 20 group showed a sequential decrease in skin viscosity, tissue oxygen saturation and partial oxygen pressure. Furthermore, the wound healing after 6 months of radiation therapy was slower than other fractionation groups. Wounds injected with cultured ASCs were almost healed by Day 12, and those treated with injection of centrifuged fat or micronized connective tissues healed faster than the untreated control group. Conclusions: The rate of cell death induced by the single-dose radiation was dose-dependent up to 2 Gy, but >2 Gy irradiation did not further enhance the cell death. Although the acute tissue injury appeared to be dependent on individual irradiation dose, a single dose of 2 Gy (commonly used in clinical therapy) was enough to affect the viability of tissue-resident ASCs and induced tissue devitalization such as impaired wound healing. ASCs and other fat-derived products harboring ASCs successfully revitalized the radiated tissues and accelerated its wound healing.
**03-SY-5 Clinical application using autologous cell-engineered chondrocytes transplantation in aesthetic and reconstructive surgery**

Hiroko Yanaga¹, Keisuke Imai², Masayuki Miyata³, Ken Matsuda³, Katsu Yanaga¹

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Regenerative surgery aims to be minimally invasive and also reduce morbidity. Autologous cartilage grafts used in rhinoplasty and ear reconstruction are beset by several limitations. To solve these problems, new autologous graft materials were needed. Therefore, we have developed a unique multilayered culture system that allows us to obtain large volumes of elastic chondrocyte expansion from a small piece of human ear cartilage.

Next, we proposed two transplantation methods for clinical application of multilayered chondrocytes. First, we started with one stage injection-transplantation and performed. We have subsequently discovered that once multilayered chondrocytes are transplanted into a human body, differentiation induction that makes use of surrounding tissue occurs in situ and a large cartilage block is obtained through cartinogenesis and matrix formation. We have named this method, "two-stage transplantation" in Plast Reconstr Surg 2009,124:817. Further, in Tissue Eng Part A. 2012;18: 2020.

These methods of cultured chondrocyte grafting could be applicable as a regenerative cartilage to a wide range of cranio-facial repair. We will also present clinical results of elastic chondrocyte transplantation to treat 316 patients safely for 16 years.

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**04-SY-1 Cell Encapsulation - A New Modality For Long-Term Cures**

Devyn Smith

Sigilon Therapeutics, USA

Sigilon Therapeutics is pioneering a new therapeutic modality - living therapeutics that function as cell factories in the body. Engineered cells that are capable of providing curative efficacy to patients require protection from the immune system. Sigilon’s Alifibromer technology protects the cells from the immune system as well as protects the milli-capsule containing the cells from the foreign body response.
Immune cell recognition of implanted biomedical devices initiate a cascade of inflammatory events that result in collagenous encapsulation of implanted materials which leads to device failure. These adverse outcomes emphasize the critical need for biomaterials that do not elicit foreign body responses. One prime example for the use of this technology is with the development of a bioartificial pancreas for the treatment of patients suffering from diabetes. Immunoisolation of insulin producing cells with porous biomaterials provide an immune barrier that is a potentially viable treatment strategy for Type1 diabetic patients. However, clinical implementation has been challenging due to host immune responses to implanted materials. To address this challenge, we have focused our efforts on the development of improved biomaterials for the use in pancreatic islet cell transplantation.

To enable the discovery of novel superbiocompatible biomaterials we have developed a high throughput pipeline for the synthesis and evaluation of >1000 material formulations and prototype devices. Here, we describe combinatorial methods we have developed for covalent chemical modification and in vivo evaluation of alginate based hydrogels. Using these methods, we have created and screened the first large library of hydrogels, and identified leads that are able to resist foreign body reactions in both rodents and nonhuman primates. These formulations have been used to generate optimized porous alginate hydrogels fabricated with tuned geometries to enhance biocompatibility. Significantly, our lead formulation has enabled us to achieve the first long-term glycemic correction of diabetic animals without immunosuppression using stem cell derived human islets.
04-SY-4  Preventing Hypoxia-induced Beta Cell Death and Dysfunction via Oxygen-generating Microbeads

Jia-Pu Liang, Maria Coronel, Minh Dang, Cherie Stabler, Deborah Chaimov

The ability to sustain mature human beta cells in culture, is one of the major challenges in cellular replacement developments therapies for type 1 diabetes. In our research, we seek to engineer physiomimetic 3D niches, in combination with microfluidics devices, for the maintenance and monitoring of human beta cells. When rat or human islets are cultured in standard tissue culture plates, our studies have observed a significant loss of islet number at post-isolation day 3 that continues to grow over the 10-day culture period. Overall islet morphology and viability was unchanged; however, functional readouts were impacted with a significant decrease in dynamically stimulated glucose-stimulated-insulin-release. It is our hypothesis that islets will remain more stable in culture if their peri-islet 3-D matrix niche is recapitulated.

To test this hypothesis, we first engineered 3-D physiomimetic decellularized ECM hydrogels. Porcine bladder and pancreas were decellularized using a combination of physical chemical and enzymatic cleaning, followed by pepsin digestion, to form ECM hydrogels that exhibited a 3D fiber network structure. Mechanical properties and proteomic analysis of ECM hydrogels were characterized and the effect of the hydrogels on rat and human islets functionality and viability was evaluated. Immunofluorescent staining (CD31, F-actin, Insulin and DAPI) was used to localize cell morphology within islet-ECM gels.

IHC and proteomic analysis revealed a high presence of BM-associated ECM proteins (collagen IV and laminin) in decellularized bladder, whereas decellularized pancreas hydrogels were comprised of more structural ECM proteins (collagen I and III). Encapsulated islets exhibited decreased aggregation and spheroid fusion, preservation of resident endothelial cells, and development of unique 3-D sprouting structures of proliferating fibroblast-like-cells that formed a connected network between the human islets. Notably, both rat and human islet function was elevated when encapsulated within ECM pancreatic hydrogels, with a 1.8 fold and 1.6 fold increase in insulin secretion during a glucose challenge, respectively.

These studies highlight the potential of decellularized ECM-based hydrogels for providing a supportive peri-islet niche for cultured islets. Future work will focus on the translational potential of these platforms, as well as the integration of this approach within our customized fluidic platforms for dynamic 3-D islet culture.

References:

04-SY-5  Engineering 3D Islet Micro Chip

Jia-Pu Liang, Kaiyuan Jiang, Smit Patel, Cherie Stabler, Deborah Chaimov

Department of Biomedical Engineering, University of Florida, Gainesville, USA

Platforms for dynamic 3-D islet culture. This work will focus on the translational potential of these platforms, as well as the integration of this approach within our customized fluidic platforms for dynamic 3-D islet culture.

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Anti-Inflammatory Properties of Corneal Stroma-Derived Stem Cells: Potential as a Topical Therapy for the Ocular Surface

Owen McIntosh, M. Lizeth Orozco Morales, Nagi M Marsit, Andrew Hopkinson, Laura E Sidney

Academic Ophthalmology, Division of Clinical Neuroscience, University of Nottingham, Nottingham, UK

Corneal stroma-derived stem cells (CSSC) show potential as a stem cell source for corneal regeneration and wound healing, by acting as bidirectional sensory “factories” that secrete trophic factors in response to an injured microenvironment. Delivering CSSC topically to an injured corneal surface, using a substrate such as amniotic membrane (AM), represents a novel cellular therapy for severe keratitis conditions that can potentially lead to blindness.

In this study, we optimised an in vitro inflammation model using human corneal epithelial cells (hCEC) treated with combinations of ethanol, lipopolysaccharide, and pro-inflammatory cytokines, interleukin 1β and tumour necrosis factor-α. The effect of this combined injury was assessed for effect on hCEC viability and proliferation, cytotoxicity, cell lysis, and further expression of pro-inflammatory cytokines. To assess the anti-inflammatory potential of the CSSC, a co-culture system was used, with and without cells seeded on AM. Expression of anti-inflammatory trophic factors by CSSC was analysed using protein arrays and ELISAs.

Co-culture of the optimised hCEC injury model with the CSSC cell therapy led to increased hCEC viability and proliferation, decreased cytotoxicity and cell lysis, and decreased levels of proinflammatory cytokines, when compared to injury alone, demonstrating the anti-inflammatory potential of the CSSC. CSSC could be easily cultured on the AM, establishing a promising method of applying the cells topically to the cornea.

CSSC demonstrate an anti-inflammatory effect with potential to be clinically translated into a topical therapy for the injured ocular surface, using a carrier such as amniotic membrane.
**05-SY-3** Advanced in vitro three-dimensional cornea tissue model  
Chiara Ghezzi¹, Tina B McKay¹, Siran Wang¹, James L Funderburgh², David L Kaplan¹

¹Department of Biomedical Engineering, Tufts University, Medford, USA, ²Department of Ophthalmology, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA

The need for human corneal tissue as replacements for grafts (keratoplasty) and for research/screening tools, continues to expand, with few new options available to meet these needs. New tissue-engineered human cornea systems are essential for both human ocular disease management as well as expanded screening tools. Our hypothesis is that tissue engineered cornea systems with appropriate mechanics, cultivation conditions and innervation, will provide functional human cornea tissue equivalents to meet such in vitro and in vivo needs. Toward this goal, we have established an in vitro cornea tissue model based on engineered silk film substrates and we have investigated the interplay of human corneal stromal stem cells, human corneal epithelial cells, and innervation. In this in vitro tissue model, we were able to recapitulate the physiological organization and phenotype of the stromal cells, the multi-layer functional features of the epithelial cells, sustained cultivation for chronic studies, and the native density and organization of nerve endings. Furthermore, the current model has been shown to be responsive to several known ocular irritants, as by pain mediators quantification and cellular recovery upon stimulation. This work should propel these new advanced in vitro, human, cornea tissue equivalents to the forefront of in vitro tissue systems to simulate human-related outcomes, including acute and chronic functions in healthy and diseased corneal states.

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**05-SY-4** Strategy for mass production of corneal endothelial substitute cells from iPS cells  
Shin Hatou

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There are over 12 million patients waiting for corneal transplants globally, while only 180,000 cases receive transplants every year. Moreover, more than half of these patients require grafts due to corneal endothelial disease (bullous keratopathy). In order to provide for the unmet millions of patients globally, one of the solutions is to take advantage of mass production of allogenic iPS cells.

We have derived corneal endothelial substitute cells from iPSCs (CECSi cells) for the treatment of bullous keratopathy. Since corneal endothelial cell (CEC) is developed from neural crest cells (NCCs), we tried to derive CECSi via NCC development from iPS cells at first. However, the stepwise protocol was a disadvantageous way for mass production, so we changed the strategy and decided to devise a large scale protocol technique that involves cryopreservation; we changed the protocol from “development via NCCs” to “direct development from iPS cells”, and to insert the process of making cryopreserved “working cell stocks” during protocol. Now the latest development protocol is simple, much easier, more efficient, and more suitable for mass production of CECSi compared to previous method.

Our cell source does not require donors or eyebanks, and therefore our product will be best suited for global distribution including blind patients in developing countries.
05-SY-5  
Genetically engineered iPSC-retina for improved retinal reconstruction after transplantation

Take Matsuyama, Hung-Ya Tu, Jianan Sun, Tomoyo Hashiguchi, Junki Sho, Genshiro A Sunagawa, Momo Fujii, Akishi Onishi, Masayo Takahashi, Michiko Mandai

Cell therapy is a promising treatment to restore cellular function in previously untreatable maladies. Currently, retinal sheet transplantation, which supplies photoreceptors and secondary retinal neurons, has been shown able to reintroduce visual function in mice with end-stage retinal degeneration. However, synapse formation between graft and host is suboptimal, as graft secondary neurons often become a physical hindrance and restrain photoreceptors from contacting the host secondary neurons. We therefore prepared two iPSC-retina with reduced secondary neurons, by using iPS cell lines where genes known to regulate maturation and survival of secondary neurons have been knocked out (Bhlhb4 and Islet1).

These KO cell lines were differentiated in vitro to iPSC-retinas with a similar retinal differentiation potency to wildtype cell lines. Immunohistochemistry and micro array analyses show that these KO retinal sheets have normal early retinal development. The differentiated iPSC-retina sheets were transplanted to end-stage rd1 mice, and tissue integration was characterized and compared by immunohistochemistry. Transplanted KO iPSC-retinas readily matured and integrated to the host, and as expected, the number of graft secondary neurons was drastically decreased in the KO lines. Synaptic contact between graft photoreceptors and host secondary neurons was confirmed by immunohistochemistry. Finally, light response of the grafted host retinas was evaluated by MEA recording and 2-photon calcium imaging of the ganglion cell activities. Both MEA recording and 2-photon calcium imaging showed that grafted cells could elicit light responses in the host ganglion cells.

Our results show that, genetically engineered iPSC-retina is a viable strategy for preparing retinal sheets for transplantation with fewer secondary neurons. These grafts can readily integrate to the host as the wildtype retinal sheets do, while improving the contact of graft photoreceptors with host bipolar cells.

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**06-SY-1** Gene2Skin: Scaffold-based delivery of therapeutic genes for enhanced skin repair

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Recent advances in tissue engineering have made progress towards the development of biomaterials with the capability for delivery of growth factors to promote enhanced tissue repair. However, controlling the release of these growth factors is a major challenge and the associated high costs and side effects of uncontrolled delivery has proved increasingly problematic in clinical applications. Gene therapy might be a valuable tool to avoid these limitations. While non-viral vectors are typically inefficient at transfecting cells, our group have had significant success in this area using a scaffold-mediated gene therapy approach for regenerative applications. These gene activated scaffold platforms not only act as a template for cell infiltration and tissue formation, but also as a 'factory' to provoke autologous host cells to take up specific genes and then engineer therapeutic proteins in a sustained but eventually transient fashion. Alternatively, scaffold-mediated delivery of siRNAs and miRNAs can be used to silence specific genes associated with pathological states in wound repair - for example suppression of M1 macrophage-mediated inflammation as well as fibrosis. This presentation will provide an overview of some of this research with a particular focus on gene-activated biomaterials for increased vascularization in skin repair and wound healing. The systems currently being employed include porous collagen-glycosaminoglycan scaffolds with proven regenerative potential for skin repair combined with non-viral vectors such as chitosan [1] and polyethyleneimine [2] which are used for controlled delivery of the individual cargo - including pro-angiogenic molecules such as VEGF and SDF-1a. These platforms can direct an enhanced regenerative response from host cells to direct healing in challenging wounds such as diabetic foot ulcer.

Acknowledgement: EU Horizon 2020 “Gene2Skin” Project


**06-SY-2** Combinatorial gene therapy for bone regeneration

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Therapeutic compensation of deficient bone regeneration is a challenging task and a topic of on-going research for novel treatment strategies. One promising approach for improvement involves non-viral gene delivery of growth factor genes to provide transient, local and sustained expression of the growth factor.

However, since the efficiency of non-viral gene delivery is still lower compared to viral systems, we first focused on the improvement of the expression system of the particular therapeutic genes, aiming for a compensation of the poorer transfection efficiency. For optimization, the native gene sequences were improved by codon optimization and insertion of a minimized intron. Transfection of multiple cell lines and mesenchymal stem cells with plasmids harbouring the improved gene sequence, such as bone morphogenetic protein 2 (BMP-2), leads to a several fold increased protein expression rate and subsequent osteogenic differentiation.

As a second step, we examined a possible therapeutic effect by combining osteogenic growth factors (bone morphogenetic proteins) with various bone-related miRNAs. Several miRNAs show bone-supportive activity and enhance the biological impact of the overexpressed BMP-2 in vitro and in an ectopic mouse model in vivo.

As a last step, we examined the combination of specific ratios and amounts of different osteogenic growth factor genes to find ideal mixes of the bone-related growth factors for the best outcome. We showed that specific formulations of ratios of different bone-related genes enhance cellular differentiation and bone tissue regeneration in in vitro approaches, as well as in a calvarial rat model in vivo. Additionally, since angiogenic growth factors have been shown to support ossification mainly through vascularization of the newly formed tissue, we also combined differenter ratios and amounts of osteogenic and angiogenic growth factors in order to accelerate bone regeneration and simultaneously inducing most suitable vascularization.

In general, combining two or more therapeutic genes can overcome potential side-effects of conventional supraphysiological dosages and lead to increased bone mass and suitable angiogenesis.
RANKL-induced M1-like macrophages are actively involved in bone formation

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Background: The traditional concept of macrophages is that M1 activity inhibits cell proliferation and causes tissue damage while M2 activity promotes cell growth and tissue repair. However, tissue macrophages may be a more multifunctional group of cells than originally appreciated with different physiologies and distinct immunological functions. It is not justified to describe M1 and M2 activity simply in a pro- or anti-inflammatory manner. Consequently, the current challenges in this field are to identify those subsets of macrophages that are specifically involved in certain conditions such as natural bone healing and remodeling process.

Objective: M1 macrophages can be found under physiological conditions without any pathological stimuli, such as lipopolysaccharide (LPS) and interferon-γ (IFN-γ). This study aimed to understand the involvement of RANKL-induced M1-like macrophages in bone formation compared with pathologically induced macrophages.

Methods: Fischer rats were used to investigate macrophage distribution in normal and injured femoral condyles in vivo. Bone marrow-derived macrophages (BMDMs) were activated with LPS+IFN-γ or RANKL to achieve M1 activation in vitro. Gene expression related to inflammation, osteoclastogenesis, angiogenesis, and migration was determined by reverse transcription-quantitative polymerase chain reaction (RT-qPCR) and fluorescence-activated cell sorting (FACS).

Results: Tissue macrophages showed distinct expression patterns at different bone regions. RANKL was found in close proximity to inducible nitric oxide synthase-positive (iNOS+) cells in vivo, suggesting an association between RANKL expression and iNOS+ cells, especially in trabecular bone. RANKL-induced macrophages showed a different cytokine secretion profile compared with pathologically induced macrophages. Both osteoclasts and M1 macrophages peaked on day 7 during bone healing. RANKL could trigger M1-like macrophages with properties that were different from those of LPS+IFN-γ-induced ones. These RANKL-activated M1-like macrophages were actively involved in bone formation.

Conclusions: Our results indicate that more M1 cells are involved in bone formation process and RANKL may play a crucial role in the phenotypic switching of macrophages. This study substantially adds to the current view on the heterogeneity of tissue macrophages and challenges the traditional concept regarding the unfavlorable role of M1 macrophages in bone formation.
**Regulation of stem cell fate using bioactive ions for tissue engineering**

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Biomaterials have been used as the mechanical support to help tissue growth, or to fix the damaged tissues, so the physical properties and biocompatibility are most important characteristics of this kind of biomaterials. With increased clinical demand on treatment of chronic wound and reconstruction of lost tissues, in particular, with the development of stem cell and tissue engineering technologies, the development of new generation biomaterials, which have the activity to activate gene expression of cells, stimulate stem cell differentiation and tissue regeneration, has attracted more and more attention. As acellular chondrogenic MSC derived grafts can initiate this bone formation we know matrix components are crucial. Collagen type X (ColX), a hypertrophy associated collagen, is involved in calcium binding and matrix vesicle compartmentalisation, however the role of ColX in EO remains unclear. How ColX affects tissue engineered (TE) chondrogenesis and subsequent bone formation is also unknown. Here we investigate the importance of ColX using lentiviral shRNA mediated gene silencing to better understand the process of MSC mediated EO.

**Methods:** MSCs were expanded and cultured in α MEM containing 10% serum, 1ng/mL FGF2, and 25µg/mL ascorbic acid. Lentivirus particles containing an empty vector, non-target control or shRNA against ColX were combined with 40µg/ml protamine to transduce MSCs followed by puromycin selection (5µg/ml). 2x10⁶ MSCs were differentiated in chondrogenic medium (high-glucose DMEM, 1mM sodium pyruvate, 40µg/mL proline, 1:100v/v ITS, 10ng/ml TGFβ3, 25µg/mL ascorbic acid, and 100nM dexamethasone), for 21 days. Samples were taken for histology, PCR, western, GAG/DNA, ELISA, migration assays and subcutaneous implantation in nude mice. Biweekly longitudinal mCT was performed for 8-10 weeks.

**Results:** ColX was effectively knocked down via shRNA gene silencing. Chondrogenic differentiation was greatly influenced when ColX was silenced as evidenced by a clear downregulation of key matrix factors and secreted molecule production. Furthermore in vitro assays show a lack of endothelial migration in response to conditioned medium generated from ColX shRNA containing pellets. Following in vivo implantation bone formation was inhibited only when ColX production was completely abolished.

**Discussion:** From a tissue engineering perspective the presence of ColX during chondrogenic differentiation is crucial for proper matrix development. When ColX is completely knocked down chondrogenic pellets have a decreased ability to make bone which further shows the importance of this collagen in MSC mediated endochondral ossification.

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**Collagen type X plays an important role in mesenchymal stem cell mediated cartilage formation and subsequent endochondral ossification**

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**Introduction:** Endochondral ossification (EO) is the process by which the long bones of the body are formed developmentally. EO can be replicated by implanting in vitro chondrogenically primed mesenchymal stem cells (MSCs) pellets in vivo. Following implantation, a bone construct is retrieved with a functional marrow cavity. However, the exact mechanism by which these pellets achieve bone formation is not fully understood. As acellular chondrogenic MSC derived grafts can initiate this bone formation we know matrix components are crucial. Collagen type X (ColX), a hypertrophy associated collagen, is involved in calcium binding and matrix vesicle compartmentalisation, however the role of ColX in EO remains unclear. How ColX affects tissue engineered (TE) chondrogenesis and subsequent bone formation is also unknown. Here we investigate the importance of ColX using lentiviral shRNA mediated gene silencing to better understand the process of MSC mediated EO.

**Methods:** MSCs were expanded and cultured in α MEM containing 10% serum, 1ng/mL FGF2, and 25µg/mL ascorbic acid. Lentivirus particles containing an empty vector, non-target control or shRNA against ColX were combined with 40µg/ml protamine to transduce MSCs followed by puromycin selection (5µg/ml). 2x10⁶ MSCs were differentiated in chondrogenic medium (high-glucose DMEM, 1mM sodium pyruvate, 40µg/mL proline, 1:100v/v ITS, 10ng/ml TGFβ3, 25µg/mL ascorbic acid, and 100nM dexamethasone), for 21 days. Samples were taken for histology, PCR, western, GAG/DNA, ELISA, migration assays and subcutaneous implantation in nude mice. Biweekly longitudinal mCT was performed for 8-10 weeks.

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**Discussion:** From a tissue engineering perspective the presence of ColX during chondrogenic differentiation is crucial for proper matrix development. When ColX is completely knocked down chondrogenic pellets have a decreased ability to make bone which further shows the importance of this collagen in MSC mediated endochondral ossification.
Molecular Mechanisms of Bioactive Glass-Induced Cell Adhesion and Osteogenic Differentiation in Human Adipose Stem Cells

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Bioactive glasses (BaGs) have been extensively used in bone tissue engineering (TE) applications but the molecular response to the BaGs is not yet fully understood. This study set out to analyze the mechanisms of cell attachment on two discs of different BaG compositions, and the adhesion-mediated early osteogenic differentiation induced by BaGs in human adipose stem cells (hASCs). Human ASCs were cultured on two silica-based BaG discs: S53P4 (23.0Na2O-20.0CaO-4.0P2O5-53.0SiO2 (wt-%)) and 1-06 (5.9Na2O-12.0K2O-5.3MgO-22.6CaO-4.0P2O5-0.2B2O3-50.0SiO2) in the absence of osteogenic supplements. We discovered that the BaG discs supported cell adhesion by enhancing integrin β1 and vinculin production, and that the mature focal adhesions were smaller but more dispersed than on cell culture plastic (polystyrene). Based on our results, both BaG-types enhanced early osteogenic differentiation by the means of alkaline phosphatase activity (ALP) and the expression of osteogenic marker genes RUNX2a and OSTERIX. The BaG composition 1-06 with lower reaction rate was discovered as a stronger osteoinducer. We also analyzed the cell signaling response to BaG interaction and found out that Focal adhesion kinase (FAK), extracellular signal-regulated kinase (ERK1/2) and c-Jun N-terminal kinase (JNK)-induced c-Jun phosphorylations were upregulated by glass contact. Additionally, inhibition of FAK, ERK1/2 and JNK reduced the BaG-induced early osteogenesis indicating the significance of these pathways in the osteogenic course of hASCs. This study enhances our understanding of the molecular mechanisms of BaG-induced early osteogenesis in hASCs and provides tools for future biomaterial designing for bone TE.

3D-Printing of multifunctional scaffolds for bone therapy and regeneration

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For therapy and regeneration of bone defects resulting from malignant bone disease, it is of great importance to develop multifunctional biomaterials for bone therapy and regeneration. Conventional biomaterials always lack multifunctional properties, limiting their application for treating and repairing bone disease (e.g. bone tumors)-initiated defects. How to design and prepare bioscaffolds with favorable microenvironments for disease therapy and tissue regeneration is one of interesting topics in the fields of biomaterials and tissue engineering. We developed several strategies, including harnessing nutrient elements, biomimetic structure and functional interface as well as thermo-therapy to construct multifunctional scaffolds by 3D-Printing method for therapy and regeneration of bone tissues. It is interesting to find that both nutrient elements and biomimetic structure of the printed bioscaffolds have important effect on the stimulation of osteogenesis and angiogenesis of stem cells, and thermotherapy plays an important role to treating bone tumors. Therefore, we put forward new concept that 3D-Printed bioscaffolds combined bone therapy and regeneration could be a new direction of bone tissue engineering.
Minimally-invasive therapies using endoscopy such as endoscopic submucosal dissection has attracted increasing attention for the treatment of digestive system cancer. Although wound healing of tissues after the dissection is important to avoid scar contracture, bleeding, and inflammation, commercially available materials lack tissue adhesiveness, biodegradability, and easy handling for delivery. In this study, we aim to develop a multifunctional colloidal wound dressing with strong adhesiveness to soft tissues after digestive system cancer treatment. Hydrophobically-modified gelatin was synthesized through the reaction between fatty aldehydes with various alkyl groups and amine groups in gelatin. We previously reported that hydrophobically-modified gelatins show strong adhesiveness to tissues (1). We prepared micro/nano-meter sized particles of hydrophobically-modified gelatin by spray drying method and thermal crosslinking method. Hydrophobized gelatin particles strongly adhered to the tissue surface under wet conditions after dissection of mucosal tissue. Adhesion strength depended on the degree of substitution and the length of alkyl groups in fatty aldehydes, indicating that hydrophobic groups increased tissue adhesiveness. This colloidal wound dressing would be promising for promoting wound healing after the dissection of digestive system cancer.
Biomaterial-driven vascularisation: strategies for developing vascularised cardiac patches

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Cardiac patches, bioengineered cardiac tissue analogues made using biomaterials populated with appropriate cell types, are a promising approach toward the repair and regeneration of injured heart muscle following myocardial infarction. However, the clinical utility of cardiac patches is limited by insufficient and delayed vascularisation. While the role of biomaterials in early cardiac patches was to deliver cardiac cells to the target area, the field has now recognised the complex information biomaterials can encode in their physical and chemical structures and the influence of this information on cell behaviour, including vascular ingrowth and patch integration. This study aims to develop silk-based biomimetic biomaterials that promote rapid vascular ingrowth for the next generation of cardiac patches.

We utilised a range of physical and biological features engineered into silk biomaterials to promote vascular ingrowth. Vascular-like hollow channels introduced into silk biomaterials played an essential role in enhancing cell infiltration and delivering oxygen and nutrients to the scaffold bulk, and promoted enhanced host tissue integration (cell infiltration & matrix deposition) and vascularisation in vivo. Perlecan, a key vascular proteoglycan enhanced endothelial cell interactions with silk biomaterials in vitro and promoted higher vessel density in a CAM angiogenesis assay. Perlecan-functionalised silk biomaterials promoted tissue and vascular ingrowth in vivo with a higher vessel density compared to non-functionalised biomaterials.

Overall, this work demonstrates the importance of physical and biological cues that can be engineered into biomaterials to enhance vascular ingrowth and ultimately biomaterial integration with the surrounding tissue.
**08-SY-1 Lessons from Translation: Engineering a pro-regenerative immune environment with biomaterials**

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The immune system is the first responder to trauma and foreign bodies such as biomaterials, yet this response and its capacity to orchestrate tissue repair has been largely ignored. Today, biomaterials can be engineered with exquisite control over physical (mechanical and geometric) properties and can present an array of spatially controlled biological cues in the form of peptides, proteins, or sugars. Until now, these scaffolds have directly targeted stem cells, vascular development, and differentiated cells to stimulate tissue formation or wound healing. Unfortunately, stem cells have not proven to be the panacea for rebuilding tissues. Translating tissue engineering technologies to the clinic, we discovered cells from adaptive immune system responded to the biomaterials. We profiled in depth the immunological response to the wound environment in combination with biological scaffolds. The adaptive immune system, specifically Th2 T cells, is required for the scaffold stimulation of wound repair. We are now investigating in detail the innate and adaptive immune response to synthetic and biological scaffolds. In parallel, we are exploiting these discoveries to design immunomodulatory materials for tissue repair. Ultimately, targeting the immune system represents a paradigm shift for the field and will bring to fruition, at least in part, the promise of regenerative medicine.

**08-SY-2 Exosome-loaded electrospun fibers: design of tissue scaffolds towards regenerative immunomodulation**

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The immune system has been identified as a key mediator in tissue regeneration by playing key roles in angiogenesis, fibrosis and tissue remodeling. Implantation of designer scaffold materials can involve both innate and adaptive immunity which could potentially be manipulated to generate pro-regenerative tissue responses. Mesenchymal stromal cells (MSCs) provide promising repair/regenerative modalities in many diseases through their paracrine products, and the exosomal secretion by MSCs in particular exhibits the immunomodulatory properties by targeting a broad spectrum of immune cells. Here, we developed a method to create electrospun fibrous scaffolds bound with MSC exosomes and investigated the immunomodulatory function of the new biological materials as cell-free constructs for tissue regeneration. The fibrous scaffolds were fabricated with morphological features to attract immune cells and the MSC exosomes were utilized to further educate the recruited cells towards pro-regenerative immune reactions. The result showed that exosomes were efficiently loaded onto the poly(ethyleneamine)-modified electrospun fibers and the electrostatic binding between exosomes and the scaffold allowed the uptake of exosomes by macrophages which were induced with M2-like phenotypes in vitro. When implanted subcutaneously in mice, the exosomes could partially be retained within the scaffolds for over 2 weeks while the presence of the injected free exosomes was shorter than a few hours. In the immunological analyses post-implantation, local M2-biased responses at the implantation site and remote Th2 immune responses and increased population of regulatory T cells in the lymphatic tissue or spleen were observed. The results suggested the immunomodulatory function of MSC exosome-loaded scaffolds and the design of functional synergy between exosomes and materials presented a new paradigm to promote tissue regeneration.
Antigen presenting cells (APCs), such as macrophages and dendritic cells, play a crucial role in orchestrating immune responses against foreign materials. Activation status of APCs can determine the outcome of an immune response towards inflammation and tissue damage or healing. Different biomaterials are used in fabrication of implantable devices, and developing drug delivery systems. These materials will be in close contact with antigen presenting cells and their different chemical and physical characteristics, such as surface topography and chemistry, could have a critical role in initiating pro- or anti-inflammatory immune responses. Different strategies aiming at modifying surface chemistry of biomaterials provide novel ways to modulate the phenotype and function of immune cells. Such modifications could enable reduction of detrimental pro-inflammatory responses and promotion of beneficiary healing responses. In this project we describe a high throughput screening strategy for identifying novel chemistries that are able to modulate macrophages and Dendritic cells populations towards distinct pro- (DC1 and M1) or anti-inflammatory (DC2 and M2) phenotypes. Such information will enable the design of new generations of “cell-instructive” materials with immunomodulatory properties which could have numerous clinical applications including in drug delivery, medical devices or vaccination.

References:

Macrophages are well known to regulate tissue healing, but T cells are emerging as key regulators in the tissue repair and regeneration processes. Indeed, T cells secrete numerous cytokines, chemokines and growth factors that can either inhibit or promote regeneration. Additionally, T cells may directly regulate the regenerative capacity of tissue-resident stem cells. For instance, it has been shown that CD4+ and CD8+ conventional T cells (Tconvs) inhibit bone regeneration in mice. In contrast, CD4+ regulatory T cells (Tregs) most likely facilitate tissue regeneration by supporting the regenerative capacity of tissue-resident progenitor cells. Moreover, bone regeneration is accelerated in T cell-deficient mice (Rag1−/−) and chimeric mice having only Tregs, while regeneration is impaired in chimeric mice having only CD4+ and CD8+ Tconvs (Prof. Martino’s lab, unpublished data). Based on these findings, it is of interest to develop novel systems that mobilize Tregs at the wound site to promote tissue healing. We screened more than 20 different cytokines for their ability to mobilize Tregs in vivo and found an epidermal growth factor receptor (EGFR) ligand to be most effective. This ligand increases Treg Forkhead box P3 (FoxP3) expression, a critical regulator of Treg development and function, but does not induce Tregs from Tconvs. Preliminary in vitro experiments also suggest that this ligand promotes Treg proliferation, is chemotactic for Tregs, and enhances Treg-mediated suppression of Tconvs. We hypothesize that it enhances the regenerative capacity of Tregs by preventing Foxp3 degradation via the Grb2/PI3K-RI/Akt/GSK-3β axis and promotes migration via activation of CXCR4-associated downstream signaling. We are currently producing recombinant versions to boost its activity, and will then test whether hydrogel delivery enhances bone, muscle and skin repair.

References:
Controlling host inflammatory/immune responses through biomaterial-based approaches are offering unique strategies for preventing infectious disease, enhancing immune acceptance in regenerative medicine, contributing to tissue repair, promoting immunity for tumor eradication and intervention in autoimmune disease. Biomaterial-based approaches include controlled release of biomolecules, biomaterials for immune cell delivery and biomaterial design for direct effects on immune cell phenotype. Central to achieving this is a need to control the balance of pro-inflammatory and tolerogenic immune states. Extracellular Vesicles (EV), including exosomes and microvesicles, are produced by most cells, circulate in body fluids, and play important roles in intercellular communication, but remain little explored as theranostics tools for tissue repair/regeneration.

EV have been reported in functions that are crucial for the key stages of tissue repair and regeneration, such as regulation of immune cell function, cell proliferation and differentiation, and also in extracellular matrix remodelling. Their presence in body fluids supports their potential to transport molecular messages to locations in the body far from the place of injury, while providing a potential source of biomarkers.

We have been working on the effect of EV secreted by immune cells upon Mesenchymal Stem/Stromal Cells (MSC), namely their migration, proliferation and differentiation. MSC are likely recruited to lesion sites by activated immune cells. Our previous work shows that human monocyte-derived Dendritic Cells (hDC) can recruit human MSC via paracrine action, and we recently established EV as the main effectors of DC-mediated MSC recruitment. Also, most recent results, using EV from a rat bone injury model, indicate that this recruitment is influenced by the effect of the injury microenvironment on DC precursors. Interestingly, DC-derived EV contain several chemoattractant molecules. Currently we are developing a biomaterial delivery system for local controlled release of naturally produced or modified EV for new bone tissue regenerative strategies.


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**O9-SY-2 Subchondral bone remodelling in osteoarthritic joints**

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**Abstract**  
Osteoarthritis (OA) is a degenerative joint disease that affects both cartilage and subchondral bone [1, 2]. With progression of OA, as a result of changes in the loading pattern, bone remodelling and resorption occur in the joint [3]. This weakens the physical environment that supports the overlying cartilage. In this study, we examined the changes in local distribution of volumetric bone mineral density (vBMD) in the subchondral bone, and the biomechanical properties of the overlying cartilage with an aim to understand the effect of subchondral bone remodelling on the overlying cartilage degeneration.

Human femoral heads were collected during total hip replacement operation due to OA. Cartilage was graded using ICRS classification, and the mechanical property of cartilage was measured by non-destructive cyclic indentation. To determine the remodelling of the subchondral bone, a peripheral quantitative CT (pQCT) was used to assess the vBMD distribution within the subchondral bone. Non-parametric Kruskal-Wallis method was used for statistical analysis (p = 0.05).

The examination of retrieved tissues revealed cartilage in different stages of degeneration, from normal to severely abnormal. Subchondral vBMD decreased with cartilage ICRS grade from 576 to 253 mg/cm³ confirming bone remodelling in all samples. Dynamic modulus of cartilage was mapped and showed a weak positive correlation to ICRS grades (3.34±0.93, 2.86±1.11, 4.64±4.37 and 5.56±1.83 N/mm for grade I, II, III and IV respectively), and a moderate positive correlation to subchondral vBMD (r=0.59), confirming the concurrence of cartilage biomechanics, degeneration and SCB remodelling.

**Acknowledgement**  
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**Reference:**  
09-SY-3  Optimization methodology for the material assignation of bioprinted scaffolds based on genetic algorithms and finite element analysis

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This paper presents an optimization methodology based on genetic algorithms, metamodels and design of experiments combined with Finite Elements Analysis (FEA) to optimize the material assignation of bioprinted scaffolds. The method optimizes the material assignation of the different bars of the strut structure to achieve the desired stiffness of the scaffold in different points of time. Therefore, the optimized design is the best combination of materials that minimizes the difference between the desired and achieved stiffness over time, so that the degradation process (4D) will be considered in the optimization process. The optimization algorithm is based on genetic algorithms and FEA simulations to evaluate the different designs proposed during the algorithm evolution. Moreover, the methodology integrates design of experiments and metamodels to estimate the simulation results, thus decreasing the number of FEA simulations and consequently reducing the optimization processing time. The mechanical properties of the materials (such as the elastic modulus) is defined in the different points of time according to experimental results of several bioinks and biomaterials. Simple configurations of struts is proposed to apply the methodology.

Acknowledgments

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09-SY-4  3D Bioprinting: opportunities and changes

Bin Zhang
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**11-SY-1**

**MEASURE: Public-Private Partnership Initiative in Japan for Research on Standardization and Validation of Methods for Tumorigenicity Assessment of Regenerative Medical Products**

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Cell therapy products (CTPs, alternatively called “regenerative medical products” in Japan), particularly those derived from embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs), show great therapeutic promise in the fields of regenerative medicine and immunotherapy. To realize their full clinical potential, there is a need for greater understanding of the mechanism of action and the safety of CTPs, for which there are several existing and emerging test methods. However, the characteristics of these test methods are not necessarily well understood by investigators. Furthermore, the regulatory landscape is not clearly defined for these emerging CTPs. Tumorigenicity is one of the biggest concerns for ESC/iPSC-derived CTPs, because undifferentiated pluripotent stem cells and cells transformed during the manufacturing process may form tumors in patients. However, there is no globally accepted consensus on the evaluation methods in vivo or in vitro.

The Forum for Innovative Regenerative Medicine (FIRM), a Japanese industry association for regenerative medicine, recently launched the Committee for Non-Clinical safety Evaluation of Pluripotent stem cell-derived producT (CoNCEPT) to provide science-based consensus for safety policy in the R&D of pluripotent stem cell-derived products. In collaboration with the Japan National Institute of Health Sciences (NIHS), CoNCEPT is conducting a public-private partnership initiative, called “MEASURE” (Multisite Evaluation Study on analytical methods for non-clinical safety assessment of human-derived REgenerative medical products), to validate the existing in vivo/ in vitro methods for detection of undifferentiated pluripotent stem cells and transformed cells. More specifically, MEASURE is currently validating six test methods, including in vivo tumorigenicity testing, and four in vitro methods, as well as biodistribution testing. In addition, MEASURE is communicating and sharing deliverables with the Health and Environmental Sciences Institute (HESI) to make its science-based consensus globally acceptable, which is critically important not only for product developers but also for regulatory authorities and patients.

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**11-SY-2**

**In vitro assays for detection of residual undifferentiated iPSCs**

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**Introduction**

The pluripotent stem cells (PSCs) are naturally tumorigenic, giving rise to teratoma, and therefore, to detect the residual undifferentiated PSCs in cell therapy products is important for minimizing the risk of PSCs-based tumorigenicity. We are developing and validating two in vitro assays to detect the residual PSCs at the multiple facilities. The first one is the highly efficient culture (HEC) assay and the other is PCR based assay. The HEC assay using the culture system, which favors the growth of PSCs, can directly detect a trace amount of PSCs. The droplet digital PCR (ddPCR) using LIN28 mRNA as a marker of undifferentiated cells, is also a powerful method to detect a trace amount of PSCs because of its sensitivity and easy handling. So far, we have conducted the preliminary studies to confirm the reproducibility of the reported method with the lowest detection limit for each assay.

**Assay protocol**

HEC assay: Cell suspensions of induced pluripotent stem cells (iPSCs) (clones; 201B7, 1231A3, ChiPSC18) were prepared using E8F or AK03N medium, Six (0.001%) and 30 (0.005%) iPSCs were spiked into 6 x 10⁵ hMSCs, and were plated onto cell culture matrix LN521 (for E8F) or iMatrix511 (for AK03N) coated 10 cm dishes. After about a week incubation, iPSC colonies were identified by alkaline phosphatase staining and the number of colonies was counted manually under microscopy. The study was conducted in 4 facilities and each experiment was carried out in duplicate and repeated for 3 times.

ddPCR assay: a cell mixture was prepared by spiking a cell line of iPSCs (ChiPSC18) into human retinal pigment epithelial cells (hRPE) from 0.0003% to 0.1%. ddPCRs were conducted after the on-site RNA purification at 4 facilities. The lower limit of detection (LLOD) of each assay was determined by using multiple lots of primary hRPE as backgrounds.

**Results & Discussion**

HEC assay: iPSCs colonies were detected under the 6 cells spiked condition for any iPSC clones and culture conditions at all the 4 facilities, therefore, it was concluded that the detection sensitivity (0.001%) was confirmed at the multiple facilities. Further investigation for the improvement of the assay including enhancement of the sensitivity and automatization of colony count process is now ongoing.

ddPCR assay: iPSCs were detected with high sensitivity at all the 4 facilities and the LLOD was expected to be 0.003% or less. We are currently fixing the protocol to initiate the main study in FY2018.
**11-SY-4**  
In vivo assays for detection of residual undifferentiated iPS cells

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[Introduction] Undifferentiated pluripotent stem cells (PSCs) contained in cell therapy products are concerned with tumorigenicity. Therefore, in order to estimate the tumorigenicity of cell therapy products derived from PSCs, it is important to establish a method for detecting the residual undifferentiated PSCs. However, appropriate in vivo test methods have not been established yet. In this presentation, we report that we attempted to establish an appropriate in vivo test method using severe immunodeficient NOD/Shi-scid, IL-2RγKO Jic (NOG) mice.

[Assay protocol] The multi-site study was conducted in five sites to preliminarily confirm test procedures. Human induced pluripotent stem cells (iPSCs) (Cellartiis® human iPSC line (ChiPSC18)), which were spiked into 10^6 Normal Human Dermal Fibroblasts and Corning® Matrigel® matrix at a dose volume of 100 µL, were injected to male NOG mice subcutaneously at dose levels of 10^2, 10^3, 10^4 or 10^5. The animals were observed for 20 weeks after injection. Parameters included clinical observations, body weights, food consumption, measurement of subcutaneous nodule size in the injection site, gross pathology and histopathology. In addition, the 50% Tumor Producing Dose (TPD50) value was calculated. The main in vivo study is ongoing at four sites according to the similar protocol to that of the preliminary study, except that dose levels of iPSCs are 10, 10^2, 10^3 or 10^4, and that both male and female NOG mice are used.

[Results & Discussion]  
In the preliminary study, formation of subcutaneous nodule in the injection site was observed in all groups. Increase of nodule size was observed from Week 2 at the highest dose group. All tumors in those nodules and/or the injection site were diagnosed "Teratoma". TPD50 values were calculated 10, 18, 18, 32, 120 or 680 in six studies, indicating the high sensitivity of the test method in detection of iPSCs. No abnormal finding was observed in clinical observation, body weights, food consumption. In the main study, the number of animals with a nodule at the site of inoculation is dose-dependent so far. Necropsy will be performed at the end of June 2018, and all results will be obtained by the end of November 2018.
11-SY-5  Evaluation of a method for biodistribution of cell therapy products

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[Introduction] Evaluating the nonclinical biodistribution (BD) of cell therapy products (CTPs) is important for predicting their efficacy and toxicity profiles. However, there is no standardized method for conducting nonclinical BD studies. Quantitative polymerase chain reaction (qPCR) methods such as real-time PCR are commonly used to evaluate the BD of CTPs. For standardization of the BD evaluation of CTPs, we characterized and validated a real-time PCR assay in a multisite study and identified important points to consider for ensuring the method's sensitivity, precision and accuracy.

[Assay protocol] We evaluated a real-time PCR assay detecting Alu sequences in human genomic DNA. Standard curve samples were selected from genomic DNA extracted from human-derived cells or synthesized DNA. The sensitivity, linearity and linear range of the assay were evaluated from standard curves. The inter- and intra-assay precision and accuracy of the assay results were determined by evaluating the quality control sets. The matrix effects of mouse genomic DNA extracted from blood and various tissues were also evaluated.

[Results & Discussion] The quantification range was mostly similar among the study sites. The amplification efficiency and linearity index (R²) calculated from the standard curves were 90% to 110% and >0.99, respectively. The coefficient of variation (precision index) and relative error (accuracy index) values were mostly within 50%. Mouse genomic DNA extracted from blood, lung and skin showed matrix effects. These results would be useful for evaluating the BD of CTPs. As a next step, we will validate and identify issues associated with DNA extraction methods and in vivo BD studies in experimental animals using a real-time PCR assay in a multisite study.

12-SY-1  Translational research of scaffold free tubular tissue modeled with Kenzan method Bio-3D Printer for cardiovascular surgery

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In advanced countries, with changing lifestyles and aging populations, there have been increasing incidences of arteriosclerotic vascular insufficiency affecting vital prognosis, such as diabetes, ischemic heart disease of maintenance dialysis patients, and peripheral artery diseases. There is high demand for transplantation surgery of blood vessels and artificial blood vessels, such as revascularization of the heart and lower limbs, and surgical construction of shunts for maintaining dialysis patients. Existing small-diameter artificial blood vessels with a diameter of less than 6 mm made of foreign substances such as PTFE (polytetrafluoroethylene) have many problems to be solved in terms of antithrombogenicity, anti-infectivity, biocompatibility, etc.

Tissue engineering and fabrication using 3D bioprinting is an emerging and rapidly expanding field with many exciting applications. We used a "Kenzan method Bio-3D Printer" to assemble multicellular spheroids to construct a three-dimensional structure predesigned on a computer system. This technique has enabled the production of tubular tissues only containing cells, without any exogenous scaffolds. Currently, we have been engaging in development that aims for the clinical application of cell-based artificial blood vessels made with this Bio-3D printer as arteriovenous shunt used for hemodialysis.

In this symposium, we will introduce the current situation, challenges, and future perspective of the clinical development of artificial blood vessels made of cells of small caliber using Kenzan method Bio-3D printers.
The Efficacy of a Scaffold-free Bio 3D Conduit on Peripheral Nerve Regeneration

Tomoki Aoyama
Human Health Sciences, Graduate School of Medicine, Kyoto University, Japan

Autologous nerve grafting is considered to be the gold standard treatment of nerve injuries. However, it has several disadvantages, including limited supply, mismatch of the caliber diameter, donor site morbidity, and neuroma formation. So several alternative treatments have been developed. For example, bridging the gap with tube-like biomaterials, combined with vascular and cell transplantation. However, the efficacy and viability of supportive cells remain to be low. Biomaterial nerve conduits are also associated with a risk of infection. To address these potential problems, we focused on the novel technology of Bio 3D printing, and created a biological, tissue-engineered, and scaffold-free conduit (Bio 3D conduit).

The efficacy of a Bio 3D conduit on peripheral nerve regeneration was evaluated in a rat sciatic nerve injury model. Bio 3D conduit was made from human dermal fibroblasts using a Bio 3D Printer Regenova® (Cyfuse Biomedical K.K.). The sciatic nerves of rats with immune deficiency was cut and bridged a 5 mm interstump gap using the Bio 3D conduits. The silicone tube was interposed in the same procedure as the control. Eight weeks after surgery, the efficacy was evaluated. Electrophysiological studies showed significantly higher muscle action potential in the Bio 3D conduit group than the silicone group. Morphometric studies showed that the Bio 3D conduit group exhibited a significantly greater myelinated axon number compared to the silicone group. No adverse event related with Bio 3D conduits was found. Now we are evaluating the efficacy of Bio 3D conduit for peripheral nerve deficit in canine ulnar nerve injury model. After the preclinical evaluation, we are planning the clinical trial for human peripheral nerve injury. We believe that the Bio 3D conduits are useful for not only the peripheral nerve injury, but several neurological disorders, including brachial plexus and cranial nerve, in which nerve grafts are needed to treat peripheral nerve defects.

Artificial trachea and esophagus using bio 3D printer

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Introduction; Most artificial organs with scaffolds have some limitations, such as inflexibility in design, lack of cytocompatibility, toxic effects, and post-transplant degradation. The present study sought to generate scaffold-free trachea and esophagus-like structures using several types of human cells by a bio-three-dimensional (3D) printing system. The rationale of the present study was to generate a scaffold-free trachea esophagela-like structure using bio-three-dimensional (bio-3D) printing technology and to assess circumferential replacement using the structure. Methods; human cartilage cells (NHACs), human fibroblasts (NHDFs), human umbilical vein endothelial cells (HUVECs), human esophageal smooth muscle cell (hESMCs) and human mesenchymal stem cells from bone marrow (MSCs) were used. By using bio-3D printer “Regenova®”, structures with different combinations of cells were made for trachea and esophagus. The structures were transplanted into F344 rats with immunosuppressant. Results; We transplanted the structures of ladder shape for trachea and simple structure for esophagus. The rats survived one month after surgery. The structures were matured in vivo with epithelial cells extended from native trachea and esophagus and with micro vessels and blood cell. Conclusions; we will show our research works of artificial organs made by bio-3D printer.
12-SY-4 Creation of functional 3D tissue for regenerative medicine and drug development

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Cyfuse Biomedical K.K., Tokyo, Japan

Cyfuse is a regenerative medicine start-up focused on creation of functional 3D tissues and organs. The proprietary "Kenzan method" skewers multiple cellular aggregates with fine needles until cells fuse entirely in a few days. Our Bio 3D Printer, "Regenova" automated this skewering process and is designed to help researchers try various cell populations and culture conditions to discover a protocol of manufacturing functional organs. The system was launched commercially in Japan and US. The development of clinical grade system is under way. The examples of cellular products include Blood Vessels, Peripheral Nerve Regeneration, and functional liver for drug discovery. Further applications with neural cells and cardiomyocytes are currently being explored by Japanese and US academia.

12-SY-5 3D Bioprinting of Regenerative Medicine and Tissue Engineered Products: U.S. Regulatory Perspective

Laura Ricles
United States Food and Drug Administration, USA

This talk will focus on the U.S. regulatory perspective of regenerative medicine and tissue engineered products, specifically focused on 3D printed medical products. While the field of bioprinting is relatively new, the FDA has been regulating 3D printed medical products for over 10 years. An overview of 3D printed products that are regulated by the FDA will be presented and scientific trends in 3D printed medical products and printing technologies, specifically focused on products containing biological materials and/or cells, will be discussed. An overview of bioprinting applications will be presented, along with technical considerations of printing biological material. Furthermore, FDA's perspective and recommendations for developing safe and effective bioprinted products will be presented.
Developing the systematic scale-out production of cells for cell therapies

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Cell based therapies fall into two broad classes, those derived from a patient’s own cells (autologous) and those derived from a donor’s cells (allogeneic). This distinction regards the product safety and efficacy model and the approaches to manufacturing, transportation and clinical delivery of the product. Manufacturing and supplying of autologous and partly allogeneic cell-based therapies are considered to have a number of specific and additional challenges to broaden its field of applications. Considering “size” of manufacturing and applying, a scale-out production is replicating the manufacturing line and is increasing the number of smaller volume batches, rather than scale-up production increasing manufacturing output by increasing the volume or number of cells processed for each batch. We are developing a small-sized platform of cellular production.

Mesenchymal stem cells (MSC) have great potential for wide range of clinical application. It is important to culture and provide large amount of MSC for injured tissue repair. Umezawa, et al reported that primary monolayer cultured cells of epiphyseal lesions in Polydactyly have great potential of cellular proliferation and cartilage differentiation (Nasu, et al. J Cell Physiol 2015). The epiphyseal cells could have some potencies to be cellular resources for cellular therapy. We are evaluating on the cellular growth using automated cell culture system with cell culture bags. The automated system could cultivate the primary cells in the closed system. Moreover, we analyzed differentiating ability of the primary cells into cartilage and tried to find biomarkers to evaluate a potential of cartilage differentiation. Preservation and transportation of the cells is one of the important process in regenerative medicine. The integrated system of temperature history on transporting cellular products was developed, and we confirmed successful tracing the products in a certain distance.

We show the model of scale-out production including an automatic cultivation, an evaluation on differential potency and a transportation stability of the primary cells. It would provide a useful example in the integration of newly developed technologies into the regenerative medicine.
**Global Business Development of Cell Sheet Engineering**

Setsuko Hashimoto
CellSeed Inc., Japan

CellSeed Inc. was founded in 2001 to commercialize an innovative and versatile technology in regenerative medicine: “Cell Sheet Engineering”.

CellSeed has been collaborating with Dr. Masato Sato, Tokai University to develop chondrocyte sheets for the treatment of osteoarthritis (OA). Clinical research of allogeneic chondrocyte cell sheet using cells from polydactyly patients is currently conducted at Tokai University Hospital. CellSeed Inc., Tokai university School of Medicine and DNA Chip Research Inc. collaborate to establish the quality assessment system of the cell sheets. By investigating the correlation between the properties such as morphology, physical properties and gene/protein expression of chondrocyte sheets and the results of the model animals transplanted with chondrocyte sheets, we want to identify the parameters that can predict the efficacy of the cell sheets.

CellSeed will take over the development activities to obtain a product approval. We are currently in the process of technology transfer from Tokai University to us which includes manufacturing of cell sheet, quality control analytical methods and clinical study protocols through a close communication with the regulatory agency, PMDA.

We have licensed out the chondrocyte sheet products to a Taiwanese company and currently we are conducting technology transfer from Japan to Taiwan. With our company’s mission to contribute to the global health care with the unique cell sheet engineering technology invented in Japan, we proactively seek for further business collaborations globally.

We recognize it very important to make a good plan to achieve a smooth technology transfer from academia and industry, as well as to the foreign countries. The latest status of our global business development will be shared.
Organ-on-a-Chip technology is a new paradigm in drug testing. The technology has as its aim to raise the physiological relevance of traditional cell culture by combining this with microfluidic techniques. Organs-on-a-Chip are 3D tissues that capture the complexity of in vivo tissues including 3D morphology, extracellular matrix embedment, multiple cell types, vascular structure and perfusion flow.

In this presentation I will introduce culture and interrogation of complex 3D tissues, such as liver, kidney, gut and brain tissue. The tissues are grown on the OrganoPlate® platform [1, 2, 3], that allow culturing of over 40 tissues in parallel. The tissues are embedded in an extracellular matrix gel and comprise both stromal tissue, bloodvessel structures as well as epithelial barriers with clear apical and basal sides. The platform can be interrogated using automated imaging equipment as well as all standard equipment including fluorescence-based assays, immunohistochemical staining, barrier integrity monitoring, transport studies, viability assays, qRT-PCR, ELISA's and many others. Last but not least, operation of the platform is thus straightforward that an end-user who is not necessarily an expert in the field of microfluidics is able to perform the study. In this presentation I will show examples of Organ-on-a-Chip tissues for use in drug safety testing and disease modelling.

**A perfused 3D artificial lymph node**

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Lymph nodes (LN) are meeting points for antigen-presenting cells and adaptive immune cells with the purpose of educating T and B cells towards antigen specificity against cancers and infectious diseases. The LNs consist of fibroblastic reticular cells (FRC) where dendritic cells (DC) can attach and present antigens to migrating naïve T cells and B cells. We focus on the T cells zone of the lymphnode [1]. We have utilized a miniaturised optically-accessible perfused bioreactor (MOAB) to mimic the microphysiological environment and functions found in LN T cell zone. The motivation for using a perfusion system is to ensure that the few antigen-specific cells (frequencies down to 1:10⁷) can interact in parallel, sequentially and dynamically with relatively few stationary cells. The MOAB has already demonstrated success on a functional human triculture system by infusing breast cancer cells to mimic metastatic colonization in bone [2]. The scaffold used is a custom-made polystyrene micro-grid fabricated by fused deposition modeling (3D Biotek) and is placed in a 10μl, 3D-printed perfusion chamber connected to a syringe pump. The construct has been evaluated by 4D confocal microscopy. We see formation of a conduit-like network of FRC on the surface of the scaffold. We found that DCs perfused through the system attach to the FRC in a manner similar to what is observed in vivo. The DCs show limited migration after attachment and tended to be concentrated in patches. We also see that introduced T cells interact with both the FRCs and the DCs, and a tendency of T cells to concentrate around DCs. The T cell motility is approximately the same as reported in vivo. We are analyzing the immunological and antigenic specificities in the system. This microphysiological immune system can be exploited towards R&D into immunotherapeutic approaches towards cancers and infectious diseases both in research and in clinics.


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Bioprinting is a young, highly interdisciplinary field. Its modern era commenced in 2000 with the work of Thomas Boland and his re-engineered Hewlett Packard desktop inkjet printer. Since then a number of other technologies have been developed utilizing extrusion, acoustic waves, laser assisted delivery and lately “liquid bioprinting”. The field has also matured from its purely academic roots into successful commercial ventures. Meanwhile the initial hype surrounding the field has substantially subsided even if not fully vanished. Long are the days when bioprinting has been hailed as a panacea for the chronic donor organ shortage, a method capable to replace dysfunctional tissue structures. At present it is mostly applied to the fabrication of sophisticated scaffold structures for tissue engineering and relatively small anatomically and physiologically relevant tissue constructs for drug development and testing and disease modeling. Overall, bioprinting has seen spectacular progress in the past two decades and a number of market analyses have predicted a bright future for the field. I will provide a hype-free overview of the technology, where it stands today, what it has specifically accomplished and what can be expected in the years to come.

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Investigation of human induced pluripotent stem cells differentiation in liver biochip

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Mature human hepatocytes are in high demand in several fields such as clinical research (e.g. cellular therapy) or pharmaceutical industries (e.g. drug screening). Unfortunately, it is challenging to obtain important quantities of cells with the required functional properties. Human induced pluripotent stem (hiPS) cell technology appears as a very promising candidate to solve this challenge but is still unable to achieve complete maturation of hepatocyte-like cells. Thus, significant adaptations of hiPS cells culture protocols are required. Here, we will present our investigation on the maturation of hepatocytes-like cells derived from human induced pluripotent stem cells in a microfluidic biochip using an alternative differentiation protocol. The differentiation of hiPS into mature hepatocyte-like cells is compared to Petri conventional protocol. To do so, we propose mRNA levels analysis, functional assay and immunostaining to control the level of maturation of our liver on chip tissue. The overall results in biochip illustrate a complex liver-like tissue formation with a greater degree of functionality than the one observed in Petri conditions.
15-SY-2 3D bioprinting of human hepatic tissue using human liver extracellular matrix as tissue-specific bioink

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Background: 3D cell printing-based liver tissue engineering has been hampered by the lack of biologically relevant bioinks able to mimic the 3D and biochemical structure of the liver. Decellularized extracellular matrix (ECM) represents an ideal source of bioink because it retains the structural and biochemical features of the native liver ECM.

Aim: The aim of this study was to evaluate the bioprintability of human liver ECM under physiological conditions, to assess the in vitro biocompatibility with human hepatic cells and to model liver fibrosis in vitro.

Methods: Decellularized human liver was lyophilized and solubilized using a protease-free protocol. Solubilized ECM was then mixed with cellulose-based bioink (CELLINK® Bioink) as support for bioprinting. Human hepatic cell lines (Hepg2 and LX2) were gently mixed with ECM bioink or CELLINK® bioink (employed as control) using a CELLMIXER® directly into a cartridge before bioprinting. Tissue printing was performed in a BIO X 3D printer under physiological conditions (low extrusion pressure at 10 kPa and room temperature). Bioprinted tissues were maintained in 3D culture up to 14 days and exposed to TGFβ1 for 6 days in order to promote an in vitro fibrogenic process. The resultant bioprinted liver tissue was analyzed by histology, viability assay and gene and protein expression.

Results: The bioprintability of human liver ECM bioink was confirmed by line extrusion and spiral tests showing the precise deposition of the material with the desired spatial and temporal control. Combination of human liver ECM bioink with liver cell lines resulted in increased cell survival and proliferation compared to cellulose-only bioink (p<0.001). Pro-fibrogenic genes and proteins including LOX (p<0.001) and pro-COL1 (p<0.001) were up-regulated in ECM liver bioink bioprinted LX2 cells after 6 days of TGFβ1 exposure. ECM bioink bioprinted HepG2 cells showed spontaneous formation of spheroids after 14 days in culture with up-regulation of albumin gene expression and protein secretion after 14 days compared to 7 days (p<0.001). The evaluation of both in vitro and in vivo biocompatibility with human primary hepatic cells is currently ongoing.

Conclusion: This is the first report describing the bioprinting of human hepatic tissue using human liver ECM as bioink. This is a key advance in the development of cell-instructive bioinks for the study of liver disease and for the development of 3D hepatic tissue for transplantation.
Hair regenerative medicine has been increasingly expected as a new approach for treatment of hair loss caused by aging, diseases, injury and medical treatments. Hair follicle morphogenesis is triggered by reciprocal interactions between hair follicle germ (HFG) epithelial and mesenchymal layers. Recent studies have shown that transplantation of a compartmentalized HFG, which was fabricated by integrating two respective 3D aggregates of mesenchymal and epithelial cells in vitro, led regeneration of hair follicles. This approach shows promising results for hair regenerative medicine, but it remains challenging to prepare a large number of HFGs particularly considering that hundreds of thousands of HFGs are necessary for a single patient. In this study, we propose a one-step preparation for large-scale preparation of HFGs with a uniform diameter and spacing via self-organization of cells. We suspended mouse epithelial and mouse/human mesenchymal cells in a culture medium and seeded in a custom-designed array plate, which had cylindrical wells of 1 mm diameter at a density of 100 wells/cm². They initially formed a randomly-distributed single cell aggregate but then spatially separated each other and exhibited typical morphological features of a HFG during three days of culture. Alkaline phosphatase activity, an indicator of the hair follicle inductivity of dermal papilla cells, was expressed in the cell aggregates. Interestingly, we demonstrated that oxygen supply through the bottom of the array plate made of oxygen permeable silicone labor was crucial for the spontaneous formation of HFGs and subsequent hair shaft generation. Unlike the previous approach, this spontaneous HFG formation in vitro facilitated the preparation of a large number of cell aggregates (~5000 aggregates/plate). Further, the HFGs transplanted intracutaneously formed hair follicles and spatially aligned hairs generated at the transplanted site after 18 days of transplantation. The regenerated hair follicles also showed the hair cycle through the rearrangement of follicular stem cells. This simple HFG preparation approach may provide a promising strategy for advancing hair regenerative medicine.

References
1. Tatsuto K. Spontaneous hair follicle germ (HFG) formation in vitro, enabling the large-scale production of HFGs for regenerative medicine. Biomaterials. 154, 291-300, 2018
Chemically Diverse, Degradable Polymeric Carriers for Nebulized Delivery of Messenger RNA to the Lung

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Nucleic acid based therapeutics have the potential to treat any disease with a protein target, however development of biomaterials to facilitate effective delivery remains a critical hurdle for clinical translation. The therapeutic utility of in vitro transcribed (IVT) mRNA is of interest due to lack of insertional mutagenesis, transient protein expression and ability to transfect non-dividing cells. Polyplex nanoparticles are based on the self-assembly of polyethylene glycol (PEG)-polyamino acid block copolymer, possessing a PEG outer layer and mRNA-containing core. This system provides excellent in vivo stability of mRNA under physiological conditions.

Cartilage degeneration diseases such as osteoarthritis (OA) is a promising target of the mRNA-based therapy. To manipulate gene transcription in cells at the cellular or molecular level, direct administration of mRNA into cells can be conducted without safety concerns. We chose a cartilage anabolic transcription factor, Runx1, to treat a mouse OA model.

Following intraarticular injection of mRNA encoding reporter protein into the mouse knee joint, widely distributed protein expression was observed in the chondrocytes in the cartilage matrix, particularly in the superficial and middle zones of the articular cartilage. By administering Runx1 mRNA into an OA joint induced by joint instability, OA progression was significantly suppressed compared to the non-treatment control. Expressions of cartilage-anabolic markers and proliferation were upregulated in chondrocytes of Runx1-injected knees. In vivo mRNA administration has the potential to be a powerful tool for introducing signaling molecules with minimal risk, and tissue regeneration may be achieved without the requirement of cell transplantation.
2D Nanoclays for Regenerative Medicine, Therapeutic Delivery and Bioprinting

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Two-dimensional (2D) nanoclays have gained unprecedented attention due to their unique atomically thin, layered, and well-defined structure. As the dimensions of 2D nanoclays are only a few nanometers thick, they interact with biological moieties in a unique way and have raised exciting questions about their interactions with cellular components. We have used next-generation sequencing technology (RNA-seq) to understand the effect of a synthetic nanoclay (nanosilicates) on human stem cells at the whole transcriptome level. Our results identify more than 4,000 genes that are significantly affected, and several biophysical and biochemical pathways that are triggered by nanosilicates treatment. This approach in understanding nanosilicates-cell interactions, illustrates how change in transcriptomic profile can predict downstream effects following nanomaterial treatment. Based on our transcriptomic data, we will demonstrate the application of nanosilicates towards bone and cartilage tissue engineering. The high surface area and charged characteristics of nanoclay is leveraged for sustained and prolonged delivery of pro-angiogenic molecules to stimulate angiogenesis. We have also evaluated the application of nanosilicates in the emerging field of 3D bioprinting to print complex organ and tissue.

Highly Branched Poly(b-Amino Ester)s as New Gene Delivery Vectors

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Gene therapy has demonstrated to be one of the most promising therapeutics. However, development of safe and efficient gene delivery vectors is still a challenge. Over the past decade, linear poly(beta-amino ester)s (LPAEs) have shown their promising for gene delivery. Nevertheless, their linear structure significantly limits the further enhancement of their gene transfection performance. Alternatively, diverse branched polymers have proven their potential as gene delivery vectors in comparison with their linear counterparts, due to the threedimensional (3D) architecture with multiple end groups. However, branched poly(beta-amino esters) have rarely been developed for gene delivery. Here, highly branched poly (b-amino ester)s (HPAEs) are synthesized as a novel type of gene vector via “A2 + B3+ C2” Michael addition from commercially available low-cost monomers. NMR and GPC results showed that the composition and structure of HPAEs can be adjusted easily by simply varying the feed ratio of different monomers. HPAEs can compact DNA to formulate nanosized polyplexes. In a variety of cell types, HPAEs were demonstrated to mediate very high gene transfection efficiency and low cytotoxicity. The efficiency was even much higher than the commercial gene transfection reagents, such as PEI, SuperFect, and Lipofectamine. In both Recessive Dystrophic Epidermolysis Bullosa (RDEB) knockout and graft mouse models, the HPAEs can deliver functional DNA to mediate high expression of functional proteins. And no obvious inflammation was observed, these results demonstrated that HPAEs could be used a highly safe and efficient gene delivery vectors for gene therapy.
**17-SY-2** Nanoclays for the delivery of regenerative microenvironments  
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Laponite nanoparticles have attracted attention in the tissue engineering field for their protein interactions, gel-forming properties and, more recently, osteogenic bioactivity. We have explored the potential to harness the gel-forming properties of Laponite to generate injectable bioactive microenvironments for osteogenesis. Injectable Laponite formulations spontaneously form stable transparent gels of 1-10 kPa stiffness upon contact with physiological fluids due to reorganization of nanoparticle interactions. Such gels can support cell adhesion, host the osteogenic differentiation of human bone marrow stromal cells and enhance osteoblast directed apatite formation. Furthermore Laponite gels demonstrate a striking ability to sequester biological molecules due to the large and highly charged specific surface area of the nano-sized particles. For example, self-assembling clay gels were observed to rapidly adsorb VEGF165 to promote an angiogenic response in local endothelial populations in vitro and in vivo and clay nanoparticle films and gels significantly enhance localised BMP2 osteoinduction in vitro and at ectopic sites in vivo. Such approaches provide exciting possibilities for the generation of developmentally potent microenvironments able to direct the growth and differentiation of stem/progenitor cell populations for tissue regeneration.

**17-SY-3** Bisphosphonate-functionalised hyaluronic hydrogel by addition of Laponite clay nanoparticles enhances a sustained release of bone morphogenetic protein-2  
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Hydrogels are widely used as carriers for bioactive molecules, such as drugs and growth factors. Bone morphogenetic protein (BMP)-2 has been extensively shown to be pivotal in the bone regeneration process over more than two decades although clinical efficacy has typically resulted in application of supraphysiological doses. To maximise the effect of BMP-2 on bone formation, a sustained and controlled release of the protein from a hydrogel was accomplished by attachment of various chemical functionalities to the hydrogel matrix. A hyaluronan (HA) hydrogel with covalently linked bisphosphonate (BP) ligands (HABP) shows a sustained release of BMP-2 in vitro [1]. Recently we have shown the efficacy of a nanoclay hydrogel, Laponite (LAP) as a drug carrier, based on the ability of the clay nanoparticle to enhance BMP-2 localisation in the gel, resulted in improved bone formation [2,3]. The objective of this study was to evaluate the effect of HABP gel with LAP (HABP-LAP) on the release profile of BMP-2 for bone regeneration. HABP, LAP, and HABP-LAP gels incorporating lysozyme, as a protein model of BMP-2 were prepared and incubated in phosphate buffered saline for 7 days. At each time point (1, 2, 6 hours, and 1, 3 and 7 days), supernatant was collected and the concentration of lysozyme in the supernatant as well as that of HABP and LAP measured. For in vivo release evaluation, HABP, LAP, and HABP-LAP gels incorporating Cy7 labelled lysozyme were implanted in the subcutis of MF1 wild mice, and imaged using an in vivo imaging system to detect the intensity of the lysozyme at day 0, 3, 7, 14, 21, and 28. In vitro release studies demonstrated 16% and 24% lysozyme release from LAP and HABP-LAP gels respectively after 24 hours, while HABP-LAP gel released less than 2% lysozyme over the same timeframe. In vivo release studies confirmed negligible lysozyme was retained in HABP gel after 24 hours of implantation. However, Cy7 labelled lysozyme incorporated into LAP and HABP-LAP gels showed a strong dye intensity even at day 28. In summary, the present findings demonstrate the development of a unique HABP – LAP hydrogel that resulted in the sustained release of protein which is crucial for bone tissue regeneration.
Extensive bone loss due to trauma or disease leads to impaired healing. Bone defects above a critical size, typically 3cm or larger in humans, exceed the natural regenerative capacity of bone and often result in non-union or delayed healing even with surgical intervention. Bone autografts and allografts, and some synthetic grafts can augment healing in small defects, but still have major drawbacks that limit their effectiveness in the treatment of larger bone defects. A number of bone substitutes in development have potential to achieve improved outcomes and are undergoing preclinical testing, but few studies have specifically investigated the in vivo tissue-material interactions that provide an important indicator to the long-term safety and efficacy of the implant.

We previously developed a bioactive ceramic named baghdadite, by doping zirconium into the crystal structure of calcium silicate. Recently, we evaluated the performance of baghdadite scaffolds implanted as tubular grafts in critical-sized mid-diaphyseal segmental defects in sheep tibia over 26 weeks. Baghdadite scaffolds achieved significant bridging (average 80%) of the 3cm defect in all samples, with substantial volume of bone infiltration and evidence of bone remodelling within the implant, in the absence of supplementation with cells or growth factors. In this study, we proceeded to evaluate specifically the in vivo tissue-material interactions and scaffold degradation at the bone-implant interface, to understand the mechanisms by which baghdadite scaffolds can induce bone formation in a large animal model in the absence of added cells or growth factors. Results obtained using focused ion beam scanning electron microscopy, multiphoton microscopy and histology indicated that the baghdadite scaffolds were highly bioactive, causing extensive bone formation that directly abutted the implant surfaces with no evidence of an inflammatory response or fibrous capsule formation. The scaffold underwent slow in vivo degradation, which influenced bone remodelling around and within the implant, and caused gradual maturation of the newly formed bone. These findings have important implications for predicting the long-term effects of baghdadite ceramics in promoting defect healing, and support the translation of baghdadite scaffolds as a new generation of bone graft substitutes with improved properties for the repair of large bone defects.
**18-SY-3 Investigating Cancer Invasiveness and Vasculature with a Biomimetic 3D Model and Patient-Specific Cancer Associated Fibroblasts**

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3D models of cancer ("tumouroids") are increasingly used as a platform to demonstrate crucial aspects of tumour microenvironments. In this study, we utilised a tissue-engineered colorectal cancer model, which recapitulates the solid tumour surroundings including matrix composition, cell spatial configuration and a primitive vascular network to study invasion, matrix remodelling and vasculogenesis. HT29 and HCT116 immortalised cell lines and CCD841 CoN normal cells were used to generate artificial cancer masses (ACM) utilising monomeric type 1 collagen (FirstLink, UK) and plastic compression with RAFT™ absorbers (Lonza, Switzerland). These were nested into a stromal compartment, acellular or containing either HDFs or patient-derived cancer associated fibroblast (CAF) samples and HUVECs additionally to laminin (Corning, UK). The resulting day 21 tumouroids were analysed at temporal time points. The "invasion" of cancer cells into the stromal compartment within the model was distinctive when compared to the non-cancerous colon normal cells. Phenotypically, the formation of spheroids within the original cancer mass and the outgrowth as cell clusters is a cancer exclusive observation, which was supported by the statistically significant upregulation of invasive markers MACC1, MMP7 and HSPE within the cancer type models. CCD841 CoN colon normal cells migrated as single cells and did not form budding cell clusters detaching from the original mass. The addition of CAFs into the stromal compartment increased invasion significantly (>10 fold) but decreased or diminished the development of endothelial networks within the tumouroid models. CAF samples were therefore added to a day 21 established endothelial network and a disruption of the networks could be observed. This was further investigated to determine molecular pathways responsible. The results indicate that the tissue-engineered colorectal tumouroid model can be utilised as a novel platform to study cancer invasion patterns and vasculogenesis by comparing to healthy colon cells and looking at specific invasive angiogenic disease markers. Making the model more biomimetic by adding patient-specific CAF samples, enables investigation of molecular pathways involved in changes of invasiveness and vasculogenesis. This indicates that CAFs play an essential role not only in invasiveness but also the remodelling of local blood vessels. Acknowledgement: EPSRC.

¹Magdeldin T. Scientific Reports. (7) 44045, 2017.
Cardiac extracellular matrix (ECM) is a tissue-specific, dynamic network providing unique structural support to cardiac tissue development, homeostasis and functionality.

The remodelling of ECM structure during the progression of cardiac diseases determines dramatic changes in its chemical composition, nanotopography and mechanophysical properties. The impact of ECM derangement on cardiac cells is the objective of intense investigation aimed at preventing the negative effects (e.g. fibrosis with cardiomyocyte trapping and loss) and restore organ functionality.

In this study, we adopted an original integrated strategy based on human cardiac tissue decellularization, atomic force microscopy (AFM), two-photon microscopy and high-resolution 3D image analysis. With these approaches, we ultimately compared decellularized ECM (dECM) structures and mechanical properties between healthy and diseased patients.

The analysis of cardiac dECM from numerous patients suffering from heart failure and from mouse infarcted tissue led us to identify common trends in the rearrangement of the tissue-specific ECM.

In particular, the combined AFM and two-photon microscopy analysis demonstrated that the ECM obtained from failing hearts encounters a consistent reduction in compliance, due to a loss in its complexity and porosity, determined by de novo collagen fibers deposition and rearrangement.

Given the role of cardiac fibroblasts in orchestrating ECM homeostasis and remodelling, we also investigated how the activation of such cells in pathological conditions can affect cardiomyocyte mechanosensing and function.

Finally, our findings on recellularization of physiological and pathological myocardial decellularized ECM suggest divergent housing performance, thus bringing further hints to unveil a possible window of action for heart cell therapies.
Emerging Techniques for Soft Tissue X-ray Micro-CT Imaging

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X-ray micro computed tomography (XµCT) enables non-destructive 3D imaging, revealing intricate features throughout volumes of up to several centimetres, at micro-scale resolution. The technique involves the acquisition of multiple X-ray radiographs whilst a sample is rotated, followed by the computational reconstruction of the imaged volume. XµCT has become well established for bone imaging due to the high X-ray attenuation of bone relative to the surrounding tissue, providing good imaging contrast. The technique was typically not suited to soft tissues due to low X-ray attenuation and limited attainable contrast; however, recent advances in imaging technology and methodology have overcome this limitation, and are opening new avenues for soft tissue imaging.

There is increasing interest in XµCT for soft tissue research, owing to synergistic leaps in capability across several areas. Improvements in source and detector technology for example have enabled nano-scale resolution, with an achievable voxel size down to 50 nm, permitting observation of sub-cellular detail. It is now also possible to XµCT image soft tissue without the need for applying contrast-enhancing stains, which have been prone to causing imaging artefacts. This is enabled by phase contrast imaging which takes advantage of the diffraction of X-rays at feature edges. Previously only available at synchrotron sources, phase contrast is now attainable using lab sources, making the technique more accessible. Developments in in situ mechanical testing equipment alongside methods to reduce acquisition times is permitting time lapse imaging, which can be used, for example, to track cell movement, or to observe deformation and stresses arising whilst an implantable undergoes mechanical force application in a wet environment. Multi-scale and multi-modal imaging are also enabling deeper interrogation of samples with regards to multi-scale architecture, chemical composition, and histological data. Further data can be obtained from the recently demonstrated elemental mapping, which identifies chemical composition directly from XµCT data.

We shall discuss the emerging techniques, which enable a wealth of qualitative and quantitative data to be obtained using XµCT for soft tissue and tissue engineering applications. Case studies will be presented to demonstrate the potential of XµCT; investigating the rotator cuff bone-tendon junction, brain vasculature, the carotid artery and intervertebral discs.

Novel CT imaging techniques for soft tissues

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This presentation will cover a case study of a novel CT imaging technology that has been developed to image dynamic tendon loading in an aqueous environment. In addition to the tendon imaging, the loading effects on the geometry of a barbed suture (inserted into the tendon) will also be discussed. Correlation of this data with finite element analysis to look at local stresses will also be presented. Other tendon imaging techniques will also be presented such as fascicle tracking in porcine tendon and ligaments and imaging of degradation monitoring of electrospun polycaprolactone over a 12month period (after insertion into a murine tendon model). As such, this presentation will give examples of the latest advances in CT imaging that can be of use for the tissue engineering and regenerative medicine field.
19-SY-3

Micro-computed tomography as a tool to improve in vitro bone tissue engineering cultures

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The future development of materials for bone regeneration depends on further translation of basic discoveries into practical applications. While it is already well understood what happens after an initial contact of cells with the scaffold material, little is known about the interactions between cells and their environment once the seeded cells start to produce tissue-specific extracellular matrix (ECM). Tissue development is a dynamic process, and the deposition of new ECM will affect the environment of the cells. The space available for cells will change and nutrient diffusion will decrease. In addition, externally applied loads - for example shear stresses applied through flow perfusion bioreactors - will also be altered. Cells are capable of sensing their changed environment, and in turn will react to these changes, for example by changing their activity or the production of ECM. Given the fact that most scaffolds geometries are highly irregular, destructive analysis methods such as histology are limited in taking the spatio-temporal development into account and can provide averaged data only on how the cellular environment changes over time. Longitudinal monitoring techniques have been proposed to support end-point read-outs by enabling following the development of an individual sample over time. One such method is micro-computed tomography, which can be used to qualitatively and quantitatively describe the formation of mineralized ECM by bone-forming cells within scaffold pores. Following the formation of mineralized ECM in response to scaffold morphology and/or mechanical loading in bioreactors has previously been investigated. At the same time, computational simulation strategies allow estimating the actual loading of cells based on the already produced mineralized ECM at any time-point measured. Combining these two methods will allow adjusting the mechanical load that is altered because of ECM production back to a level that is optimal for tissue formation. This powerful combination of methodologies will not only allow estimating the initial reaction of cells to a scaffold, but also how scaffold designs affect tissue formation over time. Being non-destructive, it further has the potential to be adapted to in vivo applications to predict how scaffolds will perform after implantation.

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19-SY-4

Micro-Tomography as a Quality Control Tool for Regenerative Medicine

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Micro-tomography (MicroCT) has been widely used in regenerative medicine and tissue engineering to characterise biomaterials’ micro-architecture features (pore size, porosity and tortuosity), as well as to obtain data to inform mathematical models of bioreactors, cell seeding efficiency, proliferation and differentiation.1

Recently, this technique has evolved from the use of X-rays to visualise dense materials in 3D, to sophisticated multi-scale imaging of constructs in 4D to target biological components at high resolution. One specific technique is the indirect imaging of mammalian (stem) cells by tagging them with (CD105) Superparamagnetic Iron Oxide particles, allowing researchers to determine cells’ location, which otherwise could not be detected with conventional microCT. Other techniques have also increased the imaging capabilities of microCT in the biological boundaries: contrast agents mark specific tissue2, and phase-contrast mode concentrates on the contrast generated by the material absorption, rather than by the x-ray attenuation3.

MicroCT of de-cellularised blood vessels, prior and post implantation, were used for material and cell characterisation, which combined with their mechanical analysis, offers accurate information on the remodeling process. These methods, along with advanced image analysis, can provide a quality control platform for regenerative medicine grafts. Quantitative analysis of high-resolution data on cell proliferation, added to information on extracellular matrix deposition and/or change on mechanical properties, can be used to predict the clinical outcome of individual constructs.

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References:

Understanding the biological and immunological characteristics of human corneal endothelial cells (CECs) is an essential key to the establishment of new strategies for treating corneal endothelial dysfunction such as Fuchs endothelial corneal dystrophy, intraocular-surgery-related bulous keratopathy, and graft failure, etc. Along this important pathway, our research group has been developing a ‘cell-injection therapy’ that involves the injection of cultured human CECs into the anterior chamber, which we expect will open the door to completely new treatment strategies. Towards this end, we have successfully induced non-proliferative CECs obtained from donor corneas to proliferate, without the induction of cell-state transition. Our findings in clinical research cases and phase 2 clinical trial have shown this approach to be very promising. However, in order to apply this novel approach to actual clinical practice, several key safety issues must first be addressed, and also assured, from the aspect of regulatory science. It is our hope that our novel procedure will receive official governmental approval based upon the aspects of the accumulated safety and efficacy data arising from a series of clinical trials.
Design criteria for biodegradable synthetic cornea

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Like other organs, cornea transplantation in most countries suffers from a severe shortfall of donated tissues. Globally, 12.7 million patients await transplantation, with only one out of 70 grafted. Even if 1-2 year graft survival is high (85-90%) for low-risk grafts, >50% grafts are rejected in corneas with severe immunopathology (inflamed or vascularised) despite immunosuppressive therapy. Corneal blindness is treated by partial or full-thickness donor allografting. Due to donor shortage, in many countries, high-risk corneas are untreated as donor corneas are prioritised for patients with highest chances for recovery.

Our aim is to develop a cell-free, collagen type I based biodegradable synthetic cornea that will allow the ingrowth of cells from the host tissue and remodelling of the implant resulting in the regeneration of a neo-cornea in vivo.

Currently, known synthetic corneas, which are capable of promoting regeneration are, however, still far from fulfilling the criteria of facile surgical implantation similar to donor transplantation and cannot be produced by cost-effective methods, thereby limiting their widespread use. Hydrogels, which could be applied for corneal regeneration, are not tough enough to withstand suturing using 10-0 nylon sutures. Hence, overlying sutures, where only the host bed was sutured, or glueing using fibrin adhesives, or a combination of both are used. However, these strategies were demonstrated to delay the epithelial coverage of the implant resulting in corneal haze formation.

The way we address the development of biodegradable, transparent and robust material is an amalgamation of the knowledge of tough synthetic hydrogels with biocompatible ECM derived soft hydrogels in close collaboration with clinical users. To evaluate if at all it is possible to fabricate a tough transparent hydrogel containing collagen, we have recently successfully chemically functionalized collagen molecules to act as reinforcing filler in a synthetic hydrogel matrix. We have in an earlier study shown that this chemically modified collagen retains its structural and biochemical properties when compared with unmodified collagen. By the incorporation of strong hydrogen bonding and balancing, solubility improvements in toughness may be achieved. This demonstration serves as proof-of-concept that designing for suturability is possible, and provides for means to develop for cost-effective processing.
Cell-free scaffolds promoting endogenous corneal regeneration

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Corneal blindness affects >23 million people globally. Worldwide, the figures are dire with 1 in 70 patients transplanted and 12.7 million waiting for transplantation. For patients with severe pathologies, it is even more problematic as they have a high risk of rejecting the grafts. The immune response is against the donor cells. Our team has now shown that biomimetic materials made from recombinant human collagen stimulate the patients’ own cells to regrow a neo-cornea using the cell-free implants as scaffolds. By incorporating an inflammation suppressing phosphorylcholine, MPC, into the scaffolds, we have regenerated corneal tissue and nerves even in patients at high risk for graft rejection. Our goal is to reach millions of patients so we developed a short, fully synthetic collagen-like-peptide (CLP) that is conjugated to inert polyethylene glycol (PEG) as a most cost-effective alternative to collagen-based implants. We show in pre-clinical studies in mini-pigs that CLP-PEG implants were functionally equivalent to the recombinant collagen-MPC ones. We also show that by modifying the matrix delivery, we are able to fabricate a "Liquid Cornea" that allows it to potentially patch damaged or perforated corneas instead of patching with cyanoacrylate glue which is toxic and neccessitates corneal transplantation.

Natural-based Approaches to Corneal Tissue Engineering: Fibrin-Agarose Scaffolds and Decellularized Xenografts

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Corneal diseases are one of the most important causes of blindness throughout the world. Corneal transplant is the main treatment but the supply of human donor corneas (HDC) is limited compared to the demand. Different artificial corneal substitutes have been generated. However, they lack biocompatibility or mechanical properties. Here, we evaluated the potential of using different natural-based materials for human corneal transplants such as fibrin-agarose scaffolds (FAS) or decellularized porcine corneas (DPC), including gamma irradiation (GI) as sterilization method.

A FAS was used to encapsulate corneal stromal cells. This tissue engineered stroma was then seeded with corneal epithelial progenitor cells to form a two-layered corneal construct. These implants are being tested in patients with corneal epithelial and stromal defects in a Phase I-IA randomized, controlled, open label, multicentre clinical trial. After 24 months of follow-up, all the patients had a complete healing of the cornea, showing a good integration of the corneal construct with no signs of rejection or infection. No serious adverse events related to the treatment were found. However, it is necessary to wait until the end of the clinical trial to obtain a robust evidence of the safety and efficacy of this new advanced therapy.

Independently, porcine corneas were decellularized using 0.1% sodium dodecyl sulfate (SDS) in water. DPC were then sterilized with 25 kGy of GI. Histological evaluation was done to confirm the decellularization efficiency and TEM to evaluate the ultrastructure of DPC. Mechanical and optical characterization was performed comparing DPC, gamma irradiated DPCs (G-DPC), native porcine corneas (NPC) and HDC. Moreover, water content evaluation, collagenase study and glucose permeability were also assessed. Recellularization of DPC and G-DPC was carried out using human corneal epithelial cells, fibroblasts and endothelial cells, independently, showing no cytotoxic effects. Afterwards, a phenotypic study revealed that HCEC expressed cytokeratin 3+12 and mucin16 on both kinds of tissues, similar to HDC. Our results confirm that SDS in water is enough to remove cells from NPC and can be sterilized with GI, without affecting the transparency of the tissue. GI changes mechanical properties of DPC mimicking those of HDC. Cell study showed that these acellular tissues can be repopulated. However, proper in vivo studies have to be performed to confirm these results.
21-SY-6  Limbal Epithelial Stem Cells from bench to bedside: Successes, Hurdles and Future in Cultivated Limbal Epithelial Stem Cell

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The corneal epithelium is constantly being rejuvenated by limbal epithelial stem cells (LESC). The corneoscleral limbus is a 2mm wide junction between the cornea and limbus that houses the LESC. Damage to these LESC results in Limbal Stem Cells Deficiency (LSCD), a condition that leads to corneal blindness as the cornea becomes opaque and vascularized. Ever since its introduction in 1997, Cultivated Limbal Epithelial Transplantation (CLET) has been proven to be an effective treatment in LSCD with an average reported success rate of 70%. In 2007, the laboratory of ophthalmology of the University of Antwerp and Antwerp University Hospital (Belgium) initiated preclinical studies with regards to limbal stem cell cultivation which resulted in a phase I/II clinical trial that showed a 67% success rate. Based on this success, our center coordinates an ongoing phase II multicenter study in Belgium since 2014. Bringing translational medicine into clinics can be challenging by running GMP-accredited laboratories, training qualified personnel, managing ATMP (Advanced Therapy Medicinal Product) logistics and hiring external QC-personnel are all expensive and labour-intensive. Furthermore, solid integration of (inter)national regulations, Good Clinical Practice (GCP) guidelines and lean-manufacturing principles is essential to successfully run an ATMP-based clinical trial. To hopefully bring more stem cell-based treatment into clinics, our research department keeps investigating emerging treatment options that tackle ocular disease. We are currently investigating the application of collagen hydrogels as a replacement to Human Amniotic Membrane as a carrier material for conjunctival and corneal epithelial stem cell transplantation. Another promising approach is the application of 3D printing in manufacturing stem cell loaded collagen constructs as a therapy for stromal corneal blindness. In vitro testing has shown the feasibility of 3D printing 200µm thick corneal constructs that show good biocompatibility with LESC and corneal Mesenchymal Stem Cells. However, 3D printed corneal constructs remain to be validated in vivo. Finally, our close collaboration with the university’s department of Hematology and Oncology, has led to the founding of ‘Antwerp Innovative Cell Solutions’ (AniCells), a spin-off institution that will act as a GMP-accredited accelerator to bring promising stem cell therapies into clinics.

22-SY-1  The KenZan method: from bench to bedside

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Tissue engineering and regenerative medicine have made major scientific, medical and technological progress in the past decade. Among them, Biofabrication is growing in the fields of tissue engineering and regenerative medicine aiming for clinical application alternatives to organ transplant or research use.

In this symposium, various Biofabrication technologies will be presented. And in this keynote talk, our original developed scaffold-free “KenZan method” and current status about clinical application mostly about blood vessels will be presented.
Additive manufacturing technologies, often referred to as 3D printing, open exciting perspectives for tissue engineering and regenerative medicine (TERM) applications. They range from producing patient-specific 3D scaffolds, which are subsequently seeded with cells, to direct processing of cell-containing materials in accordance to a computer assisted design (CAD) models, the so-called bioprinting. Among the myriad of possible approaches multiphoton lithography (MPL) stands out as a technology enabling true 3D structuring at subcellular resolution. In this contribution the recent progress of MPL development for TERM applications, as well as the challenges this technology is facing on the way to becoming a widely accepted tool, will be discussed.

References:
22-SY-4 Sorting Extracellular vesicles and rare cells from blood

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Circulating tumor-derived extracellular vesicles (EVs) has emerged as a promising source for identifying cancer biomarkers in cancer early detection. However, the clinical utility of EVs has thus far been limited by the fact that most EV isolation methods are tedious and non-standardized and require bulky instrumentation such as ultracentrifugation (UC). Here, we report a size-based EV isolation tool called ExoTIC (exosome total isolation chip), which is simple, easy-to-use, modular and facilitates high-yield and high-purity EV isolation from biofluids. We demonstrate that ExoTIC is a modular platform that can sort a heterogeneous population of cancer cell line EVs based on size. Further, we utilize ExoTIC to isolate EVs from cancer patient clinical samples, including plasma, urine and lavage, demonstrating the device’s broad applicability to not only cancers, but other diseases as well. Finally, the ability of ExoTIC to efficiently isolate EVs from small sample volumes opens up avenues for preclinical studies in small animal tumor models and for point-of-care EV-based clinical testing from finger prick quantities (10–100 μL) of blood.

23-SY-1 Clinical Outcomes of Knee Osteoarthritis Treated With an Autologous Protein Solution Injection

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Transplantation of synovial mesenchymal stem cells onto repaired meniscus with degenerative tear

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PURPOSE: The meniscus is a crescent-shaped fibrocartilaginous tissue in the knee and has a role in load distribution. For degenerative meniscus tears, a method of enhancing healing potential may overcome limitations of the current treatments which include arthroscopic partial meniscectomy. Mesenchymal stem cells (MSCs) derived from synovium are attractive for meniscus repair because synovial MSCs have a high proliferative and chondrogenic potential. We evaluated clinical outcomes of synovial MSC transplantation onto repaired menisci with degenerative tears at 2 years and assessed adverse events.

METHODS: We enrolled patients with clinical symptoms that included a catching sensation, crepitation sensation, and/or a feeling of instability in addition to pain caused by complex degenerative meniscus tears to which a meniscectomy was generally applied. Two weeks after the repair of the torn meniscus, autologous synovial MSCs were transplanted onto the menisci of 5 patients whose ages ranged from 34 to 57 years old. Lysholm knee score, Knee Injury and Osteoarthritis Outcome Scale (KOOS) and Numerical Rating Scale (NRS) were evaluated at 4w, 12w, 24w, 1y and 2y. MRI examinations were performed at 12w, 24w, 1y and 2y, and 3D reconstructed meniscus images were analyzed for height of the meniscus. Adverse events were monitored continually at each visit.

RESULTS: The total Lysholm knee score, KOOS for “pain”, “daily living”, “sports activities”, and numerical rating scale significantly increased at 2 years (p<0.05, n=5). Sequential 3D MRIs showed a disappearing meniscus tear with time and the height of the meniscus was significantly increased at 2 years (p<0.05, n=5). Though three adverse events were recorded; increase of CRP, joint effusion, and localized warmth of the knee, these could have been due to the meniscus repair surgery.

CONCLUSIONS: Transplantation of synovial MSCs onto repaired menisci with degenerative tears was effective in terms of clinical evaluation scores and MRI. There were no adverse events that led to treatment discontinuation. For degenerative meniscus tears, synovial MSCs enhanced healing potential and overcame limitations of currently available treatments.
Second-generation cell-based therapies in the continuum of knee care

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The promise of autologous cell-based therapies is to provide natural cytokines and/or stem cells early in the disease process with a goal of ameliorating treatment regimens. To date, many therapies have fallen short of that promise often because the mechanism of action is not well understood and thus they are used in all indications, in all types of patients, and have little clinical evidence supporting their benefit. The next generation of cell therapies developed must be designed for specific indications and specific patient populations. While at first glance this limits their use, this approach will vastly expand the level I evidence demonstrating a clear benefit for the patients in select indications.

One next generation product currently being investigated is nSTRIDE® Autologous Protein Solution (APS) (Zimmer Biomet). nSTRIDE APS is a product designed specifically to counteract the inflammatory proteins found inside an osteoarthritic knee while simultaneously providing anabolic proteins which may stimulate chondrocyte proliferation (1). nSTRIDE APS is classified as an Autologous Anti-Inflammatory (AAI) as the mechanism of action is focused on the activity of anti-inflammatory cytokines produced predominately from white blood cells.

Inflammatory proteins interleukin-1 (IL-1) and tissue necrosis factor alpha (TNF α ) work together to stimulate chondrocytes to produce the enzyme matrix metalloproteases-13 (MMP-13) which breaks down cartilage matrix. APS has been shown to inhibit MMP-13 production from IL-1β and TNF α -stimulated chondrocytes in a culture dish (2) and then consequently, to reduce release of matrix degradation products from IL-1β and TNF α -stimulated cartilage explants (1). Additionally, chondrocyte proliferation is observed in the chondrocyte treated explants, suggesting a potential mechanism of repair. APS has also demonstrated pain reduction in large animals with naturally occurring-OA (3;4) and in early clinical trials (5;6).

Therapeutic angiogenesis by Vascular endothelial growth factor (VEGF) delivery suffers from 2 major limitations: at least 4 weeks of delivery are required to avoid vessel regression, but sustained and uncontrolled expression can cause angioma growth. We previously found that the maturation factor Platelet-Derived Growth Factor-BB (PDGF-BB) can prevent aberrant angiogenesis by VEGF gene delivery [1]. To overcome safety concerns of gene transfer, here we investigated the delivery of VEGF and PDGF-BB proteins from a state-of-the-art matrix-bound system, based on the transglutaminase (TG) reaction to bind the modified factors into fibrin hydrogels [2].

TG-engineered VEGF164 and PDGF-BB were cross-linked into fibrin hydrogels and different doses of each, both together or no factors (control) were injected in limb muscles of SCID mice. We found that: 1) gels were completely degraded within 10 days in all conditions; 2) by 2 weeks, PDGF-BB completely normalized aberrant angiogenesis by high TG-VEGF doses, yielding only mature and functionally perfused capillary networks, with PDGF/VEGF ratios as low as 1:20; 3) 10 days of TG-PDGF-BB co-delivery with both low and high VEGF doses caused stabilization and long-term persistence of new vessels, whereas >90% regressed with VEGF alone; 4) in a mouse hindlimb ischemia model, co-delivery at 1:20 ratio (50 μg/ml VEGF), but not either factor alone, caused 2-fold increases in both microvessel density and collateral arteries after 4 weeks, fully restoring blood perfusion.

Therefore, controlled co-delivery of TG-VEGF and TG-PDGF-BB proteins provides a convenient (off-the-shelf), safe (no genetic modification) and clinically applicable approach for therapeutic vascular growth with a short-term 10 days’ treatment.

References

Acknowledgments
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The fibrin gel immobilization scheme presented is the subject of patents upon which J.A.H. is named as inventor and has been licensed by a company in which J.A.H. is a shareholder.

Microfabricated tissues for medical discovery and therapy

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The field of tissue engineering emerged a decade ago, and has progressed significantly ever since. However, commercialized tissue engineering products are still very scarce, while the skyrocketing cost in both drug discovery and clinical therapy contribute to the unsustainability of our healthcare system. With this in mind, the healthcare market demands new products that deliver improved care at reduced costs. In drug discovery, tissue engineering could offer more realistic tissue models with organ-level function to improve the predictive power of drug testing in the pre-clinical stage, hence reducing the costs in clinical trials. In clinical therapy, sophisticated tissue engineering products with built-in vascular system will accelerate its clinical translation. Both challenges, although spanning two distinct fields, require us to develop more sophisticated tissue assembly strategies.

Microfabricated smart biomaterials can provide structural support and spatial control for tissue assembly resulting in tissue models or implantable tissues with realistic physiological functions. These tissues can be complemented with the built-in capacity for vascular perfusion and rapid assembly. In this seminar, I will discuss the recent breakthroughs we have made in scaffold fabrication and how it resulted in a number of technologies, specifically AngioChip, Tissue Velcro, InVADE Plate, and TopoMembranes for drug screening and tissue repair applications. Building on this progress, I will also discuss the path forward. Successful development of novel tissue assembly strategies will expand our toolbox to propel drug discoveries and clinical therapy towards greater precision, reduced translational costs, and improved efficacy.

Engineering a Three-dimensional Tissue Using a Perfusable Vascular Collagen-based Bed

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Engineering thick tissues can facilitate novel myocardial therapy methods and in vitro models for pharmaceutical research. Our laboratory fabricated cell sheets by lowering the temperature of grafted temperature-responsive cell culture surfaces and attempted to fabricate thick tissues by layering these sheets. However, in vitro scaling up of thick tissues is limited due to the lack of vessels supplying oxygen and nutrients and removing waste molecules. Herein, we report a novel strategy for constructing perfusable vascularized tissues using abioreactor with collagen-based vascular bed.

Co-cultured cell sheets comprising normal human dermal fibroblasts or adipose-derived stem/stromal cells and GFP-expressing human umbilical vein endothelial cells (GFP-HUVECs) were prepared. In these sheets, GFP-HUVECs automatically formed vascularized networks in normal endothelial media. The cell sheets were then placed on the collagen gel with vascularized GFP-HUVECs and microchannels perfused with the culture medium. After 7 days of cultivation, microscopic observations demonstrated that the GFP-HUVECs formed tubular structures in the co-cultured cell sheets on the collagen gel. To examine blood flow in tissues, fluorescent macrobeads were perfused through the microchannels. The blood flow indicated connections between the cell sheet and collagen gel vasculatures. Perfusable vascularized tissues were then constructed in vitro using this cell sheet technology and the perfusion bioreactor system. To fabricate a three-dimensional tissue, additional cell sheets were repeatedly layered over each other. This new multi-layer construct spontaneously integrated to form a vascularized three-dimensional tissue.

These results demonstrated a method for the fabrication of in vitro engineered three-dimensional tissue using perfusable vascularized cell sheets. This technology may help in restoring damaged cardiac tissue and successfully producing accurate cardiac tissue models for pharmaceutical investigations.
Vascularized Tissue Engineering Using the Arteriovenous Loop – The Path from the Small Animal Model to the Preclinical Large Animal Model

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The vascularization of tissue-engineered constructs is one of the key elements in Tissue Engineering studies. As long as the constructs are small enough for nutrition via diffusion, traditional approaches combining a scaffold, cells and growth factors, can be highly successful. However, in view of the translation from bench to bedside, there is great demand for developing large-dimension tissue replacements. The vascularization of such constructs and the possibility to anastomose these constructs to the host’s vessels is of utmost importance.

For vascularized tissue engineering we used the arteriovenous (AV) loop model. An artery and vein are anastomosed into a loop vessel which is further placed into an implantation chamber with a scaffold, cells and growth factors. The chamber is closed and implanted for several weeks for the generation of a fully vascularized tissue-engineered construct. We mainly focused on the generation of vascularized bone tissue in the rat and for further preclinical testing in the sheep model. Different matrices and cells, e.g., from endothelial progenitor cells over mesenchymal to adipose-derived stem cells, were implanted with the aim to grow soft tissues, such as lymphatic or muscle tissue. Evaluation methods included 3D-imaging, immunohistochemistry and real-time PCR.

We successfully generated vascularized bone, lymphatic and muscle tissue in the rat AV loop model. Because of the axial vascularization by a single vascular AV loop, these constructs can be transplanted into the defect area and anastomosed to the local vascular system, leading to optimal conditions for healing after transplantation. As preclinical model we established the sheep AV loop model. As proof-of-principle we were able to transplant a large tissue-engineered construct consisting of a stable bone substitute, mesenchymal stem cells and endothelial progenitor cells, in a critical size defect with dimensions resembling the clinical setting. Compared to controls, immunohistochemistry and 3D-imaging demonstrated that there is an accelerated remodeling in the defect area in the AV loop groups.

On the basis of our experimental data in the preclinical large animal model, we believe that the AV loop model has great potential as an ‘on-demand’ product for clinical translation. The chamber and the scaffold size can be customized according to the patients’ individual requirements. This opens up new perspectives for the therapy of large tissue defects.

Non-Invasive and Real-Time Measurement of Microvascular Permeability in Intact Lungs

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Microvascular leak, or the escape of fluid through the capillary walls, is a phenomenon witnessed in multiple disease states including sepsis, systemic capillary leak syndrome, and engraftment syndrome. In organ engineering, where intact organs are decellularized and repopulated with new cells, regaining a functional barrier is the most crucial step towards creating an implantable organ. All previous methods of measuring microvascular permeability were either destructive, end-point, or relied on extrapolations from cultured cells in separate systems.

We present here a system that enables real-time measurement of microvascular permeability of intact rat lungs. Our unique system design enables direct measurement of average alveolar and capillary wedge pressures, in addition to real-time tracking of all flow paths within the organ. To demonstrate the functionality of this system, we first tracked changing internal resistances and flow patterns in decaying native rat lungs. Next, we characterized decellularized lungs under a variety of mechanical conditions to explore their effects on microvascular permeability. Finally, we measured changes in permeability within engineered lungs seeded with microvascular endothelial cells.

We demonstrate the ability to resolve the onset of microvascular and pleural leak with sub-minute resolution and quantify changing vascular resistances during organ decay. We describe the physiology of decellularized lungs and the inherent properties of the extracellular matrix as a function of perfusion rate and applied external pressures. Lastly, we quantify the effect of endothelial cells changing internal vascular and barrier resistances throughout a culture period.

The bioreactor described here facilitates repeatable measurements of average internal pressures and flow paths, enables calculation of lumped internal resistances including microvascular barrier, and performs measurements non-invasively and in real time during culture. This system marks considerable progress in bioreactor design for intact organs, and hopefully can be used to garner physiologic insight into native, decellularized, and engineered organs.

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Conflict of Interest: LEN is a founder and shareholder in Humacyte, Inc, which is a regenerative medicine company. Humacyte did not influence the conduct, description, or interpretation of the findings in this work.
Microfluidic technology has been extensively used to isolate rare cells such as circulating cancer cells for liquid biopsy. Of late, there is an emerging use of this technology to also enrich circulating microvesicles and exosomes found in the bloodstream of cancer patients. The challenge here is in having an effective approach to capture these biomarkers in their most native forms. Exosomes are a special type of circulating vesicles that lies between 50 nm and 200 nm and owing to their size, they can be very difficult to capture and will require many hours of centrifugation. The current labor intensive process impedes the true potential of exosomes and microvesicles for various applications including disease diagnosis and tissue repair and regeneration. We have develop several microfluidic platforms that uses centrifugal forces to separate these tiny vesicles within minutes. These platforms allow for a significantly reduced speed and can automatically sort circulating vesicles by sizes into different microcompartments for subsequent retrieval. It is hope that these microfluidic platforms, which can be easily used on a table top centrifuge, will enable these tiny vesicles to be easily captured within minutes from very minute samples and in their well preserved native forms as compared to current ultracentrifugation technique which takes hours, requires large amount of samples and may compromise the integrity of these nano and microvesicles.

Circulating tumor-derived extracellular vesicles (EVs) has emerged as a promising source for identifying cancer biomarkers in cancer early detection. However, the clinical utility of EVs has thus far been limited by the fact that most EV isolation methods are tedious and non-standardized and require bulky instrumentation such as ultracentrifugation (UC). Here, we report a size-based EV isolation tool called ExoTIC (exosome total isolation chip), which is simple, easy-to-use, modular and facilitates high-yield and high-purity EV isolation from biofluids. We demonstrate that ExoTIC is a modular platform that can sort a heterogeneous population of cancer cell line EVs based on size. Further, we utilize ExoTIC to isolate EVs from cancer patient clinical samples, including plasma, urine and lavage, demonstrating the device’s broad applicability to not only cancers, but other diseases as well. Finally, the ability of ExoTIC to efficiently isolate EVs from small sample volumes opens up avenues for preclinical studies in small animal tumor models and for point-of-care EV-based clinical testing from finger prick quantities (10–100 μL) of blood.
Osteoblast-derived extracellular vesicles as sites of mineral nucleation: application for tissue regeneration

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Of the 7.9 million fractures that occur annually in the United States, approximately 5-10% have impaired or delayed healing. Accelerating or facilitating fracture healing will lead to improvements in patient care. By harnessing the regenerative capacity of osteoblast-derived extracellular vesicles (EVs) we sought to develop an acellular yet biological therapy for this application. EVs were isolated from mineralising osteoblasts at weekly intervals over a total period of 3 weeks. Relative EV size and concentration was defined using nanoparticle tracking analysis, dynamic light scattering, transmission electron microscopy (TEM) and CD63 ELISA. Temporal changes in the EV proteome were analysed using liquid chromatography tandem-mass spectrometry (LC-MS/MS). The capacity of EVs to induce osteogenic differentiation in MSC cultures was assessed against a clinical gold-standard, BMP-2. Mineralisation was evaluated using alizarin red calcium staining and alkaline phosphatase (ALP) quantification. Mineral phase and quality was analysed using X-ray fluorescence (XRF) and infrared spectroscopy (IR). EVs (CD9+ /CD63+ /CD81+) were found to significantly enhance ALP levels, mineralisation rate and mineral volume beyond BMP-2. XRF elemental mapping identified significantly enriched areas of calcium and phosphorus co-localisation in EV treated MSC cultures. IR analysis of the mineral phase demonstrated the presence of octacalcium phosphate (OCP). Principal component analysis and accompanying TEM-coupled energy dispersive X-ray spectroscopy (EDX) indicated the mineral component was principally localized with EV phospholipid membrane, suggesting that this functions as a site of nucleation. The number of EVs generated, as well as their protein content and size was found to strongly correlate with osteoblast differentiation status. All EVs displayed a bimodal size distribution (week 1 and 2, 44nm and 164nm; week 3, 59nm and 220nm) with significantly fewer EVs were generated as mineralisation advanced (week 3). Proteomic analysis of EVs revealed the presence of bridging collagens, calcium chelating proteins and extracellular binding proteins. The relative intensity of proteins related to these biological processes was significantly reduced at week 3. Our data suggests that EVs function as initial sites of mineral nucleation within the developing extracellular matrix and hold considerable potential as an acellular yet biological approach to regenerative medicine.
MSC Exosome as a Cell-Free Therapy for Cartilage Repair

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The therapeutic efficacy of mesenchymal stem cells (MSCs) is increasingly attributed to its secretion of trophic factors, particularly the exosomes. Exosomes are secreted membrane vesicles thought to function primarily as intercellular communication vehicles to transfer bioactive lipids, nucleic acids (mRNAs and miRNAs) and proteins between cells to elicit biological responses in recipient cells. For MSC exosomes, many of these biological responses translated to a therapeutic outcome in injured or diseased cells. Here, we developed a scalable manufacture of human MSC exosomes from an immortalized MSC line. These exosomes displayed a modal size of 100nm and expressed the exosome markers (CD81, ALIX and TSG101). In immunocompetent rat models of osteochondral defect and chemically- and surgically-induced osteoarthritis, weekly intraarticular injections of MSC exosomes (100µg) effectively regenerated the osteochondral defects and alleviated the pain and joint degeneration, respectively. Exosome-mediated tissue repair was associated with enhanced cellular proliferation, reduced apoptosis and suppressed inflammation with increased M2/M1 macrophage polarization. By means of proteomic analysis that revealed >850 unique gene products, several enzymes that could regulate a wide array of biochemical and cellular processes were identified. Among them, exosomal CD73/ecto-5’-nucleotidase was found to regulate chondrocyte survival, proliferation and migration through adenosine-mediated activation of AKT and ERK survival kinases. Taken together, MSC exosomes mediate cartilage regeneration via a multi-faceted mechanism of enhancing proliferation, migration and matrix synthesis, attenuating apoptosis and modulating immune reactivity. These findings provide the basis for future use of human MSC exosomes as a cell-free therapeutic for cartilage repair in patients.
**25-SY-7 On the Potential of Paracrine Signals for Advanced Therapies**

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Among the various possible embodiments of Advanced Therapies and in particular of Tissue Engineering the use of temporary scaffolds to regenerate tissue defects is one of the key issues. The scaffolds should be specifically designed to create environments that promote tissue development and not merely to support the maintenance of communities of cells. To achieve that goal, highly functional scaffolds may combine specific morphologies and surface chemistry with the local release of bioactive agents. Many biomaterials have been proposed to produce scaffolds aiming the regeneration of a wealth of human tissues. We have a particular interest in developing systems based in biodegradable polymers. Those demanding applications require a combination of mechanical properties, processability, cell-friendly surfaces and tunable biodegradability that need to be tailored for the specific application envisioned. Those biomaterials are usually processed by different routes into devices with wide range of morphologies such as biodegradable fibers and meshes, films or particles and adaptable to different biomedical applications. In our approach, we combine the temporary scaffolds populated with therapeutically relevant communities of cells to generate a hybrid implant. For that we have explored different sources of adult and also fetal stem cells. We are exploring the use of adult MSCs, namely obtained from the bone marrow for the development autologous-based therapies. We also explore the paracrine signalling of cells to generate effective differentiation of the stem cells into the lineages of interest.

This talk will review our latest developments of co-cultures with stem cells and also extracellular vesicles for advanced biomedical devices and therapies.

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**26-SY-1 Vasculogenic differentiation of dental pulp stem cells in tissue engineering**

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Dental pulps contain unique stem cells that orchestrate tooth development and pulp tissue regeneration. We have shown that dental pulp stem cells (DPSC) are capable of differentiating into dental tissue-forming cells (e.g. odontoblasts) while exhibiting the capacity to differentiate into endothelial cells that generate functional blood vessels to support the metabolic demands of tissue regeneration. The clinical translation of stem cell-based therapies requires the understanding of mechanisms that control the differentiation of these stem cells. In this presentation, we will discuss mechanisms employed by DPSC to generate new dentin and functional blood vessels. We will also discuss the prospect for a mechanism-based therapy that exploits the differentiation potential of DPSC in dental pulp tissue regeneration. We believe that the development of safe strategies that foster efficient differentiation of stem cells upon transplantation into the root canal is a key step towards the use of these cells in dental pulp tissue engineering for the treatment of immature necrotic permanent teeth.
Dental pulp regeneration therapy for the pulpless tooth has recently attracted much attention, and many studies using tissue engineering approach are underway. However, there remains several concerns for the technologies reported, such as long period to regenerate the pulp or increase in the risk of inflammation and infection by the usage of scaffolds. Here, we propose a novel technology for pulp regenerative therapy to transplant three-dimensional (3D) constructs of human dental pulp stem cells (DPSCs) to the pulpless tooth. In this approach, rod-shaped 3D DPSC constructs were fabricated in vitro by shaping sheet-like cell aggregates prepared by culturing DPSCs on a thermo-responsive hydrogel. When the 3D cell constructs obtained were cultured to induce odontogenic differentiation, the cells remained viable even after prolonged culture, and localization of dentin sialophosphoprotein after 5 days and mineralized deposition after 10 days in the outer layer of the constructs were observed. In contrast, the expression of Nanog, a stem cell marker, was significantly increased in DPSCs in the inner layer of the constructs. These facts indicate that 3D DPSC constructs possess self-organization ability and can serve as a viable transplant tissue for pulp regeneration. Then, we filled the root canal of human extracted tooth with DPSC constructs and implanted it subcutaneously in immunodeficient mice. It was found that pulp-like tissue, with rich blood vessels, were formed within the root canal after 6 weeks of implantation. Histological analyses revealed that transplanted DPSCs differentiated into odontoblast-like mineralizing cells at the sites in contact with dentin while human CD31-positive endothelial cells and STRO-1-positive stem cells were found at the center of regenerated tissue. Furthermore, blood vessel-rich pulp-like tissue can be formed using DPSCs without requiring scaffolds or growth factors. Since the technology shown here allows us to prepare DPSC constructs with variable sizes and shapes, it is promising to enable tailor-made pulp regeneration therapy for individual tooth.
Regenerative silk ligament: scale up and regulatory strategy of a textile engineered silk implant for tissue regeneration of injured human ACL (anterior cruciate ligament)

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In the USA around 370,000 annual ACL ruptures in the predominately young population (age 18-30) following sport injuries cause a lot of pain and long term troubles for the affected patients. The well established standard procedure of autologous tissue transplantation stabilizes the knee and allows sport activities after 9-12 months. However, harvest of autologous tissue such as patellar tendon or semitendinosus (the most frequently used autograft) weakens other body areas and long term data show an increased rate of arthroses (up to 50% after 10 years) associated with ACL reconstructions.

Based on numerous preclinical data and data from a 12-month sheep study the scale-up as well as regulatory approval process of a novel textile engineered silk implant as ACL graft have been initiated. It is planned to enter a clinical trial and strive for regulatory approval as a medical class 3 implant. The sheep data (1) have revealed an approximately 50% degradation of the purified medical silk, which acts as scaffold for the regrowth of a new endogenous ligament. The formation of oriented collagenous tissue fibers including vascularization proves a ligament tissue regeneration for the first time.

Scale-up and implementation of a commercial process require a defined set of specifications of commercial silkworm (Bombyx mori) silk, a biological raw material sourced from the textile industry as well as adequate analytical methods to characterize the depletion of sericin in the course of the production process.

Based on first full scale samples, the defined biocompatibility program has to be executed, in the EU following the ISO requirements of the notified bodies, in the USA determined by the FDA. Upon submission of the full technical documentation as well as the biocompati-bility data, approval of a clinical trial can be achieved in order to demonstrate the clinical efficacy of the silk based ACL graft compared to the gold standard method in two different randomized groups. As primary endpoint the knee stability will be tested by an apparatus supported Lachmann test. In this study, besides the clinical benefit the patient safety is an important goal. The results of this study will be used for achievement of regulatory approval.

**Statement of Purpose:** Peptides and polypeptides are versatile tools in biotechnology, especially in drug/gene delivery area. The individual character of each amino acid can be combined, resulting in huge advantage for targeted delivery systems (Numata 2015). Cationic components make efficient gene delivery agents due to membrane-destabilizing, DNA-condensing and pH buffering properties. A group of molecular transporters such as cell penetrating peptides (CPPs) are short peptide sequences that vary significantly in sequence, hydrophobicity, polarity, and have the remarkable capacity for membrane translocation. Meanwhile, targeted delivery to essential organelles such as the mitochondria can be achieved with mitochondria-targeting peptides (MTPs), which are typically N-terminal motifs predicted to form amphipathic helixes and enriched in positively charged basic residues. To enhance the stability of the peptide, we also added silk sequence into the peptide-based carrier (Numata et al. 2010). The combination of silk, cationic, cell-penetrating and mitochondria-targeting components reported here is an exciting new design parameter that enabled cellular uptake, localization and expression of exogenous DNA in the mitochondria. In this presentation, gene delivery into tumor cells and the mitochondria of animal cells by using the peptide-based system are introduced.

**Mitochondria-targeting:** 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. Although disease symptoms can be managed with various medications, there are currently no treatments for mitochondrial disease.

In the present study, we applied our new design parameter to devise a gene carrier for effective targeting of mitochondria in mammalian cells. To incorporate multiple functionality while maintaining the simplicity of design, we combined DNA-binding, cell-penetrating and mitochondria-targeting domains into a single molecule (Chua et al. 2015). Mitochondria targeting peptide (MTP)-based carriers with different characteristics (amino acid composition, length, molecular weight, hydrophilic/hydrophobic properties) were designed and mixed with DNA at various ratios to form complexes. Transfection efficiency of the carriers was evaluated using human embryonic kidney 293 cells (Chua et al. 2016). Successful cell/mitochondrial transfection was verified by detecting the expression of the reporter gene (encoding Renilla luciferase enzyme or green fluorescent protein) using Renilla luciferase assay or confocal laser scanning microscopy. The most transfection-effective peptide sequence and conditions were selected for cell viability studies and time course evaluation of transient gene expression in transfected mitochondria. Important transfection parameters such as the effect of serum addition as well as peptide and DNA concentrations were also optimized.

**Conclusions:** My group developed a simple and yet effective peptide-based gene carrier, which is designed for the intracellular delivery and subsequent expression of exogenous DNA in the target cells or organelles. This polypeptide-mediated technology will expand the potential of gene therapy and also genome editing-related fields.

27-SY-3  Functionalization of silk fibroin matrices using recombinant spider silk fusion proteins for developing bioactive wound dressing

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Silk is considered to be a potential biomaterial for tissue engineering applications. Silk fibroin (SF) isolated from silkworms is available worldwide at an affordable cost. However, SF does not contain bioactive functions. Spider silk proteins on the other hand, can be functionalized by recombinant technology and produced in fusion with bioactive domains; the recombinant procedures are however expensive and large scale production is challenging. Herein, we present a facile methodology to combine the two types of silk for fabrication of functional constructs at affordable cost for wound healing applications. Nanofibrous mats made of natural silkworm SF were fabricated and used as a bulk material which were subsequently top-coated with recombinant spider silk (4RepCT) fusion proteins. The inherent silk self-assembling property between the two silk types was exploited to develop functionalized spider silk coated SF mats as potential wound dressing. The SF mats were coated with spider silk fused with antimicrobial peptide (AMP), fibroblast growth factor (FGF) and cell binding loop of fibronectin (FN) domains. The intended functions, i.e. antimicrobial action, growth factor stimulation and improved cell adhesion could be demonstrated by the functionalized silk mats examined in vitro. Further, in-vivo wound healing assessment carried out on diabetic rabbit model demonstrated accelerated rate of wound healing by mats coated with both FN and AMP spider silk fusion proteins in comparison to its non-coated counterparts. In addition, wound healing rate of coated mats was found to be higher than that of commercially available Duoderm dressing used as positive control in the study. The work confers encouraging results thus paving way towards fabrication of an affordable bioactive SF-spider silk based wound dressing with multiple bio-functions.

27-SY-4  Silk fibroin scaffold loaded with hydrogen sulfide-releasing agent for bone tissue regeneration

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The process of bone tissue repair is critically improved by the use of biomaterials which can promote bone formation and the establishment of an optimal osteoinductive environment, including angiogenesis. Among the sources of biomaterials, silkworm cocoons have been extensively exploited for the extraction of silk fibroin (SF), a natural fiber with an excellent biocompatibility and good mechanical properties. Hydrogen sulfide (H₂S) is a naturally occurring gasotransmitter endogenously produced within mammalian cells. H₂S was recently shown to promote bone formation in vitro and in vivo; when administered pharmacologically, it prevented bone loss in various models of bone wasting diseases. The aim of the present study was to realize an engineered SF scaffold combined with the H₂S-donor GYY4137 (GYY), designed to provide a local and prolonged release of H₂S and stimulate bone formation. Porous SF scaffolds were produced by solvent casting and particulate leaching method and loaded with GYY at concentrations of 1 and 5% w/v. The newly generated scaffolds (SF-GYY) preserved chemical and physical properties, as assessed by SEM, FT-IR, NMR and different thermal analyses techniques. SF-GYY scaffolds dose-dependently released H₂S in solution as revealed by amperometric analysis. To ascertain biocompatibility, scaffolds were loaded with NIH/3T3 cell line and human mesenchymal stromal cells (MSC) and cultured for 24h; LDH assay showed no cytotoxicity by both control SF and SF-GYY scaffolds. To investigate the capacity to support bone formation, scaffolds were cultured in a perfusion bioreactor (U-Cup, Cellec Biotech) for up to 3 weeks and subject to immunohistochemical staining and to a broad analysis of osteogenic gene expression by PCR array. The presence of GYY dose-dependently increased areas of ALP+, mineralized matrix production compared to control SF scaffolds. Moreover, analysis of gene expression for 84 osteogenic genes revealed that , in the presence of GYY, 89% and 68% of genes were up-regulated, respectively, at day 7 and day 21 in culture compared to control scaffolds. Furthermore, SF-GYY showed markedly up-regulated VEGFA expression both at the gene and protein level. In conclusion, SF loaded with H₂S-donors may represent a novel strategy to enhance osteoinduction and neo-angiogenesis of scaffolds for bone tissue engineering.

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Heterotypic Effects within Cardiac Microtissue Environments on Human iPS-derived Cardiomyocyte Phenotype and Function

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Human pluripotent stem cell-derived cardiomyocytes (hPSC-CM) exhibit immature phenotypes and physiologic properties compared to adult CM, highlighting the need for effective methods to mature hPSC-CM in vitro. CMs naturally develop in close proximity to multiple non-myocyte cell populations, suggesting the presence of other cells is necessary for proper hPSC-CM maturation and function. In this study, we examined the effect of incorporating cardiac fibroblasts (CF) or endothelial cells (EC) in cardiac microtissues on the physiologic function and phenotypic state of CM. CM were differentiated from hPSCs via modulation of Wnt signaling followed by lactate purification. Engineered cardiac microtissues were generated using highly pure (>85%) hPSC-CMs alone or combined with CFs or ECs (3:1 ratio) in agarose microwell molds to form cardiac microtissues. 3D cardiac microtissues were maintained for up to 30 days after formation. Calcium imaging was performed throughout culture using an iPSC line harboring a genetically encoded Ca2+ sensor, GCaMP6. Single-cell RNA-sequencing of dissociated microtissues was performed at early and late time points, along with immunostaining of whole mount and histological samples. Although individual cardiac microtissues exhibited autonomous spontaneous beating activity, synchronous Ca2+ handling between microtissue groups were not observed. Single-cell RNA-Seq analysis could easily distinguish distinct cell types and phenotypic states of the heterogeneous cell populations coming from the differentiated cell populations and 3D heterotypic microtissues. Consistent with Ca2+ results, CM cultured with CF as microtissues for 7 days exhibited a more pronounced phenotypic shift than for CM-EC or CM alone microtissues; CF and EC also had significant phenotypic shifts after culture with CM in microtissues. Ongoing analysis will determine specific molecular shifts due to heterotypic 3D microtissue culture in the CM and non-myocyte populations. Overall these studies demonstrate the ability to interrogate the phenotypic and functional impacts of complex heterotypic interactions that mimic the native cardiac tissue environment.

Design of a 3D Organotypic Model to Study Monocyte Extravasation to the Osteoarthritic Joint using a Combined Computational/Experimental Approach

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Inflammation plays a crucial role in osteoarthritis (OA). In OA joints, an increased number of monocytes extravasates to the synovium, driven by chemokine gradients. Microfluidics allows generating 3D models recapitulating in vitro the in vivo conditions typical of specific biological processes. We exploited this feature to engineer a 3D organotypic model of the joint to investigate monocyte extravasation. We designed a multi-compartment chip recapitulating different districts: synovium, postcapillary venules, synovial cavity, and cartilage. Synovium and cartilage were modeled as gel compartments flanked by a channel hosting synovial fluid. The synovium compartment enclosed a channel, mimicking a synovial postcapillary venule, for endothelial cell and monocyte injection. Tapered posts were used to confine gel and maximize the surface available for cells to cross the endothelial wall. Computational fluid dynamic (CFD) modeling was applied to optimize the endothelial channel, determining shear stress distribution, and the gel compartments, evaluating the kinetic of chemokine gradient formation. The endothelial channel was designed 200 μm wide and 100 μm-high, suitable to obtain a physiological 0.1 Pa shear stress using a 30 μL/h flow rate. Gel compartments were designed 400 μm-wide, suitable to obtain a stable chemokine gradient in 30 min, as experimentally confirmed monitoring FITC-dextran diffusion from the synovial fluid channel to the synovium compartment. Fibrin-embedded synovial fibroblasts and chondrocytes from OA patients were injected in the respective compartments, testing several culture conditions to select cell and fibrin densities able to limit cell outgrowth while promoting cell viability. To resemble the synovial venule, an endothelial monolayer was generated culturing endothelial cells in the 200-μm wide channel. To assess monolayer integrity, we verified the ability to block the diffusion of FITC-dextran across the endothelial wall in both directions, accordingly with CFD simulations. Finally, we validated the newly developed model by injecting monocytes in the endothelial channel and quantifying their extravasation in response to known concentrations of MCP-1. We designed and developed the first microfluidic model recapitulating the spatial organization of articular joint. This platform will be used in the next future to study monocyte extravasation and test potential chemokine-targeting drugs to counteract monocyte recruitment.
**29-SY-3 Cell Spheroids as Building Blocks for Scaffold-free Biofabrication**

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Much of the progress recently obtained in biofabrication relies on the development of a variety of biomaterials dedicated to tissue engineering. Among these are the ‘scaffolds’ or ‘bioinks’ used for 3D bioprinting, but there are several limitations of this approach. Instead, to create larger 3D tissue-like structures, the cells themselves might be directly used, counting on their own ability to produce the necessary extracellular matrix. To increase the efficiency of such bio-assembling, round cell aggregates (‘spheroids’) have been successfully considered as suitable building blocks. Spheroids are also intensely studied as tissue and tumor models in vitro, and for enhanced cell therapy in vivo. Therefore, a large body of knowledge about spheroids could be repurposed towards Tissue Engineering. In this Symposium, we will discuss this trend with examples centered on placing the spheroids in contact for fusion in larger structures, on an array of microneedles (the ‘Kenzan’ method), as performed by Cyfuse’s Regenova Bio 3D Printer. For a more comprehensive understanding of the status, and the potential of his emerging field, I will also discuss several other methods and results relying on cell spheroids as building blocks.

**29-SY-4 Stochastic spatial and temporal population-based model for the co-emergence of vascular patterns**

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Stem cells differentiation is often examined as cell responding to biochemical signals in two-dimensional (2D) cultures. However, the in vivo cellular microenvironment is a much more complex 3D environment with multiple cell types and materials. To that end, we have developed a computational model that incorporates multiple cell types, as well as, biochemical and physical cues in directing stem cell differentiation in order to explore the relationship and co-emergence and spatial patterning of two cell types; endothelial cells (EC) and vascular smooth muscle cells (vSMC) from a common vascular progenitor cell. Our spatio-temporal computational model consists of three differential equations for three changing cell populations; VPC (X_A), EC (X_B), and vSMC (X_C). Within these equations, we account for cell division (δ_i), differentiation (α_i), cell death (μ_i), motility (J_i). Additionally, functions that measured cell density and signaling from adjacent cells were added as functions of motility, cell division, and differentiation. The growth of the cell populations are tracked overtime. Empirical data was first used to estimate values for the variables, followed by regression analysis techniques to find true values that are analogous to experimental conditions. Lastly, phase space sweeps were conducted to determine which variables lead to the development of micropatterns. The current model is able to reproduce experimental patterns using a few different input combinations. Simulations suggest that the patterning is primarily to due to self-sorting after differentiation rather than neighboring cell-directed differentiation.

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**31-SY-1** Challenge toward iPSCs based cell therapy and modeling of CNS disorders and Current Status of Regenerative Medicine in Japan

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Since the development of iPSCs-technology by Shinya Yamanaka’s Lab in 2006, there is increasingly interest in its application to medical science, including i) cell therapy and ii) disease modeling associated with drug development. Here, I wish to introduce our research on these two aspects.

1. **iPSC-based cell therapy for Spinal Cord Injury**

   In our previous preclinical studies, when neural stem progenitor cells (NS/PCs)-derived from hiPSCs were transplanted into mouse or non-human primate spinal cord injury (SCI) models, long-term restoration of motor function was induced without tumorigenicity, by selecting suitable hiPSCs-lines. However, NS/PCs derived from certain iPSC-lines gave rise to late-onset tumorigenicity after transplantation. Here, to preclude these risks before clinical application, we developed molecular characterization of hiPSCs and hiPSC-derived NS/PCs together with transplantation to injured spinal cord of immune-deficient mice. We investigated global methylation status of tumorigenic hiPSC-NS/PCs and found that aberrant hypermethylation of a tumor suppressor gene was induced along the passage. Based on these findings, we are establishing production and selection method of clinical grade NS/PCs stocks-derived from human iPSC stocks generated from HLA-homozygous super-donors by CiRA. As a fail safe system to prevent the hiPSC-NS/PCs, we found that pretreatment with γ-secretase inhibitor decreases the proliferative capacity of transplanted hiPSC-NS/PCs, triggers neuronal commitment, and improves the safety of hiPSC-based approaches in regenerative medicine. In 2017, we submitted the detailed protocol of clinical research (Phase I-IIa) trials for treatments of sub-acute phase SCI using hiPSCs-derived NS/PCs to IRB.

2. **iPSCs-based modelling of human neurological diseases**

   There is an increasing interest in disease modelling and drug development using iPSCs-technologies. So far, we have established IPS cells from the patients of about 40 human psychiatric/psychiatric disorders and characterized their pathophysiology, including Alzheimer disease, Parkinson disease, ALS, Rett syndrome, Pelizaeus-Merzbacher disease lissencephaly, CHARGE syndrome, retinal pigmentosa, Pendred syndrome, and mental disorders such schizophrenia. In this talk, I will mention about our recent progress of disease modelling and drug development using iPSC-technologies, particularly drug development for ALS using a library of FDA-approved drugs.

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**31-SY-2** Endogenous and artificial scaffolds for neuronal migration and regeneration in the injured brain

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In many animal species, new neurons are continuously generated by neural stem cells at the wall of brain ventricles throughout life. These new neurons form chain-like aggregates and migrate towards the olfactory bulb (OB), which is one of the longest and fastest journeys undertaken by neurons in the brain. After brain injuries, these new neurons migrate toward the injured area, where they differentiate into mature neurons. In the post-stroke adult brain, new neurons form chain-like aggregates and migrate along blood vessels, which are thought to increase their migration efficiency. The chain formation and blood vessel-guided migration of new neurons critically depend on β1-integrin signaling. Moreover, artificial laminin-containing scaffolds promote the neuronal chain formation and migration toward the injured area. Radial glia are polarized embryonic neural stem cells, which guide newly generated neurons by providing their fibers as a migratory scaffold. Radial glial fibers are maintained for an extended period in the injured neonatal mouse brain and provide a scaffold on which V-SVZ-derived new neurons migrate toward the injured cerebrocortical cortex. N-cadherin-mediated cell-cell contact promotes RhoA activation in the new neurons and maintains their directional saltatory movement along radial glial fibers. Inserting radial glial fiber-mimetic scaffold into the brain promotes new-neuron migration toward the lesion and facilitated neuronal regeneration and neurological recovery. These findings have revealed the functional significance of blood vessels and neonatal radial glia as scaffolds for neuronal regeneration after brain injury. We propose novel therapeutic strategies for repairing the injured brain using endogenous neurogenesis in the V-SVZ.
Microglia-mediated neuroinflammation is one of the most significant hallmarks in a variety of central nervous system (CNS) diseases: traumatic brain injury, stroke, and especially neurodegenerative diseases such as Parkinson’s disease (PD), Alzheimer’s disease (AD), and amyotrophic lateral sclerosis (ALS). As the resident macrophage cells in CNS, microglia could polarize towards either the neuron-destructive pro-inflammatory M1 phenotype or neuron-regenerative M2 phenotype. Thus, microglial polarization is considered as a promising therapeutic target for CNS repair and regeneration. The gold nanoclusters (AuNCs) are novel fluorescent materials with excellent biocompatibility, which have been attracting attentions in biomedical applications such as biolabeling, bioimaging, gene/drug delivery, targeted cancer treatments, etc. AuNCs made up of 10 to 100 atoms have ultra-small size (< 3 nm) and therefore have no difficulty passing through the blood-brain barrier (BBB); previous study has found AuNCs accumulated in microglia in vivo, and the current study aims to examine the possible effects of AuNCs uptaking on microglia immune response and the consequence effects on neuronal regeneration. The results showed that under induced neuroinflammatory conditions, AuNCs application effectively suppressed microglia response by converting microglia from the M1 phenotype towards the M2 one in vitro, which was achieved through regulation of autophagy/apoptosis balance and inhibition of NFkB signaling. Moreover, the AuNCs-uptaking microglia provided an suitable microenvironment for neuron repair, as the secreted factors of which improved the neuronal differentiation of neural stem/progenitor cells (NSPSCs) while reduced the differentiation of astrocytes and therefore limited the formation of unwanted glia scars. Therefore, our study provides a novel approach for treatments against neuroinflammation in CNS diseases.

Stroke, traumatic brain injuries, and other neurodegenerative diseases lead to loss of brain tissue and the associated disruption of neuronal signaling. Cellular injections of dissociated stem cells have been pursued as a potential treatment for such neurological conditions, however, this method is not necessarily suitable for the repair of large brain defects. Engineered neural tissues may be a viable alternative, but cell death due to inflammation is a prominent concern in neuronal grafting studies. The aim of this study was to design a brain implant that can mitigate neuroinflammation post-transplantation in vivo, and thereby improve graft survival. We pursued motor cortex transplantation of 3D engineered brain tissue constructs loaded with corticosteroid and examined the effect of acute cerebral inflammation on transplant survival. Brain constructs were prepared by seeding E18 rat cortical neurons expressing GFP on silk fibroin scaffolds. The neuron-seeded silk constructs were then embedded in a fibrin hydrogel loaded with 8.7mM methylprednisolone (MP). Constructs were transplanted into a cavity in motor cortex created by aspiration (2 mm diameter, 2 mm depth) and covered with a fibrin gel +/- MP (Tufts IACUC, M2015-28; Upenn, 805600). After 3 days, animals were perfused, and brains were extracted for analysis of graft viability and the expression and secretion of inflammatory markers. We achieved controlled release of MP from the silk constructs over 12 days in vivo. Next, improved survival of 3D bioengineered tissue constructs loaded with MP was observed in vivo compared to constructs without MP during the acute phase of inflammation. Increased survival correlated with decreased levels of pro-inflammatory cytokines (IL-1β, IL-6, TNF α, CCL2) and a shift of immune cells from a pro-inflammatory towards an anti-inflammatory state with upregulation of CD163, IL-4 and IL-10 expression. Moreover, when silk constructs were exposed to pro-inflammatory cytokines in vitro, we observed a trend toward improved neuronal survival in the presence of Bindarit, a small molecule inhibitor of CCL2 that modulates inflammation through canonical NF-κ B pathway. The potential involvement of the NF-κ B pathway and STAT transcriptional factors in regulation of neuroinflammation after transplantation is also discussed. We thank the NIH (grants R01NS092847, R01NS094218, S10OD021624) and the Department of Veterans Affairs (grant IK2RX002013 to HIC) for support of this work.
A new concept for articular osteochondral defects repair is to in situ regenerate cartilage and subchondral bone simultaneously. Thus, one of the major challenges in this field is the structural design of a biomimetic scaffold that satisfies this specific requirement. A biomimetic osteochondral scaffold with continuous multilayers architecture and gradient compositions from articular cartilage layer to subchondral bone layer was fabricated by a microsphere-based SLS technique. Our results demonstrated that the resultant gradient hierarchical scaffold featured highly interconnected porosity and desirable mechanical properties as well as excellent biocompatibility. In vivo animal evaluation further verified that the scaffold could simultaneously induce regeneration of cartilage and subchondral bone, and that the newly formed tissue manifested multiple tissues types including articular cartilage and subchondral bone. Consequently, our current work realizes the simultaneous regeneration of two kinds of tissues, cartilage and subchondral bone, only by using one scaffold without addition of any cell and biological growth factor, which greatly advances the potential application of bioinspired multilayer scaffolds to regenerative medicine. We called this finding as “one scaffold, two tissues”.

Reference
**32-SY-3** Diameter of PCL fibers produced by means of additive manufacturing affects mechanical properties and response of human mesenchymal stem cells

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Microextrusion-based additive manufacturing (AM) is widely used for fabrication of 3D thermoplastic scaffolds for tissue engineering. The process is based on deposition of polymer melt in form of fibers in a layer-by-layer fashion. The fibers with diameter ranging from approximately 100 µm to over 1 mm can be deposited under various angles and distances from each other. This yields different architecture and porosity of the scaffold and affects their various properties, e.g. permeability, stiffness and cell performance. However, little is known about the effect of the diameter of single fibers building a thermoplastic scaffold on their mechanical properties and cellular response.

The aim of this study was to investigate the effect of diameter of PCL fibers produced by means of AM on their mechanical properties and response of human mesenchymal stem cells (hMSC).

The PCL fibers with diameter of 250 (G25), 410 (G22) and 1070 µm (G17) were fabricated using Bioscaffolder and investigated by means of static tensile test, atomic force microscopy (AFM) and wide angle X-ray scattering (WAXS). Human mesenchymal stem cells (MSC) were seeded on the PCL fibers either as single-cell suspension or multicellular spheroids and cultured in expansion, osteogenic and chondrogenic media. During differentiation, alkaline phosphatase (ALP) activity, extracellular matrix mineralization and glycosaminoglycans (GAG) synthesis were measured.

Diameter had a significant effect on stiffness of the fibers. The highest modulus equal 287±31 MPa was measured for fibers with the smallest diameter, and the lowest (112 ± 3MPa) for the biggest ones. This trend was confirmed by AFM. Differences in the stiffness could rise from crystallinity which increased from 48% for G17 to 55% for G25 fibers. Thicker fibers yielded approximately 2 times higher hMSC seeding efficiency but slower colonization by cells migrating out of multicellular spheroids. Moreover, ALP activity and mineralization were increased on the G17 fibers. This shows that fiber diameter is another parameter which has to be carefully considered during design of a scaffolds envisioned for tissue engineering of bone and cartilage fabricated by microextrusion AM.

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**32-SY-4** Development of programmable drug delivery capsule with 3D bioprinting

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Breast cancer is one of the most common types of cancer in women. In 2017, more than 250,000 women in the United States were diagnosed with breast cancer, and 14% of women who died of cancer had breast cancer. Chemotherapy is used to reduce the size of the tumor before surgery or to prevent cancer recurrence and other metastases after surgery. Combination therapy using multiple types of anticancer drugs was usually used for killing cancer cells and reducing the chance of causing drug resistance. However, the conventional systemic approach has limitations.

First, it cannot effectively deliver a therapeutic concentration of the drugs at the tumor site. And anticancer drugs can be accumulated in organs and cause severe side effects. In addition, patient compliance is low because of frequent injections to maintain its concentrations. In this aspect, the local delivery approach is promising to solve these difficulties. It can directly and effectively deliver proper dose to the tumor site through a single injection or implant.

Therefore, these systems can minimize systemic side effect of anticancer drugs and improve the patients’ quality of life.

3D bioprinting has various advantages in developing a local delivery system for controlled release of therapeutic agents. This automated technique can fabricate microstructures with multiple materials. And the material properties and the geometric characteristics of the system can be used to control the release rate of the drugs. In addition, the use of materials containing various types of drugs can be used together to develop a system for combination therapy. In this research, 3D bioprinting was applied to create an implantable system for controlled release of multiple types of drug for combination therapy. A hydrogel-based bio-ink was designed to deliver the drug. Then, it is co-printed with biodegradable polymer composing drug capsule.

Correlation between geometrical parameters of the capsule and release characteristics of the drug were carefully investigated. The result showed that the release profile of therapeutic agents can be precisely controlled with the geometry. And multiple types of drug were successfully loaded into a single system with the automated method. The system is expected to be widely used for combination therapy of various type of cancer.

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A translational tissue engineering technology platform for skeletal muscle repair and regeneration: From biologically relevant animal models to regenerative rehabilitation

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Despite the rather well documented capacity of skeletal muscle to repair, regenerate, and remodel following injury, more severe injuries, such as those involving the loss of a substantial portion of muscle, with simultaneous destruction/loss of multiple other tissue components are not capable of full regeneration on their own. These injuries involve a degree of frank tissue loss that exceeds the endogenous regenerative capacity of muscle, resulting in permanent cosmetic and functional deficits. Such injuries are referred to as volumetric muscle loss (VML) injuries. VML and VML-like injuries can result from a variety of causes including trauma, surgery, inflammation and a variety of congenital and acquired diseases and disorders. Current treatment options are insufficient and are often associated with poor functional and cosmetic results. This unmet medical need has stimulated research to develop new technologies for treatment of VML injuries. We have pioneered a Tissue Engineered Muscle Repair (TEMR) technology platform for treatment of VML injury. TEMR has been described in several publications, and there are 4 awarded US patents covering various aspects of TEMR production and implantation. Briefly, the TEMR construct is designed to be an autologous, sheet-like, bioengineered skeletal muscle implant for functional reconstruction or repair of VML injuries. The TEMR construct is created by seeding ≈1 million muscle progenitor cells (MPCs)/cm² onto a decellularized bladder acellular matrix (BAM scaffold) followed by 10 days of cell proliferation and differentiation, and then 5 days of bioreactor preconditioning in vitro (i.e., 10% cyclic mechanical stretch). We have developed biologically relevant rodent models where the size of the surgically-created VML injuries (>2 cm²) scale well to the proposed first-in-man use for secondary revision of unilateral cleft lip. Moreover, TEMR implantation significantly improves functional recovery (∼60-90% restoration of muscle function (contraction)) within 2-3 months of implantation in both immune competent and immune incompetent rat and mouse VML injury models to both the latissimus dorsi (LD) and tibialis anterior (TA) muscle; with a durability out to six months. More recently, we have incorporated biomechanical studies to evaluate the impact of injury and rehabilitation on functional recovery following application of regenerative therapeutics.
Clinical applications of skeletal muscle TE for urinary incontinence

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Current treatment options for Stress Urinary Incontinence (SUI) include mainly non-surgical therapy (including behavioral therapy e.g. bladder training, fluid/dietary modification), drug therapy and surgical therapy. These therapies only offer short-term relief and the overall success is often limited by complications (invasiveness of surgery, damage to surrounding tissues, leading to increased urinary infection rates) or side-effects (drugs, tissue damage by non-degradable biomaterials). There is urgent clinical need for novel treatment modalities. Advanced SUI therapy approaches show promising results towards correcting the underlying problem using the patient’s own cells. The quality of the regenerated tissue is of critical importance for its accurate function and thus, many efforts have been applied to improve in vivo cell survival and tissue development.

Early clinical trials using stem, or progenitor cells for the treatment of SUI in both male and female patients have encouraging functional results with minimal adverse effects. However, the precise identification, isolation and transplantation of these cells seems more complex than expected. Some points seem to be fundamental for the long-term success of this therapy: functional distinction between stem cells and their progenitor cells; the transition of the stem cell between quiescent and active stages; control of the microenvironment and stem cell niche; provision of additional factors to the cellular component for improving engraftment and survival/differentiation. Although many challenges remain to be addressed for the optimal clinical implementation of this technology, novel stem-cell-based multisystem-therapies are an exciting potential therapy for voiding dysfunction.

MSCs delivered using structurally optimized hydrogels enhance skeletal muscle regeneration by paracrine mechanisms

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Treating severe skeletal muscle injuries is a pressing clinical challenge in orthopaedics and traumatology. Cell therapy involving the transplantation of mesenchymal stromal cells (MSCs) have shown benefits in pre-clinical and clinical studies. This is largely attributed to paracrine factors secreted by MSCs that can influence cellular behaviour and orchestrate tissue regeneration. Our work has focused on two aspects: 1) enhancing paracrine function using biomaterials, and 2) elucidating the mechanisms by which MSCs improve muscle regeneration.

Since MSCs are responsive to their environment, we hypothesized that cells cultured on biomaterials with varying structural cues could modulate the MSC secretome. MSCs were cultured in 3D hydrogels (alginate modified with RGD peptides) that were either microporous (pore size 100-125 µm) or nanoporous (pore size 5-10 nm). MSCs in microporous gels secreted significantly higher quantities of bioactive cytokines compared to encapsulated ones. In conditioned media (CM) experiments, microporous gel-CM strongly improved in vitro myoblast functions including proliferation and migration. The differences were attributed to N-cadherin mediated cell-cell interactions between MSCs that were promoted in the microporous gels, but were prevented in the nanoporous gels. Thus, hydrogels that promoted cell-cell interactions enhanced the paracrine function of MSCs.

Next, we performed in vivo experiments using a clinically relevant muscle injury model that, when left untreated, leads to long-term decline in muscle function and severe fibrosis. Transplantation of microporous gels containing autologous MSCs and the growth factors IGF-1 and VEGF led to significant tissue remodelling (scar tissue reduction, new myofiber formation, angiogenesis in injured regions) and improved muscle function (fast and slow twitch forces). Delivery of growth factors alone was not beneficial. The regenerative effects of MSCs on injured muscle were attributed to local immunomodulation by delivered cells. Compared to controls, muscles that were treated with MSCs showed a significantly reduced number of CD8+ cytotoxic T-cells and an accumulation of CD4+ regulatory T cells, and antibody based depletion of these immune cell populations confirmed our hypothesis.

In summary, MSCs delivered in structurally optimized hydrogels potently enhanced in vivo muscle regeneration by locally modulating immune cell populations via paracrine mechanisms.
Muscle loss associated with traumatic injury can result in significant functional impairment, and restoration of this lost tissue represents a challenging clinical problem. The use of human induced pluripotent stem cells (hiPSCs) constitutes a promising strategy for the generation of patient-specific, implantable tissues to repair damaged skeletal muscle. However, a lack of reliable methods with which to generate expandable human muscle myoblasts capable of forming functional myotubes has so far limited their use for muscle replacement therapy. We have recently established robust techniques for generating fusion-competent iPSC-derived myoblast lines, as well as methods to drive them to form functional skeletal muscle myotubes. We have also demonstrated the ability to enhance the maturation of primary muscle cells through the use of biomimetic matrix nanotopography, and have successfully generated structurally organized 3D skeletal muscle tissues based on this technology. Integrating hiPSC-derived myoblasts with our biomimetic 3D culture platform, we have generated a cell-dense 3D tissue model, with biologically-relevant extracellular matrix, to promote accurate cell-cell and cell-matrix interactions in skeletal muscle tissues in vitro. Furthermore, we have investigated the capacity for engineering the culture microenvironment, including electrical stimulation and topographical modulation of culture substrates, to promote hiPSC-derived myogenic construct maturation prior to in vivo implantation. These matured engineered tissues were implanted into the tibialis anterior muscle of a mouse model of non-regenerative volumetric muscle loss, and their ability to ameliorate loss of function was then investigated. Human PAX7::GFP expressing myogenic cells were used to evaluate the integration of our hiPSC-based tissue engineered muscle patch with murine host tissue. The methods derived in this study detail powerful new tools for more effective human tissue modeling in vitro, as well as new strategies for restoring skeletal muscle form and function in patients suffering from the after effects of severe traumatic injury or congenital muscle wasting conditions.
Heart transplantation is the definitive treatment for patients with severe heart failure due to ischemia-related disease and dilated cardiomyopathy. However, a lack of donor organs remains a longstanding and serious problem worldwide. Regenerative therapies are being investigated as an alternative approach and present new possibilities for the repair of a damaged heart. Direct implantation of dissociated cells has already been used clinically as a method to improve heart function by regenerating the myocardium and blood vessels, however it is often difficult to control the form, dimensions or the position of implanted cells. In an attempt to solve these problems, the research on reconstructing functional three-dimensional cardiac grafts using tissue engineering methods has now been indicated as a treatment. Our laboratory has proposed an original tissue engineering technology called “cell sheet-based engineering” that stacks cell sheets to reconstruct functional 3-D tissues. The challenge to engineer organ-like tissues is an exciting new avenue to regenerative therapies. A major obstacle in myocardial tissue engineering remains insufficient oxygen perfusion into engineered myocardial constructs, which limits construct thickness to approximately 100µm. Therefore, to fabricate thicker and functional cardiac tissues, new technologies to control blood vessel growth are currently needed. We have developed a technique to engineer cardiac tissues with perfusable blood vessels in vitro. Using resected tissue with a connectable artery and vein as a vascular bed, overlay triple-layer cardiac cell sheets produced from co-culture with endothelial cells, and support the tissue construct with media perfused in a bioreactor. We showed that endothelial cells connect to capillaries in the vascular bed and form tubular lumens, creating in vitro perfusable blood vessels in the cardiac cell sheets. Besides, thicker engineered tissues can be produced in vitro by overlaying additional triple-layer cell sheets. Moreover, the vascularized cardiac tissues can be transplanted with blood vessel anastomoses. This technique may create new opportunities for in vitro tissue engineering and has potential therapeutic application. As a possible advanced therapy, we are now applying to fabricate large scale engineered structures that can function with the potential for circulatory support.

Lactate is a metabolite of glycolysis, commonly produced by cells consuming glucose. However, growing evidences suggest new roles for this molecule, as it has shown to act as a signalling molecule in many tissues, even able to influence gene expression and epigenetic programs and regulate tumorigenesis or vascularization1,2. In this work, we explore the effects of lactate on cardiac cells for tissue engineering applications. Our results demonstrate that lactate enhance cardiomyocyte proliferation by increasing the number of ki67 positive cells. Additionally, the expression of the stem cell marker c-kit is increased in the presence of lactate, which also modulates different cell cycle related proteins, supporting thus the idea that this molecule can be able to reprogram cardiomyocytes towards a more immature stage. Cardiac fibroblasts also show a dose-dependent response to lactate by modifying their secretome and reducing collagen production, hence promoting a suitable environment for cardiac regeneration and reducing tissue fibrosis. Ex vivo culture of mouse hearts revealed the ability of lactate to increase survival of cardiomyocytes as well as to prolong the beating capacity of the cardiac tissue after 4 days. Histological analysis of the hearts also confirmed the in vitro results.

With all these new evidences of the action of lactate, we cultured cardiac cells on a 3D scaffold based on collagen and elastin, allowing engraftment and beating of the cardiac tissue. The response of such system to external electrical stimulus was evaluated using a pulsatile electric field stimulation, showing a proliferative and more immature behaviour of the tissue in the presence of lactate. Cardiac cells also showed expression of specific lactate receptors and transporters, such as MCT1, MCT4 and GPR81. The correct development of sarcomeric electric field stimulation, showing a proliferative and more immature behaviour of the tissue in the presence of lactate. Cardiac cells also showed expression of specific lactate receptors and transporters, such as MCT1, MCT4 and GPR81. The correct development of sarcomeric structures was confirmed, as well as the coupling and presence of intercalated disks.

In conclusion, lactate arises as a novel and feasible option to promote cardiac regeneration, and therefore lactate-releasing scaffolds are a suitable strategy for cardiac tissue regeneration.

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2. Schneider C. C. Cell Physiol Biochem 30, 1547-1556, 2012
Scaffold-mediated repair of tendon injuries in large animal models that mimic human tendon disease -- the good, the bad and the ugly

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Tendon injuries affect tendons either outside (extra-thecal) or within (intra-thecal) a synovial environment, such as a tendon sheath, bursa, or joint, and are common in horses and humans. Natural healing occurs by fibrosis although this is frequently associated with dysfunctional repair. The presence of synovial fluid in intra-thecal tendon injuries also has adverse effects on healing. The effectiveness of current treatments has been questioned and so there is interest in developing biological treatments to enhance repair. This has led to the wide-spread use of mesenchymal stem cells (MSCs) in equine clinical practice but its effectiveness has been limited by a rapid loss of cells when implanted directly into tendon, and an inability of cells injected into an adjacent synovial cavity to adhere to intra-thecal tendon defects. For these reasons, we have investigated scaffolds to address these specific issues with the hypotheses that scaffolds will improve cell retention following intra-tendinous implantation and improve intra-thecal tendon healing by sealing tendon defects from adverse effects of synovial fluid.

Scaffolds for extra-thecal tendon healing: We have measured cell retention when cells are combined with injectable hydrogel and polyacrylamide gels. Cells were highly viable (>95%) in both injectable gels but their use in vivo in naturally-occurring equine tendon injuries did not improve cell retention and the hydrogel induced reaction at the injection site.

Scaffolds for intra-thecal tendon healing: A bilayered electrospun and woven polydioxanone (PDO) scaffold was tested in an intra-thecal critical tendon defect model in female adult sheep. Three months after cell-free scaffold implantation there were no local or systemic signs of excessive inflammation. All the tendon lesions healed with a mild local inflammatory reaction and with minimal-to-mild adhesion formation. Significant proliferative fibroblast infiltration was observed within and immediately adjacent to the implanted scaffold which was accompanied by an extensive network of new blood vessel formation. Although fibroblasts were the most abundant cell type, CD45+, CD4+ and CD14+ cells were also present with a few foreign body giant cells.

In conclusion, while caution should be exercised when using injectable gel scaffolds for intra-tendinous injection, our data would support the use of solid absorbable scaffolds to seal intra-thecal tendon defects to enhance intra-thecal tendon healing.

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How long do implanted mesenchymal stem cell exist at injury site in tendon tissue? a cell tracking study

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Introduction:
Injured tendon tissue can be regenerated by signaling molecules secreted by implanted mesenchymal stem cells (MSC), rather than by differentiation of implanted MSC themselves into new tenocytes. Either of these putative mechanisms, could be optimized if a high number of implanted MSCs could be attracted to the injured tissue and survive for sufficient time to induce tendon regeneration. In this study, we have tried to address the fundamental question for how long do implanted MSCs persist at the injury site in tendon.

Materials and methods:
Genetically labelled bone-marrow derived MSCs were injected into naturally occurring tendon lesions (n=14) and also surgically induced experimental lesions (n=6) in equine superficial digital flexor tendon. Lesions were classified into acute and chronic phase pathophysiologically. The tendon tissue at the site was harvested at 1, 3, 7 and 14 day post-implantation. Standardised sections were prepared from tissue taken with ultra-sound guidance at of cell implantation. The percentage of sections in which fluorescence was observed was taken as the "positive rate", and recorded for each horse. Criteria of "positive" was not considered as the number of cells, rather the overall level of fluorescence. The average "positive rate" at each successive time point was compared.

Results:
MSCs were observed mainly in or close to the endotenon regardless time point and healing phase. The "Positive rate" was 59.0, 93.8, 67.2 and 1.9% at 1, 3, 7 and 14 days respectively. Although the positive rate at 7 day was high because of the criteria not considering the actual number of positive cells, positive cells were extremely few, and indicated that few MSCs survived post-implantation.

Discussion:
We concluded that most implanted MSCs disappeared quickly from the injury site between at 3 and 7 days post-implantation. Interestingly, implanted cells were mainly located within or near the endotenon; even at day1 and 3. This may suggest that the MSCs diffused vertically and laterally through the tendon tissue via the endotenon or the boundary between tendon bundle and endotenon, by physiological pressure by injection. Analysis of an ultrasound video at time of injection showed a flow of implanted cells to the surrounding tissue, via the needle tract or damaged areas of epitenon. Such flow might continue on the tensional equine tendon even by walking after treatment. This might lead to the reduction of implanted cells at lesion.
Adult mesenchymal stromal cells (MSCs) isolated from bone marrow and other tissues have been extensively tested in the treatment of bone and cartilage repair and in osteoarthritis. In addition, fully allogeneic therapy has been effective in the treatment of graft-versus-host disease and other conditions. However, there are many aspects of the biology of MSCs that are poorly described, and a more exhaustive characterization is necessary to exploit these cells fully in the context of tissue repair. These include (1) development of new cell-specific markers, (2) deciphering the therapeutic mechanism of action and unravelling the paracrine signals that contribute to tissue repair, (3) understanding clonal heterogeneity in cultured populations, (4) ensuring that batch variability is controlled and (5) understanding the nature of host immunomodulation by transplanted MSCs and allogenicity.

While the preclinical and clinical testing of MSCs has advanced rapidly over the last few years, the development of efficient and standardised methods for their isolation and characterization has not. Most attempts to isolate these cells depend on adherent culture systems with a significant risk of preparing heterogeneous or poorly characterized populations. The uncertain nature of primary isolates of MSCs, the possibility of culture-induced heterogeneity and the lack of highly specific markers raise issues of regulatory compliance that may impede clinical testing. There is a risk that emerging clinical data will be compromised by this lack of standardization and that current methods of cell isolation may not result in the most therapeutically effective cells.

Adult articular cartilage has limited intrinsic repair capacity and current medical treatment options provide only symptomatic relief without significantly altering the disease progression or restoring cartilage integrity. In contrast, fetal mammals in the first 2 trimesters of gestation are capable of regenerating injured articular cartilage. In addition, fetal cells transplanted into an adult organism have been shown to retain their regenerative potential in skin, liver, tendon and cartilage models.

The aim of the presented study was therefore to identify key factors responsible for scarless regeneration in fetal versus fibrocartilaginous repair in adult articular cartilage as potential candidates to enhance adult healing.

In this study, we therefore compared fetal and adult protein regulation in response to cartilage injury by secretome proteomics using a standardized cartilage lesion model in musculoskeletally mature, healthy sheep and fetal lambs (gestational day 80) with approval by the animal welfare and ethics committees. In addition, we compared growth and differentiation properties of fetal and adult chondrocytes and mesenchymal stem cells to determine cellular contributions to the cartilage healing process. Time points for sampling for adults were based on the three stages of healing (inflammatory phase, reparative phase, remodelling phase). Fetal tissues were harvested at days 1, 3, 5, 7, 14 and 28 to establish the time line of regeneration.

Low variance across replicates in mass spectrometry data indicated good standardization and reproducibility of our experimental setup. Analysing the adult and fetal response to injury and their differences, revealed differential regulation of (i) proteins associated with immune response and inflammation, (ii) proteins specific for cartilage tissue and cartilage development and (iii) proteins involved in cell growth and proliferation. Fetal cells maintained better proliferation and chondrogenic differentiation in vitro.

Together these results encourage further work toward biomimetic solutions to favourably shift the adult cartilage healing milieu to a more fetal phenotype to induce regeneration by recapitulating cartilage ontogeny.

MSC Therapy for Osteoarthritis: Status and Obstacles
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Regenerative Medicine Institute, National University of Ireland Galway, Ireland

Adult mesenchymal stromal cells (MSCs) isolated from bone marrow and other tissues have been extensively tested in the treatment of bone and cartilage repair and in osteoarthritis. In addition, fully allogeneic therapy has been effective in the treatment of graft-versus-host disease and other conditions. However, there are many aspects of the biology of MSCs that are poorly described, and a more exhaustive characterization is necessary to exploit these cells fully in the context of tissue repair. These include (1) development of new cell-specific markers, (2) deciphering the therapeutic mechanism of action and unravelling the paracrine signals that contribute to tissue repair, (3) understanding clonal heterogeneity in cultured populations, (4) ensuring that batch variability is controlled and (5) understanding the nature of host immunomodulation by transplanted MSCs and allogenicity.

While the preclinical and clinical testing of MSCs has advanced rapidly over the last few years, the development of efficient and standardised methods for their isolation and characterization has not. Most attempts to isolate these cells depend on adherent culture systems with a significant risk of preparing heterogeneous or poorly characterized populations. The uncertain nature of primary isolates of MSCs, the possibility of culture-induced heterogeneity and the lack of highly specific markers raise issues of regulatory compliance that may impede clinical testing. There is a risk that emerging clinical data will be compromised by this lack of standardization and that current methods of cell isolation may not result in the most therapeutically effective cells.
**35-SY-1**
New Era of Periodontal Regenerative Therapy
- From Cytokine Therapy to Stem Cell Therapy-

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Enhancing the biological potential of mesenchymal stem cells within periodontal ligament and stimulating the periodontal regeneration are recognized as being clinically possible. Basic Fibroblast Growth Factor (FGF-2) is known to stimulate the proliferation, migration and differentiation of a variety of cell types and to strongly induce angiogenesis. In randomized controlled double-blinded clinical trials conducted in Japan, a significant difference in % increase in alveolar bone height at intrabony defectsof the periodontitis patients was demonstrated by standardized radiographs between Placebo Group and 0.3%-human recombinant FGF-2 Group at 9 months after the treatment(1,2). This FGF-2 drug has finally become commercially available (Regroth®) in Japan. We have also demonstrated that auto-transplantation of adipose-tissue derived multilineage progenitor cells (ADMPC) together with fibrin gel into intrabony defects of periodontitis patients stimulated the neogenesis of alveolar bone in the applied sites at 9 months after the transplantation(3,4). These suggest that not only cytokine therapy but also stem cell transplantation therapy is a promising option to stimulate periodontal regeneration in the near future. In this symposium, I would like to demonstrate the data of mode of actions, efficacy and safety of FGF-2 therapy and ADMPC transplantation therapy and discuss the possible applications of these therapies to craniofacial regeneration therapy in the near future.

References

Acknowledgments
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The establishment of safety and efficacy evaluation for allogeneic periodontal ligament derived multipotent mesenchymal stromal cell sheet with next-generation sequencer

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Periodontitis results in the destruction of tooth-supporting periodontal tissue, and would not heal spontaneously. Various approaches have been introduced to regenerate periodontal tissue, however, the efficacy was limited, especially in severe cases. Our institute performed a single-arm and single-institute clinical study and observed the safety and efficacy of autologous periodontal ligament-derived mesenchymal stem cell (PDLMSC) sheets in 10 patients with moderate to severe periodontitis. These therapeutic effects were sustained during a mean follow-up period of 60 months, and there were no complications. To expand this treatment, we shifted from autologous to allogeneic transplantation. In this study, we analyzed the next-generation sequencing data of PDL-MSCs (owned by Tokyo Women’s Medical University), other MSCs (in a database), and ES cells (in a database) and chose the MSC-specific marker genes and genes preferentially expressed in healthy and proliferative cell line for efficacy evaluation. These genes will be used in clinical trial for choosing PDL-MSCs that are more effective for regenerative therapy. In addition, to evaluate the microbial contamination of cell sheet products, we analyzed 242 ENCODE human RNA-seq data and evaluated pathogen identification from the RNA-seq data generated from human sample using a computational pipeline. Moreover, to confirm the accuracy of these pipeline, we analyzed DNA- or RNA-seq data generated from human samples, which were spiked either bacteria or mycoplasmas, respectively. As a result, these pipelines have shown able to detect the pathogen genomes from human samples using DNA-seq, which is consistent with the PCR results. Further studies are needed in order to investigate whether these new methods will be able to become an alternative method for detecting microbial contamination.

Tissue-engineered autologous cartilage for cleft lip-nose patients

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Patients with cleft lip-nose have been treated with autologous transplantation using rib cartilage, nasal septum cartilage, auricular cartilage, iliac bones or fascia lata, or alloplastic materials including silicone and hydroxypapitate. The present autologous grafts have shortcomings in morphological and mechanical incoherence, while the alloplastics have been associated with foreign body reactions. We explored an alternative approach using tissue-engineered autologous cartilage for correction of the nasal deformity, in multicenter clinical trials. Eleven patients, aged 17-29 years, suffering from cleft lip-nose, were identified as candidates for open rhinoplasty using this cartilage. A auricular cartilage biopsy was obtained from each patient. Chondrocytes were grown in culture, and placed within porous biodegradable scaffold made of poly L-lactide, which shape was rod-like with 5 cm long, 6 mm wide and 3 mm thick. About 7 weeks after the biopsy, the tissue-engineered constructs were transplanted into the nasal bridge by open rhinoplasty. We confirmed safety, based on degree and frequency of adverse events. Moreover, we analyzed cephalogram and functional outcomes to know its usefulness. Follow-up range was 12-24 months (mean 18 months). Each tissue-engineered cartilage fulfilled the defined release criteria for transplantation. The transplantation of the tissue-engineered cartilage in all the patients was performed just as planned. We did not experience any serious adverse events that were related to the tissue-engineered cartilage. Nose shapes improved in all the patients, and more than 2 mm of nose augmentation maintained, as measured in cephalogram. The inconvenience generally improved during the post-surgical course. In conclusions, the tissue-engineered autologous cartilage, produced with autologous auricular chondrocytes placed within poly L-lactide scaffolds can be safely and advantageously used for patients who need correction of cleft lip-nose.
Periodontitis is a chronic inflammatory disease that leads destruction of tooth-supporting tissues, periodontal tissue. Due to unsatisfied efficacy of current periodontal regenerative therapies, ‘true’ periodontal tissue engineering has to be established by development of new cell-based therapy.

We previously revealed that autologous transplantation of adipose tissue-derived multi-lineage progenitor cells (ADMPC) significantly induced the periodontal tissue regeneration in preclinical studies using experimental periodontitis models on beagle dogs. Then we moved on to a clinical study to evaluate the efficacy and safety of ADMPC transplantation for periodontal tissue regeneration.

We conducted an open-label, single-arm exploratory phase I study in accordance with the Ministry of Health, Labor and Welfare guidelines on clinical stem cell research and Act on the Safety of Regenerative Medicine. The protocol was reviewed and approved by the ethical review committee at Osaka University Dental Hospital. 12 periodontitis patients who provided written informed consent were enrolled in this clinical study. ADMPC was isolated from subcutaneous adipose tissue and cultured in a cell processing isolator at Osaka University Dental Hospital, Center for Translational Dental Research. Each patient underwent flap operation in accordance with the modified Widman procedure during which autologous ADMPC were transplanted to the bone defect with a fibrin carrier material. Until 36 weeks after the transplantation, we performed a variety of clinical examinations including periodontal tissue inspection and standardized dental radiographic analysis. Thirty-six week follow up demonstrated that ADMPC transplantation resulted in reduction of probing pocket depth, improvement of clinical attachment level and neogenesis of alveolar bone. The therapeutic efficiency was observed not only in 2- or 3-walled vertical bone defects but also in more severe periodontal bone defects. In terms of safety, no severe transplantation-related adverse events were observed in any cases. These results suggest that autologous ADMPC transplantation can induce periodontal regeneration. Further clinical study is required not only to validate the efficacy but also to reveal the indications of the ADMPC transplantation.
The substantial burden of myocardial infarction, with nearly 790,000 annual incidences and 14% mortality rate, has been evidently apparent on nationwide health. Over the past decades, hydrogel-based biomaterials have been at the center of attention for developing functional cardiac tissues to induce regeneration of infarcted myocardium. However, unlike the tight electrically coupled architecture of cardiomyocytes (CMs) within the native myocardium, hydrogel matrices typically do not offer conductive microenvironments. This deficiency can interfere with electrical signal propagation within the hydrogel construct, leading to slow conduction velocity and unsynchronized contractions. Furthermore, CMs typically have low adhesion affinity to hydrogels that can negatively influence the cellular retention and spreading, which results in poor expression of sarcomeric α-actinin (SAC) and connexin43 (Cx43) gap junctions.

In this work, we developed a photo-crosslinkable hybrid hydrogel composed of gelatin methacrylate (GelMA) incorporated with gold nanorods (GNRs) for cardiac tissue engineering applications. The embedded GNRs enhanced electrical conductivity and mechanical stiffness of the GelMA hydrogels. Cardiac tissues were formed by seeding of neonatal rat CMs on both GelMA-GNR and pristine GelMA. GelMA-GNR hydrogels significantly improved the retention and spreading of CMs, which potentially are attributed to the enhanced stiffness and cell anchoring sites provided by the GNRs. Increased cellular retention and spreading led to the expression of uniaxially aligned and extended sarcomeres along with homogenously dispersed Cx43 gap junctions. Moreover, electrically conductive GelMA-GNR tissues exhibited synchronized Ca\textsuperscript{2+} sparks and contractions as well as reduced voltage excitation threshold. Next, we introduced micro-grooved topographies to induce anisotropic native-like organization of CMs on GelMA-GNR constructs. Concrete patterns of anisotropic myofiber assembly with enhanced expression of SAC and Cx43 were predominantly observed on GelMA-GNR micro-topographies. Overall, the proposed GelMA-GNR constructs, featuring enhanced electrical conductivity and tissue contractile properties, offer great potentials for engineering functional cardiac patches as a treatment for myocardial infarction.
37-SY-3 3D Bioprinted Functional and Contractile Cardiac Tissue Constructs

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Wake Forest Institute for Regenerative Medicine, Wake Forest School of Medicine, USA

Bioengineering of a functional cardiac tissue composed of primary cardiomyocytes has great potential for myocardial regeneration and in vitro tissue modeling. However, its applications remain limited because the cardiac tissue is a complex and highly organized structure with unique physiologic, biomechanical, and electrical properties. In this study, we undertook a proof-of-concept study to develop a contractile cardiac tissue with cellular organization, uniformity, and scalability by using three-dimensional (3D) bioprinting strategy. Primary cardiomyocytes were isolated from infant rat hearts and suspended in a fibrin-based bioink to determine the printing capability for cardiac tissue engineering. This cell-laden hydrogel was sequentially printed with a sacrificial hydrogel and a supporting polymeric frame through a 300-μm nozzle by pressured air. Bioprinted cardiac tissue constructs had a spontaneous synchronous contraction in culture, implying in vitro cardiac tissue development and maturation. Progressive cardiac tissue development was confirmed by immunostaining for α-actinin and connexin 43, indicating that cardiac tissues were formed with uniformly aligned, dense, and electromechanically coupled cardiac cells. These constructs exhibited physiologic responses to known cardiac drugs regarding beating frequency and contraction forces. In addition, Notch signaling blockade significantly accelerated development and maturation of bioprinted cardiac tissues. Our results demonstrated the feasibility of bioprinting functional cardiac tissues that could be used for tissue engineering applications and pharmaceutical purposes.

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37-SY-4 Electrically Conductive Multi-Dimensional Scaffolds with Controllable Architecture for Skeletal Muscle Engineering

Megane Beldjilali Labro, Alejandro Garcia, Firas Farhat, Murielle Dufresne, Fahmi Bedoui, Jean-François Grosset, Cecile Legallais, Rachid Jellali

A major ongoing challenge in the skeletal engineering field is the design of scaffolds able to mimic the native skeletal muscle to provide in vitro proliferation, differentiation and alignment of elongated myotubes. Indeed, the analysis of literature data suggests that both exogenous chemical and physical factors can play an effective role in inducing cellular alignment in engineered tissues. The aim of this study was to investigate the synergistic effects of topographical, mechanical and electrical stimulations cues on skeletal muscle cells’ development.

To achieve our goal, we propose to develop a multi-scale scaffold, using electrospun fibers of poly(ε - caprolactone (PCL) with electrically conductive elements and photolitographed micropatterned Poly(ethylene glycol) (PEG) hydrogel. The culture of C2C12 myoblasts cell line was carried out over a period of seven days by applying or not uniaxial stretch and/or electrical stimulation. The cells were exposed to cyclic stretch (5% stretch at 1Hz) for 2 h per day. Carbon electrodes connected to a generator were used to apply a continuous electric current of 90 mV/mm for 1 h per day. Particular attention was paid on the quantification of cell proliferation, alignment and fusion index, assessed by fluorescent staining, MTT assay, and RT-PCR.

We thus examined whether geometrical multi-scale scaffold could guide morphogenesis of skeletal muscle cells. We showed that the geometrical cues can significantly influence the alignment and differentiation process of C2C12. When associated with electrical and/or mechanical stimulations, an increase was displayed in myogenic differentiation and myotube alignment.

We studied more appropriate environments, which induce cell alignment and elongation for myogenesis by combining physical stimulation and hybrid material scaffolds. In the long run, we highlighted the possibility of a potent, effective approach to regulating muscle tissue regeneration.

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**37-SY-5** The effect of increasing dosage of atmospheric dielectric barrier plasma discharge (DBD) on the surface chemistry and topography on electrically conductive electrospun PLCL/PANI biomaterials

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**Introduction**

Plasma surface treatments such as DBD are routinely used to enhance cellular attachment and differentiation in tissue engineering. Electrically conductive polymers have become increasingly important as a method of promoting cellular response and monitoring real time cell culture in vitro. Here we investigate the effects of increasing DBD plasma processing on the surface physical and chemical properties of electrospun PLCL/PANI an electrically conductive polymer.

**Methodology**

Electrically conductive PLCL/PANI (4:1) composite polymer was manufactured from 10% w/w PLCL and 3mg/ml PANi in chloroform/DMF solvent. Polymers were electrospun at 12 cm distance and 20kV to produce a randomly aligned 50µm disc. This was exposed to increasing dosages of DBD plasma under atmospheric conditions. After a 48-hour resting period the samples were characterised using wettability analysis, SEM, AFM, FTIR and XPS.

**Results**

Randomly aligned, electroconductive electrospun PLCL/PANI matrices 50µm of thickness, with an average fibre diameter of 1.75µm were manufactured. The effect of the increasing DBD treatment caused a dose dependant statistically significant enhancement in their wettability until the point where surface melting was observed. SEM revealed a dose dependant beneficial change in fibre morphology and topography at low DBD dosages up to 500w. However, higher dosages above this caused polymer fibre fractures and surface melting changes to be observed. FTIR analysis following DBD plasma treatment showed no chemical changes to the bulk properties of the polymer. AFM showed alterations in the topography of the individual surface fibres. Chemical XPS analysis showed an increase in surface oxygenation in plasma treated samples a desirable quality in a cell culture biomaterial.

**Conclusions**

Electrically conductive polymers have many applications in tissue engineering. The problems associated with synthetic medical polymers are their inherent hydrophobicity and poor surface chemistry. The research presented here clearly shows that atmospheric DBD plasma treatment alters the surface topography to improve surface wettability. Despite DBD being a cold plasma technology thermal damage to the individual fibres was observed at higher DBD dosages. 500w DBD treatment is the optimal DBD dosage to surface modify electrospun PLCL/PANI matrices balancing the need for improved surface wettability against polymer thermal degradation.

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**38-SY-1** Bioengineered stem cell niches

Vunjak Novakovic, Bohao Liu, John O'Neill, Jinho Kim, Kacey Ronaldson-Bouchard

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Tissue engineering is becoming increasingly successful with authentically representing the actual environmental milieu of the development, regeneration and disease. The classical paradigm of tissue engineering involves an integrated use of human stem cells, biomaterial scaffolds (providing a structural and logistic template for tissue formation) and bioreactors (providing environmental control, and dynamic sequences of molecular and physical signaling). This biomimetic approach results in an increasingly successful representation of the environmental milieu of tissue development, regeneration and disease. Living human tissues are now being engineered from various types of human stem/progenitor cells, and tailored to the patient and the condition being treated. A reverse paradigm is now emerging with the development of platforms for modeling of integrated human physiology, using micro-tissues that are derived from human induced pluripotent stem cells and functionally connected by vascular perfusion. In both cases, the critical questions relate to our ability to recapitulate the cell niches, using bioengineering tools. This talk will discuss the biomimetic approach as the common underlying principle for tissue engineering, and the design principles for cell niches in regenerative medicine, modeling of disease, and drug screening applications. The specific examples include engineering of clinically sized, anatomically correct living bone, whole lungs and bones, whole lungs and a cardiac patch for heart repair.

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Substrate Stiffness Influences Doxorubicin-Induced Growth Suppression via p53 Activation

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The TP53 gene, which encodes p53, is one of the most well-characterized tumour suppressor genes. p53 dysfunction is usually associated with treatment failure and poor outcome in cancers. Recently, accumulating evidences have shown that extracellular matrix (ECM) stiffness in mechanical microenvironments affects cancer cell behaviours like proliferation, metastasis, and resistance to chemotherapy. However, it is still unclear if mechanical microenvironments interfere with p53 activation. To address this issue, we evaluated the effect of the anti-tumour drug, doxorubicin, on the growth inhibition of breast cancer MCF-7 cells cultured on gelatin-conjugated acrylamide gels having different elasticities.

Cell growth inhibition by doxorubicin treatment was reduced when the cells were cultured on soft substrates. As an underlying mechanism, we found that culturing cells on soft substrates diminished the doxorubicin-induced activation of p53 by attenuating the YAP-mediated expression of ROCK2. Notably, the actin cytoskeleton needed to be disassembled for the downregulation of the YAP-ROCK2-p53 axis on soft substrates. Our results reveal that substrate stiffness and actin cytoskeleton integrity are key factors that influence the tumour suppressing function of p53.
**38-SY-4 Tissue Adhesive, Rapid Forming, and Sprayable ECM Hydrogel for Stem Cell Delivery**

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We report on a tissue adhesive hydrogel based on novel recombinant tyrosinase mediated crosslinking for in situ stem cell delivery system. The adhesive hydrogels were fabricated by the site-directed coupling of tyramine-conjugated hyaluronic acid (HA_t, 1% w/v) and gelatin (3% w/v) (HG_gel) with novel tyrosinase derived from Streptomyces avermitilis (SA_Ty). The enzyme-based crosslinking by SA_Ty was fast, with less than 50 seconds for complete gelation, and the SA_Ty based crosslinking significantly enhanced the physical properties and adhesive strength of the hydrogel with the native tissue samples. Furthermore, by optimizing the injection conditions, we tailored the enzyme-based crosslinking hydrogels to be injectable and sprayable with a medical syringe and commercial airbrush nozzle, respectively. An in vivo analysis of the adhesive hydrogel showed a negligible immune reaction. Finally, we demonstrate that this novel enzyme-based crosslinking hydrogel has a robust potential for reconstituting stem cell niche upon in situ injection.

**38-SY-5 Multiphoton 3D microprinting of protein microstructures and micropatterns for cell niche studies**

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In native tissues, cells reside in a specialized niche, sustaining their lifelong maintenance and determining their pattern of differentiation. The major constituents of cell niche include neighboring cells, soluble factors, extracellular matrix, topological features and mechanical information. These cell niche factors interact among one another simultaneously and present important signals to living cells. Reconstituting a cell niche in a highly regulated manner in vitro is critical for understanding how cells sense and interact with their niches and rationalizing scaffold and protein chip design for future applications. We have established a multiphoton photochemical crosslinking-based platform technology, which is an emerging 3D printing-based biofabrication technology with numerous advantages such as submicron resolution, non-heating and non-contact procedure, free-form fabrication and spatial control ability. The non-contact laser-based crosslinking makes it possible to functionalize the surface of extremely soft 3D microstructures at selected locations with specific densities without damaging their topological features. Here, the ability of the two-photon microfabrication platform in fabricating complex protein micropatterns with precisely controlled voxels, topological structures and porosity, mechanical properties, extracellular matrix niche factors, the ability to decouple the mechanical and matrix niche factors and the recent effort to fabricate single cell 3D microniche will be reviewed. Moreover, a number of example applications of the protein microstructures and micropatterns such as single cell traction force measurement and screening of multiplex cell niche factors will be given. Finally, the outstanding challenges in developing a truly programmable cell niche platform with user-defined specifications and spatially controlled heterogeneity will be discussed.
Yosuke Hiraoka, Hiroyuki Ida, Monika Patel, Hiroshi Tsukamoto

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Gelatin has been widely used in medical fields such as DDS, medical device and regenerative medicine including cell transplantation because of its high biocompatibility and biodegradability (1, 2). When it is applied to clinical step, it is important to remove endotoxin and inactivate viruses. In general, those issues can be resolved by high temperature processing or filtration. But, those techniques cannot be used for gelatin, because physical property is reduced by heat treatment and viscosity of gelatin solution inhibits filtration.

Here, we developed highly purified gelatin by new approach used alkali solution. Resulted endotoxin level was lower than 10 EU/g and virus inactivation was confirmed. On the other hand, the physical property was not changed. In addition, we confirmed that it showed negative results in safety tests including cytotoxicity, sensitization, intracutaneous reactivity and pyrogenicity. We named this innovative gelatin “beMatrix gelatin”. It has the potential to rapidly expand gelatin-based regenerative therapy.

(1) Young S1, Wong M, Tabata Y and Mikos AG. Gelatin as a delivery vehicle for the controlled release of bioactive molecules. J Control Release 109(1-3), 256, 2005


Clinical Experiences of Tissue Engineered Vascular Grafts in Pediatric Heart Surgery

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In 2001, we developed the first tissue engineered vascular graft (TEVG) for use in congenital heart surgery and confirmed its significant potential via an initial clinical trial. The primary graft-related complication was stenosis. The rational design of improved, second-generation TEVGs will be predicated on our understanding of the cellular and molecular mechanisms underlying the formation of TEVG stenosis. We reported our results using a murine model to investigate neovessel formation, the process by which a biodegradable tubular scaffold seeded with BM-MNC transforms into a living vascular conduit with the ability to grow, repair, and remodel, in which the neovessel forms from host-derived cells rather than from the autologous seeded cells. We suggested that the seeded cells participate in neovessel formation via a paracrine mechanism Taken together these findings suggest that macrophage infiltration into the TEVG is critical for neovessel formation and also provides a strategy for potentially predicting, detecting and inhibiting stenosis in TEVGs.

Tissue engineering provides a theoretical method for creating an improved vascular conduit. The feasibility of tissue engineering large caliber, autologous vascular conduits for use as venous interposition grafts for congenital heart surgery has been demonstrated in both large animal and in a human clinical trial. Early results suggest that the use of TEVAs is both safe and efficacious. The carefully designed clinical trial under the supervision of the FDA is now underway in the United States. The development of a readily available vascular graft constructed from autologous tissue that also has growth potential has dramatic implications for the field of congenital heart surgery.
Clinical translation of bone regeneration using rhFGF-2-impregnated gelatin hydrogel for early-stage osteonecrosis of the femoral head

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Osteonecrosis of the femoral head (ONFH) is a multifactorial disease that can cause femoral head collapse, pain, gait disorders. ONFH is common among young people in their 30s and 40s. In the clinic, although patients are diagnosed, 70%-80% of untreated patients experience femoral head collapse and have to undergo total hip arthroplasty. In the past decades, minimally invasive regenerative therapy has been desired for the early stages of ONFH. Cell therapy, proteins, and other bone substitutes have been proposed, and various types of cell therapies using autologous marrow cells or stem cells are already being attempted, though they have not yet become standardized. Noncellular therapeutic strategies using growth factors have also been proposed; however, verification in animal experiments has made little progress, primarily because of the absence of an animal model for femoral head-specific necrosis and secondarily because of the lack of a technique to locally deliver the growth factor. To help address this problem, we reported a new rabbit model in which early ONFH progresses to femoral head collapse and secondary osteoarthritis, similar to that in humans. In this model, we showed that a single local injection of recombinant human fibroblast growth factor (rhFGF)-2-impregnated gelatin hydrogel, which has superior slow-release characteristics, suppresses the progression of femoral head osteonecrosis. To translate this research to humans, the first in human clinical application of controlled release rhFGF-2 for precollapse ONFH patients was performed at our hospital starting in March 2013. Ten ONFH patients up to precollapse stage 2 underwent a single local administration of 800-μg rhFGF-2 gelatin hydrogel and were followed up. Primary outcomes included adverse events and complications. Secondary outcomes included changes in Harris Hip Scores, VAS pain scores, UCLA scores, radiological changes as determined via X-ray, CT, and MRI images. There were 14 adverse events (five patients). Patients completely recovered from all adverse events without problem. The surgery was performed with a minimally invasive technique (1 cm of skin incision), and walking was allowed from the day after surgery. Mean clinical scores improved significantly compared with before surgery. There was only one case of femoral head collapse, and it had the greatest necrosis volume fraction. The other nine cases did not involve ONFH stage progression, and collapse was prevented. CT and MRI images confirmed bone regeneration in the ONFH. Clinical application of rhFGF-2 gelatin hydrogel for precollapse stage of ONFH was feasible and safe. Our research is ongoing, further phase II multiple center study has been started in January 2016. Recruitment of 64 patients and clinical application of rhFGF-2 has been completed.

The regeneration facilitating effects of basic fibroblast growth factor impregnated biodegradable gelatin hydrogel as a novel therapeutic strategy for facial nerve paralysis

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Introduction: Temporal bone fracture occasionally leads to severe facial paralysis. Although incomplete or delayed onset of facial paralysis following temporal bone fracture calls for conservative management, the prognosis of severe facial paralysis is still poor even if facial nerve decompression is performed. Basic fibroblast growth factor (bFGF) is known to promote nerve regeneration following denervation and is currently commercially available as a medication in Japan. We reported that bFGF-impregnated biodegradable gelatin hydrogel facilitated regeneration of the damaged facial nerve as a result of its sustained release in an experimental study and the patients with severe Bell’s palsy and other facial paralysis.

Objective: The purpose of this study was to determine the regeneration-facilitating effects of bFGF-impregnated biodegradable gelatin hydrogel in patients with temporal bone fracture.

Methods: Seven patients with temporal bone fracture after more than 2 weeks following the onset of severe or complete facial paralysis corresponding to degree of denervation exceeding 90% by electroneuronography were treated with the new procedure. The facial nerve was exposed via the mastoid. A bFGF-impregnated biodegradable gelatin hydrogel was placed around the exposed nerve. Regeneration of the facial nerve was evaluated by the House-Brackmann (H-B) grading system. The outcomes were compared with the authors’ previous study, which reported outcomes of the patients who underwent conservative treatment.

Results: The recovery rate of the novel treatment was significantly better than the conservative treatment.

Conclusion: Advantages of this novel treatment are efficacy in the cases of severe or complete facial paralysis following temporal bone fracture. To the authors’ knowledge, this is the first clinical report of the efficacy of bFGF using a new drug delivery system in patients with temporal bone fracture.
41-SY-5 Tissue engineering technology of bFGF slow release system for various plastic surgical reconstructions

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Cartilage tissue is characterized by its poor regenerative properties, and the clinical performance of cartilage grafts to replace cartilage defects has been unsatisfactory. Recently, cartilage regeneration with mature chondrocytes and stem cell have been developed and applied in clinical settings. However, there are challenges with the use of mature chondrocytes and stem cells for tissue regeneration, including high cost associated with the standard stem cell isolation methods and decreased cell viability due to cell manipulation. Previous studies demonstrated that cartilage can be regenerated from chondrocyte clusters that contain stem cells. Based upon some of the existing techniques, the goal of the present study was to develop a novel and practical method to induce cartilage regeneration. A micro-slicer device was developed to process cartilage tissues into cubic micro-cartilage in a minimally-invasive manner. We evaluated the size of micro-cartilages and demonstrated that 100-400 µm micro-cartilage are optimal for generating high cell yield with collagenase digestion. In addition, canine autologous intra-fascial implantation of the composites of micro-cartilage and absorbable scaffold with a slow release system of basic fibroblast growth factor (bFGF) was carried out which demonstrated cartilage regeneration. Our results demonstrated that the extent of bFGF diffusion depends on the size of micro-cartilage, and that cartilage regeneration was induced most effectively with 200 µm micro-cartilage via SOX5 up-regulation. The technique was applicable in the field of Plastic and Reconstructive Surgery. Some of our clinical cases for hand surgery, facial fracture, and ear and eye socket reconstructions are to be demonstrated.

42-SY-1 3D fabrication of bioinspired structured bone substitutes for bone regeneration

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The repair and regeneration of large bone defects under load has remained a major clinical challenge for orthopaedic surgeons. In addition, the development of suitable synthetic scaffolds for use as tendon and ligament (T/L) prosthesis to treat T/L ruptures remains a significant challenge. This presentation will describe our strategies in synthetic platform to develop cell-free therapeutics to promoting bone and tendon regeneration in these challenging situations.

This presentation will describe our 1) three dimensional (3D) printed scaffold with different architectures and their effect on the rate and quality of bone formation in vivo. This work demonstrated that manipulating pore size and permeability, as a function of scaffold architecture, provides a useful strategy for enhancing bone regeneration outcomes. 2) engineering solution to develop synthetic T/L prostheses, based on fibre-reinforced hydrogels that mimic the hierarchical structure of the native human tendon. The composition of this new tendon and ligament allows for high strength and good in vivo tissue integration. Our technologies open avenues for skeletal and soft tissue regeneration in various clinical applications.
**Bone tissue engineering and regenerative medicine 2.0**

---Paradigm shift from “Proof-of-concept” to “Proof-of-value”

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Bone tissue engineering (BTE) strategy provides a promising approach for large bone defect treatment. Despite its first clinical application report in 2001, BTE strategy still stays as a laboratory technique rather than a regular clinical practice, with very limited clinical impact so far. In order to understand the major bottlenecks factors that hinder the fast clinical translation of BTE technology, the development of tissue engineering technology will be compared with computer technology in order to illustrate the influence of “Proof-of-concept (POC)” and “Proof-of-value (POV)” oriented research strategy on the translation of new technology. Generally, it can be regarded as the BTE 1.0 development stage for the past thirty-year’s R&D effort. BTE 1.0 stage is the POC oriented with the goal to prove the scientific feasibility and clinical efficacy and safety of BTE concept. In order to facilitate its wider clinical application and the final translation from a lab technique into a routine clinical therapeutic practice, we believe, in the subsequential BTE 2.0 stage, the focus of our research should be shift from POC to POV, whose mission is to improve and achieve sufficient clinical and commercial value of BTE strategy to replace the current technique. I will discuss and illustrate the Low-Value points of current BTE strategy, such as unavailability off-the-shelf, high cost, complicated manufacturing process and so on and share with you our POV research efforts and thoughts on how to conquer these problems one by one. We believe, similar to the development of computer industry, the next stage of POV-orientated R&D effort (BTE 2.0 stage) will be the most critical and essential stage in order to boost up the final translation of BTE strategy into the real clinical technique, and facilitate the commercialization and maturation of the new industry of tissue engineering and regenerative medicine.

**Hybrid-spheroids incorporating ECM mimicking nanofibers for tuning stem cell functions**

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Engineering 3D tissue construct has been an interesting approach as in vitro tissue surrogates to restore functions of damaged organs and/or advanced in vitro models for new drug development. Herein, we developed a micrometer scale hybrid-spheroid of stem cells by incorporating engineered extracellular matrix (ECM). The ECM mimicking fibers were prepared from fragmentation of electrospun fibers of biodegradable polymers and the fibers with approximately 100 - 150 mm in length were then combined with stem cell, which resulted in stable spheroidal constructs. The electrospun fibrous mesh of poly (ι-lactic acid) (PLLA) was aminolyzed to prepare fragmented fibers (FFs), which was then subsequently coated with cell-interactive functional groups. The surface modification can be further used to make mineral rich environment or reservoir for inductive signals for stem cells. The stem cells within the hybrid-spheroids showed significantly higher proliferation and viability. We used several types of mesenchymal stem cells for the study including cells from turbinate surgery and fat tissue, human turbinate mesenchymal stem cells (hTMSCs) and human adipose derived stem cells (hADSCs), respectively. Within the sphereoid, cells and fibers were homogenously distributed, and TEM analysis confirmed the cells were tightly bound to the fragmented fibers with maintaining cell-cell contacts. These characteristics led to significant growth factor secretion, and preservation of stemness under growth media. The mineral-deposited FFs induced osteogenic differentiation of hADSCs under general media. The sphereoids were then experimented for in vivo transplantation for bone regeneration. Collectively, fabrication of hybrid-spheroid with FFs can be promising strategy as a carrier for stem cells with improved survival and target-dependent direction of stem cell differentiation for therapeutic outcome.
### Temporal control of 3D tissue morphogenesis in artificial cellular microenvironments

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Xerostomia is often a consequence of autoimmune disease, radiation therapy or aging, and is related to dysphagia, aspiration pneumonia and dysarthria, which strongly impair the individual’s life activities. Therefore, it is crucial to understand salivary gland tissue development for developing new treatment methods for such diseases.

Submandibular gland tissue (SMG) shows unique morphological changes during its development. This morphological change, so called branching morphogenesis, can be seen in a variety of tissues including lung, kidney, mammary gland, pancreas and so on. This morphogenesis is regulated by the cross talk between epithelium and mesenchyme cells. In this tissue development, a variety of external and internal stimuli guide or organize the functional changes of cells in SMG tissue. In this context, we built platform by using hydrogel that can supply external mechanical stimuli which would be valuable to modulate SMG tissue growth in vitro [1].

By using this in vitro culture system, we investigated the presence of macrophages and the effect of macrophage colony stimulating factor (MCSF), a key regulator of macrophage differentiation, on salivary gland branching morphogenesis. Interestingly, we found that MCSF is one of the key factors for salivary gland tissue development, by regulating FGF-7 and FGF-10 expression and neuronal network development. We also applied the in vitro tissue synthesis model to confirm the effects of MCSF on the early stages of epithelial bud formation, and found that blockade of MCSF with specific antibody attenuates salivary gland development and neuronal innervation.

Thus, it was confirmed that our system is valuable as a new engineering approach to obtain new mechanisms of gland tissue development.


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### Treatment of hypoxic brain injury by exosomes from adipose-derived stem and progenitor cells

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Perinatal cerebral hypoxic-ischemic (HI) injury is the major cause of neonatal mortality during childbirth and resulted severe neurological deficits in survivors. The neurovascular unit (NVU) composes the main architecture of brain which is severely damaged to trigger the pathogenesis after injury. Adipose-derived stem cells (ASCs) is an ideal source for cell-based therapy with similar characteristic to the bone marrow mesenchymal stem cells. Transplantation of endothelial lineage cells (ELCs) can prevent the vascular damage and blood-brain-barrier disruption. Neural differentiation of stem cell provides alternative source for neural lineage cells (NLCs). ASCs can sense the microenvironmental cues for differentiating into ELCs using laminar shear stress and toward NLCs on chitosan-coated surface. Microenvironments cause cells to modulate its microRNAs (miRs) for signal transduction and differentiation. We recently discovered the synergic of ELCs and NLCs combination to prevent neonatal rat pups from HI brain injury. In this study, we further investigated the mechanism of miRs in ASCs differentiation and ELC-NLC interactions for the neurovascular regeneration. The miR expressions in ASCs, ELCs, and NLCs were profiled to identify new miRs and their direct target genes that regulate cell differentiation in response to microenvironments. The properties of secreted exosome were characterized by nanoparticle tracking analysis and transmission electron microscopy. When treating the conditional medium to the pro-inflamed cells, different medium from stem or progenitor cells showed various therapeutic outcomes. The isolated exosomes were applied to injured rats by local injection with embedding in hydrogel or systemic circulation via tail vein injection. The combination of ELC-NLC showed best inhibition of inflammation responses and prevention of cell death in damaged endothelial and neural cells. Thus, the exosomes from therapeutic cells is an important mediator to prevent brain injury.
Aging and a sedentary lifestyle lead to a reduction in bone quantity and quality, a decrease in muscle mass and strength, and postural instability, which increases the risk of skeletal fractures. Stem cells are vitally involved in tissue regeneration and homeostasis in later life. In adults, the mesenchymal stroma contains tissue-specific multipotent stem cells, MSC, that are found throughout the body. MSC have the potential to regenerate damaged tissue and thus represent an attractive candidate for clinical cell therapy. Differentiation potential and maximal lifespan of MSC depend on donor’s age and in vitro culture conditions. Whether these changes are due to alterations in the stem cell itself or caused by changes in the niche is being investigated.

It has been shown by us and others that as we age, the stringent controls on regulatory networks that guide MSC biology gradually become worse. Human and animal model studies show that aging influences two aspects of MSC: a decrease in the bone marrow MSC pool and a biased differentiation to adipocytes at the expense of osteoblasts. In advanced bone age, both the mass and mineral density of the cortical and spongy bone are steadily decreasing while more and more fat cells are being produced in the bone marrow. This phenomenon is termed “adipogenic switch”. We therefore focused on characterizing pathways driving MSC aging and propagating the adipogenic switch. It is believed that deviations within the MSC microenvironment, such as chronic inflammation, adverse secretory phenotype by senescent cells as well as age-specific exosomal excretion ultimately lead to these adverse manifestations.

Cellular senescence has evolved from an in vitro model system to study aging to a multifaceted phenomenon of in vivo importance since senescent cells in vivo have been identified and their removal delays the onset of age-associated diseases in a mouse model system. In order to understand how senescent cells that accumulate within organisms with age negatively impact on organ and tissue function, we have started to characterize miRNAs and RNA modifying proteins that are differentially expressed in early passage versus senescent cells and their functional role in the context of cellular and organismal aging. Thereby, we identified circulating miRNAs as bona fide members of the senescence associated secretory phenotype (SASP) that are transferred from senescent cells to their microenvironment or even the systemic environment. These miRNAs are transported via extracellular vesicles and recipient cells taking them up are altered in their cell fate, including altered osteogenic differentiation of mesenchymal stem cells.

In summary, we present evidence of the importance of specific miRNAs and highlight their potential use as biomarkers of aging and age-associated diseases like especially osteoporosis, or even as therapeutic tools and targets.
Using a Novel Histone Deacetylase Inhibitor to Enhance Human Bone Marrow Stromal Cell Osteogenic Differentiation In Vitro

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For functional tissue engineering, it is key to effectively control lineage specific differentiation of stem cells. The limitations of current strategies (e.g. gene therapy and iPSCs) has led to the search for alternative methods – such as epigenetic approaches which are capable of controlling stem cell fate without altering the genome. A novel histone deacetylase (HDAC) 3 selective inhibitor, MI192 has been widely used for cancer and rheumatoid arthritis therapeutics. This study aimed to investigate the potential of using MI192 to enhance in vitro osteogenesis of human bone marrow stromal cells (hBMSCs).

The effect of different MI192 concentrations on hBMSCs viability was assessed via AlamarBlue quantification. Following MI192 treatment, hBMSCs were cultured in osteoinductive conditions either in monolayer or a 3D micro-tissue model. Osteogenesis was confirmed by qPCR, biochemistry, in-cell western (ICW) assay and histological analysis.

A time-dose dependent decrease in cellular viability was observed following MI192 treatment where concentrations of MI192 (≥ 20 µM at 24 hrs; ≥ 10 µM at 48 hrs and ≥ 1 µM at 72 hrs), significantly reduced hBMSC viability compared to untreated cells (P ≤ 0.01). However, after 50 µM MI192 pre-treatment for 48 hrs followed by 14 days of osteogenic induction, there was a significant enhancement in alkaline phosphatase specific activity compared to untreated cells (P ≤ 0.001). This pre-treatment condition significantly upregulated hBMSC gene expression for osteogenic markers (Runx2, ALP, OCN and BMP2) during 21 days osteogenic culture compared with untreated cells (P ≤ 0.001). Similarly, ICW showed that MI192 pre-treatment significantly enhanced Runx2, ALP, OCN and BMP2 expression in hBMSCs throughout 28 days osteoinductive culture (P ≤ 0.01). Importantly, Von Kossa staining showed that MI192 pre-treatment substantially enhanced mineralisation after 28 days osteogenic culture.

MI192 pre-treated hBMSCs were cultured as pellets and assembled in a 3D printed PEGT/PBT scaffold (micro-tissue) and cultured in osteogenic medium for 6 weeks. MI192 pre-treatment extensively enhanced ALP, OCN, BMP2 and Col1α protein expression and extracellular matrix mineralisation within the micro-tissues compared to the untreated group.

These results show that MI192 is capable of promoting hBMSC osteogenesis in vitro, indicating the potential of this novel epigenetic approach for controlling hBMSC osteogenesis for bone augmentation.
**43-SY-5** Human induced pluripotent stem cells-based strategies for bone tissue engineering and regeneration during aging

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Background and aim: Compromised bone tissue physiology and the decreased function of bone forming cells during aging limit the native tissue capacity for healing. Thus, regeneration of bone defects in the elderly patients presents significant clinical and scientific challenges. We have previously reported on engineering of bone tissue substitutes from human induced pluripotent stem cells (hiPSCs) (de Peppo et al. 2013), an autologous source of potentially rejuvenated osteogenic progenitors available for any patient in unlimited quantities. The aim of our current project is to explore hiPSCs as a source of secreted factors and extracellular matrix for cell-free bone tissue engineering and to test the effects of specific components secreted by the hiPSC-progenitors on primary bone marrow stromal cells from traumatology patients of different ages. Methods: hiPSCs were expanded and differentiated into embryonic-like mesenchymal progenitors (hiPSC-MPs) with high proliferation and osteogenic differentiation potential, as in our previous studies. hiPSC-MPs were expanded in culture and used for the preparation of conditioned media (CM) as well as for in vitro engineering of extracellular matrix (ECM) layers. Major cytokine and growth factor components of the CM were determined using a Luminex array. ECM structure and composition were identified and compared before and after decellularization procedure by immunofluorescent staining (collagens type I and IV, fibronectin, laminin). Using a library of primary BMSCs exhibiting diverse proliferation and differentiation potentials, we found that hiPSC-MP-derived CM and ECM modulate the primary BMSC responses in a patient-specific manner. In particular, some aged BMSC lines only exhibited increased proliferation and osteogenic differentiation (alkaline phosphatase activity, osteogenic gene expression) when cultured in the presence of hiPSC-MP secreted components. Conclusions: Our studies suggest that hiPSC-MP-derived microenvironment components can modulate the regenerative potential of adult/aged BMSCs. Further investigation of patient-specific responses is expected to offer insight into the underlying mechanisms and guide the approaches for the engineering of bone tissue substitutes to enhance the regenerative responses in elderly patients. References: de Peppo GM et al. Proc Natl Acad Sci USA, 2013

**10054**

**44-SY-1** In Vivo Bioreactors for Craniofacial Tissue Regeneration

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Craniofacial defects are challenging to repair and result in significant patient morbidity. As a convergence science, tissue engineering represents an exciting paradigm to improve outcomes in craniofacial reconstruction. The in vivo bioreactor approach can be leveraged to create customized bony free tissue flaps of geometry specific to each individual defect. Using a large animal model, we have demonstrated the feasibility of using in vivo bioreactors to generate tissue-engineered flaps successful in reconstruction of large mandibular defects. These bioreactors can be 3D printed and are seeded with acellular scaffold without the need for additional growth factors. With this approach, we have also found that synthetic scaffold can be used to seed these bioreactors to reduce further donor site morbidity associated with autologous graft-based scaffold. In a sheep model of disease, we have demonstrated that different large mandibular defects, including the angle (1 x 4 cm) and superior diastema (0.5 cm), can be reconstructed using mineralized tissues generated from in vivo bioreactors. In addition, as these tissue-engineered flaps were generated within the patient, they are fully vascularized and can be anastomosed with local vasculature in the head and neck to ensure tissue viability after reconstruction. The in vivo bioreactor is an exciting new technology that holds promise for improving healthcare outcomes in the repair of large craniofacial defects.
In situ tissue regeneration has been shown a promising tissue engineering and regenerative medicine strategy for several applications, spanning from hard to soft tissues. Typically, in this approach a biomaterial with designed surface properties is implanted in a host which acts as an in vivo bioreactor for tissue maturation. After maturation, the engineered graft can remain in the implanted location if coincident with the final one or can be transplanted into the final site of interest.

Here, we present an in situ tissue regeneraton approach for the biofabrication of vascular grafts. The graft was engineered through the design of the surface properties of a polymeric rod that was used as a template for tissue regeneration. Different surface properties of the polymeric rods were screened in vitro and in vivo for enhanced vascular extracellular matrix (ECM) formation and macrophage polarization towards a tissue healing phenotype. Rods with optimal ECM formation and macrophage polarization showed to support also vascular tissue maturationn in small and large animal models.
44-SY-5
Spatially Patterned Atheroprotective Vascular Grafts for Enhanced Patency

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With 25 million American suffering from at least one clinical manifestation of atherosclerosis, and approximately 500,000 deaths annually attributed to coronary artery disease, there is a pressing need to better understand the biological basis for the development of atherosclerosis. Our previous work demonstrated that spatial patterning cues from extracellular matrices (ECMs) play an important role in modulating endothelial angiogenic phenotype including inhibiting adhesion of lipogenic proteins and monocytes in the circulating blood that leads to atherogenesis. Therefore, we hypothesized that vascular grafts containing longitudinally oriented spatial patterning will promote aligned re-endothelialization to provide atheroprotective function and improved graft patency, when compared to vascular grafts without cell patterning.

Spatially patterned vascular grafts (0.5cm length, 1mm diameter) were fabricated from electrospun 30% (w/v) poly (ε-caprolactone) in chloroform using a dual layer approach. For aligned grafts, PCL sheets were constructed with the aligned layer on the luminal side and the randomly-oriented layer on the outside. The orientation was reversed for randomly-oriented grafts. Grafts were heparinized using 2% (w/v) heparin in 0.05M 2-(N-morpholino)ethanesulfonic acid buffer pH 5.6. SEM of aligned inner layer shows uniform fibril alignment of fibers with 7-10mm diameters and a randomly-oriented outer layer with fibers of 15-20mm diameters. Heparin-conjugated grafts demonstrated a release rate of 202 μg per scaffold over a 48h period. Transplantations of heparinized acellular grafts into the rat carotid artery demonstrate patency by B-mode echo 24h and 7 days post transplant. Studies are currently underway to assess the long term patency and endotheliaztion of grafts transplanted into the rat carotid artery. Findings from these studies will provide novel insights into the mechanisms of resisting atherogenesis that are mediated by spatial cell patterning, and have important translational potential in the generation of atheroprotective vascular grafts that enhance graft patency.
Regeneration of elastic fibers after wound repair is poor, which limits the function of the repaired tissue. Biomaterials containing elastin may be used as elastic tissue replacement or to enhance the biosynthesis of elastin. It has been shown, for instance, that elastin degradation products can signal through the elastin receptor complex, which could result in elastin synthesis, cell proliferation and chemotaxis of cells. Elastin in biomaterials may also provide these biological effects upon implantation.

When applying elastin in biomaterials, one option is to use elastin purified from tissue. This can either be applied in its insoluble form or as solubilized elastin. Insoluble elastin is more difficult to handle than solubilized elastin, and is more prone to calcification. Insoluble elastin can be isolated from e.g. ligamentum nuchae, whereas solubilized elastin is obtained by hydrolysis of the insoluble elastin, thereby fragmenting the insoluble material. Methods used to make extracellular matrix-based scaffolds for regenerative medicine are discussed, incorporating either insoluble elastin fibers or solubilized elastin.

In vitro studies showed that insoluble elastin enhanced primary human keratinocyte proliferation, whereas solubilized elastin promoted proliferation of primary human fibroblasts. In addition, results from animal experiments upon subcutaneous implantation of these scaffolds showed that the application of solubilized elastin enhanced both angiogenesis and synthesis of elastic fibers in vivo. Biomaterials with solubilized elastin did not calcify in young rats, where biomaterials with elastic fibers showed calcification in young -but not adult- animals. Upon application of a skin construct containing solubilized elastin onto rats, augmented blood vessel formation was observed at early time points and an increased number of elastic fibers at later time points. In addition, less contraction and fewer myofibroblasts were detected.

These results show the potential of elastin purified from tissues in biomaterials.
**45-SY-3** Multifunctional, JNK-2 Gene Silencing Nanotherapeutics for Elastic Matrix Regenerative Repair in Abdominal Aortic Aneurysms

Anand Ramamurthi, Sarah Carney, Andrew Camardo, Thomas Broekelmann, Robert P. Mecham

Growth of abdominal aortic aneurysms (AAAs) is driven by degradation of elastic fibers due to overexpressed matrix metalloproteases (MMPs), where inability to repair or regenerate elastic fibers leads to further weakening of the aorta. Therapies must be pro-elastogenic and anti-proteolytic to arrest or regress AAA growth. Previously, we showed doxycycline (DOX) delivered via biodegradable poly(lactic glycolic acid) (PLGA) nanoparticles stimulated elastic matrix neoassembly and crosslinking in aneurysmal smooth muscle cell cultures with effects mediated by DOX inhibition of regulatory protein c-Jun-N terminal kinase (JNK). DOX inhibition of the JNK2 isoform incited attenuation of MMP-2 and upregulation of TGFβ1 resulting in reduced proteolysis, increased elastogenesis and increased lysyl oxidase (LOX)-mediated crosslinking of elastin, respectively. Since DOX and other JNK-2 inhibitor drugs are non-specific, we've investigated JNK-2 gene silencing in this study through JNK-2 siRNA delivery from nanoparticles. Exogenously delivered siRNA probes stimulated anti-proteolytic effects downregulating MMP-2 and -9 while increasing expression of lysyl oxidase like 1 (LOXL1) which catalyzes the formation of crosslinks in elastin. Due to the inability of exogenous siRNA to penetrate the cell membrane without a carrier, PLGA nanoparticles have been utilized as a transfection vector with cationic polyethyleneimine (PEI) incorporated into the polymeric matrix to increase anionic siRNA encapsulation efficiency. The addition of PEI facilitates increased electrostatic attraction between the cell membrane and nanoparticle surface causing greater localization of treatment delivery and potentially aiding in the diffusion of siRNA probes from the PLGA matrix. The PLGA nanocarriers were surface-functionalized with cationic amphiphiles previously shown to stimulate elastin crosslinking and provide anti-MMP effects due to combined effects of positive charge and long chain acyl groups presented by the amphiphiles. Our results in aneurysmal SMC cultures suggest that JNK-2 siRNA delivered from the NPs was biologically functional and stimulated regenerative elastogenic outcomes while simultaneously inhibiting proteolytic degradation of the elastic matrix. Our results also show synergy in elastin crosslinking and anti-MMP effects between the released siRNA and functionalized polymer nanocarriers. The results are promising towards a regenerative nanotherapy to arrest small AAA growth.

**45-SY-4** Elastin in cardiovascular biohybrids

Petra Mela

Elastin constitutes a fundamental component of the extracellular matrix (ECM) of many tissues (e.g. cardiovascular, lungs, skin, tendons) as it confers the physiological function of stretching and recoiling besides modulating cellular processes. The difficulty of isolating and processing the native elastin has led to the development of synthetic human tropoelastin and elastin-like-polymers. Among them, elastin-like recombinamers (ELRs) have emerged as a relevant class of materials for biomedical applications. They are obtained by recombinant technology and consist of the repetition of amino-acid sequences inspired by the VPGVG sequence of the human elastin with the additional possibility of inserting functional motifs to obtain desired bioactive properties (e.g. cell adhesion ability, protease sensitivity). ELRs show the advantages of an engineered material because of the exhaustive control over their composition by recombinant technology, while mimicking the biological performance of the elastin (i.e. elastic behavior, cytocompatibility, low thrombogenicity and the inverse temperature transition (ITT)). Here we show our efforts towards the realization of cardiovascular implants based on ELRs. A range of fabrication techniques is used to tune the structural properties to the intended application. Cell-based as well as cell-free strategies are explored where ELR are used in combination with other biomaterials and technical components.
The onset of a wound-healing process after skin damage leads to a scar tissue that gradually restores the skin barrier. The structure of this new skin lacks the elasticity of the original skin because a disruption in the elastin network and a higher collagen production. It is of great interest to manufacture scaffolds that provide the lost elasticity. A method to obtain fibrous scaffolds based on a clickable elastin-like recombinamers (ELRs) system that crosslink in situ during the electrospinning process itself, without need any further treatment to stabilize them and completely stable under in vitro and in vivo conditions could be of great interest on the skin tissue engineering field. Electrospun fibers usually need a post-treatment to stabilize them. Many of the crosslinking methods make use of solvents or organic compounds that are harmful to cells. We present the first system for obtaining fibers from clickable ELRs that crosslink during the flight of the fiber from the tip of the needle to the collector electrode. These ELR-click fibers do not need any further stabilization treatment and are completely stable under in vitro conditions. A wrinkled fiber morphology was observed in the SEM images, and both random and oriented fiber orientations, with a high degree of alignment and coherence, have been produced making use of a rotational electrode. An average tensile strength at failure value of 0.59±0.08 MPa after a maximum percentage of strain of 247.5±36.08%, with a mean Young modulus of 1.73±0.95 MPa was obtained, fitting with the ones found in the literature for fibers made of elastin or blends of elastin and other materials. These ELR-click fibers open up the possibility of incorporate different functionalities into each ELR that will contribute to the final overall fiber functionality. The cytocompatibility of these ELR-click fibers has been clearly proved by adhesion and proliferation studies during in vitro experiments in which the growth of keratinocytes and fibroblasts, on the ELR-click fibers is similar and follows the same trend as for the positive controls. The affinity of these two cell lines for the ELR-click fibers has also been proven, as can be seen from the videos recorded for both cell types during culture. Optical fluorescence images and a histological analysis of the growth of these two cell lines indicate the possibility of using scaffolds based on ELR-click fibers for wound dressings in skin tissue engineering applications.
Adipose-derived stem cells (ASCs) and stromal-vascular-fraction (SVF) promote nerve regeneration. Biodegradable artificial nerve conduits are used to treat peripheral nerve injuries, but their efficiencies are lower than that of autologous nerve graft. This study developed biodegradable hybrid nerve conduits containing ASCs and SVF and evaluated their facial nerve regenerating ability in rat models. Exposing the buccal branch of the facial nerve under anesthesia, a 7-mm nerve defect was made. SVF and ASCs were individually poured into nerve conduits with type I collagen as a scaffold (ASCs and SVF groups). The conduits were grafted to the nerve defect. As the control, the defect was bridged with PGA-collagen nerve conduit without cells. Re-innervation was confirmed at 13 weeks after transplantation by retrograde tracers, and regenerated nerves were evaluated physiologically and histologically. Compound muscle action potential of SVF group was significantly higher in amplitude than that of the control group (1.6 ± 1.2 mV vs. 0.7 ± 0.4 mV), though there were no significant differences between the amplitude values of the control and ASCs groups (0.7 ± 0.4 mV vs. 1.1 ± 0.8 mV) or those of ASCs and SVF groups (1.1 ± 0.8 mV vs. 1.6 ± 1.2 mV). The center of cross-section of the regenerated nerve was observed histologically. Toluidine blue stained specimens showed that the number of myelinated fibers of SVF group was highest (1278 ± 757) followed by those of ASCs group (913 ± 497) and of the control group (595 ± 528), no significant difference was observed among three groups. Electron microscopy showed that the axon diameter of SVF group was largest (4.5 ± 1.6 μm), followed by ASCs group (4.0 ± 1.3 μm), and the control group (3.7 ± 1.3 μm). SVF group had the largest fiber diameter (5.5 ± 1.7 μm) followed by ASCs group (5.1 ± 1.4 μm) and the control group (4.5 ± 1.5 μm). ASCs group had the highest myelin thickness (0.6 ± 0.1 μm), followed by SVF group (0.5 ± 0.1 μm) and the control group (0.4 ± 0.1 μm). Identical excellent promoting effects on nerve regeneration were observed in ASCs and SVF groups. Upon clinical application, SVF had a lower hurdle than ASCs, because only enzymatic process was required for its preparation. SVF could be more applicable for inducing nerve regeneration for treating nerve defects due to injuries.
The efficacy of a scaffold-free Bio 3D conduit developed from dermal fibroblasts on peripheral nerve regeneration in a canine ulnar nerve injury model

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Background
Autologous nerve grafting is widely accepted as the gold standard treatment for segmental nerve defects when direct repair cannot be achieved. However, to overcome its inevitable disadvantages, alternative methods such as the tubulization technique have been developed. Several studies have investigated the ideal nerve conduits modified with supportive cells, scaffolds, growth factors, and vascularity. Previously, we confirmed that biological scaffold-free conduits fabricated from human dermal fibroblasts promote nerve regeneration in a rat sciatic nerve injury model.1 The purpose of this study is to evaluate biological scaffold-free conduits that are entirely composed of each animal’s own dermal fibroblasts using a large animal model.

Materials and Methods
Six male beagle dogs were used in this study. Eight weeks before surgery, dermal fibroblasts were harvested from their groin skin and grown in culture. Bio 3D conduits were assembled from proliferating dermal fibroblasts using a Bio 3D printer. The ulnar nerve in their forearm was exposed under general anesthesia and sharply cut to create a 5 mm interstump gap, which was bridged by the prepared 8-mm Bio 3D conduit. Ten weeks after surgery, nerve regeneration was investigated.

Results
Electrophysiological studies revealed that compound muscle action potentials (CMAPs) of the hypothenar muscles and motor nerve conduction velocity (MNCV) were detected in all animals. Macroscopic observation showed regenerated ulnar nerves. Histological and morphometric studies confirmed the existence of many myelinated axons through the Bio 3D conduit. A small amount of hypothenar muscle atrophy was confirmed.

Conclusions
Hypothenar muscles were re-innervated by regenerated nerve fibers through the Bio 3D conduit. The scaffold-free Bio 3D conduit fabricated from dermal fibroblasts is effective for nerve regeneration in a canine ulnar nerve injury model. This technology would be useful for peripheral nerve injury and segmental nerve defects in clinical settings.

References

The Impact of Autologous Nerve Graft Phenotype and Extracorporeal Shockwave Therapy on Peripheral Nerve Regeneration in the Rat

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Although regeneration after autologous nerve grafting (ANG) has been the target of scientific curiosity since the beginning of modern medicine, not much progress in accelerating this tedious process has been made. An explanation could be the experimental model chosen. Most research groups use the sciatic nerve defect as a model for ANG, dismissing the influence of nerve graft phenotype on regeneration. We hypothesize that this mismatch has a negative influence on motor axonal regeneration and that extracorporeal shockwave therapy (ESWT) can ameliorate this effect. Our first aim was to establish a femoral nerve defect model reflecting the phenotypical difference of ANGs in the clinic. Second, we aim to evaluate the effect of ESWT on nerve regeneration using this model in vitro and in vivo. Adult male rats were divided in groups of at least 8 animals. A 6 mm autologous nerve transplantation was performed using either homotopic (matched) or heterotopic (mismatched) grafting. The treatment groups received ESWT once after wound closure. Regeneration was evaluated functionally, histologically, and by qRTPCR and western blot.

In vitro: Phenotypical differences were obvious between Schwann cells (SCs) derived from motor and sensory branches of the femoral nerve. Motor derived SCs exhibited less proliferation, and higher expression of myelination markers. ESWT increased proliferation and expression of pro-regenerative phenotype markers such as c-jun in all SCs.

In vivo: Motor nerve derived grafts showed less than 50% expression levels of pro-proliferative markers (Ki67,p75) in early stages of neuronal regeneration. Furthermore, electrophysiological and histological evaluations indicate slower regeneration of motor axons in the mismatched setting when compared to the homotopic (motor to motor) grafting. ESWT significantly increases expression of pro-regenerative genes at early timepoints and of markers for re-myelination (Cadm3) and homeostasis (TrkB) 6 weeks after injury in both groups. Furthermore all ESWT treated animals showed faster functional recovery when compared to untreated animals, indicating amelioration of negative effects of phenotypical mismatch. This study shows that ESWT is able to accelerate peripheral nerve regeneration in a successfully modified femoral nerve model which reflects the clinical reality after autologous nerve transplantation. This study provides support for the use of ESWT after surgical repair of peripheral nerve injuries.
**46-SY-5** Impact of Stem Cells Secretome and ECM-Like Hydrogels in Axonal Growth and Spinal Cord Injury Repair

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The low regeneration potential of the central nervous system (CNS) represents a challenge for the development of new therapeutic strategies. Mesenchymal stem cells (MSCs) have been proposed as a possible therapeutic tool for CNS disorders, namely due to the beneficial actions of their secretome. Indeed, the latter possesses a broad range of neuroregulatory factors that promote an increase in neurogenesis, inhibition of apoptosis/glial scar, immunomodulation, angiogenesis, neuronal and glial cell survival, as well as relevant neuroprotective actions into different pathophysiological contexts. Considering their protective action in lesioned sites, MSCs, and their secretome, might also improve the integration of local progenitor cells in neuroregeneration processes. In this sense their use could represent an important vehicle for the establishment of future CNS regenerative therapies. In the present talk the role of MSCs, and their secretome, on phenomena such as in vitro and in vivo neuronal/glial survival will be addressed. Additionally, their possible applications, alone or combined with ECM-like hydrogels, for Parkinson’s Diseases and Spinal Cord Injury regenerative medicine will also be presented. Finally, new trends on how to modulate the secretome MSCs will also be presented.

Keywords: Mesenchymal Stem Cells, Secretome, Central Nervous System, Regenerative Medicine

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**46-SY-6** Synergistic Local Delivery of Gene Recombinant Stem Cells and Methylprednisolone after Severe Rat Spinal Cord Injury

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Central nervous system diseases, such as spinal cord injury (SCI), always experience complicated dynamic pathology and are mostly incurable due to the limited self-repair capacity. Functional restoration after SCI calls for comprehensive solution for the loss of neurons, absence of extra cellular matrix and severe inflammation in the local lesion cavity, which has ranked among the most challenging projects. Stem cells are broadly considered promising therapeutic candidates for various diseases. Gene transfection of stem cells and stem cell-based nerve repair strategies have received extensive attention. This study is aimed to propose an effective solution by developing a composite implantation system and locally deliver the gene recombinant stem cells as well as drugs. Firstly, BDNF gene recombinant mesenchymal stem cells (MSCs) are prepared via a systemic study of non-viral transfection system. On the other hand, the anti-inflammatory drug methylprednisolone (MP) is the only FDA-approved drug for clinical treatment of SCI, but its application has been limited by the systemic toxicity. To overcome this problem, a novel preparation of MP, MP gelatin microsphere (MPGM), is developed in this study for local implantation. Hyaluronic acid is utilized to fabricate a scaffold which is further modified by an adhesive peptide PPFMLMKGSTR. After investigation of cytocompatibility and nerve repair functions of the scaffold, gene recombinant MSCs and MPGM are encapsulated to construct a composite implant. The composite system exhibits sustained drug release profile for more than 150h and elicits superior regenerative effect after implantation. Inflammation is alleviated by not only the drug-loaded implant, but also the implant with blank microspheres. Through investigations on different implants, the study also reveals the respective roles of gene modification, MSCs, MP and the scaffold in nerve tissue repair.
Silk proteins have emerged from the fabric of the textile world into a growing suite of biomaterials and medical utility over the past few decades. This progression started with new fundamental insights into this unique protein, and subsequently evolved into new materials with clinical impact. We will review the historical, research and translational steps that have supported new medical materials and devices based on silk. One key to this emergence has been to modify the native protein using new processing methods and chemistries to engineer new material features. Some of the strategies developed to morph silk, as a high molecular weight amphiphilic protein, into new biomaterials with new properties will be discussed. The utility of some of these new material formats in 3D printing, biomaterial scaffolding, tissue engineering and regenerative medicine will be presented. The needs for tunable, degradable, robust biomaterials for a range of medical goals remains high, and silk proteins offer a unique suite of options to help address these needs.

Cerium oxide nanoparticles (CNPs) have robust redox catalytic activity, owing to their unique physicochemical properties, such as surface area and chemistry regulated by the alternating [Ce3+]/[Ce4+] ratio. These properties significantly contributed to the bioactivity and antioxidant properties of CNPs, such as the scavenging of reactive oxygen species (ROS). We synthesized CNPs that have uniform and high catalytic properties, as observed by 3,3',5,5'-Tetramethylbenzidine (TMB) assay, with dominant Ce4+ oxidation state, that is known to be more therapeutically efficient. The CNPs were observed to be easily uptaken by the cortical neurons. CNP groups showed significant reduction of ROS in vitro (cortical neurons), and in the contused spinal cord of rats. Furthermore, acute inflammation was decreased and anti-apoptotic molecules were down-regulated, which in turn led to enhanced locomotor function. Therapeutic effects of CNPs evidenced in the current study suggest their use as alternative treatment for spinal cord injury (SCI).
To Improve Regeneration of Corneal Endothelial Cells Using the Taurine/Silk Fibroin Film

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Taurine (Ta) promote the expression of connective tissue growth factor that it can repair the damaged tissue. Also, Ta is known as non-toxic. So, Ta is suitable for corneal reconstruction from tissue engineering. And, Silk fibroin (SF) is used as a tissue engineering scaffold because it has good biocompatibility and it can be produced easily. In this study, Different ratios of Ta (0, 0.25, 0.5, 1 and 2mM) blended film scaffolds were fabricated well with SF. Fabricated Ta/SF films were analyzed using SEM, contact angle, transparency, FTIR, MTT assay, mRNA expression, etc. In results, we can observe that 0.25mM loaded Ta/SF film have good cell proliferation. Also, Ta/SF film support cell growth and these form hexagonal morphology with well-maintained bio-functions. Thus, it can be used the Ta/SF film as a suitable alternative for cornea transplantation because it is having the good water mobility.

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Natural biomaterials and emerging 3D cancer models: engineering the microenvironment

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Over decades, solid evidences recognize the fundamental role of the tumor microenvironment in disease progression, and biomaterials provide the means to engineer it. Natural biomaterials, namely hyaluronic acid, silk, gellan gum and collagen, display unique properties allowing them to create 3D niches that mimic the chemical, morphological and mechanical characteristics of the native tumour microenvironment. Biomaterials are used to recapitulate physiologically-relevant models to investigate new aspects of cancer. Under this approach, the glycosaminoglycan hyaluronic acid (HA) is used to evaluate the impact of its chemical features (e.g. molecular weight) on tumour cell migration and invasiveness (gastric cancer cells, MKN45). HA, in its solubilized form does not have a significant impact in cell behaviour; but when surface immobilized (i.e. ECM-like presentation) is able to modulate cellular activity. This is one of the most important condition, which needs to be met when designing HA-based tumour models. Further, silk-gellan gum hydrogels can notably adjust the biochemical and mechanical cues of multi-cellular cancer stroma (oateosarcoma Saos2 cells and adipose derived stem cells) to elucidate the mechanisms that drive the formation of spheroids. Freeze-dried silk fibroin scaffolds co-seeded with normal mammary fibroblast (HMFP) and non-metastatic breast cancer cells (MCF-7), or invasive cancer cells (MDA-MB-231) generate 3D breast cancer-like gene expression; defining the main features of the tumor microenvironment in vitro. The latter can be integrated within a microfluidic device to obtain a more realistic cancer model. In this regard, collagen hydrogel encapsulating lung tumor m-organoids (A549) and endothelial cells (HUVEC) is used to develop a tumour blood-vessel-on-a-chip to mimic the invasiveness of cancer cells into the human vasculature. All these engineering approaches using biomaterials reflect diverse phenomenon of cancer physiology, which may enhance cancer therapeutic efficacy in the future.

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Sustained Local Release of NGF from a Chitosan-Sericin Composite Scaffold for Treating Chronic Nerve Compression

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Chronic nerve compression (CNC), a common form of peripheral nerve injury, always leads to chronic peripheral nerve pain and dysfunction. Current available treatments for CNC are ineffective as they usually aim to alleviate symptoms at the acute phase with limited capability toward restoring injured nerve function. New approaches for effective recovery of CNC injury are highly desired. Here we report for the first time a tissue-engineered approach for the repair of CNC. A genipin cross-linked chitosan-sericin 3D scaffold for delivering nerve growth factor (NGF) was designed and fabricated. This scaffold combines the advantages of both chitosan and sericin, such as high porosity, adjustable mechanical properties and swelling ratios, the ability of supporting Schwann cells growth, and improving nerve regeneration. The degradation products of the composite scaffold upregulate the mRNA levels of the genes important for facilitating nerve function recovery, including glial-derived neurotrophic factor (GDNF), early growth response 2 (EGR2), and neural cell adhesion molecule (NCAM) in Schwann cells, while down-regulating two inflammatory genes’ mRNA levels in macrophages, tumor necrosis factor alpha (TNF-α), and interleukin-1 beta (IL-1β). Importantly, our tissue-engineered strategy achieves significant nerve functional recovery in a preclinical CNC animal model by decreasing neuralgia, improving nerve conduction velocity (NCV), accelerating microstructure restoration, and attenuating gastrocnemius muscles dystrophy. Together, this work suggests a promising clinical alternative for treating chronic peripheral nerve compression injury.

Enzymatically-crosslinked silk fibroin hierarchical scaffolds for osteochondral tissue engineering

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Osteochondral (OC) regeneration has been facing several limitations in orthopedic surgery due to the complexity of simultaneously repair of articular cartilage and subchondral bone defects. The possibility of using bilayered scaffolds to interconnect two different SF layers as a monolithic and hierarchical structure for OC purposes, was the main focus of our most recent studies. Novel porous bilayered scaffolds, integrating a SF chondral-like layer and a SF-nano calcium phosphate (SF-nanoCaP) subchondral-like layer, were proposed for OC regeneration showing ability to support in vivo cartilage regeneration in the SF layer, and subchondral bone ingrowth in the SF-nanoCaP layer [1]. Recently, horseradish peroxidase (HRP)-crosslinked SF hydrogels emerged as a promising approach to create fast-formed SF structures, with controllable mechanical properties and swelling ratios according to the crosslinker concentration and β-sheet crystalline structure [2,3]. Biofunctional monolithic and hierarchical porous scaffolds composed of a HRP-crosslinked SF chondral-like layer (HSF layer) fully integrated into a HSF/ZnSr-doped β-tricalcium phosphate (β-TCP) subchondral-like layer (HSF-dTCP layer), were developed as bilayered structures (BdTCP) for OC tissue regeneration. Similar bilayered structures produced without ionic incorporation in β-TCP (HSF-TCP layer) were used for comparative purposes (BTCP). Human osteoblasts and human articular chondrocytes were co-cultured on the bilayered scaffolds, showing good cell adhesion and proliferation in the respective scaffold compartments. Osteoblasts were able to produce a mineralized extracellular matrix (ECM), whereas chondrocytes showed GAGs deposition in the chondral-like layer. In brief, the hierarchical structure and suitable mechanical properties of the bilayered scaffolds proposed make these structures possible solutions for OC defects treatment. The enzymatic crosslinking system has shown to improve the stability and structural adaptability of the BdTCP and BTCP scaffolds, desired for long-term OC implantation purposes.

References:
Advanced mechanical analysis for protein-based biomaterials

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Mechanical properties of biomaterials are critical factors to define their application in tissue engineering. We employ routine mechanical analysis including tensile tests and creep tests for soft films and hydrogels, and dynamic mechanical thermal analysis to deliver molecular structural information of these biomaterials in the form of film or fibre. Silk fibroin and elastin are fibrous structural proteins and widely exploited for biomaterial use. A hybrid protein hydrogel from silk and elastin has been fabricated to incorporate the features of both proteins. Quasi-static tensile tests show 75 elastin/25 silk hybrid hydrogel has an elastic modulus of 4 MPa, similar to that of native human corneal tissue. Cyclic tensile tests at 37°C prove that the elasticity of the hydrogel can be retained after 100 cycles under 0.1 MPa mechanical stress. 0.3 MPa is the stress threshold to maintain elasticity under long service of creep cycles. The glass transition analyses using DMTA on dry films of silk fibroin, elastin and their hybrids reveal that silk fibroin is an ordered protein, whereas the structural order of tropoelastin can be tuned by ethanol treatment. The structural order of the hybrid can be changed by the composition of the two proteins. FTIR structural analysis confirms that the increase of structural order is due to an increase in the b-sheet content. The advanced mechanical analysis can provide a comprehensive mechanical performance report prior to the in vivo use of the biomaterial, which would be valuable for the development and approval of new biomaterials.

In Situ Tissue Regeneration: A Translational Strategy in Regenerative Medicine

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Cell-based approaches using tissue engineering and regenerative medicine techniques have provided new opportunities for repairing various tissue pathologies. We have followed a strategy that involves the use of biocompatible matrices with cells. Target cells, obtained from donor tissue, are expanded in culture, attached to a support matrix, and re-implanted into recipient for recovery of tissue function. Although this strategy has been demonstrated to be effective in creating functional tissue experimentally and clinically, this approach requires a donor tissue biopsy and ex vivo cell manipulation prior to application in vivo. Simplifying these processes would provide a more efficient means of developing biological substitutes for functional tissue restoration. It has been demonstrated that almost every tissue in the body contains some type of stem or progenitor cells. These cells are believed to be part of underlying regenerative machinery that is responsible for daily maintenance and repair of injured tissue. The presence of an underlying regenerative mechanism in the form of tissue-specific stem and progenitor cells suggests that there may be a potential opportunity to bias the host response towards repair and replacement of tissue defects. This may be achieved by maneuvering host stem and progenitor cells using target specific scaffolds. The concept of in situ tissue regeneration using the body’s own biological resources and potential tissue applications will be discussed.
There is a large variety of musculoskeletal diseases and injuries that could benefit from developing new technologies in regenerative medicine. Regenerative engineering can be defined as the convergence of advanced materials science, stem cell science, developmental biology, and clinical translation. Stem cells (SCs) treatment hold great repair potential through systemic and local delivery. However, a major challenge is to improve therapeutic cells’ delivery and targeting using standard protocols easily scalable. We have studied different strategies and biomaterials to increase the SC characteristics as therapeutic agents, with applications in different diseased and injured models.

We used SCs from classic bone marrow origin together with novel cortical bone and cartilage tissue resident SCs to study and improve their stemness and therapeutic characteristics. We developed treatments to improve their homing ability, and created functionalized biomimetic materials to increase their immune-suppressive potential and support in situ proliferation and differentiation, showing osteoinductive and chondroinductive properties both in vitro and in vivo.

We demonstrated successful SC isolation from the different sources and were able to maximize their migratory potential towards the sites of inflammation and increase the production of molecules associated with immunosuppression. When inflamed, SCs showed a greater anti-inflammatory capacity. Moreover, the biomaterials showed osteoinductive and chondroinductive properties ideal for musculoskeletal applications.

We believe these treatments to be the new direction in order to develop cellular therapies to treat musculoskeletal inflammatory conditions. The transient enhancing of SCs potential induced by different substrates could be a new tool for new therapies with limited side effects.

Repairing damaged joint cartilage remains a significant challenge due to its low metabolic rate and avascular properties. Treatment involving micro-fracture, tissue grafting or cell therapy provides some benefit, but seldom regenerates lost articular cartilage. Providing a point-of-care solution that is cell and tissue-free has the potential to transform orthopaedic treatment for such cases. Key to this strategy is the development of implantable devices that closely mimic host tissue while providing a high degree of functional integrity. Glycosaminoglycans such as sulfated variants like heparan sulfate (HS), are well-suited for this purpose because they provide a matrix that enhances the pro-chondrogenic activities of resident growth factors found at sites of articular damage. Here, we evaluated the ability of a HS device to repair large osteochondral defects (4 mm in diameter, ≈1mm in depth) in the femoral trochlea of 19 New Zealand white rabbits over a 12-week period. Using SPR-based analytics, we show that high-affinity complexes are formed between HS and many notably reparative proteins such as TGFb1, BMP-2, FGF-2, PDGF-BB, and rhVEGF165. Macroscopic ICRS I scores shows that osteochondral defects treated with HS generate repair tissue that fills the defect and integrates with surrounding host tissue, unlike control treatment. Detailed histological assessment using ICRS II scoring clearly demonstrated abundant new subchondral bone following HS treatment that reached the level of the tidemark in adjacent host bone, but not in control. Importantly, regenerated hyaline cartilage in theochondral layer was only observed following treatment with HS. Although hybrid cartilage was found in nearly all defects, more chondrocyte features were observed with HS treatment. Such HS-based devices that enhance the activity of endogenous reparative factors represent the next-wave of tissue-engineering constructs with significant therapeutic utility.

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**48-SY-4**

**CXCR4 Antagonism Endogenously Mobilises Stem and Progenitor Cells and Rescues Non-union Development**

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**Introduction:** A significant number of fractures develop non-union. Stem cell therapy may be beneficial in their treatment, however this requires acquisition, culture and delivery of stem cells. Stem cell homing and migration is regulated through SDF-1 and its receptor CXCR4. Studies have demonstrated endogenous mobilisation of different populations of stem and progenitor cells by administering growth factors with a pharmacological antagonist of CXCR4; AMD3100(1). Hypothesis: Endogenous mobilisation of stem and progenitor cells via administration of growth factors and AMD3100 will enhance bone formation and avoid delayed/non-union.

**Methods:** A 1.5mm femoral osteotomy in adult female Wistar rats was stabilised with an external skeletal fixator. After osteotomy, saline/PBS (P) VEGF (V), IGF-1 (I) or GCSF (G) (100ug/kg, 0.5ml/100g i.p.), were administered daily for 4 days. On day 5, a single 5mg/kg i.p. dose of AMD3100 was given. Control group (C) did not receive growth factors or AMD 3100. At 5 weeks, the femur was retrieved and microCT scanned and bone volume within the osteotomy gap measured. Radiographic union was graded as union, partial or non-union.

**Results:** Compared to group C (n=7), group P (n=5) had a significant increase in bone volume (P=0.01) 8.9±2.2um³ (control 4.3±3.1um³) and trabecular thickness (P=0.03). Group I (n=6) also had a significant increase in bone volume (P=0.035) 5.1 ± 4.2um³ and trabecular thickness 0.062 ± 0.008um (control 0.042 ± 0.01um) (P=0.01). Group V (n=8), showed a non-significant increase in bone volume; 5.22 ± 1.7um³ and trabecular thickness 0.048±0.007um. Group G (n=5) showed a significant decrease in bone volume (2.5±2.6um³) (P=0.048). Radiographic union assessment showed for group C: 42% non-union, 29% partial, 29% union. For group P: 20% non-union, 80% union. Group I: 33% non-union, 17% partial, 50% union. For group V 25% non-union, 25% partial, 50% union, and group G: 40% union, 60% non-union.

**Conclusion:** AMD3100 alone and IgF1 AMD3100, showed the greatest increase in bone formation, presumably through mobilisation of beneficial combinations of stem and progenitor cells. GCSF-AMD3100, which is expected to mobilise hematopoietic progenitors only, inhibited bone healing.


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**48-SY-5**

**A neuropeptide, Substance-P, directly induces tissue-repairing M2 like macrophages by activating the PI3K/Akt/mTOR pathway even in the presence of IFN γ**

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Macrophage polarization plays an important role in tissue damage and repair. In this study, we show that Substance-P (SP) can directly induce M2 polarization of inflammatory macrophages. SP induced the differentiation of GM-CSF-differentiated pro-inflammatory macrophages into alternatively activated phagocytic M2 like macrophages (M2SP) through direct activation of the PI3K/Akt/mTOR/ S6kinase pathway and induction of Arginase-1, CD163, and CD206, all of which were nullified by pretreatment with the neurokinin-1 receptor (NK-1R) antagonist RP67580 and specific signaling pathway inhibitors. M2SP were distinct from IL-4/IL-13-induced M2a and IL-10-induced M2c subtypes; they did not show STAT activation and exhibited high phagocytic and endothelial adhesive activity. Furthermore, SP had a dominant effect on M2 polarization over Interferon gamma (IFN γ ), a potent M1-skewing cytokine, and effectively induced the M2 phenotype in monocytes and the human THP-1 cell line. Finally, adoptively transferred M2SP migrated to a spinal cord injury (SCI) lesion site and improved functional recovery. Collectively, our findings show that SP, a neuropeptide, plays a role as a novel cytokine by inducing tissue-repairing M2SP macrophages and thus may be developed for pharmacological intervention in diseases involving chronic inflammation and acute injury. Acknowledgements: This work was supported by NRF2016M3A9B4917320 and Ministry of Health and Welfare (HI13C1479) given to Dr YSon.

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A Regenerative Medicine Approach for the Treatment of Volumetric Muscle Loss in Humans

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Although mammalian skeletal muscle has a modest degree of regenerative capability, the loss of large amounts of tissue within an individual muscle group exceeds this endogenous regenerative potential. Volumetric muscle loss (VML), defined as a loss of 20% muscle mass, results in significant loss of function and lifelong morbidity, and has no effective treatment options. Free and pedicle muscle grafts are only marginally successful and associated with donor site morbidity. Stem cell-based therapies have uniformly failed to match the hope and expectations of new functional muscle formation. However, a recent report of 13 patients with VML treated with an inductive bioscaffold composed of xenogeneic extracellular matrix (ECM) showed the formation of new vascularized, innervated, functional skeletal muscle that markedly improved clinical outcomes. Widespread clinical adoption of this regenerative medicine approach requires the transfer of scientific principles from the benchtop to the caregiver, a multi-center clinical trial that validates results of the 13-patient cohort study, adoption by key opinion leaders, and establishment of appropriate reimbursement mechanisms. The general principles of translating regenerative medicine concepts to the clinical setting will be discussed.

Controlled Release of Proteins and PRP for Tissue Regeneration

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For tissue regeneration therapy based on the natural self-healing potential of patients, it is practically indispensable to manipulate the inherent ability of cells for their proliferation and differentiation which physiologically contributes to the self-healing potential. As one trial to achieve cell-based tissue regeneration, cells present in the body should be activated to promote their potentials of tissue regeneration. If a key protein is supplied to cells at the right site, the right time period or the suitable concentration, their potential will be enhanced to naturally induce cell-based tissue regeneration. Biodegradable hydrogels have been explored for the controlled release of growth factors, chemokines, and platelet-rich-plasma (PRP), to succeed in the protein-induced cells activation for regeneration therapy of tissues. The hydrogel system can not only release one type of drug, but also two types of drugs in different concentrations or time profiles. Such a dual release of drugs could further enhance the potentials of cells proliferation and differentiation for tissue regeneration and repairing. When cells are not present around the target site to be regenerated, it is practically necessary to enhance the cells recruitment to the target site. For example, the controlled release of a chemokine protein can enhance the in vivo recruitment of stem cells, followed by the local functional activation of cells recruited by another drug released for an enhanced cell-based tissue regeneration. It is no doubt that inflammation is one of the essential host responses to pathologically modify the process of tissue regeneration. Tissue regeneration was naturally promoted by positively regulating the inflammation process through the local release technology of an anti-inflammatory drug. The positive regulation of inflammation further enhanced the therapeutic efficacy of tissue regeneration which is induced by the biomaterials technology of drug release. This paper introduces significance in the controlled release of proteins and PRP for tissue regeneration.
**51-SY-4**  
**Macrophage cells secrete factors that orchestrate bone repair**  
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Duke University, USA

The pace of bone repair declines with age and studies using parabiosis and bone marrow transplantation show that young hematopoietic cells can rejuvenate fracture repair in old mice. This study examined the cell types and factors responsible for rejuvenation of fracture healing. Using conditioned media, we found that young macrophages produce factors that promote osteoblast differentiation of old bone marrow stromal cells. Heterochronic parabiosis using young mice in which macrophages can be depleted and bone marrow translation experiments showed that young macrophages could rejuvenate fracture repair. Proteomic analysis of the secretomes using azidohomoalanine incorporation identified differential proteins secreted between old and young macrophages. One protein that was secreted at higher levels in young cells was Low-density lipoprotein receptor-related protein 1 (Lrp1). Depleting Lrp1 in young cells abrogated their ability to rejuvenate fracture repair, and treating old mice with recombinant Lrp1 improved fracture healing. Here we show that macrophages and proteins they secrete orchestrate the fracture repair process, and young macrophage cells produce proteins that rejuvenate the fracture repair process.
Identification of synergistic stimuli to guide spheroid function

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Mesenchymal stem cells (MSCs) have tremendous potential for use in cell-based therapies of tissue repair and regeneration due to their proliferation, multilineage potential, proangiogenic capabilities, immune regulatory and anti-inflammatory potential, and relative lack of ethical concerns. Despite the promise of MSC-based therapies, previous studies have reported that the high death rate and poor engraftment of cells in ischemic conditions reduces the efficacy of stem cell therapy. Compared to individual cells, the formation of MSCs into spheroids enhances their survival, proangiogenic and anti-inflammatory potential. However, the fabrication and delivery of MSC spheroids to promote tissue repair is not a "one size fits all" approach, and effective strategies to optimize spheroid function for these applications are lacking. We design strategies to optimize MSC function, whether through preconditioning in specific microenvironments or transplanting with engineered materials to dictate cell function in situ. Once formed, we examine the capacity of biomaterials used as cell carriers to further guide cell function. The characteristics of the material are key in guiding cell participation in tissue repair, representing an active area of research in our laboratory. In this talk, I will highlight ongoing efforts by our laboratory to develop MSC spheroids for use in treating large bone defects and accelerating wound closure.

Contrasting tissue and blood proteins for tissue regeneration

Jeremy Mao
Columbia University, USA

During millions of years of evolution, life-threatening wounds must be healed by innate factors or survival of the individual is threatened. Certain proteins and peptides are probably stored locally in various tissues or centrally in the circulating blood for the repair or healing of wounds. Much remains unknown about 'healing' factors that are stored in peripheral tissues or centrally in the blood. For a given tissue, are de-cellularized matrices more effective for regeneration than individual, constitutive molecules? If a given tissue's extracellular matrix proteins heal multiple other tissues, what are the pivotal constitutive proteins/peptides responsible for healing? What are the pros and cons of storing healing proteins/peptides in a given tissue vs. centrally in the blood? This presentation will explore novel factors in blood and local tissues by contrasting them with known factors that play roles in tissue regeneration. High throughput technologies such as proteomics, metabolomics and RNA Seq are used to screen new molecules that may promote regeneration of multiple tissues. Molecular pathways are explored to elucidate how new molecules function in relation to known proteins. Tissue-specific and blood proteins have broad implications in the healing of multiple tissues and organ systems, and development of molecular therapies.
Cardiovascular disease is the leading cause of death in the United States. Tissue engineered blood vessels (TEBVs) offer the potential to develop new treatments for vascular disease. TEBVs have been used clinically as vascular grafts, and may also serve as 3D human disease models to screen potential therapeutics. The majority of approaches to vascular tissue engineering utilize cells seeded on or within exogenous scaffold materials, resulting in homogenous tubular structures. However, most vascular diseases are localized in nature, and involve injury and remodeling to cells and extracellular matrix (ECM). Therefore, we developed an alternative approach to generating TEBV, by using engineered cellular self-assembly to create functional, 3D tissue rings from cells and cell-derived ECM. The self-assembly system allows one-step 3D tissue ring fabrication by seeding a cell suspension into custom agarose wells. Cell rings self-assemble within 24 hours, and are strong enough to harvest within 1-3 days after seeding. After 7-14 days in culture, the tissue ring format is well suited for quantitative functional analysis of cell-derived tissues (e.g., uniaxial tensile testing, wire myography). To date, we have created and analyzed self-assembled vascular tissue rings using primary human smooth muscle cells, mesenchymal stem cells, and induced pluripotent stem cell (iPSC)-derived human vascular smooth muscle cells from healthy subjects or patients with supravalvular aortic stenosis (SVAS). In addition, we have found that self-assembled tissue rings can serve as modular building units to create tubular tissue constructs. We have also shown that gelatin microspheres can be mixed and co-seeded with cells during assembly to achieve growth factor delivery within individual ring segments. Finally, modular assembly and fusion of individual tissue ring units enables creation of tubular tissue with focal heterogeneities, which may enable modeling of vascular lesions. In addition to vascular grafts, the system can be used to create other tissue types, including cartilage/tracheae. In summary, modular tissue fabrication by self-assembled ring assembly and fusion may represent a new approach for the engineering of complex, living multi-tissue constructs.
**52-SY-5 Delivering instructive cues to development-mimetic cellular condensations for modular engineering of complex tissues**

Eben Altsberg  
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High-density cultures of cells can mimic immature condensates present during many developmental and healing processes. Presenting specific soluble signals, such as growth factors, exogenously in tissue culture media can regulate cell behavior in these cultures and promote new tissue formation. However, shortcomings of this approach include transport issues, limited spatial control over signal presentation, and required repeated dosing in the media. We have engineered technology that overcomes these challenges by incorporating biomaterial particles containing bioactive signals within the cellular condensations, which permits localized spatial and temporal control over the presentation of these regulatory signals to the cells. In this talk, I will present our research using this strategy to engineer a variety of tissues, including bone, cartilage and trachea. The capacity to deliver diverse signals, including growth factors, plasmid DNA and in vivo mechanical loading, for driving new tissue formation will be demonstrated. In addition, the value of this technology for engineering a wide range of tissue shapes, including spheres, sheets, rings and tubes, and for regenerating tissue in cartilage and bone defect models will be shown. Finally, the utility of providing cell-instructive bioactive factors from biomaterials in a controlled manner for the assembly of modular tissue units to engineer complex constructs comprised of multiple tissue types will be explored.

**52-SY-4 Smart hydrogel platforms for high-throughput production and culture of size-controlled multi-cellular spheroids**

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Cell therapy has been highly spotlighted as an alternative therapeutic approach for incurable diseases. However, there are some limitations in direct injection of cell suspension since it is compromising cell-cell and cell-extracellular matrix (ECM) interactions. To enhance regenerative potential, multi-cellular spheroids have been widely used. This similarity in recapitulation of 3D micro environment within the spheroid leads to increased cellular functions such as stemness, differentiation potential of stem cells, anti-inflammatory effect or high retention rate after injection. Various methods to make spheroids have been developed such as using hanging drop plate, spinner flask or ultra-low attachment plate, however, mass production and controlling size of spheroids at the same time have been difficult to achieve. For example, methods focused on mass production such as using spinner flask showed limited control in the size of spheroids and methods aiming at controlling the size of spheroids such as hanging drop are too laborious to fabricate large number of spheroids.

To overcome these limitations, we developed smart thermally responsive hydrogels combined with chemical micropatterning technology for mass productive spheroid fabrication and culture with controlled size. We used mesenchymal stem cells from three different sources and we could harvest spheroids from micro cell patches by simply changing temperature without any damage. The size of spheroids can be modulated by change in the size of micropatterns, demonstrating narrow distribution of size of the spheroids with high viability. These spheroids increased secretion of ECM proteins, up-regulation of stemness markers and angiogenic factors. Finally, we investigated the potential for therapeutic application according to injectability and cryopreservation. In conclusion, our new approach would be a feasible method as a scaffold-free strategy for regenerative medicine.
**53-SY-1** Regeneration of Functional Tissue-engineered Anterior Cruciate Ligament

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The need for tissue engineering of the anterior cruciate ligament is apparent from the unsatisfactory outcomes of current treatment modalities for such ruptured tissues. There are limiting factors in current practices that include donor site morbidity, immunological foreign body response, disease transmission, donor shortages, donor-recipient compatibility, infection, and implant fatigue failure. The strategy for treatment thus lies in reducing these problems while developing a closer or similar substitute to that of the native ligament. Such is the rationale for the interest of the field towards providing a tissue engineering solution. In the pursuit of functional tissue engineering of the anterior cruciate ligament, several groups had identified certain factors as essential. They include cell source, various aspects of the scaffold, and the stimulatory biochemical/mechanical cues. It is therefore important in tissue engineering of the anterior cruciate ligament to understand the interplay of these different stimuli that guide cells, specifically MSCs, towards differentiation and tissue growth. Our group has focused on the use of silk as scaffold material in the regeneration of anterior cruciate ligament since silk fibroin, a protein component of natural Bombyx mori silk bears equivalence to collagen in supporting cell attachment, inducing appropriate morphology and growth. Building on our concept of the knitted scaffolds, we have developed a hybrid scaffold combining knitted silk and free-dried silk sponge to produce a scaffold type that is not only mechanically viable for ligament tissue engineering, but also facilitates long term degradation that matches that of tissue regeneration. We have shown in vitro that these scaffolds support mesenchymal stem cells (MSCs) proliferation and differentiation; while in vivo, they are effective in regeneration functional ACLs in rabbit and pig models.

**53-SY-2** Scaffold-less Tissue Engineering Technologies To Repair Anterior Cruciate Ligament Injuries

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Injuries to the anterior cruciate ligament (ACL) often require surgical reconstruction utilizing tendon grafts in order to restore knee function and stability. Current available graft options for ACL repair, however, are associated with reports of recurrent knee instability and failure to return to sport, and have continued risk for the development of early onset osteoarthritis. These may be attributed to stiffer biomechanical properties of available grafts compared to native ACLs as well as poor integration of the graft with the host. Our laboratory has designed and fabricated a scaffold-less, multi-phasic, tissue engineered bone-ligament-bone (BLB) constructs that demonstrate host integration and advancement toward native ligament mechanical properties and phenotype. We have performed 6 different studies using this BLB graft for repair of ACL tears in a sheep model. These studies range in recovery time from weeks, to study the acute regeneration of the ligament tissue to a 2-year study to evaluate the long-term efficacy of the BLBs in our sheep ACL reconstruction model. Our studies show that using the BLB as a replacement graft for ACL reconstruction improved outcomes compared to current graft options. Furthermore, we have shown that the use of frozen BLBs resulted in outcomes indistinguishable from those of fresh BLBs following six months implantation. The ability to use frozen BLB grafts that do not have live cells will help to facilitate regulatory approval and clinical translation of our technology. In addition, our two-year results exhibited a continued advancement of the engineered tissue towards native ACL with an approximate 30% increase in restoration of contralateral modulus compared to our six-month recovery study. This presentation will highlight the data from our six sheep studies and show the efficacy of our scaffoldless tissue engineered technology for the repair of a torn ACL.
**53-SY-4  Anterior Cruciate Ligament Regeneration using the Silk-based “RegACL” Scaffold**

Andreas Teuschl¹,², Patrick Heimel¹,²,³, Xavier Monforte¹,², Sylvia Nürnberger³,⁴, Stefan Tangl³,⁴, Martijn van Griensven⁵, Heinz Redl¹,², Thomas Nau¹,³

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Tissue engineering to treat ACL injuries has gained increasing research interest in the last decade. In order to replace the clinical use of autologous tissue transplantations various approaches using smart biomaterials alone or in combination with cell-therapeutic strategies and/or growth factors are currently investigated. In this article a silk-fiber based scaffold called “RegACL” braided in a wire-rope design and its in vitro testing and in vivo characterization are presented. The optimal scaffold for ACL regeneration is regarded to be biocompatible and biodegradable to allow tissue ingrowth, but also needs to provide optimal mechanical properties. Maximum strength and elasticity are supposed to be comparable to native ACL tissue in order to (1) promote ligament tissue formation and guarantee (2) immediate primary stability in vivo. In this regard, the crucial steps in the development of RegACL are summarized including the proprietary method to remove sericin from the silk-based graft and the encouraging results from a 12 month sheep study. In this study a cell-therapeutic approach in a one-stage surgical procedure (cell isolation, cell application and graft transplantation done in a one single step) enhanced ACL regeneration for the 6 months observation time point compared to the scaffold alone group. Interestingly, this beneficial effect could not be detected for the 12 month groups. In both groups, with and without applied cells, the results were comparable which argues for the use of RegACL as an off-the-shelf product. Recent µCT-data showed that cells infiltrating the scaffold from surrounding tissues not only produce new ligament tissue but also form a gradual tissue interface from the bony insertion parts to the intraarticular part. Moreover, fibrous infiltration from regenerated ligament tissue into new-formed bone could be detected in this 12 month sheep study. Taken together, these results strongly indicate that the reconstruction and repair of ruptured ACL is possible with the presented silk-based RegACL.

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**Engineering Tissue Connectivity**

Helen H. Lu

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Musculoskeletal motion is orchestrated by synchronized interactions between multiple tissue types and the seamless connectivity of bone with soft tissues such as tendons, ligaments or cartilage. In particular, ligaments join bone to bone while tendons connect muscle to bone. Moreover, many of these soft tissues transit into bone through a biphasic fibrocartilaginous interface, which serves to minimize the formation of stress concentrations while enabling load transfer between soft and hard tissues. Given their critical role in physiological motion, complexity of these functional tissue units have become a design consideration for tissue engineered orthopaedic grafts. Using the classic ligament-to-bone enthesis as an example and inspired by current understandings of the native interface structure-function relationship, the design and fabrication of complex scaffolds for multi-tissue integration will be discussed, with the focus on strategic biomimicry and functional tissue integration.
We have developed cell-sheet-based tissue engineering and autologous cell sheet transplantation has been already clinically applied to regenerative therapy for cornea, heart, esophagus, cartilage, gingiva, ear and lung diseases. Furthermore, functional three-dimensional (3D) tissues have been successfully fabricated by stacking cell sheets for future regenerative therapy and drug screening. Functional 3D tissue fabrication contains many manufacturing processes including cell isolation, cell culture, cell sheet fabrication, cell sheet stacking, preservation and transportation. We have already established a novel, flexible and automated manufacturing facility “Tissue Factory” for cell-sheet-based products. By Tissue Factory, multilayered skeletal myoblast sheets were successfully fabricated from a piece of skeletal muscle tissues. For scaling-up of functional 3D tissues, additional manufacturing processes are inevitable for huge cell expansion and introduction of perfusable blood vessels. Regarding iPS cell expansion, we have developed 3-D suspension bioreactor system, which realize minimization of damage on embryonic bodies with original rotary wings. Continuous perfusion system maintains the lactate concentration and pH during the expansion and differentiation process. Reproducibility of human iPS cell-derived cardiomyocytes has been now established. On the other hand, for vascularization in 3D tissue, we have developed multi-step cell sheet layering technique and perfusion bioreactor system for continuous oxygen and nutrition supply. In vitro perfusable blood vessel fabrication and scaling-up of bioengineered 3D tissues have been realized, however these complex 3D tissue fabrication is still faced with the problem of reproducibility. Introduction of automatic fabrication system may also minimize the variety. Moreover, real time monitoring of engineered tissue viability and real time controlling of perfusion culture condition with feedback system may increase the reproducibility of these functional 3D tissue fabrication. In this presentation, the development of manufacturing processes for cell-sheet-based products are introduced and their manufacturability is discussed.
**54-SY-3** A pneumatic pressure bioreactor for applying dynamic mechanical loads to cell culture plates

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TECHNOLOGY: We have constructed a bioreactor which applies uniform mechanical loading to standard well plates via an un-used element of the culture environment: the gas phase above it. Cells are subjected to hydrostatic loading by repeatedly compressing the gas phase above the culture plate. The pneumatic pressure reflects physiological loading cycles that cells such as osteoblasts and osteocytes actually experience in the body, which at less than 300kPa are easily and safely achievable in a research environment. The universal pressure change allows complex shapes, structures and even moving constructs to experience uniform loading.

EXPERIMENTAL: Human MSCs in collagen hydrogels were cultured under standard conditions or under pneumatic loading at 300kPa at 1Hz for one hour per day in an osteogenic media. The percentage of the hydrogel which became mineralised after 28 days (as determined by microCT) was found to be <1% in the controls, but over 75% in the bioreactor cultured hydrogels. Mineralisation was distributed throughout the hydrogel but centred on dense nodules, which stained positively for calcium. QPCR at 28 days revealed significant fold-changes in genes associated with osteogenesis (RUNX2, ALP, OPN, COL1, OCN).

CONCLUSION: Pneumatic loading is a potent differentiation stimulus, capable of driving significant bone formation in tissue engineered scaffolds. By using an environmental loading rather than biochemical stimulus to guide differentiation, we can reduce costs, overcome regulatory hurdles and generate mechanically conditioned tissues for treating osteochondral defects.

IMPACT: Mechanotransduction has been shown to have roles throughout biology – from bone healing to cancer progression, regulating the circadian clock which affects healing and drug responses, and be vital in the very earliest stages of body patterning in the embryo. Despite this, almost all research in vitro is performed with no mechanical loads whatsoever.

This versatile bioreactor can conveniently and effectively load any cell or tissue that can be grown in a well-plate. It is applicable to any cell or tissue type that would experience pressure changes in vivo: chiefly musculoskeletal tissues, but also e.g. retina, brain (inflammation) and bladder. The bioreactor can also provide up to -90 kPa negative pressures, allowing this technology to stimulate e.g. lung epithelial cells. *We are especially keen to develop this bioreactor via novel collaborations arising from this conference*.

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**54-SY-4** A 3D Printed Microfluidic Bioreactor to Engineering Biphasic Musculoskeletal Construct

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**Introduction:** Tissue engineered constructs coupled with high-throughput bioreactors can be used as innovative in vitro preclinical models of tissue functions and disease pathogenesis for drug screening and toxicity assessment [1]. Here, we present an innovative 3D printed bioreactor for biphasic constructs, enabled with optical access for direct monitoring of cellular responses.

**Materials and Methods:** Fluid circuits for the bioreactor were optimized by modeling (ANSYS Fluid Flow, CFX), then tested using food coloring for biphasic constructs, enabled with optical access for direct monitoring of cellular responses. Experimental tests confirmed modelling predictions [3], showing minimal mixing in the media stream for each tissue component. Successful chondro/osteogenic differentiation of hMSCs in the two sides of the constructs was confirmed by histological staining and optically by fluorescence of the Col2 sentinel cells in the half of the construct exposed to chondrogenic medium.

**Results and Discussion:** Cells were successfully stained and imaged by the dynamic live assay within the bioreactors where they maintained viability and active metabolism, confirming the possibility of non-destructive monitoring within the system. For the biphasic bioreactor, experimental tests confirmed modelling predictions [3], showing minimal mixing in the media stream for each tissue component. Successful chondro/osteogenic differentiation of hMSCs in the two sides of the constructs was confirmed by histological staining and optically by fluorescence of the Col2 sentinel cells in the half of the construct exposed to chondrogenic medium.

**Conclusions:** We validated the use of the microbioreactor to perform a continuous, non-destructive, real-time monitoring of cellular responses without breaking sterility. This is especially relevant for biphasic constructs that require specific media for each component [4].

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Tendon injuries are a major healthcare concern in humans but they also occur spontaneously in other species including horses and dogs. Exercise and age are common risk factors in all affected species and involves a multifactorial component leading to cumulative degeneration. Injuries can occur outside the tendon synovial sheath (extra-thecal) or within the synovial environment (intra-thecal) of the tendon synovial sheath, bursa or joint. Tendon injuries repair naturally by fibrosis resulting in dysfunctional tissue. Intra-thecal injuries in particular repair poorly due to the presence of synovial fluid which, in our ex vivo models has adverse effects on tenocyte viability.

A number of laboratory animals are used to model human tendon disease with a variety of approaches to induce disease (physical, chemical or biological). These have been valuable in our understanding of pathophysiological processes, disease mechanisms and for genetic investigations but there is significant difference in the similarity of induced with naturally-occurring disease in pathobiology. Promising results of tendon regeneration have often not translated into the clinic. Therefore the clinical and experimental data from naturally-occurring disease in large animals may be informative.

Extra-thecal injuries to the human Achilles tendon are functionally and clinically equivalent to the horse superficial digital flexor tendon which we have used as a model for both in vivo and in vitro studies. Our results have supported regulatory mechanisms for regenerative medicine trials in human Achilles injuries. In contrast, intra-thecal injuries of the human rotator cuff represent a significant challenge to model because a quadruped’s shoulder is anatomically and functionally different from the human shoulder. Current small experimental models using shoulder tendons are clinically less appropriate as they are neither compressed nor intra-synovial. We have developed a novel model of the sheep deep flexor tendon (DDFT) as it mimics the functional similarities between human rotator cuff injuries in the human and the horse DDFT as there is a similar biomechanical environment (compression), intra-synovial location, and failed healing with persistent pain. This model has shown features of failed healing consistent with clinical outcomes observed in human intra-thecal injuries and our data supports its use as a relevant model for investigating engineered scaffolds for cell-based therapies.
Sheep and horses are well established and frequently used large animal models pivotal to bridge the translational gap from bench to bedside. At the same time they are patients with naturally occurring disease, sharing many important pathophysiologic characteristics between them and humans. However, regulation of inflammation and hence the inflammatory response may differ between different species, impeding generalization of results. Enhancing our understanding of these species-specific differences is essential to improve the translational output of studies using animal models.

We have therefore analysed and compared proteome alterations induced in PBMCs of sheep, horses and humans upon inflammatory activation using nanoLC hyphenated with high-resolution mass spectrometry. PBMCs were isolated from whole blood collected from healthy sheep, horses and humans as well as from sheep and horses following laparotomy (each n=3) with approval from local ethics committees. Species dependent variations were applied to the Ficoll isolation protocol to balance the shift of species dependent cell size and density. Homogeneity of primary PBMCs was assessed by haemocytometry and DiffQuick staining. To induce inflammation the PBMCs were stimulated using different inflammatory cues (LPS, IL-1, TNF-α) and incubated for 4h in autologous plasma followed by culturing for additional 3h in serum-free medium for proteome analysis. Comprehensive assessment of proteomes was performed through subcellular fractionation into secreted, cytoplasmic and nuclear proteins. Proteins were digested using a variation of the FASP protocol. For proteome analysis a label free bottom-up shot gun proteomics approach applying high-resolution orbitrap mass spectrometry was applied. Peptides were separated with nano-flow UHPLC and analysed with a QExactive orbitrap mass spectrometer. Protein identification, quantification and statistical data evaluation was performed using MaxQuant and Perseus software. Results were compared to PBMCs obtained from sheep and horses with a natural inflammatory response following laparotomy.

Inflammatory reactions of PBMCs were compared between the three species and similarities and species-specific alteration were recorded. This work is offering a comprehensive overview for in depth understanding of species specific inflammatory response differences in order to improve the translational output of studies using animal models in the future.

Extensive annulus fibrosus (AF) radial tears lead to intervertebral disc (IVD) herniation, which gives rise to subsequent radicular pain when a nerve root in the spinal canal is compressed. While unrepaired defects in the AF are associated with postoperative reherniation and high IVD degeneration prevalence, current surgical strategies are limited to symptomatic treatment of pain and disregard the structural integrity of the AF. For all these reasons, this study is focused on the design of an electrospun implant composed of polycaprolactone (PCL) for AF closure and repair. We have shown that this implant mimics the oriented and multi-lamellar fibrous structure of the native AF and we have assessed its ability to properly close an AF defect, maintain normal IVD biomechanics and prevent further disc degeneration in a sheep in vivo model.

Oriented and non-oriented PCL mats were successfully produced by electrospinning and presented similar average fiber diameters of 1.12 ± 0.42 nm and 1.03 ± 0.36 nm, respectively. Oriented PCL mats possessed a tensile modulus (50 ± 1 MPa) matching the one of a native AF lamella (~ 47 MPa), as determined by uniaxial tensile mechanical analysis. In vitro experiments demonstrated the colonization of PCL mats by human and ovine AF cells and the deposition of a type I collagen rich extracellular matrix. Ex vivo experimentations (ovine spine) were performed to evaluate i) various shape of AF defect (circular punch or rectangular box) and ii) implant fixation in the defect (suture, glue, or external patch). We selected to create a scalpel-induced box defect and to secure a 10-layer PCL implant in the defect by gluing an external PTFE patch to the adjacent vertebral bodies. In vivo study was then carried out on 6 sheep where 5 lumbar discs were exposed using a left retroperitoneal approach. Box defects (2 x 5 mm and 2 mm depth) were created in the outer annulus of 4 discs (L1/2 to L5/6), followed by randomized distribution of conditions including oriented or non-oriented implants, unrepaired group and healthy disc controls. All groups received an external PTFE patch. Post-surgical X-ray and MRI examinations were performed every month to evaluate disc degeneration (Pfirrmann scoring, disc height). Explantations were performed at 1, 3 and 6 months, followed by immuno-histological analysis. These data highlight that multi-layer PCL electrospun mat is a promising biomaterial for AF repair and prevention of further IVD degeneration.
Current therapies are not successful to consistently restore the original properties of a functional tendon. Hence, there is an urgent need to find alternatives for effective tendon regeneration. Recent developments on magnetically assisted strategies within the remit of cell based therapies offer novel possibilities to stimulate biological processes as mechanotransduction or cell differentiation and to develop smart responsive tissue substitutes anticipating improved therapies.

In this work, we propose to develop complementary magnetically activated approaches to target and direct tenogenic commitment of human adipose stem cells (hASCs) using magnetic nanoparticles (MNPs) technologies. In our first approach, we triggered tenogenesis in hASCs through the stimulation of mechano-receptors involved in the TGF-β /Smad2/3 signaling pathway. The ActRIIA receptor was targeted using anti-ActRIIA functionalized MNPs, which were externally activated using an oscillating magnetic field up to 14 days. Remotely activated MNPs tagged hASCs showed enhanced expression of tendon related genes and synthesis of a complex tendon-like ECM matrix. These results demonstrated the possibility to trigger the downstream Smad2/3 pathway in hASCs via mechanomagnetic stimulation, leading to tenogenic responses.

Secondly, a tissue-engineered patch resourcing to magnetic cell sheet technology was developed using a subpopulation of hASCs positive for Tenomodulin (TNMD), previously identified with increased tenogenic potential over crude population. TNMD+ hASCs combined with MNPs assisted the formation of magnetically responsive tenogenic living constructs after 7 days in culture with good mechnoelasotc properties and a tendon-like rich ECM. Moreover, magnetic cell sheets form cohesive magnetic responsive tissue-specific ECM patches with the potential to be explored as tendon healing promoters by the actuation of external magnetic fields.

In summary, the magnetic activated strategies developed provided insights on the biological and biophysical mechanisms of tenogenesis that are pivotal in engineering tendon TERM solutions as part of an integrated approach targeting tendon regeneration.

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 INTRODUCTION Advanced therapeutic medicinal products for wound healing applications are an evolving option, aiming to improve wound healing outcomes by reducing wound healing closure time by delivering cells, medical compounds and biologics. Matrix-rich tissue equivalents can be fabricated in vitro by employing biophysical, biological and biochemical cues. Our work is focused on the accelerated production of matrix-rich tissue equivalents with the utilisation of macromolecular crowding (MMC) which has been shown to enhance matrix deposition in vitro.

EXPERIMENTAL METHODS In this work, we investigated the effect of MMC on the fabrication of a matrix rich tissue equivalent for a wound healing application. A collagen-based film has been utilised for the fabrication of a modular, matrix-rich cell carrier for wound healing. The structural, mechanical and thermal properties of the material were assessed with electronic microscopy, uniaxial mechanical testing and differential scanning calorimetry respectively. Human dermal fibroblasts and stem cells (bone marrow and adipose tissue derived) were expanded up to passage 3 in DMEM and MEM media, supplemented with 10% fetal bovine serum and 1% penicillin / streptomycin. For the enhancement of extracellular matrix deposition, a macromolecular crowding agent (Carrageenan) was utilised at all time points. Matrix deposition was assessed with immucytochemistry. A splinted wound healing model in athymic nude mice was utilised to assess wound healing in vivo. Wound healing closure ratio was assessed on day 3.7 and 14. Tissues were harvested 14 days post implantation for histology.

RESULTS AND DISCUSSION Extracellular matrix deposition has been enhanced at all time points when carrageenan was used as a MMC agent in the in vitro regime. The 30k/cm2 cell seeding density was found the most suitable for the fabrication of a cell and matrix rich construct in the 7 day culture period prior in vivo implantation. Modular constructs grown in vitro in the presence of carrageenan, facilitate improved and accelerated wound healing in vivo when implanted in the athymic nude mouse model.

CONCLUSION Collagen-based matrix-rich tissue equivalents for cutaneous tissue engineering fabricated with collagen and MMC facilitate the enhanced matrix deposition in vitro, and improved and accelerated in vivo wound closure. Further assessment is under way.

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**Keynote: Accelerating translation of peripheral nerve tissue engineering approaches into real products by choosing meaningful pre-clinical models**

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Each year researchers around the world come up with new innovations in biomaterials and tissue engineering approaches thought to improve peripheral nerve regeneration through bioartificial implants. Looking at the number of FDA / CE approved products for clinical use, it has only changed minimally during the last two decades and the new developments, especially tissue engineered approaches, have only rarely been translated from bench to bedside. The reasons for this may be as manifold as the innovative engineering ideas and some may originate from a non-appropriate pre-clinical study design. This paper aims to pinpoint the most valuable in vitro and in vivo models to be used for convincing clinical scientists that a clinical evaluation of new development is worth to be performed. Therefore, they need to be provided with data clearly indicating that the new development has a high potential to substitute or even replace autologous nerve grafting in long distance repair of transected peripheral nerves. Here, like in all pre-clinical research, it is equally important to consider the 3R strategy to shorten animal use in research and to produce meaningful results at the same time. It also needs to be considered that results obtained from study designs that are comparable among diverse research groups are more meaningful than results obtained utilizing barely used modifications of these techniques. This paper will demonstrate that biocompatibility studies conducted exclusively with glial cell lines, although they may reveal some important information, have minimal value in demonstrating that a biomaterial is very promising for the fabrication of nerve guidance channels. For a more substantial indication of the biomaterial properties in vitro studies evaluating the behavior of primary nerve cells (Neurons, Schwann cells) are needed. Furthermore, a final comprehensive pre-clinical in vivo evaluation in at least one challenging animal models is unavoidable. And again meaningful results from in vivo studies need to evidence that regeneration of axons occurs across a substantial (critical) distance and leads to successful reinnervation of distal targets (including indicated specificity of this reinnervation) and to the most important functional recovery. Evaluation of functional recovery as well is critical because it is only predictive if function reaches a level that would also be meaningful for the patient to be treated with such a novel implant.

**The Impact of Autologous Nerve Graft Phenotype and Extracorporeal Shockwave Therapy on Peripheral Nerve Regeneration in the Rat**

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Although regeneration after autologous nerve grafting (ANG) has been the target of scientific curiosity since the beginning of modern medicine, not much progress in accelerating this tedious process has been made. An explanation could be the experimental model chosen. Most research groups use the sciatic nerve defect as a model for ANG, dismissing the influence of nerve graft phenotype on regeneration. We hypothesize that this mismatch has a negative influence on motor axonal regeneration and that extracorporeal shockwave therapy (ESWT) can ameliorate this effect. Our first aim was to establish a femoral nerve defect model reflecting the phenotypical difference of ANGs in the clinic. Second, we aim to evaluate the effect of ESWT on nerve regeneration using this model in vitro and in vivo. Adult male rats were divided in groups of at least 8 animals. A 6 mm autologous nerve transplantation was performed using either homotopic (matched) or heterotopic (mismatched) grafting. The treatment groups received ESWT once after wound closure. Regeneration was evaluated functionally, histologically, and by qRT PCR and western blot.

In vitro: Phenotypical differences were obvious between Schwann cells (SCs) derived from motor and sensory branches of the femoral nerve. Motor derived SCs exhibited less proliferation, and higher expression of myelination markers. ESWT increased proliferation and expression of pro-regenerative phenotype markers such as c-jun in all SCs.

In vivo: Motor nerve derived grafts showed less than 50% expression levels of pro-proliferative markers (Ki67, p75) in early stages of neuronal regeneration. Furthermore, electrophysiological and histological evaluations indicate slower regeneration of motor axons in the mismatched setting when compared to the homotopic (motor to motor) grafting. ESWT significantly increases expression of pro-regenerative genes at early timepoints and of markers for re-myelination (Cadm3) and homeostasis (TrkB) 6 weeks after injury in both groups. Furthermore all ESWT treated animals showed faster functional recovery when compared to untreated animals, indicating amelioration of negative effects of phenotypical mismatch. This study shows that ESWT is able to accelerate peripheral nerve regeneration in a successfully modified femoral nerve model which reflects the clinical reality after autologous nerve transplantation. This study provides support for the use of ESWT after surgical repair of peripheral nerve injuries.
**56-SY-4** CRISPR-based activation of adipose stem cell endogenous gene expression for peripheral nerve regeneration

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Peripheral nerve regeneration is a complex and delicate process that requires coordinated functions of neurotrophic factors and neuronal cells. CRISPR activation (CRISPRa) has been adapted as a powerful tool with the potential for multiplexed endogenous gene expression. Here we developed a hybrid baculovirus (BV) vector to express a p300-fused CRISPRa SAM system that enables simultaneous epigenetic activation of multiple genes. We demonstrated that the expression of p300-CRISPRa SAM system in rat adipose-derived stem cells (ASC) enabled high level activation of neurogenesis-related genes (e.g. BDNF, GDNF and NGF). In addition, the p300-CRISPRa-mediated expression of neurotrophic factors stimulated the migration of Schwann cell and neurite extension and guide the remyelination process in vitro. Importantly, implantation of the hybrid BV-engineered ASCs into sciatic nerve transection site in rats significantly improved the nerve regeneration as judged from the enhanced functional recovery, integrity of nerve conduction, electrophysiological functionality, axon regeneration and remyelination. These data demonstrate that the baculovirus-delivered p300-CRISPRa system can activate endogenous gene expression and enables functionalization of rat ASC for peripheral nerve regeneration.

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**56-SY-5** Centella Asiatica Induced Mesenchymal Stem Cells (MSCs) Promote Regeneration of Critical Defect of Peripheral Nerve in an Animal Model

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Introduction: No studies have been done on the effects of C. asiatica induced mesenchymal stem cells (MSCs) on in vivo nerve regeneration. The aim of this study was to investigate the effect of natural conduit seeded with neural differentiated MSCs (ndMSCs) (using C. asiatica) on the regeneration of critical defect of sciatic nerve in an animal model.

Methodology: ndMSCs were differentiated using 400µg/ml of C. asiatica, and nerve conduit was constructed by seeding MSCs or ndMSCs into decellularized artery conduit. Then, 1.5 cm sciatic nerve gap in Sprague-Dawley rats was bridged using reversed autograft (RA)(n=3) (positive control), MSCs conduit(n=4) and ndMSCs conduit(n=4). Functional studies (pinch test and nerve conduction studies) were performed every 2 weeks up to 12 weeks. The conduits were examined histologically at 12 weeks post-implantation for presence of Schwann cells and axonal regeneration (anti-S100B, anti-P75 NGFR, anti-GFAP, anti-MBP and anti-NF 200 antibodies). TEM analysis was done to analyse the number and thickness of myelinated axons. Gastrocnemius muscle weight was measured to examine the extent of muscle atrophy.

Results: Immunocytochemistry analysis revealed that MSCs had differentiated into neural lineage cells. Implantation of ndMSCs conduit found to improve rats’ sensory sensitivity in a similar manner with RA group. At 12th week, nerve conduction velocity (NCV) found to be the highest in ndMSCs group (1.1667 ± 0.243). Axonal regenerations were also enhanced in ndMSCs group and RA group, as seen by the expression of MBP protein. Furthermore, the myelin sheath thickness of ndMSCs conduit (0.7610 ± 0.009) were in similar range with RA (0.7644 ± 0.007) compared to MSCs conduit (0.7060 ± 0.019). Examination of muscle atrophy found that there were no significant differences in the three groups.

Conclusion: ndMSCs conduit showed promising effects on nerve regeneration and functional restoration similar with RA group. These observations demonstrated the neuroregenerative properties of C. asiatica and its potential as a new alternative strategy to the treatment of critical nerve defect.
56-SY-6  Effect of MARCKS peptide functionalized intraluminal collagen fibre-based conduit on a critical gap model of peripheral nerve injury

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Identifying key molecules that increase regeneration has been a long-term goal of tissue engineering. Our detailed proteomic analysis of the material-based regenerative response showed that Myristoylated alanine-rich protein kinase C substrate protein (MARCKS) was upregulated in autologous graft sciatic nerve injury repair but not in biomaterial graft implants. This study compared the efficacy of the functionalized intraluminal fibre collagen conduit with autograft (the current “gold standard”) in regeneration of sciatic nerve critical gap injury.

ELISA was used to compare upregulation of MARCKS in both non-critical (10 mm) and critical gap (15 mm) injuries. Effect of MARCKS functionalized conduits was assessed using force measurements and compound nerve action potential. Sixty rats with critical gap injury were used for functional regeneration analysis; groups as follows: (a) autograft, (b) hollow collagen conduit, (c) fiber conduit, (d) MARCKS functionalized conduit (low dose, 0.05 mg/ml), (e) MARCKS functionalized conduit (high dose, 0.2 mg/ml).

ELISA results combined with the proteomics data suggest that expression of MARCKS can significantly upregulated during peripheral nerve regeneration. Assessment of functional recovery in TA and EDL muscles showed that recovery in autograft group was significantly greater than that of the hollow conduit and plain fiber conduit. In terms of maximum twitch and tetanic force, MARCKS peptide treated groups showed significant differences in EDL groups. Results showed that conduits functionalized with MARCKS peptide enhanced functional recovery following critical gap nerve injury. These results will be confirmed with histological and proteomics analysis. Also, knockdown study for MARCKS protein on rat DRG neurons will be performed to show effect of MARCKS protein on molecular regeneration process of peripheral nerve.

57-SY-1  FLIM-PLIM microscopy toolkit for tissue engineering of the intestinal organoids

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Three-dimensional (3D) tissue models are important for tissue engineering, stem cell research and cancer biology, advancing basic and translational sciences. In particular, small intestinal organoids with complex cell composition and displaying many functional properties of native intestinal epithelium are highly heterogeneous and often need to be studied individually. Modern live cell imaging approaches, such as fluorescence (FLIM) and phosphorescence (PLIM) lifetime imaging microscopies, provide an opportunity to quantitatively, directly and in a minimally invasive way study cell cycle, real-time oxygenation, viscosity, Ca²⁺, pH and fluxes of other relevant biomarkers. However, reliable calibration, brightness and efficient bio-distribution are among the main requirements for FLIM and PLIM probes.

Here, we outline the recent developments in FLIM and PLIM imaging methods, helping to directly label cell proliferation and oxygenation in mouse intestinal organoids. This allows identifying and studying the dynamics of the stem cell niche, its metabolic activity and relate it to effects of drug action. Using analysis of organoid real-time oxygenation, we demonstrated the presence of trans-epithelial O₂ gradients and significant variability of oxygenation in culture. The application potential of cell proliferation by FLIM was demonstrated in study of metformin effect. We discuss how the method can be complemented by introducing scaffold materials and additional imaging parameters e.g. luminal autofluorescence, labeling lipid droplets or specific cell types, in order to facilitate more efficient tissue engineering.

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Marker-independent monitoring tools for online inline cell and tissue analysis

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Regenerative medicine offers unique opportunities for developing new, potentially personalized, therapeutic approaches to treat and ultimately prevent life-threatening diseases. This includes strategies for the replacement, repair, and regeneration of tissues and organs damaged by disease and/or traumatic injury. It is the fabrication of replacement tissues and organs that here is called tissue engineering. This represents a rapidly growing interdisciplinary field within regenerative medicine involving biology, chemistry, physics, engineering and medical sciences. A major focus of tissue engineering is the creation of ex vivo manufactured tissues and organs, even multi-organ systems, in order to explore fundamental questions of (stem) cell, extracellular matrix and developmental biology. These in vitro manufactured systems can also be used as sophisticated tissue and organ test systems to either reduce or even replace the need to use test animals. The monitoring of tissue-engineered constructs during their in vitro maturation or post-implantation in vivo is highly relevant for test system or graft quality evaluation. While traditional methods for studying (stem) cell and extracellular matrix components in engineered tissues and organs such as histology, immunohistochemistry or biochemistry require invasive tissue processing, resulting in the need to sacrifice the in vitro-engineered structures, multiphoton imaging and Raman microspectroscopy allow the non-invasive, marker-free monitoring. Moreover, Raman spectroscopy can also be used to generate biochemical profiles of living cells. Different cell phenotypes and cell fate decisions can be therefore assessed based on their Raman spectroscopic signature. My presentation will present an overview of our work on the utilization of marker-free monitoring modalities for basic and applied biomedical research and disease diagnosis.

Tracking tissue turnover and scaffold degradation in 3D constructs

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Three dimensional (3D) construct which mimics native tissues structure is the central part in tissue engineering. Two dynamic activities occur in opposite directions simultaneously within tissue engineering constructs. Biodegradable scaffolds are gradually decomposed, whilst neotissues are regenerated to fill the space of pores within scaffolds. For the prolonged culture process, non-destructive monitoring techniques allowing for real-time, live imaging of the constructs and associated scaffold degradation are highly valuable and demanded. Monitoring scaffold degradation is an essential area as degradation has been shown to have a significant effect on tissue turnover within constructs. Collagen is a key extracellular matrix. Its synthesis rate directly and indirectly reflects the metabolism of cells in the construct. Sometime its synthesis rate becomes the key marker of a specific tissue. In this study, we introduce two new techniques enabling to follow the aforementioned processes. The first technique is to tag polymers with biocompatible fluophors, which turns scaffolds into fluorescence. The intensity of the tagged fluorescence change correlates with the weight loss of the scaffold, and the degradation rate. To quantify the effect of scaffold degradation on the cell activities, we defined a turnover index for the correlation of biomaterial degradation and cell based extracellular matrix synthesis. The work showed that the degradation of a range of biomaterials can influence cell behaviour including proliferation and gene and protein expression. Slower degrading biomaterials were shown to increase cell proliferation when compared to faster degrading biomaterials, whereas faster degrading biomaterials were shown to increase osteogenic protein deposition when osteoblasts were used. A neocollagen fluorescent labelling technique using azide-proline was used to quantify the amount of collagen produced by fibroblasts within the conjunctival tissue model, which measured the collagen formation rate in response to various external stimulations up to two weeks for the same samples. Thus, these new techniques offer convenient tools for optimization of culture condition and mechanism study.
57-SY-5  Longitudinal Evaluation of Tissue Engineered Articular Cartilage using Non-destructive Multimodal Imaging: Effects of Latent Transforming Growth Factor- β1

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Regulatory guidelines for tissue engineered products require stringent characterization during production and necessitate the development of novel, nondestructive methods to quantitate key functional parameters for the translation of these products to the clinic. This study investigated the potential of a fiber-based, multimodal probe combining multispectral fluorescent lifetime imaging (FLIm) and ultrasound backscatter microscopy (UBM) to measure changes in the biochemical and mechanical properties of tissue engineered articular cartilage.

A scaffold-free, self-assembling process was used to generate neocartilage treated with active transforming growth factor-beta1 (TGF-β1), latent TGF-β1 (LAP), or without TGF-β1 (CTL) over a 28d culture period. Longitudinal FLIm-UBM tracking of neocartilage development was performed twice per week. In addition, matched samples were assessed nondestructively for their biochemical content (collagen and proteoglycan), their mechanical properties (tensile and compressive), and by histological staining. Single and multiple variable regression models were performed to evaluate relationships among optical, biochemical, and mechanical properties.

Structural parameters were quantified from UBM data and a novel homogeneity index was calculated combining FLIm and UBM parameters. Structural parameters were quantified from UBM data and a novel homogeneity index was calculated combining FLIm and UBM parameters.

Longitudinal optical assessment detected the changes in both the biochemical composition and mechanical properties of cartilage that occur during matrix maturation over 28d. LAP increased neocartilage extracellular matrix uniformity resulting in a 2.3-fold increase in compressive modulus over TGF-β1 and CTL samples to achieve native values. Both LAP and TGF-β1 conditions upregulated collagen crosslinking and increased tensile properties 5-fold over CTL. There were strong linear correlations between 1) FLIm LT (532-565 nm) and both proteoglycan content and compressive modulus and 2) FLIm LT (375-410 nm) and collagen content and tensile modulus. LAP significantly increased structural homogeneity 3.8-fold over CTL.

Nondestructive assessment of tissue engineered neocartilage, using a combination of FLIm and UBM in a single probe, proves successful as a quantitative method to determine tissue biochemical composition and mechanical function; this multimodal tool holds great potential for research, industrial, and clinical settings.

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**58-SY-1** Bone mineral matrix: An underappreciated regulator of metastatic breast cancer?

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Bone metastasis is a leading cause of death in patients with breast cancer, but the underlying mechanisms are poorly understood. While much work focuses on the molecular and cellular events that drive breast cancer bone metastasis, it is mostly unclear what role bone extracellular matrix (ECM) properties play in this process. Bone ECM primarily consists of mineralized collagen fibrils, which are composed of non-stoichiometric carbonated apatite (HA) and collagen type I. The physicochemical properties of HA nanocrystals can vary significantly by anatomical location, age, and pathology. However, whether bone regions typically targeted by metastatic breast cancer feature distinct HA materials properties is largely unclear. Furthermore, it is unresolved whether physiologically mineralized collagen fibers differentially affect tumor cell behavior relative to non-mineralized collagen fibers although epidemiological evidence suggests that reduced bone mineral density increases the risk to develop bone metastasis. This talk will discuss how a combination of high-resolution X-ray scattering analysis with large-area Raman imaging, backscattered electron microscopy, histopathology, and microcomputed tomography can be used to characterize HA in mouse models of advanced breast cancer in relevant skeletal locations. Furthermore, it will be highlighted how physiologically mineralized collagen may be used as a biomimetic platform to study how the mineralization state of skeletal collagen I may regulate tumor cell interactions within the bone microenvironment and which functional consequences may emerge for the pathogenesis and treatment of bone metastasis.

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**58-SY-2** 3D bioprinting of brain tumour models: multi-lineage glioma constructs to investigate tumour biology and drug responses

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Brain tumours are amongst the most deadly forms of cancer in both adults and children; even with the best current treatment, the great majority of brain cancer patients die from the disease within a year of diagnosis. An urgent need if we are to improve this situation is for better experimental models of the disease, both to deepen our understanding of the biology of these tumours and for successful pre-clinical testing of anticancer drugs. Recently, laboratory methods have been developed to provide cells representing many healthy and diseased states, with appropriate genetics and initial gene expression patterns. However, our inability to recreate the 3D environment of the body means that these cells behave very differently that they do in the body.

We are using 3D bioprinting with modified cell-laden alginate matrices incorporating hyaluronan and collagen, in order to create tumour-like structures containing both glioma stem cell lines and glioma derived stromal cells. Our results to date show that the printing process can be performed at very high cell densities with minimal effects on cell viability. We also see that cells proliferate after printing and that rapid cell-cell adhesion can be promoted with appropriate matrices. We are able to print glioma stem cells with and without stromal cells in separate layers within tumour-like 3D constructs with a smallest feature size (resolution) of approximately 300 micrometers. This capability to spatially position cell populations and other components within a 3D tumour construct allows new experimental approaches by controlling individual parameters of the tumour microenvironment and should provide reproducible human models for preclinical drug testing.
58-SY-3
Glycosaminoglycan-based biohybrid hydrogels for mimicking tumor microenvironments in vitro

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Glycosaminoglycans (GAGs) govern important functional characteristics of the extracellular matrix (ECM) in living tissues. Incorporation of GAGs into biomaterials opens up new routes for the presentation of signaling molecules for exploring mechanistic aspects of tumor formation and progression under defined constraints, as well as for individualized anticancer drug screening.

To systematically explore the related options, we have introduced a platform of biology-inspired hydrogels containing multi-armed poly(ethylene glycol), GAGs and peptides (1,2,3). GAG-hydrogel based 3D culture models were used to study breast and prostate tumor vascularization in vitro. Multiple cell types were shown to be less sensitive to chemotherapy when compared with two dimensional (2D) cultures and displayed tumor regression comparable to that displayed in vivo(5,6). Matrix metalloproteinase-sensitive GAG-based hydrogels functionalized with adhesion ligands and pro-angiogenic factors were furthermore shown to be instrumental for the ex vivoanalysis of acute myeloid leukemia development and response to treatment (7).

Beyond that, immunotherapeutic organoids were developed by housing human mesenchymal stromal cells (MSCs), gene-modified for the secretion of an anti-CD33-anti-CD3 bispecific antibody (bsAb), in macroporous GAG-based cryogel scaffolds. The constructs were demonstrated to serve as a transplantable and low invasive therapeutic machinery for the treatment of acute myeloid leukemia in a mouse model (8).

References

58-SY-4
A 3D tumour microenvironment regulates cancer cell growth and gene expression patterns

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Peritoneal invasion through the mesothelial cell layer is a hallmark of ovarian cancer metastases. Using tissue engineering technologies, we aimed to recreate an ovarian tumor microenvironment replicating this aspect of disease progression. 3D co-cultures consisted of ovarian cell-laden hydrogels and mesothelial cell-layered melt electrospun written scaffolds. These 3D constructs were characterised by proliferation and transcriptomic analyses and used as intraperitoneal xenografts. Increased cancer cell proliferation in 3D co-cultures was validated using patient-derived cells and linked to greater tumor burden in vivo. Transcriptome-wide expression analysis identified IGFBP7, PTGS2, VEGFC and FGF2 as bidirectional factors deregulated in 3D co-cultures compared to 3D mono-cultures, which was confirmed by immunohistochemistry of xenograft and patient-derived tumor tissues and correlated with overall and progression-free survival. This pre-clinical cancer model allows for the complexity of the disease as seen in patients and advances our understanding of the role of the tumor microenvironment in ovarian cancer progression.
The activities of JSRM are not limited to only publishing an academic journal that is common with general academic societies and also include other diverse activities, such as actively making policy proposals as a community, engaging in voluntary research/development and research promotion, and exploring new avenues of clinical research in collaboration with patients and citizens. The content of the representative policy proposal, the “Yokohama Declaration”, has had significant impact on the actual content of the “Act on the Safety of Regenerative Medicine” and “Pharmaceutical and Medical Devices Act” enacted in 2014 and has attracted worldwide attention as a premier approach to legal regulations. Moreover, the putting forth of such a policy proposal itself from an academic society was highly acclaimed worldwide, and in the 2014 World Stem Cell Summit, JSRM received the International Leadership Award, which was a global first for an academic society. After 2016, JSRM acquired competitive funds called “Regenerative Medicine National Consortium” from the Japan Agency for Medical Research and Development (AMED) and has since been able to utilize public funding for activities such as the support of clinical research, development of human resources, development of clinical research data systems, coordination of academia–industry translations, and interactive communication with patients/citizens, resulting in remarkable achievements including undertaking over 40 consulting engagements in support of clinical research and conducting educational workshops for most of the nationally accredited regenerative medicine committees. In particular, development of the “Good Post-marketing Study Practice” standard compatible database and “National Regenerative Medicine Database (NRMD)” is gaining attention as another global first in the attempt to acquire real world evidence from all clinical cases of regenerative medicine and cell therapy.

The activities of UK Catapult for commercialisation of Cell and Gene Therapy was established in 2012 as a centre of excellence in innovation, with a vision of the UK to be a global leader in the development, delivery and commercialisation of cell and gene therapy. We help cell and gene therapies to be safer, more effective, scalable and affordable.

Our mission is to drive the growth of the industry by helping cell and gene therapy organisations across the world translate early stage scientific research into full-scale commercialization by: taking products into clinical trial, de-risking them for further investment; providing clinical expertise and access to NHS clinical partners; providing technical expertise and infrastructure to ensure products can be made to GMP and delivered cost effectively; providing regulatory expertise to ensure that products can get to the clinic safely, in the shortest amount of time; providing opportunities for collaboration, both nationally and globally; and providing access to business expertise, grants and investment finance so that commercially viable products are progressed and investable propositions are generated.

We have the infrastructure and a team of specialists across the cell and gene therapy life cycle including non-clinical, clinical, process development, manufacturing, regulatory, health economics and market access expertise. We are based on Guy’s Hospital in central London, with over 180 cell and gene therapy experts, state-of-the art development and viral vector laboratories. We have also built a large-scale GMP manufacturing centre in Stevenage to help bring cell and gene therapies to market in the UK and internationally.

I will discuss how we collaborate with stakeholders (Industry, Government and University) by showing some case studies and will summarize the current UK cell and gene therapy clinical trial activities and industry growth from 2012 to 2017. Additionally, I will share information upon new UK initiatives including Advanced Therapy Treatment Centres and the New UK Accelerated Access Pathway. Finally, I will talk about the next 5 years of activity that CGT Catapult plans between 2018-2023. CGT Catapult works with the UK’s innovation agency “Innovate UK” who is part of UK Research and Innovation, a non-departmental public body funded by a grant-in-aid from the UK government. For more information please visit ct.catapult.org.uk or visit http://www.gov.uk/innovate-uk
61-SY-3  A collaborative public/private partnership driving commercialization in regenerative medicine

Michael May
CCRM, Canada

CCRM is a Canadian-based commercialization and translation centre accelerating the development of revolutionary new therapies in the field of regenerative medicine and cell/gene therapy. Dr. May’s presentation will highlight the key features of the CCRM model, including stakeholder networks, specialized teams and dedicated infrastructure, as well as showcase the major outcomes and lessons learned after six years of operation. Finally, he will describe how collaboration and global scaling of the model is needed to create a sustainable catalyst at the interface between academia and industry that generates both health and economic benefits for all stakeholders.

61-SY-4  TBA

Jeff Ross
Miromatrix, USA

TBA
**62-SY-1**  Quality Control of 3D Printed Resorbable Implants: The 3D Printed Airway Splint Example

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Resorbable scaffolds delivering biologics has been a cornerstone of regenerative medicine research for over three decades, promising personalized medicine in the form of patient specific reconstructive approaches. However, translation of this research to clinical care, even resorbable materials alone minus biologics, has occurred at an extremely slow pace. This is due in part to the economics of manufacturing patient specific devices and in part due to the regulatory requirements that must be addressed to achieve clinical translation. This is especially difficulty in academics given the model of academic research is orthogonal in many ways to the steps necessary for translation. Specifically, regulatory bodies like the FDA require all device development be done in a design control framework that is a centerpiece of quality control. There are, however, few published examples of academic design control approaches.

In this talk, we present an example of design control process for a 3D printed patient specific resorbable device that has been implanted in 15 patients. In this talk, we first outline the design control components required for any device: user needs, design inputs, design process, design outputs, design verification and design validation. We then detail how we have implemented each design control component for the airway splint, from developing user needs to design validation in clinical use. We specifically focus on challenges in the design control process that result from patient specific devices created through 3D printing, including the increased use of computational modeling to assess devices to specific batch testing of 3D printed devices to assess manufacturing quality of the 3D printing process. We then suggest a template that can be used as a basis for design control of general 3D printed patient specific resorbable devices, including how this design control template can fit into a general quality control process.

**62-SY-2**  Cardiovascular Tissue Engineering using 3D Printing Technology

Narutoshi Hibino

Division of Cardiac Surgery, Johns Hopkins Hospital, USA

Cardiovascular disease is one of the leading causes of death worldwide despite the variety of medical, mechanical, and surgical strategies. We have developed novel 3D printing technologies that could change the practice of cardiovascular disease treatment, including patient-specific 3D printed tissue engineered vascular graft and bio 3D printed cardiac tissue. I will discuss insights of these new 3D printing technology as well as challenges we need to overcome for future clinical application and commercialization.
Quality aspects of regenerative medical products
Yoshiaki Maruyama
Pharmaceuticals and Medical Devices Agency, Tokyo, Japan

On 2014, regulatory reform was carried out to improve access to new therapeutic innovations in regenerative medicine in Japan. Two laws known as "The Act on the Safety of Regenerative Medicine (Safety Act)" and "The Pharmaceuticals and Medical Devices Act (PMD Act)" came into effect on November 25 2014. Safety Act regulates medical professionals’ practices and clinical studies related to regenerative medicine, which had previously been under the jurisdiction of the Medical Practitioners’ Act and the Medical Care Act. PMD Act which revised Pharmaceutical Affairs Law, allows patient early access to promising therapies, using conditional and time-limited approval schemes (as "accelerated approval") for regenerative medical product. Accelerated CMC development is extremely important for accelerated approval. Quality control of biotechnological/biological products, which are high molecular compounds with complex structure possessing a low level of homogeneity, are good reference for human cellular and tissue-based products (hCTPs). At present, ICH guidelines for quality of biotechnological/biological products such as Q6B and ICH Q5 series can be applicable for quality control of hCTPs. If the final product consists of cells and noncellular components such as a matrix, medical materials, scaffolds, support membranes, fibers, and beads, clearly describe in detail the quality and safety of the noncellular components. Provide any relevant information concerning the noncellular raw materials, taking into consideration their type and characteristics, the form and function in the final product, and evaluation of the quality, safety, and efficacy from the standpoint of the presumed clinical indication. If using materials that are absorbed by the body, perform the necessary tests on the degradation products to address safety concerns. Guidelines for medical devices describe the test methods, and provide justification for the use of the noncellular raw materials. The presentation will give a short introduction to Japan regulatory framework for regenerative medical products and related guidelines. The key consideration for developing the regenerative medical products from experience of the consultation and review by Pharmaceuticals and Medical Devices Agency (PMDA) will be addressed.

Biofabrication: a new toolset to study biology in 3D with applications in regenerative medicine and in vitro models
Lorenzo Moroni
MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University, The Netherlands

Organs are complex systems, comprised of different tissues, proteins, and cells, which communicate to orchestrate a myriad of functions in our bodies. Technologies are needed to replicate these structures towards the development of new therapies for tissue and organ repair, as well as for in vitro 3D models to better understand the morphogenetic biological processes that drive organogenesis. To construct tissues and organs, biofabrication strategies are being developed to impart spatiotemporal control over cell-cell and cell-extracellular matrix communication, often through control over cell and material deposition and placement. Here, we present some of our most recent advancements in biofabrication that enabled the control of cell activity, moving towards enhanced tissue regeneration as well as the possibility to create more complex 3D in vitro models to study biological processes.
Stem cells are attractive sources in regenerative medicine. Human pluripotent stem cells (hPSCs), such as embryonic stem cells (hESCs) or induced pluripotent stem cells (hiPSCs), are one of the promising cell sources to be used in the future. Studies have indicated that stem cells require specific microenvironment for maintaining their pluripotency and differentiation abilities. Therefore, developing appropriate biomaterials for stem cell culture is necessary. In this study, we targeted on three aspects in order to develop an optimal hiPSCs and cultivation method for its applications: (1) Xeno-free extraction and cultivation of human mesenchymal stem cells (hMSCs) from tissue samples as reprogramming cell sources. (2) A safer reprogramming method (Sendai virus vector) instead of typically method (lentivirus or adenovirus vector) was applied to host cells for reprogramming into hiPSCs. (3) Xeno-free conditions for hiPSCs cultivation. We applied PVA-IA hydrogel, poly (vinyl alcohol-co-vinyl acetate-co-itaconic acid), and immobilized extracellular matrices (ECMs) or ECM-derived nanosegments for specific cell cultivation. In PVA-IA-oligopeptide immobilized dish, we can provide both physical and biological properties on the surface by adjusting hydrogel stiffness from 10 to 30 kPa and grafting specific oligopeptides on it. We compared our biomaterials with other commercialized ECM substrates (recombinant vitronectin-coated dishes, Synthemax II and CellStart coated dishes). Our results showed high stability of reprogrammed cells grew under PVA-IA grafted substrates having optimal elasticity and comparable with commercialized ECM (recombinant vitronectin) coated substrates. PVA-IA grafted substrates performed the highest reprogramming efficiency among other xeno-free conditions (0.03-0.21%). hiPSCs generated in this study can culture on our designed biomaterials for over 10 passages and showed pluripotent protein expression (Oct4, Sox2, Nanog, and SSEA-4). hiPSCs cultured after 10 passages can differentiate into cells derived from three germ layers (endoderm, mesoderm and ectoderm) in vitro by embryoid body formation as well as in vivo by teratoma formation. Our final goal is targeting on specific linages differentiation directly from hiPSCs on our biomaterials in xeno-free conditions for in future clinical usage.

References
1. A. Higuchi et al., Chemical Reviews, 2013, 113, 3297.
One of the most complex biological processes in human life is the repair and regeneration of a damaged lesion. Mainly, the chronic wounds healing for individuals suffering of various lethal genetic diseases are a challenging, costly, and even deathful - e.g., individuals suffering from a deadly genetic disease. Complex mechanistic pathways, specific to the distinct stem cells (SCs) populations, are activated in response to their regeneration, and therefore, stem cell (SCs) therapies have been proposed for the recovery of these wounds. The SCs have high capability for proliferation and release of soluble growth factors and cytokines that stimulate new vessel formation and modulate inflammation.

Herein, we used the bone marrow mesenchymal stem cells (BMSCs) based multifunctional nano-based grafts for the treatment of chronic wounds. The nano-grafts (NG) were synthesized using an original method and have a composition of biocompatible fibers, hydrogels, and nono-encapsulated antibiotics and growth factors. The NG were used as delivery systems of the BMSCs and has shown significant wound contraction in DB/DB mice model mimicking diabetic ulcers. The treatment efficiency was confirmed both by histology and immunohistochemistry results and showed rapid healing of diabetic skin wounds by promoting granulation tissue formation, angiogenesis, extracellular matrix secretion, wound contraction, and re-epithelialization.
Injectable Cell Delivery Constructs and Conductive Hydrogels for Myocardial Tissue Engineering

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Cell transplantation via direct intramuscular injection is a promising therapy for patients with ischemic diseases. However, following injections, retention of transplanted cells in engrafted areas remains problematic, which can be deleterious to cell-transplantation therapy. In this presentation, a thermo-responsive hydrogel system composed of aqueous methylcellulose (MC) blended with phosphate-buffered saline is constructed to grow cell sheet fragments and cell bodies for the treatment of myocardial infarction (MI). The as-prepared MC hydrogel system undergoes a sol–gel reversible transition upon heating or cooling at approximately 32°C. Via this unique property, the grown cell sheet fragments (cell bodies) can be harvested without using proteolytic enzymes; consequently, their inherent extracellular matrices (ECMs) and integrative adhesive agents remain well preserved. In animal studies using rats and pigs with experimentally created MI, the injected cell sheet fragments (cell bodies) become entrapped in the interstices of muscular tissues and adhere to engraftment sites, while a minimal number of cells exist in the group receiving dissociated cells. Moreover, transplantation of cell sheet fragments (cell bodies) become entrapped in the interstices of muscular tissues and adhere to engraftment sites, while a minimal number of cells exist in the group receiving dissociated cells. Moreover, transplantation of cell sheet fragments (cell bodies) significantly increases vascular density, thereby improving the function of an infarcted heart. These experimental results demonstrate that the developed cell sheet fragments (cell bodies) may function cell-delivery constructs by providing favorable ECM environments to retain the transplanted cells locally, consequently improving the efficacy of therapeutic cell transplantation. Additionally, injectable self-doping conductive hydrogels that improve electrical coupling of isolated cardiomyocytes and restore heart function after MI will be reported.
Synergy of ECM type and elasticity on the self-beating cardiomyocyte differentiation of iPS

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Stem cell-based myocardial regeneration therapies have emerged as alternative strategies to heart transplantation for various heart diseases. However, to use the autologous beating cardiomyocytes is one of the biggest problems. Here we investigated the synergetic effect of extracellular matrix type and its elasticity of the culture substrates on the cardiomyocyte differentiation of induced pluripotent stem cells (iPSs) in vitro. The differentiation process consists of multiple consecutive steps, and each step must require different culture substrates. We then studied the process by separately evaluating the four steps: (1) cardiac marker gene expression, (2) contractile gene expression and self-beating, (4) beating, and (4) beating duration. To this end, rat neonatal cardiomyocytes (NCMs) were evaluated in addition to iPS cells. These cells were cultured on substrates with different natures, i.e., an elastic substrate (Es) with the modulus of 9, 20, or 180 kPa, and hard tissue culture polystyrene dishes (TCPS) coated with collagen (Col), gelatin (Gel), or fibronectin (FN). The results revealed that the effective niches in each step were very different. The cardiac marker gene (GATA4, Tbx5, MEF2C) expression of iPSs was very high on the TCPS coated with FN or Gel, whereas on the FN-coated Es (especially with the 9 kPa modulus), the undifferentiated marker gene (Nanog) expression of iPSs was maintained. The expression of the contractile genes α-MHC, TnC1, and TnT2 and the self-beating of the NCMs were very high on the TCPS coated with FN or Gel, whereas on the FN-coated Es (especially with the 9 kPa modulus), the undifferentiated marker gene (Nanog) expression of iPSs was maintained. The expression of the contractile genes α-MHC, TnC1, and TnT2 and the self-beating of the NCMs were very high on the TCPS coated with FN or Gel, whereas on the FN-coated Es (especially with the 9 kPa modulus), the undifferentiated marker gene (Nanog) expression of iPSs was maintained. The results suggest that a single culture substrate is not suitable for preparing self-beating cardiomyocytes and are useful for understanding and designing cardiac differentiation niches for regenerative medicine.
The Effect of Mechanical Training on Human iPS cell-derived Cardiac Tissue Sheet Stacks

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Background: Cell sheet technology has been previously applied in our lab, with scaffold-free 3D tissues generated by stacking cardiac tissue sheets (CTSSs) showing promising therapeutic effects in vivo, as well as using CTSSs to develop an in vitro model of cardiac arrhythmia. While functionally promising, these three-dimensional tissues are still structurally immature and fragile to show sufficient force generation. To overcome this, we applied cyclic stretching forces during cell sheet stack culture in order to drive tissue maturation. Methods: CTSSs were prepared by differentiating cardiomyocytes and mural cells from 201B6 iPS cells and co-culturing them on temperature-responsive culture plates. Three layers of CTSSs were then stacked and allowed to merge with a biocompatible adaptor prior to loading the stacks on a mechanical training device to undergo cyclic stretching. CTSS stacks were then analysed for cellular viability, protein expression and force generation. Results: Our results showed that cyclic stretching clearly induced cellular alignment in both mural cells and cardiomyocytes, which is the first step towards cardiomyocyte maturation and improved mechanical properties, while keeping cellular viability above 95% (158/164 (96.4%) stretched; 134/137 (99.8%) suspended control; and 120/120 (100%) attached control). To a lesser extent, the static stress derived from tissue suspension also induced cellular alignment. This indicator of tissue maturation was accompanied by an increase in the expression of cardiac troponin I in cardiomyocytes. Additionally, force generation studies showed a tendency for an increase in Young's modulus and active force in the stretched samples (8.4kPa and 0.02mN, respectively) compared to the suspended control (1.0kPa and 0.007mN) and the attached control (7.2kPa and 0.006mN). Conclusion: The application of cyclic mechanical training on CTSS stacks drives cellular alignment on cardiomyocytes and mural cells, improves the tissue's mechanical properties and could potentially drive cardiomyocyte maturation, as shown by an increase in CTnI expression.


Heterotypic Effects within Cardiac Microtissue Environments on Human iPS-derived Cardiomyocyte Phenotype and Function

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Human pluripotent stem cell-derived cardiomyocytes (hPSC-CM) exhibit immature phenotypes and physiologic properties compared to adult CM, highlighting the need for effective methods to mature hPSC-CM in vitro. CMs naturally develop in close proximity to multiple non-myocyte cell populations, suggesting the presence of other cells is necessary for proper hPSC-CM maturation and function. In this study, we examined the effect of incorporating cardiac fibroblasts (CF) or endothelial cells (EC) in cardiac microtissues on the physiologic function and phenotypic state of CM. CM were differentiated from hPSCs via modulation of Wnt signaling followed by lactate purification. Engineered cardiac microtissues were generated using highly pure (>85%) hPSC-CMs alone or combined with CFs or ECs (3:1 ratio) in agarose microwell molds to form cardiac microtissues. 3D cardiac microtissues were maintained for up to 30 days after formation. Calcium imaging was performed at early and later time points, along with immunostaining of whole mount and histological samples. Although individual cardiac microtissues exhibited autonomous spontaneous beating activity, synchronous Ca²⁺ transients were observed within each construct and all of the tissues could be externally paced. One week after formation, the magnitude and kinetics of Ca²⁺ handling were greater for microtissues with CF compared to CM alone or with ECs, suggesting accelerated maturation. However, by 30 days, differences in Ca²⁺ handling between microtissue groups were not observed. Single-cell RNA-Seq analysis could easily distinguish distinct cell types and phenotypic states of the heterogeneous cell populations coming from the differentiated cell populations and 3D heterotypic microtissues. Consistent with Ca²⁺ results, CM cultured with CF as microtissues for 7 days exhibited a more pronounced phenotypic shift than for CM-EC or CM alone microtissues; CF and EC also had significant phenotypic shifts after culture with CM in microtissues. Ongoing analysis will determine specific molecular shifts due to heterotypic 3D microtissue culture in the CM and non-myocyte populations. Overall these studies demonstrate the ability to interrogate the phenotypic and functional impacts of complex heterotypic interactions that mimic the native cardiac tissue environment.
**65-SY-2** Generation of canine and feline pluripotent stem cells for application in regenerative medicine

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Induced pluripotent stem cells (iPSCs) have the property of differentiating into various cell types and can be generated from somatic cells. Hence, it is considered to be a powerful tool in disease modeling and transplantation therapy. Establishment of canine and feline iPSCs would enable us to generate various differentiated cells to cure several canine and feline refractory diseases, including diabetes, liver disease, chronic kidney disease, etc. It might also be possible to apply the knowledge gleaned from veterinary medicine to human medicine.

In our study, we generated canine iPSCs (ciPSCs) using lentiviral vectors encoding human OCT3/4, SOX2, KLF4, and C-MYC. The ciPSCs presented in a tightly domed shape, and expressed a critical pluripotency marker and a normal karyotype. Additionally, the ciPSCs differentiated into cells derived from all three germ layers via the formation of an embryoid body. They also differentiated into megakaryocytes (MKs) and platelets on OP9 stromal cells supplemented with growth factors. The ciPSC-derived MKs were hyperploid and transformed into proplatelets. Electron microscopy revealed that the generated platelets had the same ultrastructure as peripheral platelets.

We further aimed to derive iPSCs from cat embryonic fibroblasts by retroviral transfection with mouse Oct3/4, Klf4, Sox2, and c-Myc. We observed flat, compact primary colonies that resembled human iPSC colonies, whose cells expressed embryonic stem cell markers, had a normal karyotype, proliferated beyond passage 45, and differentiated into all three germ layers in vitro. Additionally, these cells had the capacity to differentiate into blood cells such as monocyte-like cells and erythroid progenitor-like cells.

Finally, we tried using the Sendai virus vector encoding human OCT3/4, SOX2, KLF4, and C-MYC for transfection. Sendai viruses can help express genes exogenously without genome insertion. Hence, we generated the canine and feline iPSC lines using the Sendai virus vector for clinical applications. Our canine and feline iPSCs maintained their pluripotent state even after removal of the Sendai virus vector. Thus, these iPSCs may serve as suitable cell sources for clinical applications in the future.
Clinical application and future directions of mesenchymal stem/stromal cell therapy for spinal cord injury in dogs

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Severe spinal cord injuries (SCI) often have a grave prognosis for motor and sensory function in humans and dogs. Common causes in dogs include vertebral fracture/luxation and intervertebral disc herniation. Clinical signs in dogs are similar to those in human patients. Therefore, naturally occurring SCI in dogs provide an ideal opportunity to develop translational research for treatment of SCI. Mesenchymal stem cells (MSCs) derived from various tissues such as adipose tissue and bone marrow have been well-documented in the last decade. However, it is still challenging to apply stem cell therapies using canine MSCs in clinics. Therefore, we have developed a new approach for stem cell therapy using novel canine MSCs, named as bone marrow peri-adipocyte cells (BM-PACs). In this presentation, I would like to show our challenges to develop stem cell therapies using BM-PACs for spinal cord injury and articular cartilage injury in dogs. We have previously isolated a cell population adhering to mature adipocytes in canine bone marrow by ceiling culture method and named this population as BM-PACs. When compared to canine bone marrow mesenchymal stem cells (BMMSCs) harvested by adhering culture of mononuclear cells in bone marrow, BM-PACs showed superior colony-forming ability, self-renewal and multipotency, and had higher expression of CD73. To date, we have further revealed BM-PACs secrete hepatocyte growth factor (HGF), which is a well-known tissue-repairing factor, in response to pro-inflammatory cytokines such as TNF-α and IL-1β. In addition, when intravenously injected into a severe spinal cord injury model in nude mice, BM-PACs migrated into the injured spinal cord and functional recovery was promoted. These results indicate that BM-PACs appear to be a feasible source for stem cell therapy for spinal cord injury in dogs through trophic effect and homing ability.

On the other hand, focusing on the eminent chondrogenic ability of BM-PACs, we are developing a method of fabricating ex vivo canine cartilaginous constructs using a 3D-bio printer and stem cell therapy for articular cartilage injury in dogs. To obtain high-quality hyaline cartilage, optimal conditions for chondrogenic induction in dogs were investigated. To date, we have revealed FBS concentration and growth factors such as basic fibroblast factor (bFGF) and growth differentiation factor 5 (GDF-5) are essential for ex vivo reproduction of canine hyaline cartilage. Currently, a 3D-canine cartilage construct is fabricating to replace a damaged canine articular cartilage.
Guideline for securing the safety of regenerative medicine and cell therapy in dogs and cats

Kazuya Edamura
Guideline Working Group, the Japanese Society for Veterinary Regenerative Medicine, Japan

In Japan, the law regarding regenerative medicine for humans has been enacted to carry out scientifically and ethically. On the other hand, no such laws or guidelines exist in the field of veterinary medicine. Thus, the number of veterinary hospitals that introduce regenerative medicine and cell therapy has been increasing, and unfortunately some incidents that damage trust have occurred. From this background, the decision was made to create guideline for regenerative medicine and cell therapy in veterinary medicine. The Japanese Society for Veterinary Regenerative Medicine and the Japanese Association of Veterinary Regenerative Medicine and Cell Therapy began by forming a guideline working group and then drafted a preliminary guideline. In addition, the advice was got from the Ministry of Agriculture, Forestry, and Fisheries of Japan and the Japan Veterinary Medical Association, and then “Guideline for securing the safety of regenerative medicine and cell therapy in dogs and cats” was completed. The guideline went into force in April 2018, and guideline on regenerative medicine and cell therapy for dogs and cats has been applied for the first time in the world. This guideline does not include approved cellular and tissue-based product for animals. Its sole purpose is to ensure the safety of regenerative medicine and cell therapy procedures performed in clinical veterinary settings. This guideline covers veterinary medical practices using somatic cells, embryos, immune cells, and stem cells that have undergone processing. This guideline emphasizes that safety should be ensured and that the benefits should outweigh the risks. In addition, it states that therapies without scientific grounds should not be performed merely at the request of an owner or veterinarian. Furthermore, this guideline also includes an outline for informed consent, quality control of cells and scaffolds for therapy, methods for transplanting and administering cells, and storage periods for cells used in therapy. The most important feature is the introduction of the first notification system for veterinary medical practices in Japan. The responsible person of veterinary hospital performing regenerative medicine or cell therapy submits a notification to the Consortium for the Advancement of Animal Regenerative Medicine (CARM) and a certificate will then be issued from CARM. In this lecture, I will provide a summary of the guideline and overview of the notification system.
Overview of liver regeneration

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The liver is highly regenerative, however chronic and acute liver damage such as hepatitis virus infection, alcohol intake and nonalcoholic steatohepatitis (NASH), etc. result in liver cirrhosis, leading to liver failure, with reduced liver function, portal hypertension, and increased risk of hepatocellular carcinoma (HCC). Regarding liver regeneration, two modes; 1) compensatory hypertrophy model (mature hepatocytes in the remaining tissue become hypertrophy followed by cell division after partial hepatectomy) and 2) stem/progenitor cell-mediated regeneration models (immature adult hepatic stem/progenitor cells are activated and contribute to the regeneration process by differentiating to hepatocytes and biliary epithelial cells) are popular. Liver transplantation is ultimate therapy for these liver failures, however recent basic and clinical approaches are elucidating the possibility of other therapies. We developed autologous bone marrow cell infusion (ABMI) therapy that improves liver fibrosis and induces liver regeneration from 2003 in human, and recently elucidated that combination therapy using mesenchymal stem cells and induced macrophages from bone marrow effectively induce fibrosis regression and liver regeneration in mice. In this mice study, we elucidated using intra-vital imaging technique that the administrated macrophages phagocyted the hepatocyte debris and produced pro-regenerative factors. Recently hepatocytes or liver buds induction from ES cell and iPSC cells are actively studying with aiming the future clinical study and new concept of chemically induced liver progenitors (CLiPs) are introduced and proposed a new direction for cell transplantation therapy. Furthermore, engineered hepatocyte sheet and mesenchymal cell sheet are also expected for future application.

Transplanting tissue-engineered epithelial cell sheets to esophageal mucosal defect for the prevention of severe strictures

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Iron-regulatory mechanism of liver and functional organoids

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Iron is crucially involved in many of the essential physiological processes of the human body, such as oxygen delivery by red blood cells, energy processes in muscles, and enzymatic catalysis of various metabolic processes. However, excessive free iron in blood is highly reactive and can cause cellular and visceral damage. Therefore, tight regulation of plasma iron is required to avoid iron-related toxicity in the body.

The liver plays a key role in iron metabolism of our body via hepcidin, an iron-regulatory peptide hormone produced by hepatocytes. An increase of plasma iron concentration activates bone morphogenetic protein (BMP) signaling pathway in hepatocytes, which is followed by expression and secretion of hepcidin. The peptide hormone binds to ferroportin of intestinal cells and induces internalization and degradation of the cellular iron exporter, which is followed by suppression of iron influx from dietary to plasma. However, the mediator between iron stimuli and activation of BMP signaling has not been elucidated yet.

In our study we demonstrated that Runx3 is the upstream regulator of BMP6 in the liver. We observed an iron-overloaded liver with decreased expression of hepcidin. Interestingly, BMP6 expression and activity of the BMP pathway were decreased in the liver tissue of Runx3 knock-out (KO) mice. A transcriptome analysis on primary hepatocytes isolated from Runx3 conditional KO (cKO) mice also revealed that iron-induced increase of BMP6 was mediated by Runx3. Similar results were observed in Runx3 knock-down experiments using HepaRG cells, which are functional human hepatocytes and HepG2 cells, which are human hepatocarcinoma cells. Finally, we showed that Runx3 enhanced the activity of the BMP6 promoter by responding to iron stimuli in the hepatocytes.

Recently, organoids have been an emerging model to study the molecular mechanisms underlying organ functions. Liver organoids established by several research groups showed various function of livers such as lipid and glucose uptake and storage, and metabolism. However, iron metabolism-related functions require hepatocyte-endothelial cell interaction, and no liver organoid showing the functions has been reported. Using 3-dimensional endothelial cell network and bi-potent hepatoblasts, we established novel in vitro culture system to mimic hepatocyte-endothelial cell interaction. This system would be useful to study the detail mechanism of iron metabolism by liver.
Platelet derivatives as cell culture supplements and as triggers of endogenous regeneration mechanisms for tissue repair

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Present cell culture medium supplements, in most cases based on fetal bovine serum (FBS or FCS), are not fully satisfactory. Platelet growth factors can support cell proliferation and differentiation and human platelet derivatives and were proposed as alternatives to FCS. Platelet derivatives used as cell culture medium supplements, are commonly provided in the form of platelet lysate (PL) within a small amount of plasma. We investigated the role played by PL devoid of plasma or serum contaminants and by serum derived from plasma free of platelet contaminants, on the viability, proliferation kinetic and differentiation potential of MSC derived from human tissue. The effect on the proliferative capability was also tested on several cell lines. Although PL (in saline solution) was capable of activating the cell proliferation machinery, the PL itself used as single additive to the medium was unable to support cell proliferation unless plasma or serum components were also present. Interestingly, in constitutively stimulated cells, such as different cell lines, or in some cultures of cells derived from fetal tissues, the addition of PL was not an absolute requirement and cell proliferation could be obtained by the simple addition of PL-free serum.

For tissue repair, major logistic and economical constraints exist to a broad application of transplantation of “ex vivo” expanded autologous stem/progenitor cells, alone or associated to carrier biomaterials. Therefore, a stem cell-based therapeutic approach could be adopted only in extreme life or organ saving situations and, to enable a large number of patients to benefit, new strategies should be considered. The triggering event in all injured tissues is the platelet activation. Platelet released factors promote innate immune cell migration to the wound site, thus creating an inflammatory microenvironment, in turn, causing the activation of angiogenesis and vasculogenesis processes. Eventually, repair or regeneration of the injured tissue occurs via paracrine signals activating, mobilizing or recruiting to the wound site cells with healing potential, such as bone marrow derived stem cells and progenitors or undifferentiated cells derived from the reprogramming of tissue differentiated cells.
Platelet lysate induces activation of chondroprogenitor cells in mature human articular cartilage

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Objective: Regeneration of damaged hyaline cartilage still remains a tremendous clinical challenge. New trends in cartilage regeneration often contemplate the use of platelet derivatives (PRP or PL) for the effect on the proliferation of human articular chondrocytes preserving their somatic differentiation potential and inducing the cartilage repair. Our study identify new pathway induced by platelet derivatives that are able to regulate articular cartilage homeostasis.

Materials and Methods: From human articular cartilage biopsy adult human articular chondrocytes (HAC) and chondro-progenitor cells (CPC) were isolated and expanded “in vitro”. The CPC population was obtained by PL (platelet lysate) treatment of human cartilage chips: after 10 days in culture the cells migrate and proliferate. Both cell populations were tested “in vitro” for clonogenic capability, for phenotype and gene expression of typical chondrogenic markers and for differentiation potential.

Results: Our data strongly suggested that fully differentiated chondrocytes possess “reserved stemness,” which are reactivated during in vitro expansion only in presence of PL, gradually displaying multipotent stem/progenitor cell characteristics. PL is able to activate and recruit CPCs positive for nestin, gremlin-1 and sox-9 genes normally expressed in dividing cells during the early stage of development.”In vivo”, cartilage matrix formation was assessed by histology after subcutaneous transplantation of HAC/CPC isolated cell culture; cells were seeded on PGA-HA scaffolds with or without PL and implanted into nude mice. The addition of platelet derivatives to cartilage grafts resulted in robustly formation of hyaline-like cartilage that showed the expression of type II collagen and Chondromodulin-1 (ChM1), a specific anti-angiogenic factor strongly present in avascular cartilage. Moreover, type X collagen and VEGF expression were not observed in the tissue graft, supporting the maintenance of hyaline cartilage phenotype and no hypertrophy tissue transition.

Conclusion: Our study show that PL induces in mature cartilage, cell activation and proliferation leading to new cartilage formation through stimulation of chondroprogenitor resident cells. These observations support the application of PL for therapeutic treatment of damaged articular cartilage.

Platelet Lysate-laden Hyaluronic Acid/Cellulose Nanocrystals Hydrogels Display Chemotactic and Pro-angiogenic Properties

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Tissue engineering (TE) constructs on any meaningful scale or complexity must incorporate aspects of the functional tissue, namely a vasculature, providing encapsulated cells the nutrients and oxygen critical for their survival, and a privileged path for progenitor cells income. However, the ability of current TE strategies to promote a fast revascularization is critically limited. Particularly in endodontic regenerative therapies, the complicated anatomy of root canal system, and the narrow apical access limit the supply of new blood vessels and pulp tissue ingrowth. This study aimed at improving the chemotactic and pro-angiogenic features of injectable hydrogels by using bioactive smart biomaterials for the controlled delivery of growth factors (GFs).

For this goal, we proposed the amelioration of a class of injectable hyaluronic acid (HA) hydrogels formed in situ, reinforced with cellulose nanocrystals (CNC), and enriched with platelet lysate (PL). Hydrogels were prepared by mixing equal volumes of 1) aldehyde-modified precursors (HA and CNC) solutions and 2) hydrazide modified HA solutions containing PL. Different weight ratios of CNC were tested. Our findings suggest that the incorporation of a-CNC enhanced the stability of the materials against hydrolytic and enzymatic degradation. Moreover, the release of the chemotactic and pro-angiogenic factors (PDGF and VEGF) from the PL-laden hydrogels showed an improved sustained profile proportional to the amount of incorporated a-CNC. The PL-laden hydrogels exhibited preferential cell supportive properties in in vitro culture conditions due to the potential interaction of encapsulated human dental pulp cells (hDPCs) with the biochemical cues provided by PL proteins. Finally, the ability of the PL-laden hydrogels to recruit dental pulp-origin cells and promote cell sprouting in hDPCs/ Human umbilical vein endothelial cell co-cultures in vitro was confirmed.

Overall, these results proved that the developed hydrogels may simultaneously act as a GFs controlled delivery system and as a supportive matrix for cell culture, recruitment, and revascularization induction, holding great potential for the regeneration of vascularized soft tissues, such as the dentin-pulp complex.

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Molecular Mechanisms of Platelet-Rich Plasma (PRP): Implications for Clinical Use

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Introduction
Platelet-rich plasma (PRP) is used in multiple medical specialties without clear understanding of its mechanisms of action1,2. PRP’s efficacy is controversial for virtually every indication, with mixed reports that are not infrequently diametrically opposed3,4. This study was designed to study PRP’s molecular mechanisms of action.

Materials and Methods
Multiple human PRP samples (N=23) were collected and isolated following IRB approval. Human bone-marrow mesenchymal stem/progenitor cells (hMSCs) and HUVEC were separately assayed for migration, proliferation and differentiation in response to different PRP concentrations. Caspase 3 and TUNEL were performed to evaluate apoptosis. Pro-inflammatory cytokines (IL-1β, TNFα, IL-6), anti-inflammatory cytokine (IL-10) and apoptotic FasL were assayed by ELISA. All quantitative data were treated by ANOVA and Bonferroni analysis, upon verification of normal data distribution with α value at 0.05.

Results
Strikingly, 50% PRP induced cell death. Cleaved-caspase3 and TUNEL assays showed abundant cell debris exposed to 50% PRP relative to ≤ 25% PRP. Excessive pro-inflammatory IL-1β, TNFα and IL-6, apoptotic FasL and abnormally high osmotic pressure were observed in 50% PRP. Nonetheless, diluted PRPs not only induced dose-dependent MSC migration, but also promoted endothelial tube formation of HUVECs, benchmarkable with VEGF.

Discussion and Conclusions
Novelty of the present findings includes cell death caused by ≥ 50% PRP due to excessive pro-inflammatory and apoptotic agents, and aberrantly high osmotic pressure, which in itself may stimulate reactive oxygen species and cause apoptosis. PRP concentrations and/or composition may need to be tailored for a given clinical indication.

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The Effects of Platelet Lysate on Human Macrophages in in vitro Mono- or Co-Culture with hBMSCs

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Macrophages are important cells of the innate immunity. They have several functions, like phagocytosis of pathogens, the initiation of inflammatory reactions, but also aid tissue repair and thus can be classified into inflammatory M1 and anti-inflammatory M2 type. Especially along with multipotent human bone marrow stromal cells (hBMSCs), macrophages activate tissue renewal and anti-inflammatory reactions. Platelets take also part in tissue regeneration processes. Once activated and degranulated they release different growth factors, which leads to tissue repair. Hence, human platelet lysate (hPL) has been considered a promising alternative serum component for cell culture. Especially in the field of tissue regeneration and the corresponding use of biomaterials, hPL is becoming more and more interesting.

In this study, we analyzed the effects of hPL, with or without the addition of heparin, as serum supplement on macrophages in in vitro mono- and co-culture with hBMSCs in comparison to human serum (hS) and fetal calf serum (FCS). We demonstrated that hPL represents a successful alternative to hS for macrophage mono-cultivation, since qPCR and flow cytometry proved spontaneous and induced differentiation into the different macrophage subtypes. The cell adhesion in culture medium with hS and hPL was comparable and higher than in medium with FCS.

The phagocytic activity of macrophages in hPL was approximately the same as in hS, but differed significantly from the results in medium with FCS.

Further, we analyzed the performance of hPL as serum supplement for the in vitro co-culture of macrophages and hBMSCs, which are commonly cultivated with different standard sera, i.e. hS for macrophages and FCS for hBMSCs. In the co-culture with hPL, both cell types showed phenotypes comparable to their respective standard culture serum. In addition, differences in gene expression and phagocytic activity were observed. Moreover, we showed that there is no need of heparin addition for macrophage in vitro culture with hPL.

With this data, we proved for the first time that hPL is a successful serum alternative for macrophage mono- and co-cultivation with hBMSCs, especially overcoming the negative effects of FCS on macrophages. Thereby, both cell types showed comparable phenotype and functional characteristics to their respective standard sera in cell culture.

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**68-SY-1** Osteogenic Programming by Inhibiting Epigenetic Suppression

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The gene expression program that supports osteogenesis is suppressed during early stages of fetal development to prevent precocious mineralization of the collagenous extracellular matrix that characterizes bone. Suppression of bone specific gene expression is mediated in part by epigenetic mechanisms involving DNA methylation and trimethylation of lysine residues in histone 3 (H3K9me3 and H3K27me3), as well as by transcription factors that control mesenchymal lineage commitment and progression. We discuss data from an extensive human musculoskeletal RNA-seq gene expression atlas (n>1000 samples) showing that specific isoforms of distinct epigenetic regulators are selectively expressed during osteoblast phenotype commitment and maturation. RNA interference, gene knockout and pharmacological inhibition studies combined with genomics approaches (e.g., ChIP-seq) revealed that blocking suppressive epigenetic regulators in mesenchymal stem cells stimulates osteogenic differentiation to promote new bone formation.

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**68-SY-2** Smart hydrogels for local and controlled delivery of RNAi molecules to cells for tissue regeneration

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RNA interference (RNAi) molecules permit post-transcriptional control over cell gene expression and function, and may have great value when applied in tissue regeneration strategies. Traditional RNAi molecule delivery approaches, which rely on incorporation into nanoparticles or liposomes and systemic delivery or bolus injections, are plagued by challenges such as RNA degradation by serum RNases and limited accumulation or rapid clearance at the target site. These issues often result in only limited and transient cellular effects. We have engineered macroscale hydrogels capable of localized, sustained and tunable delivery of RNA (i.e., siRNA and miRNA) to cells incorporated within hydrogel and to those in the surrounding microenvironment. Systems will be presented in which delivery may be regulated by tailoring hydrogel degradation, controlling affinity interactions with the RNA, tethering RNA to the hydrogel backbone, and applying external “on-demand” stimuli. The capacity to spatially control delivery of RNA and subsequently spatially control changes in gene expression will be demonstrated. Finally, the promise of this approach to deliver RNA relevant for tissue engineering bone *in vitro* and *in vivo* will be presented.
**68-SY-3** Sustained non-viral delivery of small non-coding RNAs to promote nerve regeneration

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Modulating the local inhibitory microenvironment at the spinal cord injury (SCI) site is a common therapeutic approach that is often insufficient to achieve desired nerve regeneration. Mature neurons have limited regenerative capacity and thus, it is crucial to stimulate this intrinsic growth ability of injured axon. MicroRNA (miR) is a class of nucleic acid that plays significant roles in regulating the local protein synthesis at growth cones, promoting their advancement into the non-permissive zones. However, the lack of effective non-viral miR delivery strategies limited the clinical application of miRs. Here, we first introduce an aligned fiber in vitro platform that provides both sustained non-viral delivery of miRs and proteins as well as topographical cues for effective miR combination screening in neurons. Successful gene knockdown in primary neurons was achieved through this platform. 4 established axonal miRs and their cocktails were screened with this in vitro platform by using neurons from both central and peripheral nervous systems. Selected miRs were subsequently incorporated into a three-dimensional (3D) aligned hybrid fiber-hydrogel in combination with Neurotrophin-3 for SCI treatment. Using a complete spinal cord transection injury rat model, robust and aligned nerve ingrowth into our 3D hybrid scaffold was observed as early as two weeks post implantation. Further, the extent of nerve regeneration in response to our miR combination was similar to our in vitro screening data. Recently, using this 3D hybrid scaffold, we developed a spinal cord organotypic culture where spinal cord slices from thoracic-lumbar region were seeded on these scaffolds to observe neuronal filament outgrowth. Interestingly, we observed similar extent of ingrowth and the presence of miRs within scaffolds significantly enhanced this phenomenon. Taken together, our results demonstrate the potential of our aligned fiber platform in providing sustained non-viral gene transfection to neurons in vitro and in vivo and facilitating nerve regeneration after SCI.

**68-SY-4** Spatiotemporally Controlled CRISPR/Cas9 Gene Editing

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The recently discovered CRISPR/Cas9 gene editing system and its derivatives have found numerous applications in fundamental biology research and pharmaceutical sciences. The need for precise external control over the gene editing events has driven the development of inducible CRISPR/Cas9 systems. We are pursuing an elegant optogenetic approach to control CRISPR/Cas9 using light-responsive components. We have demonstrated the feasibility of chemical modification of CRISPR/Cas9 and achieved light-mediated DNA cleavage upon 1-min exposure to light in vitro. Safe and efficient intracellular delivery of such constructs represents a major problem, and we have developed a biodegradable polymer-based platform that can deliver Cas9/gRNA complexes into a variety of cell lines with a 30-fold higher efficacy than commercially available transfection reagents. Finally, we have achieved light-mediated GFP reporter gene as well as an endogenous gene editing in HEK293 cells, whereas modified CRISPR/Cas9 showed no detectable gene editing in the absence of light irradiation. Our approach will allow for two-photon photoactivation and multiplexing of different targets enabling the use of this strategy in 3D matrices for tissue engineering applications.

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Duchenne muscular dystrophy (DMD) is a muscular disorder, affecting up to 1 in 3500 births worldwide. The disease is caused by the almost complete absence of dystrophin protein due to out-of-frame mutations in the DMD gene. The loss of dystrophin is characterized by progressive muscle weakness and wasting, eventually causing skeletal and cardiac muscle degeneration, respiratory and cardiac failure, and death before the age of 30. Currently, there are no effective cures available for DMD; therefore, therapy is limited to management of symptoms, including physiotherapy and steroids medication. Antisense oligonucleotides (AONs) exon-skipping is one of the most promising therapeutic strategies for DMD, designed to skip a specific DMD exon, which produces a shortened transcript but a functional dystrophin protein. The use of carriers can be an efficient approach for AON delivery, particularly in order to increase AONs stability and to enhance cell uptake. Here we designed a biomaterial strategy for encapsulation of therapeutic AONs in hydrogels as a part of controlled release system that can improve the pharmacokinetic properties of the AONs. Specifically, we used PEG-fibrinogen (PF) hydrogel-based microspheres for the delivery of the AONs in the treatment of DMD. The hypothesis was that this microsphere device will greatly decrease the overall administered dosage, thus reducing any adverse effects associated with their administration. The specific AONs used were intended to facilitate dystrophin expression and the device was used to achieve controlled administration of the AONs in vivo. We investigated the effects of increased cross-linking density of the hydrogel on AONs encapsulation and the release rate from the hydrogel. The resulting PEG-fibrinogen microspheres were spherical, with a diameter of approximately 90 µm and with a biphasic release profile of AONs modified by the cross-linking density of the hydrogel. Cellular uptake and localization of AONs in mouse C2C12 muscle cells demonstrated that AONs released from the microspheres well penetrated into the cells. In vivo experiments were performed by intra-atrial and intramuscular injections in mdx mice. Histological and protein expression analysis were performed after 30 and 60 days, to demonstrate altered dystrophin expression as a result of the therapy. The results indicate that this delivery system could represent a potential therapy for DMD.

Introduction:
Electrical stimulation (ES) offers excellent control on cell growth, orientation, and intracellular calcium level. Electrical signals have the capability to induce intracellular calcium concentration which helps in bone remodelling and mineral deposition. Capacitive coupling (CC) stimulation involves two parallel electrodes placed above and below, or left and right to the well plate, with no direct contact of electrodes to the culture medium, hence the effect of toxicity, pH changes and current fluctuation can be minimised. With appropriate electrical regime, homogenous electric field (EF) can be generated. This allows equal amount of stimulation for every cell regardless of the position in the culture vessel [1].

Methodology:
A customised CC stimulation bioreactor is designed and simulated using COMSOL Multiphysics. The EF in culture medium and titanium disc were also simulated to obtain an applied voltage for the actual stimulation.

Titanium disc was sonicated in degreasing solution followed by sterilisation before conducting the experiments. The bioreactor was stimulated continuously for 14 days, under incubation at 37°C & 5% CO₂, with medium change every 3 days. Human bone marrow derived mesenchymal stem cells lineage (passage 8) was employed in the experiments.

Results and Discussion:
A very homogenous EF was simulated across the culture medium and titanium disc. The corresponding applied DC voltages to EF were as followed: 15.0 V for 100 mV/mm and 30.0 V for 200 mV/mm. Simulated and actual experimental current were both zero. No visible effect on pH changes under this ES regime.

Higher proliferation rate and cell orientation were observed. AlamarBlue assay suggested that the cell metabolism rate in 200 mV/mm was doubled to that of non-stimulated and 100 mV/mm. The significant increase in cell metabolism rate may be an effect of activated electrical signal across the titanium disc and bioreactor, and hence triggering greater cell proliferation. Live/Dead Imaging showed that hMSCs cultured on the discs were aligned in a direction and greatly stretched across the discs - either multiplying themselves, or differentiating into osteoblastic cells. Alkaline Phosphatase (ALP) assay revealed that the ALP activity was highest in 200 mV/mm, denoting an early marker of bone mineralisation and osteogenic cell differentiation under the higher EF.

References:
Physical gradients play a major role in the regeneration of peripheral nerves [1,3]. Collagen, the predominant structural protein in nerve ECM, is commonly used as a physical support for cells to make repair scaffolds [2]. To improve nerve repair approaches, it is critical to understand the cell-substrate interaction and the changes in cell behaviour induced by the stiffness. However, little is known about the effect of a mechanical gradient on neurite extension [3]. In this study, the stiffnesses of two RAFT-stabilised Collagen Gradients (RsCGs) were characterised. The behaviour of NG108-15 neural cells was studied in response to these RsCGs. RsCGs were fabricated using rat tail collagen type I (First Link, UK). A standard protocol [4] was used to generate neutralised collagen solutions within 3D printed moulds designed to generate gradients. The gels were stabilised using RAFT® absorbers (Sartorius/Lonza) to produce RsCGs. Each gradient was separated into three regions with a predefined collagen density range (67-100 mg/ml). Atomic force microscopy (AFM) was performed to map the stiffness profile across different regions of RsCGs. NG108-15 cells (500 cells/mm²) were seeded onto the upper surface of RsCGs in serum-free DMEM. Cultures were maintained for 2 days in a humidified incubator. Neurite extension was detected using immunostaining and fluorescence microscopy and analysed using ImageJ.

AFM results showed a stiffness variation correlated to the change in collagen density. 45 ± 10% cells elongated neurites on lower density gradient versus 28±4% elsewhere. The mean neurite length is 100±3.6 µm. They were more neurites elongating towards a lower density gradient. In conclusion, collagen constructs have been engineered to include well-defined density gradients for studying neural cell behaviour in vitro. This has revealed information about how neurites respond to gradients with specific ranges of stiffness. Furthermore, results indicated that neurite sprouting and orientation can potentially be altered in response to physical gradients within collagen gels in vitro. These data will inform a mathematical model to simulate how neurite extension might be enhanced and controlled, providing new information to aid in the design of nerve repair scaffolds.

4. JB Phillips et al, Tissue Engineering, 2005

Engineered skeletal muscle tissues in three-dimensional (3D) cell culture platforms that resemble the complex native muscle structure and organization can be used as in vitro models to study muscle physiology and metabolism [2]. As a secretory organ, the skeletal muscle produces and releases myokines in response to contractile activity under different physiological conditions. These proteins have hormonal effects on several organs, including the muscle itself. Therefore, the development of functional tissues made up of highly aligned and mature myofibers in a 3D environment could provide valuable insight into muscle metabolism studies, especially if they are integrated with immunosensing technologies that monitor myokines and other secreted molecules.

Here, we present 3D skeletal muscle constructs, fabricated by encapsulating C2C12 cells in a photocrosslinkable Gelatin Methacrylate and Carboxymethylcellulose Methacrylate (GelMA:CMCMA) hydrogel scaffold. These hydrogels, assembled onto the upper part of transwell permeable supports, present a microgrooved topography that promotes cell alignment and differentiation [4]. Electrical stimulation (ES) was then applied to the engineered tissues during cell culture using biocompatible carbon electrodes integrated in the transwell system to induce tissue contraction. Cell alignment, differentiation, and ES effects were assessed by calculating the orientation angle and fusion index of immunostained myotubes expressing Myosin Heavy Chain (MHC).

Moreover, the transwell configuration allows the combination of our cell culture platform with a magnetic microbead-based immunosensor. By having specifically functionalized magnetic microbeads in suspension within the lower compartment and in direct contact with target myokines, very low limits of detection can be achieved. Overall, this device is a promising approach toward organ-on-a-chip technologies, which would present a major advance in the preclinical research of metabolic and degenerative diseases.

References:
Muscle tissue engineering is an attractive approach to produce an in-vitro tissue model to better understand skeletal muscle physiology and the mechanisms of muscle diseases. We previously reported that aligned myotube constructs were produced using a micropatterned thermoresponsive substrate. In this study, to functionize this engineered tissue, we developed a new culture system using a fibrin-based gel. Aligned myotubes prepared on the micropatterned surface were transferred onto a fibrin-based gel for further maturation. As a result, after 3 weeks of incubation on the gel, the myotubes showed sarcomere structures essential for muscle contraction. In fact, these myofibers contracted significantly by electrical pulse stimulation (EPS). When simulated at 1.0 Hz frequency (voltage: 10 V, duration time: 3 ms), the myofiber sheet showed twitch contraction, whereas the tetanic contraction was observed at 15 Hz frequency. These contractile behaviors indicate that the myofiber sheet was functional physiologically.

This study also demonstrated that the EPS-induced muscle contraction was prevented chemically by the addition of ryanodine into the medium. With 50 mM of ryanodine, the contracting displacement decreased to approx. 60% after 1 min, and then the contraction and relaxation completely stopped within 5 min. In addition, higher doses of ryanodine prevented more quickly the muscle contraction. These results suggest that the ryanodine affected physiologically muscle contraction through its specific interaction with ryanodine receptor in the myofibers. These results allowed us validate their potential use of the myofiber sheets in predictive studies of muscle physiology. Also, this engineered tissue is possible to be used to better understand the relationships between mechanical stress and myogenesis including muscle growth and regeneration. In this study, continuous EPS (1h stimulation at 1 Hz frequency with 3 h rest) was repeatedly applied for 2 weeks to allow the myofibers exercise. This periodic exercise actually enhanced the contractile ability of the myofibers. Since the physiology of skeletal muscle is directly related with mechanical stress, this tissue model will become a platform for the biological studies of skeletal muscle including muscle metabolism, muscle atrophy and muscle regeneration.


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Cardiovascular diseases are responsible for the greatest proportion of deaths in the Western countries. A major cause of heart dysfunction following heart attack is the extensive remodeling of the extracellular matrix, which leads to the formation of fibrotic scar and death of heart muscle cells (cardiomyocytes, CMs) - a collective process known as myocardial remodeling. Diagnosis and treatment of this progressive pathological remodeling process are a top priority in the modern healthcare.

The current research aims to engineer a novel 3-dimensional (3D) human patterned heart construct based on a unique nano-technological approach of heart cells manipulated by magnetic force. Novel magnetic nanoparticles (MNPs) were first developed to target human CMs. Prussian blue assay, electron microscopy, confocal microscopy as well as cell viability assays confirmed that these MNPs can efficiently and selectivity bind to the human heart cells and preserve their phenotype and viability. Next, a simple, cost-effective, one-step approach for fabrication of a patterned and magnetic heart tissue was demonstrated by manipulating the magnetically labelled heart cells to the desired orientation in a 3D collagen hydrogel under the influence of magnetic field. The biochemical, morphological, and electrical properties of the patterned 3D heart tissue were fully characterised by histological, gene expression, and advanced electrophysiological methods (multi-electrode array and optical mapping). The current data indicate that the 3D construct preserves characteristics similar to the native heart tissue, including cell-cell and cell-matrix interactions. In-vivo studies using pre-clinical cardiac MRI and two-dimensional echocardiography demonstrated patch location within the healthy rat heart and showed increase in cell retention with no alteration to normal cardiac function.

This state-of-the-art platform can be translated to treat the remodeled area in the heart by restoring structure and function to the damaged heart tissue; and marks a major breakthrough in magnetic targeting therapy for lesions localized deeper in the body. The outcome of this novel research has the potential to become a vital component in therapeutic approaches to cardiovascular diseases.
A microscale biomimetic platform for generation and electro-mechanical stimulation of 3D cardiac constructs

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Unraveling the mechanisms involved in cell differentiation and tissue generation and maturation remains a great challenge in the field of tissue engineering. In vitro cardiac models are potent tools to investigate in an economically sustainable way the cardiac differentiation of stem cells and myocardium tissue development. Here we present an innovative micro-bioreactor designed to provide 3D cardiac microtissues with a controlled biomimetic environment for biochemical, electrical and mechanical conditioning.

The platform consists of 3 functional elements: i) a culture compartment, composed by a 3D cell-laden hydrogel region flanked by 2 side channels for medium perfusion, ii) a pressure-actuated compartment providing cyclic mechanical stretch to cells, and iii) a pair of stainless-steel electrodes. The design was optimized through finite element (FE) modeling, in terms of electric fields and current density intensities. The platform was experimentally characterized by recording the electric current when monophasic or biphasic signals (5V/cm, 2ms at 1Hz) were provided. Preliminary validation was performed culturing fibrin-embedded human bone marrow stromal cells (hBMSC) under electrical stimulation with biphasic waves (0.6V or 3V, 2ms duration, 1Hz), evaluating cell viability and preferential orientation. The platform was then exploited to guide tissue maturation of neonatal rat cardiomyocytes (NRCMs) subjected to the same stimulation.

The micro-bioreactor provides microtissues with a controlled biomimetic environment. FE analysis demonstrated uniformity of electric field and current density, with intensities of 5V/cm and 60mA/cm² respectively. Such values were confirmed by experimental characterizations. hBMSCs cultured up to 7 days showed high viability and exhibited a preferential orientation coherent with electric field. Electrically stimulated NRCMs microtissues showed enhanced electrical connections, evidenced by the spontaneous synchronous beating, the higher maximum capture rate achieved (5.58 ± 0.3 vs 4.4 ± 0.2 of ctrl) and the higher expression of markers characterizing cardiac maturation (TnT and Cx43). Current investigations are aimed at observing the combined effects of electrical and mechanical stimulations.

We developed a micro-bioreactor allowing the evaluation of biochemical, mechanical and electrical stimulation cues, either alone or combined, on cardiac cell maturation, which represents a promising tool for developmental biology studies.

Translational Research of Cultured Corneal Endothelial Cell Transplantation

Naoki Okumura

The corneal endothelium maintains corneal transparency via its pump and barrier functions. Consequently, its decompensation causes severe vision loss due to corneal haziness. Fuchs’ endothelial corneal dystrophy and decompensation of corneal endothelium post cataract surgery are the leading causes of corneal endothelial dysfunction. At present, corneal transplantation is the only therapeutic option for treating corneal endothelial dysfunction.

Modern corneal transplantation techniques provide excellent clinical outcomes post surgery. However, we are constantly focused on generating new therapeutic modalities with higher effectiveness and less invasiveness. In 2013, we obtained the approval from the Japanese Ministry of Health, Labour, and Welfare to initiate a first-in-man clinical trial of cell-based therapy to treat corneal endothelial dysfunction in Japan. Our initial clinical results seem to indicate that this treatment is both safe and effective.

The ultimate goal of translational research is to connect innovative research ideas with subsequent commercial products. In this presentation, translational research aimed at the treatment of corneal endothelial dysfunction will be introduced. In addition, I will provide our example as a platform for debate about translational research.
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**71-SY-2**

**Femtosecond laser cut corneal stromal lamellae for the bioengineering of endothelial grafts**

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With only one donor cornea available in the world for 70 requests, the shortage of transplants is overwhelming (1). One third of grafts are currently used to perform endothelial keratoplasty that selectively replace the posterior surface of the diseased cornea. The replacement of these donor grafts by endothelial bioengineering (injectable cells or reconstituted graft) would therefore make it possible to reserve donations for indications requiring a whole cornea and which remain very numerous.

Our university team of ophthalmologist-researchers develops solutions to recycle corneas disqualified by banks and usually destroyed. In France, a self-sufficient grafting country with 4500 keratoplasties/year, about 2000 corneas are destroyed for insufficient endothelial quality while they are microbiologically safe. We have developed an original process that allows 10 to 12 ultra thin lamellae to be cut from these corneas using a femtosecond laser (2). These transparent and corneo-compatible lamellae are decellularized from their keratocytes and stored over the long term. They are ready to use to be re-cellularized with human corneal endothelial cells or their functional equivalent. The reengineered grafts can be manipulated like an ultra-thin DSAEK or DMEK graft to be implanted in the patient. In parallel we have also developed and patented a corneal bioreactor which allows the functionality of endothelial bioengineering products to be tested on human corneas by measuring their ability to control hydration and corneal thickness and to survive over time. This unique platform complements animal experimentation.

**References**


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**71-SY-3**

**Co-ordinated generation of multiple ocular cell lineages and fabrication of corneal epithelial cell sheets from human IPS cells**

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The eye is a complex organ whose highly specialized constituent tissues are derived from different primordial cell lineages. The retina, for example, develops from neuroectoderm via the optic vesicle, the corneal epithelium is descended from surface ectoderm, whilst the iris and collagen-rich stroma of the cornea have a neural crest origin. Recent work with induced pluripotent stem cells (iPSCs) in culture has revealed a previously under-appreciated level of intrinsic cellular self-organization, with a focus on the retina and retinal cells, and eventually, lead to a start of clinical research for iPSC-derived retinal cells transplantation. Moreover, we and others have demonstrated the in vitro induction of a corneal epithelial cell phenotype from pluripotent stem cells (iPSCs) in culture has revealed a previously under-appreciated level of intrinsic cellular self-organization, with a focus on the retina and retinal cells, and eventually, lead to a start of clinical research for iPSC-derived retinal cells transplantation. Moreover, we and others have demonstrated the in vitro induction of a corneal epithelial cell phenotype from pluripotent stem cells. These studies, however, have a single, tissue-specific focus and fail to reflect the complexity of whole eye development. Furthermore, no established method for isolation of corneal epithelial lineage has existed. Here, we demonstrate the generation from human induced pluripotent stem cells of a self-formed ectodermal autonomous multi-zone (SEAM) of ocular cells. In some respects the concentric SEAM mimics whole eye development because cell location within different zones is indicative of lineage and this is broad, spanning the ocular surface ectoderm, lens, nerve-retina, and retinal pigment epithelium. The approach also has translational potential and to demonstrate this we show isolation of corneal epithelial progenitor cells from the ocular surface ectodermal zone of the SEAM with a cell sorter using combination of antibodies for specific cell surface markers and expansion of them ex vivo to form a functional corneal epithelial tissue that recovers function in an experimentally-induced animal model of corneal limbal stem cell deficiency.
71-SY-5  Regulatory perspectives on developing investigational regenerative medical products in Japan

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In Japan, regenerative medical products, i.e., cell therapy and gene therapy products, are an independent product category under Pharmaceuticals and Medical Devices Act (PMD Act). PMD Act, enacted in November 2014, introduced “time limited, conditional approval for marketing of regenerative medical products”. In the clinical development of regenerative medical products, they must meet certain requirements on quality and non-clinical evaluation before being administered to human subjects in clinical trials to ensure safety. In order to facilitate clinical development of regenerative medical products, it is important to understand these requirements at an early stage of product development, and to understand process control that affect product quality and characteristics. These considerations support product quality that affect patient safety and clinical performance of the product. During clinical development, verification of manufacturing process will inform the process control, further enabling stable production of the investigational regenerative medical product.
Novel Management of Larger Bone Defect: Combination of Biomaterials and Distraction Osteogenesis Technique

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Introduction: Distraction osteogenesis (DO) techniques have been widely accepted and practiced in orthopaedics, traumatology, and craniofacial surgery over the last two decades. The major limitation of DO is relatively long period required for new bone consolidation. Here, we investigated whether the application of biomaterials, including polycaprolactone (PCL) and hydroxyapatite (HA) cylinder or composite microspheres could be used to reduce the treatment time and enhance bone formation in DO.

Study 1: A 1.0cm tibial shaft was removed in the left tibia of 36 rabbits and divided into three groups: Group A, the defect gap shortened for 1.0-cm; Group B, the defect gap was filled with 1.0-cm porous HA/TCP cylindrical block; Group C, The 1.0-cm defect gap was reduced 0.5cm and the remaining 0.5-cm defect gap was filled with 0.5-cm HA/TCP block. The tibia was then fixed with unilateral lengthener; for groups A and C, lengthening started 7 days after surgery at 1.0 mm/day, in two steps. Group A received lengthening for 10 days and Group C for 5 days, there was no lengthening for Group B. All animals were terminated at day 37 following surgery. The excised bone specimens were subject to micro-CT, mechanical testing and histological examinations. BMD and the mechanical properties of the regenerates were significantly higher in Group C compared to Groups A and B. MicroCT and histological examinations also confirmed that the regenerates in Group C had most advanced bone formation, consolidation and remodeling compared to other groups.

Study 2: PCL/HA (20 mg), PCL (20 mg), or PBS were then locally administered into the distraction gap in SD male rat DO model at the end of distraction period and 4 weeks later the animals were terminated. Weekly x-rays, MicroCT, mechanical testing, histology, and immunohistochemical examinations were performed to assess the quality of the newly bone. The bone volume/total tissue volume, bone mineral density, and mechanical properties of the newly formed bone were significantly higher in the PCL/HA group compared to the PCL and PBS groups. Histological and immunohistochemical analyses confirmed improved bone formation and vascularization in the PCL/HA group.

Conclusions: The combined use of biomaterials such as HA/TCP blocks or PCL/HA composite microspheres in DO is a novel approach for promoting bone regeneration and consolidation, their clinical applications may reduce the treatment time, pain and suffer of the patients.

Dawn of Cybernic Treatment
-- Functional Regenerative Treatment by Cybernic System HAL

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Effect of isotropic gravity on cytoskeletal and nucleoskeletal structures of human mesenchymal stem cells

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An understanding of fundamental mechanisms underlying the cellular behaviors to mechanical properties of culture environment will extend our knowledge regarding the regulation of cell functions. The present study aimed to investigate the effect of isotropic gravity on actin cytoskeleton and nucleoskeleton of human mesenchymal stem cells (hMSCs) using a three-dimensional clinostat, an isotropic gravity generator, Gravite®. Fluorescent staining of F-actin revealed that cells in unidirectional gravity culture displayed numerous actin stress fibers in both the basal side and apical side of the nucleus and their actin stress fibers mainly oriented along the longitudinal cell axis. However, the stress fibers in cells cultured in isotropic gravity condition were present along the basal side of the cells but not at the apical side of these nuclei. In addition, the paxillin which is a focal adhesion protein at lamellipodia was phosphorylated in the periphery of the cells cultured in isotropic gravity condition, although expression of phosphorylated-paxillin in cells cultured in unidirectional gravity condition was not observed. Finally, to assess changes in the spatial organization of nuclear lamin, we compared the 3D organization of a major nuclear lamin component, lamin A/C. Lamin A/C was detected at the periphery of the nucleus in all culture conditions. Cells in unidirectional gravity showed that lamin A/C was located mainly on the apical side and largely absent from its basal side, whereas cells in isotropic gravity culture showed that lamin A/C was dominantly localized at the apical and basal side of the nucleus. These results indicate that the isotropic gravity culture affects the formation of cytoskeletal, focal adhesion, and nucleoskeletal structures through dynamic hMSC behaviors.

Cells are able to sense certain environmental changes induced by gravity and will respond accordingly. Although responses to changes of the gravity environment have been intensively studied, little is known about how cells react to isotropic gravity. The aim of this study was to investigate the effects of isotropic gravity on human mesenchymal stem cells (hMSCs) using a three-dimensional clinostat (Gravite®) for generating isotropic gravity. After hMSCs were subject to isotropic gravity for 24 h, the cell morphology, cytoskeletal formation, and nucleoskeletal structures were investigated. Time-lapse observation revealed that cells in unidirectional gravity culture presented unidirectional migration with a stretched morphology, while cells in isotropic gravity culture showed multidirectional migration with oscillation in direction of cell movement. We found that cells in unidirectional gravity culture maintained their shape with formation of fibronectin fibrils in the bodies, introducing to the stabilization of focal adhesion with enriched stress fibers. However, cells in isotropic gravity culture showed partial contraction with degradation of fibril structures in the trailing edge. In addition, the paxillin which is a focal adhesion protein at lamellipodia was phosphorylated, leading to active lamellipodium protrusions. In addition, phosphorylated-paxillin spots in the cells cultured in isotropic gravity were more intensive at cell periphery but being less in the cell bodies than those in unidirectional gravity. Furthermore, a major component of the nuclear lamin, lamin A/C of cells in unidirectional gravity culture was mainly located at apical side than basal side, representing a basal-to-apical polarization of lamin A/C. However, cells in isotropic gravity culture showed localization of lamin A/C at both of apical side and basal side. These results demonstrated that isotropic gravity affects the changes in nuclear lamina organization through spatial reorganization of cytoskeleton.

Interactive effects of cell therapy and rehabilitation in brain injury model

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The patients with central nervous system (CNS) diseases such as stroke, spinal cord injury, and parkinson’s disease suffer from persistent neurological defects. In general, they have taken rehabilitation such as exercise therapy to facilitate their functional recovery. Recently, cell therapy has been gaining attention as a novel treatment of CNS diseases. Animal experiments and clinical trials revealed that cell therapy achieved the recovery of CNS diseases. Principle of rehabilitation and cell therapy shared same goal for improving their function. However, there are a few reports about the effect of rehabilitation after cell transplantation for brain injury. Here, we report the cell transplantation into a brain injury model followed by treadmill exercise training. We designed traumatic brain injury model mice and mouse embryonic stem-derived neural stem/progenitor cells (NSC) were used for cell transplantation. Model mice were divided into the following five groups according to the treatment received: only NSC transplantation after brain injury (group T); only treadmill exercise after brain injury (group E); NSC transplantation and treadmill exercise after brain injury (group TE); the absence of treatment after brain injury (group C) and only skin incision (sham operation; group S). Mice in groups T and TE were injected with NSC intravenously on day 7 after brain injury. Starting at 24 h after transplantation, the mice in groups E and TE were placed on the treadmill and were made to run for 20 min as a rehabilitation procedure. In results, mice in the group T and the group E showed more improvement in motor functional analysis compared with mice in the group C. Moreover, mice in the group TE exhibited the most significant improvements in motor functional analysis and electrophysiological analysis (motor-evoked potential) among five groups. These results demonstrated that rehabilitation is important for functional recovery after cell therapy.
Generating artificial human skeletal muscle would be instrumental for investigating muscle pathophysiology and developing novel therapies. However, most bioengineering platforms are challenged by the limited expansion potential and differentiation ability of tissue-derived myogenic cells. Moreover, there is an increasing need to develop patient-specific, multilineage, complex skeletal muscle platforms to model severe and incurable muscle disorders. These obstacles negatively impact the translational potential of these platforms to develop novel therapies for muscle diseases. To overcome these limitations, we generated three-dimensional (3D) artificial skeletal muscle tissue from human pluripotent stem cells, including induced pluripotent stem cells (iPSCs) from patients with Duchenne, limb-girdle and congenital muscular dystrophies. 3D skeletal myogenic differentiation of pluripotent cells (both transgene-driven and transgene-free) was induced within hydrogels under tension to provide alignment of myofibres. Artificial muscles recapitulated key characteristics of human skeletal muscle tissue and could be implanted into immunodeficient mice. Importantly, pathological cellular hallmarks of severe incurable muscular dystrophies could be modeled with high fidelity using this novel 3D platform. Finally, we show generation of fully human iPSC-derived complex multilineage models, containing key isogenic cellular constituents of normal skeletal muscle, including vascular endothelial cells, pericytes and motor neurons. These results lay the foundation for a human skeletal muscle organoid-like platform for regenerative medicine, complex disease modelling and drug development.
**73-SY-2 Establishment of a long-term culture system for Muscle stem cells**

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Muscle stem cells (MuSCs, or Satellite cells) have essential roles in homeostasis and regeneration of skeletal muscles. Although MuSCs are considered as a potent source for muscle regeneration therapies for the muscular dystrophy, the difficulty to expand MuSCs in vitro without reducing their regenerative and self-renewal capacity still remains. Recently, we have reported that Klf5, a zinc-finger transcription factor is an essential regulator of skeletal muscle differentiation and regeneration. MuSCs isolated from the muscle progenitor cell specific Klf5 knockout mice (Klf5cKO) showed higher proliferating rate and decreased expression of myogenic differentiation markers compared with the control MuSCs. We performed the transcriptomic analysis and found several signaling cascades were upregulated in Klf5cKO MuSCs. By the treatment of a small compound which stimulates one of the signaling pathway increased in Klf5cKO MuSCs, we succeeded to promote long-term expansion of Pax7 expressing MuSCs. The expanded human MuSCs were capable of promoting the regeneration in NOG-mdx mice. These results represent an efficient method to expand functional MuSCs for the stem cell therapy.

**73-SY-3 Core transcription factors promote induction of functional Pax3-positive skeletal muscle stem cells**

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The use of adult skeletal muscle stem cells (MuSCs) for cell therapy has been attempted for decades, however it still encounters considerable difficulties. MuSCs derived from human induced pluripotent stem (hiPS) cells are promising candidates for stem cell therapy to treat Duchenne muscular dystrophy (DMD). Here we report that 4 transcription factors, selected by comprehensive screening of different MuSC populations, enhance the derivation of PAX3-positive myogenic progenitors from hiPS cells, using medium that promotes the formation of presomitic mesoderm. These induced PAX3-positive cells contribute efficiently to the repair of DMD-damaged myofibers and also reconstitute the MuSC population. These studies demonstrate how a combination of core transcription factors can fine-tune the iPSC cell-derivation of muscle stem cells capable of contributing to the repair of adult skeletal muscle.
Cell therapy is one of desired method for treating intractable muscular diseases, such as Duchenne muscular dystrophy (DMD). Instead of adult satellite cells, generating satellite cells from induced pluripotent stem cells (iPSCs) would have advantage for application of cell therapy, because of their unlimited proliferation potentials. Here, we demonstrated the effective stepwise differentiation method from human iPSCs to engraftable muscle stem cells without transgene induction. In the first step, we induced the dermomyotome-like population over 90% efficiency. In the second step, we induced myotome-like population that is identified as Myf5 positive cells, which showed highly myogenic differentiation potential in vitro. Gene expression profile of purified Myf5+ cells demonstrated that the expression of Pax7, a marker of satellite cells, was significantly increased in Myf5+ cells at the late stage of differentiation. To assess the regeneration potential, we transplanted the Myf5+ cells at the late stage of differentiation into immunodeficient DMD-model mice. The Myf5+ cells could be engrafted in more than one hundred of host myofibers and regenerate the diseased muscles with producing dystrophin. Moreover, a part of the engrafted cells settled as a satellite cells in vivo with expressing Pax7. Finally, we confirmed the recovery of muscle function after transplantation. Taken together, we demonstrate that the transplantation of the human iPSC-derived muscle stem cells with step-wise differentiation can be effective for DMD with amelioration of muscle function. Our results facilitate to establish the cell therapy of muscular diseases using iPS cell-derived muscle stem cell. 

References:
A bioengineered skeletal muscle tissue as an alternative for autologous tissue flaps, which mimics the structural and functional characteristics of the native tissue for reconstructive surgery. Rapid progress in the cell-based tissue engineering principle has enabled in vitro creation of cellularized muscle-like constructs; however, the current fabrication methods are still limited to build a three-dimensional (3D) volumetric muscle construct with a highly viable, organized cellular structure with the potential for a future human trial. Here, we applied our integrated tissue-organ printing (ITOP) strategy to fabricate an implantable, bioengineered volumetric skeletal muscle tissue (mm$^3$ – cm$^3$) composed of human primary muscle progenitor cells (hMPCs). The bioprinted skeletal muscle tissue showed a highly organized multi-layered muscle bundle made by viable, densely packed, and aligned myofiber-like structures. Our in vivo study presented that the bioprinted muscle group reached 82% of functional recovery in a rodent model of volumetric muscle loss (VML) injury at 8 weeks of post-implantation. In addition, immunohistological examinations indicated newly formed organized skeletal muscle tissue with vascular and neural integrity. We demonstrate the potential of the use of the 3D bioprinted skeletal muscle with biomimetic features that can reconstruct volumetric large-scale muscle defects.

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To simulate the structure and function of natural vessels, a bilayer tubular graft was fabricated via coaxial electrospinning and dynamic liquid electrospinning. The tubular graft was based on poly(l-lactide-co-caprolactone)/collagen (P(LLA-CL)/COL) fibers and yarns, and loaded with heparin/anti-CD133 antibody (HEP/CD133) in lumen. Compliance test results showed the graft provided satisfactory compliance, which was comparable with human saphenous vein and better than the commercial ePTFE. Furthermore, the release of HEP/CD133 was sustained for almost 40 days, and the fibers and yarns still maintained their structures. The released heparin contributed to lumen anticoagulation and the anti-CD133 antibody promoted the recruitment of endothelial progenitor cells (EPCs). Smooth muscle cells (SMCs) proliferated significantly better on the yarns than the fibers, and penetrated throughout the entire yarns in the outer layer. In vivo evaluations through H&E, Masson’s trichrome and immunohistochemical staining were performed after implanting the grafts in nude mice for 2 months, demonstrating that the bilayer vascular graft promoted vascular-related tissue regeneration. The monolayer endothelium (CD 31 labelled) and aligned smooth muscle tissues (α-SMA labelled) regenerated on the bilayer graft, which presented similar components and structure with the native vessels. Hence, the functional bilayer graft can be a promising candidate incorporating with multi-functions including anti-thrombus, endothelium formation and SMCs penetration for vascular tissue engineering.

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In vivo engineered extracellular matrix scaffolds with aligned microchannels enhanced tissue regeneration and functional integration

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Regeneration and functional integration of tissue repairing materials with host tissue remains a challenge for regenerative medicine. Extracellular matrix (ECM) materials possess excellent biocompatibility and potential tissue regeneration activity in comparing with the synthetic materials. However, the decellularized ECM scaffolds often failed to support cell infiltration and cellularization, thus leading to poor tissue regeneration and severe calcification. Here, we developed an in vivo engineering technique to prepare ECM scaffold. A fibrous polymer template was implanted subcutaneously in rat for a few weeks to allow the formation of a hybrid tissue. Upon harvest, the explants were subjected to decellularization and removal of the polymer, and leaving ECM scaffolds with the predesigned shapes, desired porous structure and good mechanical strength. Three types of ECM scaffolds were prepared with this method, and they exhibited rapid cellularization, vascularization and excellent tissue regeneration in rat tibialis anterior muscle, abdominal aorta and left sciatic nerve, respectively. The ECM scaffolds composed of only natural collagen, elastin and GAG, demonstrated minimal immune response in analyzing the M2/M1 ratio and expression of inflammation related cytokines. In summary, this approach demonstrated the versatility and flexibility in preparing state-of-the-art ECM scaffolds for repairing the difficult tissue damage or loss.

Key Words: in vivo tissue engineering; subcutaneous implantation; fibrous PCL; extracellular matrix
Neutrophils rapidly swarm to an implanted biomaterial and can modulate tissue regeneration through several mechanisms, like the abundant release of biomaterial-preconditioning neutrophil extracellular traps (NETs), which can induce fibrotic tissue formation around electrospun polydioxanone (PDO) templates. We previously showed that NET release is differentially modulated by the template fiber diameter, with large diameter (LD) fibers significantly decreasing NET release compared to small diameter (SD) fibers, which translated to improved marginal tissue integration in vivo. In this study, we investigated if electrospun SD and LD PDO templates could function as a local delivery system of Cl-amidine, an irreversible inhibitor of peptidyl arginine deiminase 4 (PAD4), to further attenuate PAD4-mediated NET release during the acute neutrophil response to biomaterials. We hypothesized that local elution of Cl-amidine from the templates would reduce NET release with the greatest effect from SD templates. SD and LD templates were electrospun with 0.4 ± 0.2 μm and 1.8 ± 0.6 μm diameter fibers, respectively, fabricated with 0.5 mg/mL Cl-amidine, and analyzed for elution of active drug. Acute NET release was then evaluated in vitro with freshly isolated human peripheral blood neutrophils and in vivo with a rat subcutaneous implant model. The elution experiments revealed that Cl-amidine eluted from the SD and LD templates in a dose-dependent manner and that 60% of the total elution occurred within 3 hours. The in vitro results indicate that NET release was significantly decreased on SD templates in a dose-dependent manner while the opposite was observed for LD templates, suggesting that NET release is occurring independent of PAD4. In addition, the in vivo results verified local inhibition of NET release in the inflammatory microenvironment through the rapid burst release of Cl-amidine from the SD templates. Importantly, LD templates with Cl-amidine significantly enhanced neutrophil invasion and survival in vivo, indicating the potential for long-term microenvironment regulation through the secretion of potent factors from the neutrophil. With an emphasis on biomaterial-guided tissue regeneration, this preliminary study demonstrates that electrospun PDO templates can function as a novel delivery vehicle for Cl-amidine to regulate acute NET preconditioning and neutrophil survival to potentially enhance neutrophil-driven tissue integration and regeneration.
Tissue dysfunction after skin wound healing still is a reality for most of the employed therapeutics. This is greatly because a reparative process rather than a regenerative one occurs. Improved therapeutic approaches are therefore a major reality in the field but are likely to be achieved with enhanced and sustained knowledge on skin pathologies and associated wound healing.

It has been increasingly recognized that Tissue Engineering principles, elements and successes can be used to bioengineer in vitro human tissue units in which the environmental milieu of tissue development, regeneration and disease progression can be studied. Under this context we have been focusing on tailoring biomaterial properties to accurately recreate cell-cell and cell-extracellular matrix (ECM) interactions representative of each skin cell niche to be able to modulate cellular crosstalk and tissue (patho)physiology. A direct comparison with microenvironments, based on cell’s own ECM, generated using cell sheet technology has allowed posing different questions regarding skin regeneration and disorders that ultimately will help to uncover associated “therapeutic niches”.

Ultimately, we envision taking advantage of the generated knowledge to propose tissue engineered substitutes with improved functionality, as well as to advance the development of new or improved therapies for skin diseases and disorders.

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In a previous study conducted in mice full thickness wounds, we were able to observe the formation of de novo hair follicles and rete-ridges like structures, when 3D constructs of human adipose stem cells (hASCs) cell sheets were applied. Together with these findings, an up-regulation of keratinocyte growth factor (KGF), in relation to the control group, was detected under the testing conditions. This data pointed out to the hypothesis that the natural adhesive character of the cell sheets promoted the direct interaction between the host and the transplanted cells. To unravel this proposed crosstalk, both a direct and indirect (mediation by KGF) interplay were analysed. A set of in vitro scratch assays revealed that the secretome of hASCs in contact with human keratinocytes (hKC) promotes cell migration and closure of the scrape. But, in the presence of KGF-antibody a diminished hKC migration was observed, suggesting that KGF might be one of the key cytokines involved in the interaction with hASCs. In order to further investigate the effect of direct communication between hASCs cell sheets and hKC at the wound margins, both 2D in vitro and a 3D ex-vivo wound models were used. In both assays, hKC migrated in the presence of hASCs, as revealed by a significantly diminished gap in the first assay but more distinctly when hASCs cell sheet constructs were transferred to a 3D ex-vivo wound model. Additionally, to clarify their possible communication via gap junctions (GJ), a calcein-AM transfer assay was performed in the presence/absence of a GJ inhibitor. By using fluorescence microscopy and flow cytometry, the transference of the dye between the two cell types was detected, but not after applying GJ inhibitor, revealing that they communicate via GJ. Moreover, connexins appear to be involved in the interaction between these cell types, while hKC expressed connexin (cx) 43 and cx26, highly expressed at the wound margins, hASCs only expressed cx43 as shown by immunocytochemistry and flow cytometry. Up to now, we demonstrated that hASCs and hKC crosstalk occurs directly through cx43 and indirectly via KGF secreted by hASCs, which indicated to promote KCs migration therefore affecting skin re-epithelialization.

(1) Cerqueira, MT, (...), Marques, AP. Biomacromolecules 2013,14,3997–4008
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Tailoring bioink biofeatures to meet skin microenvironments specificities

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Skin tissue engineering strategies typically involve the recreation of the dermis and epidermis layers each containing single key cell players, such as the fibroblasts and keratinocytes, respectively. The regenerative potential of these constructs upon transplantation has been limited, conceivably, due to the absence of other skin components, such as the vascular, immune and nervous parts, and the appendages. The emergence of bioprinting has generated the possibility to create skin analogues with higher degree of complexity not reached by other methods. Nonetheless, to recreate the different skin components in a 3D bioprinted construct, one must develop different bioresponsive bioinks that fulfill the requirements of different skin cells in terms of biocues, physico-chemical and mechanical features. Under this context, we developed different gellan gum (GG)-based bioinks. GG was biofunctionalized with fibronectin-derived RGD as a bio-signal to human adipose stem cells (hASCs) and human dermal fibroblasts (hDFbs), and with CCN1-derived T1 and collagen IV-derived HepIII for dermal microvascular endothelial cells (hDMECs) and human primary keratinocytes (hKCs) biorecognition, respectively. Bioinks viscoelasticity were tailored by altering the amounts of GG (0.75-1.25%) and functionalized GG (0.375-0.625%) without compromising its printability upon dispensing. Bioinks with higher polymer amount formed hydrogels with higher viscoelasticity and stiffness, and lower swelling. hASCs and hDFbs showed their typical morphology and phenotype within the RGD-biofunctionalized hydrogels with high but not low stiffness. Additionally, cells were able to deposit characteristic extracellular matrix components and keep their typical secretome. hKCs and hDMECs adhered respectively within Hep III- and T1-containing hydrogels independently of the stiffness. hKCs expressed early (k5) and mature (k10) markers along the cell culture period and organized in an epidermal-like layer. hDMECs ramified structures were observed in hydrogels with higher stiffness. These results bring us one step closer to the bioprinting of a complex skin analogue as GG-based bioinks can be easily tailored to match the specificity of different skin cell types.


76-SY-1

Differentiation of human iPSCs into kidney and pancreatic lineages towards regenerative therapy

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Kidney diseases and diabetes cause both medical and medicoeconomical problems worldwide. Although kidney, whole pancreas and pancreatic islet transplantations are an effective therapeutic strategy, the insufficient donor organ/tissue supply is a major obstacle to these interventions. Regenerative medicine strategies using human induced pluripotent stem cells (iPSCs) or embryonic stem cells (ESCs) are among the candidate approaches to solve the problems. Based on the knowledge of developmental biology, we are currently developing the stepwise differentiation methods for kidney and pancreatic lineage cells from human iPSCs/ESCs. In addition to conventional treatments using growth factors and cytokines, chemical biology approaches have been introduced to identify novel differentiation-inducing factors by performing high-throughput screening (HTS) of low-molecular-weight chemical compounds. We are also examining the therapeutic potential of kidney and pancreatic lineage cells generated from human iPSCs/ESCs by transplantation into mouse models of renal diseases and diabetes. We have demonstrated that the transplantation of human iPSC-derived renal progenitors ameliorates acute kidney injury (AKI) in mice induced by ischemia/reperfusion injury. We have also succeeded in lowering blood glucose levels in diabetic mice by transplanting human iPSC-derived pancreatic cells. However, the methods to generate kidney and pancreatic lineage cells from human iPSCs/ESCs have not yet been fully established. Further elucidation of the developmental mechanisms and establishing the efficient differentiation methods from human iPSCs/ESCs into the two organ lineages will be required for the development of regenerative medicine strategies. In this presentation, I would like to summarize the current status of kidney and pancreatic regeneration researches using human iPSCs/ESCs including our results and discuss the future perspective of iPSC technology-based regenerative treatment of kidney diseases and diabetes.

Osafune Kenji
Functional hypothalamus and pituitary induction \textit{in vitro} from human pluripotent stem cells

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The hypothalamic-pituitary system is essential for maintaining homeostasis and life by controlling systemic hormones. We have established techniques that allow the generation of functional adenohypophysis and hypothalamus from human pluripotent stem cells.

Pluripotent stem cells, such as embryonic stem (ES) cells and induced pluripotent stem (iPS) cells, differentiate into neuroectodermal progenitors when cultured as three-dimensional floating aggregates under serum-free conditions. Recent results have shown that strict removal of exogenous patterning factors during the early differentiation period induces efficient generation of rostral hypothalamic-like progenitors, which means the hypothalamic position in the cerebral nervous systems is characterized by the most rostral and most ventral. As a result of recapitulating such condition \textit{in vitro}, the ES cell-derived hypothalamic-like progenitors generated rostral-dorsal hypothalamic neurons, in particular magnocellular vasopressinergic neurons, which release hormones upon stimulation.

We have subsequently succeeded in inducing both ventral hypothalamic and oral ectodermal tissues simultaneously. Self-organization of Rathke’s pouch, pituitary primordium namely, occurred at the interface of the two epithelia \textit{in vitro}. After long culture, various endocrine cells including corticotrophs and somatotrophs were produced from the Rathke’s pouch-like structures. The induced corticotrophs efficiently secreted adrenocorticotropic hormone (ACTH) in response to corticotropin-releasing hormone (CRH). In addition, we found that, \textit{in vitro}-generated corticotroph cells were able to rescue hormone levels, physical activity levels and survival when grafted into pituitary-resected hypopituitary mice. Thus, we have generated a useful methodology for the production of functional human pituitary tissue.

Our culture methods above are characterized by replication of stepwise embryonic differentiation. Therefore, these methods could potentially be used as developmental and disease models, as well as for future regenerative medicine. We will show our latest trial for establishing \textit{in vitro} neuronal degenerative disease model using disease specific iPS cells.

Curvature Promotes Podocyte Differentiation in a Biomimetic Cell Culture Platform

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Here we present a micro-physiological cell culture system that promotes differentiation of podocytes in a technology for \textit{in vitro} physiology and drug testing applications.

Podocytes are an epithelial cell in the kidney that wrap around glomerular capillaries to provide barrier function during blood filtration. About 1 liter of blood is filtered through the kidneys every minute. When the filtration process is disrupted during chronic kidney disease, limited treatment options exist. As a result, kidneys are the most transplanted organs around the world, with around 80% of waitlists worldwide being for kidneys.

Many kidney diseases are related to podocyte dysfunction, making this cell type a target for \textit{in vitro} studies. However, there is a lack of \textit{in vitro} models. Podocytes \textit{in vitro} face a limited ability to differentiate. Appreciable displays of maturation hallmarks such as arborated morphology, foot process formation and interdigitation, and expression of slit diaphragm proteins such as nephrin, remain difficult to achieve, even with chemical supplements and co-culture conditions. However, the role of geometric cues in supporting cell development were unknown.

\textit{In vivo}, podocytes experience convex curvature. We hypothesized that a topography that mimics curving glomerular capillaries would provide a stimulus to promote podocyte differentiation \textit{in vitro}.

We engineered a platform that resembles the round capillaries of the glomerulus by using spherical glass beads to generate a microhemispherical topography. These platforms were inserted into 24-well plates, and used with standard cell culture and analysis techniques. We cultured podocytes for 9 days on the topographic platform and compared them with the flat counterpart. In all experiments, the topographic platform yielded cells with more arborized morphologies, greater upregulation of nephrin, and protein localization. We were also able to observe differences in barrier function by translating the topographic platform into a membrane format. In addition, this versatile system was amenable to testing the podocytes with molecular insults and therapeutic compounds, thereby presenting a wide range of use.

This work demonstrated the successful implementation of biomimetic curvature into a tissue engineering system, allowing us to engineer higher-fidelity podocytes, with the potential to create cell-based models for study and drug screening applications.
**Background:** Currently there are no effective treatments for the progression of fibrosis resulted from chronic kidney disease (CKD). MicroRNAs (miRNAs) are emerging as potential therapeutics to reduce fibrosis. However, a lack of reliable methods to achieve targeted delivery of miRNAs with sustained expression or suppression represents a major roadblock in translating miRNA therapy to effective treatment. We have developed a novel self-assembled nanoparticle (SAnP) delivery system that, when functionalised with a targeting ligand, is capable of delivering miRNA into specific cells within the kidney via receptor-mediated uptake.

**Methods:** An in vitro Epithelial to mesenchymal transition (EMT) was induced in cultured MDCK cells using TGF-β (10ng/ml). Thereafter, these cells were treated with miR29 mimic (1nM), either packaged into the SAnP system or as “naked” miRNA. A renal fibrosis model was created by subjecting the mouse to a Unilateral Ureteral Obstruction (UUO model). The miR29 mimic (0.1mg/kg), packaged into the SAnP system or as “naked” miRNA, was directly delivered into the renal parenchyma of the UUO model at the time of the obstruction, within an injectable hydrogel. Mice were euthanized 7 days after UUO. Kidneys were collected and processed for histology analysis.

**Results:** In Vitro: In cultured MDCK cells (expressing receptor “X”) uptake of Cy-3 was only detected in cells transfected with SAnP-Cy3-miRNA, confirming receptor-mediated uptake hence the specificity of our SAnP delivery system. TGF-β induced EMT was partially blocked in MDCK cells by SAnP-delivered miR29 mimic, as treated cells maintained the cobblestone-like morphology and preserved E-cadherin expression. In Vivo: By incorporating SAnP into a tailored degradable, injectable hydrogel reservoir, we were able to deliver Cy3 into the renal capsule to ensure controlled temporal delivery of genetic material. At 7 days post UUO, SAnP-delivered miR29 mimic was able to substantially reduce tubular dilatation, perivascular infiltration and collagen deposition (24% decrease based on Picrosirius Red staining). Delivery of “naked” miR29 mimic did not show any beneficial effect in the UUO model.

**Conclusion:** Our data demonstrate that combining our receptor-mediated, targeted nanoparticle delivery system with an injectable hydrogel, SAnP-delivered miR29 can inhibit the EMT transition in vitro, and significantly reduce the development of renal fibrosis in the UUO model.
Development of 3D Printed Biodegradable Polyurethane Nanohybrid Scaffold for Heart Valve Regeneration

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Currently available mechanical and bioprosthesis heart valve are still unable to reproduce the functional heart valve and multiple resizing operations are necessary in paediatric patients. The aim of this study is to develop 3D printed biodegradable polyurethane nanohybrid 3D scaffold for heart valve regeneration. Porous biodegradable 3D scaffolds were fabricated from POSS terminated aliphatic polyester urethane urea (PEUU-POSS) solution using indirect 3D printing. Physical characterisations of the 3D scaffolds were studied by analysing the structural morphology, wettability, mechanical properties and degradation. For in vitro study, human dermal fibroblasts (HDFs) were cultured for 14 days on the 3D scaffolds. In vivo study by subcutaneous implantation in rats was examined at week 4 and 12. Statistical analysis was done by Prism 6 software (n=6, p<0.05). The SEM morphology shows hierarchical porous structure with the pore size distribution from 500 - 800 µm macropores and 10 - 50 nm micropores. The scaffold surface is hydrophobic (57⁰ ± 3.7⁰). Although the porous structure reduced the stiffness and the strength of the scaffold, with ultimate strength (0.27 ± 0.012 MPa) and Young's modulus (0.23 ± 0.009 MPa) lower than native human heart valve, the excellent compliance remain with super high strain (up to 600%). In 10 weeks, exposure to oxidative buffer resulted in the most mass losses (>3%) and only minor hydrolysis degradation (<1%). The scaffolds tested in vitro and in vivo were found to exhibit positive cellular activity for both cell proliferation and angiogenesis. These results revealed that the biodegradable PEUU-POSS scaffold by 3D printing provides potential soft and compliant substrate for cell niches in heart valve tissue regeneration while withstand the systemic pressure, haemodynamic resistance and high shearing in heart valve before regeneration of extracellular matrix takes over the degradation of scaffold.
**77-SY-3** Human Hair Proteins as Raw Materials for Tissue Engineering and Regenerative Medicine Applications

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The potential of using human hair derived proteins for Tissue Engineering and Regenerative Medicine (TERM) applications has been well described in the literature. The heterogeneous pool of proteins, including keratin intermediate filament proteins and keratin associated proteins, has been demonstrated to be versatile in fabricating a range of templates that can be used as drug carriers or as scaffolds for tissue regeneration. Of note, our group has demonstrated the ability of a keratin-alginate sponge to induce efficient tissue ingrowth and vascularization in a rodent subcutaneous implantation model. In a preliminary burn model in pigs, we found the efficacy of these sponges to be comparable to commercially available collagen based dermal equivalents. On top of functional studies, we are also keen to establish fundamental understanding of the behavior of this material, and its potential to assemble into novel structures and find new applications. We recently showed that hair extracted protein fractions can act as antioxidants to protect cells in oxidizing environments. Purified keratin intermediate filament proteins could also self-assemble or interact with partner materials to produce novel 2D and 3D structures. This presentation will describe some of our recent findings in these aspects. Human hair is an abundant and easily accessible source of keratins and keratin associated proteins, which are an intriguing pool of biomaterials with great potential from both materials science and biochemical perspectives, for applications in TERM.

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**77-SY-4** An Injectable Multi-Responsive Hydrogel as Self-Healable Tissue Adhesive Exhibiting On-demand Dissolution Capacity

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**INTRODUCTION:** Tissue adhesive hydrogels have attracted great attention due to the appealing haemostasis wound closure property compared with the conventional suture or stapling technique. However, the current tissue adhesive materials are suffered from poor responsive properties which largely confines their application scenarios. Therefore, a series of hyaluronic acid based hydrogels with multi-responsive properties was developed as a self-healing tissue adhesive for wound closure exhibiting on-demand dissolution capacity.

**RESULTS & DISCUSSION:** The hydrogel is composed of aldehyde functionalized hyaluronic acid (HA-CHO) and a disulfide bond containing crosslinker. The hydrogel possesses five responsive properties, namely 1) Chemical responsive; 2) pH responsive; 3) Strain responsive; 4) UV responsive; 5) Enzyme responsive. The dynamic covalent chemistry is accounted for three responsive properties (chemical, pH, and strain responsive properties). The enzyme responsive property is due to the nature of HA chain and UV responsive property is achieved by the incorporated disulfide bond in the crosslinking site. All the hydrogel formulations showed fast gelation rates within 1 min which indicates the excellent injectable capacity. The results of AlamarBlue and Live/Dead assays demonstrated excellent biocompatibility for 3T3 and human keratinocytes. Bio-glue, a commercial tissue adhesive, was selected as the control counterpart. The hydrogel with high Mw of HA-CHO showed a significantly higher lap shear strength. This biocompatible hydrogel system holds a great potential to develop into commercial bio-adhesive product.

**CONCLUSIONS:** We have developed a new series of multi-responsive hydrogel system that can act as strong tissue adhesive. The newly fabricated hydrogels showed good biocompatibility. Moreover, the hydrogel showed strong tissue adhesive property can even superior than a commercial tissue adhesive product - BioGlue⁴, indicating the potential value for the biomedical applications.


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Silk proteins have emerged from the fabric of the textile world into a growing suite of biomaterials and medical utility over the past few decades. This progression started with new fundamental insights into this unique protein, and subsequently evolved into new materials with clinical impact. We will review the historical, research and translational steps that have supported new medical materials and devices based on silk. One key to this emergence has been to modify the native protein using new processing methods and chemistries to engineer new material features. Some of the strategies developed to morph silk, as a high molecular weight amphiphilic protein, into new biomaterials with new properties will be discussed. The utility of some of these new material formats in 3D printing, biomaterial scaffolding, tissue engineering and regenerative medicine will be presented. The needs for tunable, degradable, robust biomaterials for a range of medical goals remains high, and silk proteins offer a unique suite of options to help address these needs.
Silk fibroin (SF) is known for its outstanding mechanical properties and excellent biocompatibility. Despite encouraging results in various tissue engineering applications an increasing number of strategies include the functionalization of SF-based grafts with other bioactive agents to further increase and modulate their efficacy in specific applications. Here three examples are presented: (1) a SF based hemostatic device which acts as carrier system for the delivery of fibrinogen and thrombin as coagulant supplements, (2) SF-meshes chemically functionalized with the plant lectin WGA (wheat germ agglutinin) to provide instant and robust cell-adhesion properties and (3) nerve guidance structures based on SF fibers chemically modified with laminin to accelerate and increase the adhesion and proliferation of Schwann cells (SCs).

(1) The incorporation coagulant supplements in SF sponges has been realized by mixing them with an aqueous SF solution, followed by molding, freeze-drying, and water annealing. In this combination system, we demonstrated the delivery of fibrinogen and thrombin while maintaining their hemostatic potential. This novel co-delivery system can be used for an improved control of bleeding which is one of the most important interventions after a traumatic injury.

(2) The covalent bonding of WGA to SF with carbodiimide chemistry led to significantly enhanced and accelerated cell attachment within only 5 minutes incubation period. Intensive characterization of the lectin-mediated cell adhesion proved enhanced protease and mechanical resistance. With this technique SF mesh were modified and tested in a rat hernia model. The in vivo results demonstrated that the animals with WGA-functionalized mesh showed superior tissue integration and remodeling. This novel material optimized for intra-operative fast cell seeding could bring a remarkable benefit in soft tissue repair.

(3) The growth of SCs are regarded as one of the most critical parts in peripheral nerve regeneration. To further increase SC growth on SF a protocol to covalently bind human laminin to SF utilizing carbodiimide chemistry was established. Cell adhesion and proliferation assays suggest beneficial effects of laminin on both, cell adhesion as well as proliferative behavior of SCs. To sum up, the tailoring of SF drastically enhances its properties as biomaterial, which will help to overcome current hurdles in various approaches of tissue engineering and regenerative medicine.
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Millions worldwide suffer from vision impairing conditions like corneal injury and corneal ectatic diseases. Silk fibroin (SF) is an emerging biopolymer, used in several investigations and possesses excellent biocompatibility, mechanical strength, ability to stabilize labile compounds, biodegradability and near perfect transparency to visible light. This combination makes SF a promising material for manufacturing ocular prostheses. Riboflavin (RF) is a photoinitiator for biomedical applications, used with UVA light to crosslink corneal collagen as an etiopathogenetic approach to keratoconus and for strengthening unstable cornea. All-trans Retinoic Acid (RA) affects ocular tissue development, eye morphogenesis, lens and cornea regeneration, and controls the phenotype and extracellular matrix composition of corneal stromal cells. RF and RA have been examined individually for their effect on corneal regeneration. To the best of our knowledge, the effect of their combined delivery has not been studied. In this study, we couple both bioactive molecules onto SF matrices. The physical properties, bioactivity and response of human keratocytes to these matrices has been evaluated.

Different concentrations of RF and of RA were respectively photo crosslinked and covalently coupled through carbodiimide coupling with 2 wt% SF. Physical characterization and preliminary cellular evaluation were used to identify the most promising concentrations of both RF and RA. Once these were determined their combined effect after loading onto SF matrices was examined. Matrix physical characteristics were evaluated by FTIR, SEM, transparency, release of bioactive molecules and mechanical tensile testing. The biological response of the cells to the matrices was evaluated using cellular adhesion assays, proliferation assays, cytoskeleton staining, gene expression analysis and immunostaining. Results indicated that incorporation of both bioactive molecules (RF and RA) improved cellular proliferation, cytoskeletal organization and expression of genetic markers associated with stroma regeneration, than sole loading of either RF or RA. These in vitro studies serve as a starting point for optimization of loading bioactive molecules on SF based matrices for formulating clinically relevant ocular prostheses.

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Bioactive Nanofibers Induce Neural Transdifferentiation of Human Bone Marrow Mesenchymal Stem Cells (BMSCs)

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Due to the delicacy and complexity of the CNS, little spontaneous regeneration, repair or healing occur. The combination of biomaterials with stem cells represents a promising therapeutic strategy to repair traumatic injuries in the central nervous system. Human BMSCs are the most translatable option amongst various sources of stem cells for their clinical application. BMSCs are also capable of transdifferentiating into a diverse family of cell types that are unrelated to their phenotypical embryonic origin. However, the specific mechanisms responsible for their neural transdifferentiation are largely unknown at present.

Inspired by the potent effect of laminin on enhancing CNS development, we reported here a robust biomaterial strategy to transdifferentiate human BMSCs into neuronal cells by using bioactive IKVAV-peptide amphiphile self-assemblies displaying a laminin-mimetic IKVAV sequence. The IKVAV-PA can form supramolecular nanofibers, and induce neuroectodermal lineage commitment after 1 week, evidenced by the upregulation of the neural progenitor gene Nestin (NES) and glial fibrillary acidic protein (GFAP). After 2 weeks, the bioactive IKVAV-PA nanofibers induce significantly higher expression of neuronal markers TUJ-1, MAP2 and neuronal nuclei (NeuN), as well as the extracellular matrix laminin. Furthermore, human BMSCs show an evident polarized cytoskeletal rearrangement with a decrease in cellular size, resembling neuron-like cells.

Taken together, the synthetic bioactive IKVAV-PA nanofibers could transdifferentiate human BMSCs into a neuronal lineage. The reported approach is simple and elegant and opens opportunities to use this synthetic bioactive material to direct endogenous or transplanted adult BMSCs into neural cells, which would greatly contribute to the functional recovery of the traumatic injuries in the CNS.
Modulation of Non-Coding RNAs to Promote Osteogenic Differentiation of Mesenchymal Stromal/Stem Cells

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Although genome sequencing projects revealed that around 97% of human DNA does not codify for proteins, the extent of transcription and the function of non-coding RNA (ncRNA), particularly those involved in disease, are still a matter of debate. MicroRNAs (miRNA) are a class of small ncRNA involved in disease that control main cellular functions, including the mechanisms required for bone formation. In contrast, the role of long ncRNA (lncRNA) in bone regeneration/repair remains largely unexplored. This study aims to modulate ncRNA levels to promote osteogenic differentiation in Mesenchymal Stromal/Stem Cells (MSC).

Recently, we identified the most significantly altered miRNAs during osteogenic differentiation. Moreover, we showed that modulation of miR-195 and miR-497 levels in MSC leads to a simultaneous effect on osteogenic differentiation potential, MSC proliferation rate, and a paracrine effect on angiogenesis in vivo. Furthermore, our results show that lncRNAs, which can interfere with transcriptional regulation by acting as enhancer RNAs or as endogenous complementary RNAs (e.g. as natural miRNA sponges), are also involved in osteogenic differentiation, particularly the transcribed ultra-conserved regions.

In conclusion, our data shows that both miRNA and lncRNA are involved in osteogenic differentiation and that their levels are timely controlled, leading to the hypothesis that ncRNAs regulate bone regeneration through modulation of MSC biology. The understanding of the role of ncRNA in MSC can boost novel gene and cellular therapies for bone repair in physiological and pathological conditions.

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Construction of Stem Cell-based Novel Multi-Functional Targeting Vehicle and Its Application in Ischemic Stroke Therapy

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Cerebral ischemic stroke is the main type of stroke, which refers to irreversible local lesion caused by blood circulation disturbance and successive ischemic-hypoxic necrosis. The high mortality rate, high morbidity and high morbidity bring the stroke into focus. However, success of clinical treatment has been rather limited now. As we all know, stem cell transplants perform well in the treatment of a variety of brain diseases. Stem cell can target to lesion region and secrete neurotrophic factor. Stem cell transplantation significantly improves neurological function, promotes endogenous neurogenesis and differentiate into neural cells. In our study, we modified mesenchymal stem cells with this targeting peptide (MSC) (PA)-CLEVSRKN in order to enhance homing capability. Meanwhile, non-viral vector that had high transfection efficiency with low cytotoxicity was used to delivery miR-133b into MSCs. This combined method could make MSCs as a promising targeting therapeutic agent with its targeting delivery ability of RNAi Genes, as well as its own therapeutic effect. Then we established middle cerebral artery occlusion (MCAO) rat model to evaluate the targeting ability and the effect therapeutic effect of Pep-133b-MSCs in MCAO rat. The results show Pep-133b-MSCs has the targeting ability for brain ischemic lesion and can deliver miR-133b to target tissue and exhibited own therapeutic effects of MSCs served as a multi-functional targeting vehicle. The study evaluated potential of MSCs as a targeting vehicle for brain diseases and prospect of MSC-based targeting therapy for ischemic stroke.
The generation of complex tissues has been an increasing focus in tissue engineering and regenerative medicine. With recent advances in bioprinting technology, our laboratory has focused on the development of platforms for the treatment and understanding of clinically relevant problems ranging from congenital heart disease to preeclampsia. We utilize stereolithography-based and extrusion-based additive manufacturing to generate patient-specific vascular grafts, prevascular networks for bone tissue engineering, dermal dressings, cell-laden models of preeclampsia, and bioreactors for expansion of stem cells. Furthermore, we have developed and characterized a library of UV crosslinkable materials with tunable mechanical properties as 3D-printable biomaterials for clinically relevant applications. These developments address important scientific challenges on both macro - for the generation of large-scale functional constructs, and micro - to regulate the cellular microenvironment - scales. A key challenge associated with the development of large tissues is providing adequate nutrient and waste exchange. By combining printing and dynamic culture strategies, we have developed new methods for generating macrovasculature that provide adequate nutrient exchange in large engineered tissues. To dissect cell-cell interactions that regulate the local microenvironment and consequently the macro-scale tissue structure, we have also developed microbioreactors capable of varying cell spacing in a dynamic culture. This enables us to systematically probe cell response to external stimuli while controlling the cell signaling modalities. Finally, the use of stem cells in regenerative medicine is limited by the challenge in obtaining sufficient cell numbers while maintaining self-renewal capacity. Our efforts in developing 3D-printed bioreactors that mimic the bone marrow niche microenvironment have enabled successful expansion of mesenchymal stem cells by recapitulating the physiological surface shear stresses experienced by the cells. This presentation will cover the diverse range of materials and processes developed in our laboratory and their application to relevant, emerging problems in tissue engineering.
81-SY-3  Multi-Scale Biofabrication Approaches for Osteo-Chondral Tissue Regeneration

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Biofabrication technologies provide the opportunity to further replicate native tissues at different length scales through the convergence of different fabrication approaches. We have adopted this for osteo-chondral defect repair. Specifically, we developed cell-laden hydrogel (pre-cursor) bioinks and used extrusion-based bioprinting processes to enable their controlled deposition, also addressing the zonal (layered) nature of the cartilage tissue. Moreover, melt electrowriting (MEW) that enables the fabrication of (sub)-micrometer scale fibers was used to generate fiber structures that were combined with hydrogels and resulted in mechanically stable tissue constructs. Importantly, the controlled deposition of these reinforcing fibers also allows for the generation hierarchical reinforcing structures, further mimicking the native tissue biomechanics. As this two-step approach still limited the freedom of design regarding the use of multiple materials and cell types, these techniques were converged into a single-step process. Mechanically stable constructs with the spatial distributions of different cell could be generated without compromising cell viability and (chondrogenic) differentiation. For the bone compartment, a low-temperature setting printable calcium phosphate paste, consisting of α-tricalciumphosphate (α-TCP), nano-hydroxyapatite and biodegradable, crosslinkable poloxamer was used and combined with the MEW and bioprinting processes. This resulted in a compressive modulus and ultimate strength in the range of cancellous bone, as well as efficient embedding of the fibers in the bone phase upon setting of the cement. This works illustrates essential steps for the fabrication of more biomimetic tissue equivalents, and highlights the opportunities provided by these emerging technologies.

Selected references:
Malda et al. Adv Mat 2013
Visser et al. Nature Comm 2015
Castilho et al. Adv Healthc Mat 2017
de Ruijter et al. Small 2018
de Ruijter et al. Adv Healthc Mat 2018

81-SY-4  3D Bioprinting: Translational Pathway to Clinical Application

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3D bioprinting has emerged as an innovative tool that enables rapid construction of complex 3D tissue constructs with precision and reproducibility. This developing area promises to revolutionize the field of medicine by addressing the dire need for tissues and organs suitable for surgical reconstruction. However, further technological development is required to use 3D bioprinting in patients. This includes improvements in hardware, software and bioinks. In addition, the pathway to bringing this technology to the clinic is not clearly defined. In this session, various challenges associated with the translation of 3D bioprinting technology will be discussed. Clinical perspectives unique to 3D bioprinted tissue structures will also be discussed.
**82-SY-1**  
**Regenerative medicine with allogeneic mesenchymal stem cells ~the current status and future prospective~**

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Mesenchymal stem cell (MSC) therapies have been used in clinical trials in various fields. These cells are easily expanded, can be acquired from medical waste such as adipose tissue and umbilical cord tissue, and have multiple functions such as anti-inflammatory effect, anti-oxidative effect, anti-fibrosis and angiogenesis. According to ClinicalTrials.gov, more than 650 clinical trials using MSCs are registered for cell therapy of many fields including liver, skin heart diseases. Furthermore, MSCs show low immunogenicity, allogeneic MSC can be used on demand. Thus, allogeneic MSC therapy has the potential to expand MSC therapy to many patients. Using these advantages, we are now performing phase I/II clinical study using adipose tissue derived allogeneic mesenchymal stem cell for HCV and NASH origin decompensated liver cirrhosis with ROHTO Pharmaceutical Co.,Ltd. I will introduce this clinical trial and ROHTO Pharmaceutical discuss from a viewpoint of company side. Except for administrating MSCs, cell sheet technologies are developed in heart disease and mobilization of MSCs using novel peptide drugs are developing. Furthermore, in this session we invited a special speaker Professor. Detlef Schuppan (University Medical Center of the Johannes Gutenberg) who is as expert of liver fibrosis. He will introduce the up-to-date research results against liver fibrogenesis and fibrolysis.

**82-SY-2**  
**Building a new treatment strategy for severe heart failure using allogeneic mesenchymal stem cells**

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We have developed autologous cell based treatment experimentally and already introduced to the therapy of severe heart failure in clinical setting as translational research. From a stand point of industrial development how to automatically culture a large number of allogenic cells with homogeneity in cell quality may be crucial and therapeutical potential in allogenic mesenchymal stem cells should be analyzed using heart failure animal model.

Basic experiments revealed that allogenic mesenchymal stem cells transplantation showed therapeutic efficacy via angiogenic cytokine paracrine effects with some immunologic tolerances in heart failure animal model.

We have also developed new drug which enhance endogenous tissue repair system by recruitment of bone marrow mesenchymal stem cells. High-mobility group box 1 (HMGB1), a necrotic cell-derived damage-associated molecular-pattern protein molecule, reportedly enhances bone marrow mesenchymal stem cell recruitment to damaged tissue for tissue regeneration. Administration of HMGB1 attenuated Left ventricle reverse remodeling in dilated cardiomyopathy hamster and rat old myocardial infarction model by recruitment of bone marrow derived mesenchymal stem cells.

In this session we will report allogenic mesenchymal stem cell transplantation and endogenous tissue repair induced by HMGB1 for treatment of severe heart failure.
Bone marrow-derived mesenchymal stem cells (MSCs) have been applied to regenerative therapy for various intractable diseases. MSCs are defined to show colony-forming unit (CFU) activity and multi-differentiating activity towards mesenchymal lineages, but also demonstrated to exert anti-inflammatory and pro-regenerative activities in vivo when administered regionally or systemically. Despite such practical progress of MSC application, the role and function of bone marrow MSCs in situ have not been fully elucidated. We have studied in vivo function of MSCs, and elucidated that MSCs migrate into circulation by sensing elevation of serum HMGB1 (high mobility group box 1), which is a nuclear chaperon protein but released from necrotic cells in the injured tissue. HMGB1 also enhances expression of CXCR4, a receptor of chemokine CXCR12 (SDF-1a), on the surface of MSCs. The circulating MSCs are then recruited around the necrotic tissue by SDF-1a, which is released from endothelial cells in the hypoxic necrotic region. The bone marrow-derived MSC in the necrotic tissue are activated by inflammatory cytokines such as TNF-a, resulting in secreting potent anti-inflammatory factors including TSG-6, and differentiate to multi-lineage cells to promote tissue regeneration. With these background, we moved to develop HMGB1 as a drug for mobilizing bone marrow MSCs into circulation to promote regeneration of intractable tissue damage. We performed a double-blinded, placebo-controlled phase I study of investigator-initiated clinical trial, and proved safety of intravenous administration of HMGB1 peptide to the adult male volunteers. We also observed HMGB1-specific induction of the PDGFRb-positive mesenchymal cells in the blood.

Mesenchymal stromal/stem cells (MSCs) show therapeutic potentials against various diseases such as immune and neurological disorders. Rohto Pharmaceutical Co., Ltd. is currently developing cell therapy products using MSCs isolated from adipose tissue or umbilical cords. The most advanced program is to treat liver cirrhosis with ADR-001 composed of adipose-derived MSCs. A phase I/II study of ADR-001 is currently conducted in Japan to evaluate the safety and efficacy. There are three key strategies to develop MSC products. Firstly, we use allogenic MSCs to develop an off-the-shelf product which has advantages for commercialization. Secondly, we use serum free medium which is prepared in-house and in compliance with the Japanese Standards for Biological Ingredients to assure the quality and safety of MSC products. Thirdly, we developed an automated cell culture and storage system to increase production capacity, to decrease cost of goods sold and to ensure lot-to-lot consistency. It is also essential to understand the mechanisms of action of MSCs against each target disease. We have various research programs including the collaboration with academia for the better understanding of the therapeutic mechanisms of MSCs. In this presentation, our activities on the development of MSC products will be introduced.
Prevention or reversal of cirrhosis has become a primary endpoint for trials in patients with chronic liver disease. Significant progress has been made in understanding the mechanisms of liver fibrosis. This includes its dynamic nature and the plasticity of all liver cell populations, especially immune cell subsets that determine fibrosis progression (fibrogenesis) or reversal (fibrolysis), opening the potential of 1) inducing pharmacological reversal even of advanced fibrosis and early cirrhosis, and 2) improving stem cell engraftment in advanced cirrhosis. It has been established that even a cirrhotic human liver can regress to a noncirrhotic stage with highly effective antiviral therapy for chronic hepatitis B or C. Moreover, pharmacological therapies have been developed that prevent further progression or speed up reversal in patients with viral and nonviral fibrotic liver diseases, in whom causal treatment comes too late or is not available, such as genetic or cholestatic liver diseases. Promising antifibrotic strategies address upstream signals, e.g., 1) interfering with hepatocyte injury and lipoapoptosis, 2) attenuating fibrogenic cholangiocyte activation, 3) changing the functional polarization of macrophages towards fibrolysis, 4) directly targeting the fibrogenic effector cells, i.e., hepatic stellate cells and myofibroblasts, 5) directly targeting major fibrogenic effector molecules like procollagen type I or certain integrins, and 4) and 5) are direct antifibrotic therapies that are applicable to any kind of fibrotic liver disease. This session will discuss an increasing number of drugable molecular and cellular targets, and specifically focus on small molecules, blocking antibodies, siRNA, or antisense oligonucleotides, preferably in nanoparticulate carrier systems, that can inhibit fibrosis progression or induce fibrosis reversal. Some of these agents have already entered phase 2-3 clinical trials, with a study design that is still based on histological readouts, but increasingly complemented by a number of biologically plausible surrogate markers, mainly serum fibrosis, fibrogenesis and fibrolysis markers, and targeted imaging of fibrosis and fibrogenesis. These markers and methods are currently validated in past and ongoing phase 2-3 clinical trials for NASH and other fibrotic liver diseases. Their availability will permit shorter and smaller proof-of-concept trials, and be critical for a decision if to continue towards a phase 3 clinical trial. Moreover, they will allow personalized therapies, with an individualized dose titration and the use of drug combinations for improved efficacy and reduced side effects. Overall, the prospects for liver regenerative therapies, even in advanced cirrhosis, look promising when antifibrotic and stem cell therapies are combined.

Select references
RNA therapeutics promise to be void of drawbacks of conventional protein and gene therapies. Limitations related to the use of growth factors such as high cost of the therapy and adverse effects including heterotopic bone formation, dysphagia, inflammation and retrograde ejaculation could be overcome. Moreover, gene therapies using plasmid DNA and virus vectors, although proven successful at preclinical levels, features a long, expensive and tortuous regulatory path forward into human clinical trials.

Recent data from gene therapy studies suggest that when BMP-2 is delivered genetically, the level and duration of gene expression need not be very high. This opens the possibility of delivering messenger RNA encoding BMP-2 instead of delivering its cDNA. There are several potential advantages of this approach. Removing the need for a viral vector reduces cost considerably. It substantially lowers the associated safety concerns including the elimination of possible insertion mutagenesis and adverse immune reactions. Moreover, the administered RNA is degraded by natural, intrinsic biochemical processes leaving no residue.

Messenger RNA is a poor drug because it is rapidly degraded, toxic to cells and inflammatory as a result of toll-like receptor activation. These limitations may be overcome by performing chemical modifications to the messenger RNA molecule. Several strategies have been developed by us in order to obtain a modified messenger RNA highly stable and with great translation potential. In addition, using optimized UTRs and relevant elements in the messenger RNA sequence has proven a beneficial impact on RNA translation and stability.

In this presentation, a summary of our research work on messenger RNA applied to bone engineering will be presented. We have devoted considerable resources to the latter using non-viral vectors to deliver BMP-2 chemically modified RNA in vitro to stem cells, ex vivo to several tissues and in rat, femoral, non-critical and critical-size defect models. The administration of BMP-2 chemically modified RNA resulted in increased osteogenesis in vitro and in successful bone regeneration in vivo.
**Objective:** The objective of this study was to develop a biomimetic hydroxyapatite:collagen synthetic graft to deliver a low dose of growth factor in a spatially and temporally controlled manner to stimulate both bone and periosteal regeneration. **Introduction:** The supraphysiological doses of recombinant human bone morphogenetic protein 2 (rhBMP2); typically used for the regeneration of large bone defects clinically in conjunction with a collagen sponge, have significant complications and inflammatory sequelae. Previous studies have demonstrated that an early burst release followed by a sustained release of the growth factor is most efficacious in stimulating bone regeneration as the early release stimulates recruitment and the formation of a stabilizing callus, while the sustained delivery results in continued mineralization and consolidation of ossified tissue. **Methods:** The scaffolds were tested in two critical sized defect models, (1) 15 mm segmental defect in the rabbit radius with the periosteum removed over an 8 week duration and (2) a delayed restoration 8 mm full thickness calvarial defect treated 4 weeks after injury and evaluated over 8 weeks. **Results:** We paired a hydroxyapatite:collagen I (HA:Col) scaffold with a collagen membrane guide and by delivering 20% the recommended dose of BMP2 from the guide alone, demonstrated successful regeneration of a critical sized segmental defect in the rabbit radius over 8 weeks. Significantly higher quality and quantity of bone was regenerated compared to the same experimental therapy with the full recommended dose, as well as the collagen sponge alone with rhBMP2. Most therapeutic solutions for filling bone defects are used after the site has been stabilized, potentially much after the time of injury. Delayed BMP2 delivery from the HA:Col scaffold was combined with early release from a periosteal collagen membrane and it was found that delayed restoration (4 weeks after injury) of critical sized rat calvarial defects was possible in a more efficacious manner and at much lower doses than the current clinical practice of rhBMP2 alone or a stiff periosteal guiding membrane. **Conclusions:** These preclinical studies demonstrate that the controlled spatial delivery of rhBMP2 at the site of the periosteum at significantly lower doses can be used as a strategy to improve bone regeneration around spatial maintaining scaffolds.
Cartilage intermediate µTissue assemblies for healing of critical-sized long bone defects

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Bone loss caused by trauma, tumors or infection is one of the major problems in orthopedics. Thus, the development of effective scaffolds is still a critical issue for researchers and clinicians. Nowadays bone tissue engineering is looking forward to new solutions and bio-inspired biomimetic scaffolds address relevant attention.

GreenBone Ortho s.r.l., has developed a patented, wood (Rattan)-derived, bone regenerative implant for extensive bone damages in non-loaded and load-bearing skeletal segments.

GreenBone is a synthetic, acellular, new generation biomimetic and resorbable bone graft for bone reconstruction. GreenBone implant is engineered to reflect anatomical and physiological bone composition and hierarchical structures: biomimetic calcium phosphate phases (HA +/- TCP and minerals), endowed with interconnected porosity and high mechanical strength. The idea was conceived at ISTEC-CNR in Faenza (Institute of Science and Technology for Ceramics – National Research Council).

In order to evaluate the safety and the bone regeneration ability of GreenBone, a load bearing large bone loss sheep study has been successfully performed (Assaf-Harofeh Medical Center Israel and Rizzoli Orthopaedic Institute Italy). GreenBone scaffold was implanted in adult sheep metatarsus, divided into 3 groups: control group (allogenic bone graft), GreenBone DR group (HA + TCP and minerals) and GreenBone ND group (HA only). 6 months after surgery animals were sacrificed for radiological, macroscopical, histological, histomorphometric and micro-CT evaluation.

GreenBone DR and ND scaffolds showed the presence of newly formed bone and an intense bone remodeling at the bone/scaffold interface and around and inside the porosity of the scaffold. In addition, DR scaffold showed a higher cellular activity with the presence of numerous osteoblasts along the newly formed bone trabeculae in comparison to all the other experimental groups. In DR group new vessel formation inside the scaffold with a high osteoclastic activity was also observed.

The findings of this study suggest that the Rattan-derived bone graft substitute GreenBone can open new prospective in the combination of mechanical strength and regeneration potential. GreenBone materials, due to their properties and consequent expected application in different skeletal segments, can play an important role in the market segments of synthetic bone substitutes, bone allograph and machined bones. GreenBone is now entering clinical development.
84-SY-1  Fabricated vascularized tissues with elastic protein

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The benefits of incorporating tropoelastin and elastin into engineered templates for tissue regeneration applications are increasingly being recognized, where wide ranging advantages are elicited through the inherent biological and mechanical characteristics of the protein. The availability of recombinant human tropoelastin makes such strategies particularly attractive. Tropoelastin incorporation, through either physisorption or covalent crosslinking, can be used to functionally modify clinically relevant synthetic polymers to promote successful integration within the body. Tropoelastin coatings protect materials against non specific molecular adhesion, promote fibroblast adhesion and proliferation, improve endothelial cell attachment and proliferation, modulate polymer thrombogenicity, prevent smooth muscle cell proliferation and neointimal hyperplasia and stimulate in vitro vascularization and in vivo blood vessel penetration. Advantageously, covalently immobilized tropoelastin is sterilizable by ethylene oxide. Incorporation of tropoelastin into scaffolds, through techniques such as electrospinning and hydrogel formation, has also been used to support and influence in vitro cell growth. The requirement for tissue matching compliance and durability is particularly critical in tissue engineered blood vessels (TEBVs) where incompatibility can lead to graft failure through aneurysm. Elastic fiber content can not only determine the mechanical properties of TEBVs but can also inhibit smooth muscle cell proliferation and so prevent graft occlusion.

84-SY-2  Silk – from Textiles to Medicine

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Silk proteins have emerged from the fabric of the textile world into a growing suite of biomaterials and medical utility over the past few decades. This progression started with new fundamental insights into this unique protein, and subsequently evolved into new materials with clinical impact. We will review the historical, research and translational steps that have supported new medical materials and devices based on silk. One key to this emergence has been to modify the native protein using new processing methods and chemistries to engineer new material features. Some of the strategies developed to morph silk, as a high molecular weight amphiphilic protein, into new biomaterials with new properties will be discussed. The utility of some of these new material formats in 3D printing, biomaterial scaffolding, tissue engineering and regenerative medicine will be presented. The needs for tunable, degradable, robust biomaterials for a range of medical goals remains high, and silk proteins offer a unique suite of options to help address these needs.
Bioprinted capillary beds as universal building blocks for multiple organ-on-a-chip applications

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Tissue engineered in-vitro models represent a valuable alternative to animal models in pre-screening of pharmacological substances and toxicity testing. In order to fabricate models that resemble native tissue function as close as possible, integration of an organized capillary network that delivers nutrients and removes metabolic waste products plays an important role. Here, we present a novel bioprinting strategy to generate capillary beds with similar structure to native tissue. The capillary beds were applied as universal building block to fabricate tissue-similars mimicking tubulointerstitium, myocardium, and liver lobules.

Capillary beds were printed maintaining high cell viability (> 94 %) and proliferation potential. Following two weeks of culture pronounced capillary formation could be observed in all bioprinted samples. Cardiomyocytes, hepatocytes, and tubular epithelial cells were successfully incorporated yielding vascularized tissue-similars mimicking tubulointerstitium, myocardium, and liver lobules. For thin tissue models (300 µm) no significant differences in marker secretion (e.g. albumin and urea) was observed between the printed and non-printed control group. However, in thick tissue models (1,800 µm) the printed capillary-network significantly improved biofunctionality compared to the non-vascularized control group.

Viability, motility, and functionality of all applied cell types were shown not to be affected by the printing procedure. Remarkably, in thick tissue models integration of capillary-like networks was shown to improve the removal of metabolic products. We conclude that capillary integration is a key step in shifting bioprinted 3D-models towards biomimetic tissue analogues.

Biofabrication of 3D Convoluted In-hydrogel Vessel-like Microfluidic Channels via Digital Light Processing Bioprinting

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Hydrogels allow to culture cells in a native-like microenvironment, which is necessary in many tissue engineering and disease modelling applications. To further recapitulate the complex organization of living tissues, advanced biofabrication technologies are fundamental to accurately control the three-dimensional (3D) architecture of cell-laden hydrogels and to tackle major challenges in the field of tissue engineering, such as the incorporation of vessel-like structures. The aim of this work is to develop a hydrogel-based strategy for digital light processing (DLP) bioprinting, to print high-resolution living constructs with embedded microfluidic, vessel-like channels. In DLP bioprinting, patterns of light are drawn layer-by-layer into a cell-laden hydrogel solution, termed bioresin, which gelates only where illuminated. With this approach free-form 3D biological structures with embedded microchannels are generated in a single-step process, without the need for sacrificial materials. Photosensitive gelatins, bearing methacryloyl and norbornene groups, were investigated as bioresins for DLP printing (Perfactory 3 Mini, Envisiontec), using a recently described visible light photoinitiator[1]. Working curve for the bioresins, optimal exposure settings and printing resolution were described. Unconfined uniaxial compression and sol-gel analysis were conducted to assess mechanical and physico-chemical properties of the printed constructs as a function of polymer content. Constructs displaying superior resolution compared to extrusion bioprinting (25-50µm), and embedding convoluted vessel-like networks, branching in the x, y and z axis (diameter down to 200µm) were successfully printed and perfused through these microchannels, which also allow cell attachment for endothelialization. DLP-bioprinted Mesenchymal Stromal cells (MSCs) were homogenously distributed across the construct, and showed high viability and proliferation up to 28 days. As an example of functionality, MSC were successfully stimulated to undergo osteogenic differentiation, and synthesized bone-like mineralized matrix into the constructs. In conclusion, this DLP-bioprinting approach allows generating high-resolution, perfusable vessel-laden constructs with complex geometries with potential application in tissue engineering, and enable a new toolset for the design of the next generation of hydrogel-based organ-on-chip devices.

Laser engraving matrix treatment for successful repopulation of decellularized tissues

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Organ and tissue decellularisation have been intensively investigated the last years with the expectation to generate optimal scaffolds for tissue engineering. The concept of this approach is to make use of the specific architecture and composition of native matrices and likewise provide an excellent environment for seeded cells. Although this could be partially fulfilled, repopulation of these materials turned out to be challenging, especially in case of dense or larger materials.

To overcome the difficulties of cell repopulation, several strategies have been developed to open up the matrix and make it more porous. In dense materials, strategies to remove ECM components such as glycosaminoglycans are frequently not sufficient. As alternative, laser strategies have been developed. Lasers are already used in diverse fields of clinics (dermatology, ophthalmology) and, depending on the laser type (CO2-, femtosecond-, Er:YAG lasers etc.), work on different physical principles and have different effects on tissue. In the field of scaffold development, lasers have the advantage to allow engrave fine structures with controlled size, distance and depth, bearing the advantage of reproducibility and high throughput production.

These methods are particularly promising for articular cartilage, which has an extremely dense matrix and may not be sufficiently perforated by glycosaminoglycan removal. Based on this example, two of the most commonly used laser techniques (CO2- and femtosecond-laser) and different application strategies are presented and advantages and disadvantages for engineering and reseeding are discussed. Furthermore, experiences on additional matrix depletion and its effect on cell adhesion and tissue integration with different cell types (chondrocytes and human adipose derived stromal cells) in in vitro, in situ (cartilage defect in osteochondral plugs) and in vivo are included in this talk.

We believe that laser treatment has a high potential for scaffold generation and is at the beginning of its application in tissue engineering. However, the success will depend on the particular way of laser application and engraving strategies.
Tissue-engineered trachea transplantation might represent a good strategy for the treatment of patients with cicatricial tracheal stenosis. We demonstrated that adult human nasal chondrocytes (hNC) display a large degree of plasticity and unique regenerative properties. The aim of this study was to investigate the capability of hNC to colonize devitalized tracheal cartilage tissues (TCT) previously exposed to laser perforation.

Nasal chondrocytes have been harvested from septal cartilage biopsies of patients undergoing rhinoplasty. Human native tracheal specimens, isolated from cadaveric donors, were exposed to devitalization, controlled superficial or deep laser-perforation (300 μm and 1000 μm depth, respectively), and, subsequently, irradiation with Iridium-192 for disinfection. hNC were expanded under established conditions and cultured onto devitalized and laser-perforated TCT. Some revitalized specimens were cultured in the presence of the inflammatory cytokine IL-1β.

We demonstrated that hNC efficiently colonized the pores of the TCT after 7 days of culture. Extent of colonization (i.e.: percentage of viable cells spanning >300 microns of tissue depth) further improved once the revitalized constructs were implanted ectopically in nude mice. IL-1β treatment enhanced cell colonization in vitro but resulted in increased extracellular matrix degradation and consequent loss of spatial organization of the TCT.

New experiments are ongoing to establish proper conditions allowing efficient epithelialization of the revitalized TCT using nasal epithelial cells (i.e., cell source that can be isolated together with hNC from the same biopsy). The engineered epithelial-cartilage composite tissues will be finally tested for their capacity to repair tracheal wall defects in rabbits.
Problems to be solved in islet transplantation

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Diabetes mellitus (DM) that leads to severe complications, such as nephropathy, retinopathy and neuropathy, needs urgent measure of treatment, since the patient number is growing rapidly worldwide. These severe complications usually develop under condition of unstable glycemic control, which is currently treated by pancreas or islet transplantation. Islet transplantation is apparently less invasive than pancreas organ transplantation and its outcome is becoming better probably due to strong immune suppression. However, donor scarcity is another problem of these transplantations. To further improve outcome of islet transplantation, we need to solve the following problems.

1. Adverse events related to immune suppression: Many researchers are searching more effective and less harmful immune suppression. Immune isolation by encapsulation can solve this problem.

2. Donor scarcity: Islet-like tissue can be made artificially by differentiation technologies from human pluripotent stem cells, etc. Xenotransplantation is another possibility.

3. In current islet transplantation, islets are infused into portal vein and expected to engraft in the liver. More than half of transplanted islets are thought to be lost due to instant blood-mediated inflammatory reaction (IBMIR) and hypoxia shortly after transplantation. Islet transplantation site other than portal vein may solve IBMIR. Various measures are studied to overcome these non-immune inflammatory responses and hypoxia. 4. In addition, problems related to islet isolation procedure are still major issues.

In conclusion, there are many problems to be solved for improved outcome of islet transplantation. However, new innovative technologies including tissue engineering and regenerative medicine will contribute to achieve better treatment for DM.
**86-SY-3** Alginate-poly-L-ornithine-alginate (APA), three layered microencapsulated neonatal porcine islets (NPIs) - Islet-derived damage-associated molecular pattern molecule (DAMPs) affects in immune regulation -

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**Background:** Although clinical allogeneic islet transplantation has become an attractive procedure for type 1 diabetes mellitus treatment, there is a severe shortage of human donors. Microencapsulated neonatal porcine islets (NPIs) xenotransplantation may be an alternative transplantation procedure. Previously, we have demonstrated that high-mobility group box 1 (HMGB1), a damage-associated molecular pattern (DAMP) molecule, was released from transplanted islets and triggered inflammatory reactions leading to early loss of syngeneic islet grafts in mice. In the present study, we hypothesized that the inflammatory reaction in the peritoneal cavity following the transplantation of microencapsulated NPIs is more severe than that of empty capsules. Additionally, we predicted that HMGB1 released from transplanted microencapsulated NPIs triggers further inflammatory reactions in mice. Finally, we hypothesized that microencapsulated NPIs xenotransplantation efficacy would be improved by treatment targeting inflammatory reactions in a mouse model.

**Methods:** 10,000 empty APA capsules or 10,000 IEQ microencapsulated NPIs were transplanted into the peritoneal cavity of streptozotocin-induced diabetic C57BL/6 mice.

**Results:** Flow cytometry (FCM) analysis revealed that TNF-α-, IL-6-, IFN-γ-, and/or IL-12-positive macrophages, neutrophils, and dendritic cells had infiltrated the peritoneal cavity after empty capsule or microencapsulated NPIs administration. Cell infiltration peaked at 3 days after transplantation. IL-6 concentrations in the peritoneal lavage fluids on 7 days after empty capsule or microencapsulated NPIs transplantation were 18.5±10.0 pg/mL and 157.4±46.3 pg/mL, respectively (p<0.001), while TNF-α concentrations were 4.6±1.4 pg/mL and 19.8±8.4 pg/mL, respectively (p<0.01). In addition, HMGB1 concentrations were 37.6±6.6 ng/mL and 117.4±8.1 ng/mL, respectively (p<0.0001). FCM analysis revealed that TNF-α- and IL-6-positive macrophages were also observed in the peritoneal cavity following intraperitoneal injection of HMGB1 itself. Anti-TNF-α antibody treatment was associated with prolonged graft survival and improved glucose tolerance 30 days after transplantation.

**Conclusions:** In conclusion, early inflammatory reactions might be therapeutic targets for the prolongation of microencapsulated NPIs graft survival. Thus, treatment-targeting inflammation might improve the efficiency of clinical microencapsulated NPIs xenotransplantation.
Regeneration of Pancreatic Islets from Pluripotent Stem Cells

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The prevalence of diabetes has been rising more rapidly over the world these years. It is a major cause of blindness, kidney failure, heart attacks, and stroke. World Health Organization projects that diabetes will be the seventh leading cause of death by 2030. While islet transplantation is a promising treatment for diabetes, securing the resource of transplantable islets remains a significant challenge. Therefore, great efforts have been made to generate pancreatic insulin-secreting beta cells from differentiation of human pluripotent stem cells (hPSCs). However, dysfunction of both alpha and beta cells in an islet results in diabetes. Hence, islet transplantation would provide better remedy for curing diabetes. We report herein a novel approach to produce functional islets from hPSCs by providing engineered tissue-specific niches to hPSCs during pancreatic islet progression. We demonstrated the formation of islet organoids after spiking stem cells with decellularized pancreatic extracellular matrix during stepwise differentiation. These organoids exhibit characteristics of human islet architecture consisting of four subsets of hormone-specific cell populations, including alpha, beta, delta, and pancreatic polypeptide (PP) cells. Flow cytometric analysis indicated that the cellular composition of islet organoids formed by these four cell types is comparable to that of human islets. These endocrine cells possess glucose-responsive insulin-secretion capability. Furthermore, immunofluorescence imaging characterization of hPSC-derived islet’s morphology revealed that beta cells are intermingled with other endocrine cell types, which makes it similar to human islet morphogenesis. The study provides a novel strategy to promote the production of functional islets.
Additively Manufactured Biphasic Construct Loaded with BMP-2 for Vertical Alveolar Bone Regeneration

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The vertical augmentation of alveolar bone is a major challenge. The primary objective of this study was to test a bio-mimetic bone tissue engineered biphasic construct containing bone morphogenetic protein-2 (BMP-2) for vertical bone augmentation in a pre-clinical ovine extraskeletal bone regeneration model. The second objective was to quantitatively assess the resorption pattern of the newly formed bone following surgical re-entry and dental implant placement.

The biphasic scaffold consisted of a robust outer shell similar to cortical bone. Within this shell, there was a highly porous melt electrospun microfiber mesh mimicking the structure of cancellous bone, permitting osteogenic and angiogenic cell ingrowth. The construct functionalized with a hyaluronic acid hydrogel containing BMP-2. The in vivo study was divided in two parts, one investigating bone formation while the second assessed bone resorption following implant placement in the newly formed bone. The vertical bone formation performance of the biphasic scaffold loaded with BMP-2 and the corresponding control groups was assessed in an extraskeletal sheep calvarial model for 8 weeks. In a separate group, regenerated extraskeletal bone received a titanium dental implant. Bone formation, bone resorption and bone to implant contact was quantitatively assessed by microcomputed tomography and histomorphometry.

We showed that the presence of the biphasic scaffold combined with various doses of BMP-2 resulted in full bone fill of the augmented space after 8 week. The biphasic scaffold was able to guide bone regeneration resulting in the homogenous formation of a mature and dense bone tissue. In addition, the augmented bone within the biphasic scaffold displayed excellent dimensional stability post-surgical re-entry and implant placement. This was in contrast to the specimens where bone was originally formed within the hydrogel + BMP-2 (but without the scaffold), whereby the augmented bone fully resorbed upon the removal of the occlusive barrier and implant placement.

We demonstrated that this highly innovative concept results in full bone fill within the extraskeletally placed construct. A major finding of the study related to the excellent dimensional stability of the newly formed bone following surgical re-entry and implant placement. This study has high potential for bench to bedside translation and will pave the way towards human clinical trials.
87-SY-4 Blood prefabricated HA/TCP induced ectopic vascularized bone via modulating the osteimmune environment

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Tissue engineering bone regeneration usually requires the addition of stem cells and growth factors, which make it an expensive strategy with complex experimental procedure derived from stem cell expansion and implantation. Blood clot has been known to contain peripheral mesenchymal stem cells and various growth factors. The successful bone healing depends greatly on the structure of blood clot and functional responses of blood cells. Therefore, we hypothesized that blood prefabrication could endow bone substitute materials with stem cells and growth factors that were sufficient for the ectopic bone formation subcutaneously, even without the addition of exogenous stem cells and osteogenic factors. An exogenous "stem cell and growth factor"-less strategy would thus been developed to improve bone substitute material's osteogenic capacity. It was found that CD29+, CD44+, CD90+, and CD45- mesenchymal stem cells could be detected from the blood cells. Blood also endowed hydroxyapatite 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). It was found that blood prefabricated hydroxyapatite induced ectopic bone-like structure. The possible mechanisms were related to 1) the modification on the fibrin network that facilitates MSCs recruitment and differentiation, 2) the modulation of the early osteoimmune environment favouring osteogenesis, and 3) the enhancement of angiogenesis and mature vascularisation. These results unveiled the multidirectional effects of blood clot on regulating osteogenesis, osteoclastogenesis, immune response, and angiogenesis, etc. Therefore, blood prefabrication can be a valuable exogenous "stem cell and growth factor"-less strategy to improve the osteogenic capacity of materials, and prefabricating the materials with blood clot before implantation should be encouraged. Future development of new generation bone substitute materials could target at modulating a favourable blood clot response for better bone regeneration.
Regenerative Microenvironment Therapy for Ischemic Organs

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Stem cell biology for organ regeneration is supported by blood and tissue associate cells in organ microenvironment. Blood regenerative associate cells are mainly composed of endothelial progenitor cells (EPCs), M2 macrophages, helper T lymphocytes and neutrophils for vasculogenesis, inflammatory regulation and microenvironmental priming. Tissue associate cells constitutes of mesenchymal stem/stroma cells (MSCs), endothelial cells and pericytes for orchestration of microenvironment in organ regeneration. Recently these cells are challenged to develop a new concept of cell therapy for regenerative microenvironment to enhance intrinsic organ regeneration or to support stem cell therapies using IPS derived cells and adult stem cells. This keynote introduces the issue regarding regenerative associate cell biologies and therapeutic applications for advanced organ regeneration.

Exosome therapy for regenerative microenvironment

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Extracellular vesicles (EVs) play important roles in intercellular communications via their content molecules, and mimic, at least in part, the roles that are played by their originating cells. Consistent with this notion, an increasing number of reports have suggested that EVs derived from mesenchymal stem cells (MSCs), which are therapeutically beneficial to a wide range of diseases, can serve as drugs to treat multiple diseases. EVs contain a variety of molecules, including proteins, microRNAs, and mRNAs, and are associated with biological processes in a content molecule-dependent manner. Here we overview the latest reports regarding the therapeutic potential of MSC-EVs by focusing on the underlying molecular mechanisms of their effects. Specifically, we feature the effects of MSCEVs in terms of their content molecules and of the tissue recovery processes endowed by these molecules. Furthermore, EV-targeting therapy for tumor metastasis will be discussed.
In this study, we have assessed the safety and efficiency of the treatment of inoperable patients with critical lower limb ischemia (CLLI) with the use of donor mesenchymal stem cells (MSC) [ClinicalTrials.gov Identifier: NCT03239535]. MSC were isolated from healthy donors' bone marrow using a standard procedure [1]. The MSC transplantation (2 million cells per 1 kg of the body weight) was performed in 18 CLLI patients (16 men and 2 women, 42-75 years old), including 5 patients of class 4, 11 patients of class 5 and 2 patients of class 6 (Rutherford classification of peripheral arterial disease) [2]. 13 out of 18 patients had been repeatedly operated at the side of the affected limb prior to the transplantation. A multi-level damage of the arterial flow was found in all the patients, 15 of them also had trophic ulcers.

In 10 patients, a single MSC transplantation into the tibial muscles was performed, 6 patients underwent 2 courses of intramuscular transplantation, with a 1 month interval between the courses. In 2 patients, we administered MSC intra-arterially, along with the arterial reconstruction to improve the outflow.

All the patients noticed a satisfactory tolerance of the cell transplantation procedure, no serious direct adverse effects were observed. No fatalities or treatment-related complications were seen during the observation period (up to 48 months). The limb preservation was achieved in 12 patients, with either complete or almost complete relief of the pain syndrome at the 30 months mean duration of observation. The mean pain index decreased from the initial value of 56.7 to 26.4 post-treatment, with the corresponding increase of the pain-free walking distance. 6 patients experienced complete healing of trophic ulcers, 3 patients – significant healing of them.

The pain was essentially reduced even in patients with the remaining trophic disorders. The unfavorable outcomes requiring limb amputation were observed in patients with the initial severe pain syndrome (more than 80 points).

CONCLUSION: Application of donor MSC from the human bone marrow is safe and efficient in CLLI, allowing limb preservation in 12 of 18 patients at the 30 months mean duration of observation. The study is to continue both for the technique perfection and for a more detailed assessment of this therapy efficiency.

REFERENCES:
Bioartificial organs are biohybrid constructs usually designed for organ supply, in case purely artificial organs fail in replacing some of the dedicated functions. They rely on the classical pillars of tissue engineering to mimic native tissues, ie cells, scaffolds and environment (both physical and chemical). Of prime importance are the bioreactors whose size will define the potential applications. In such bioreactors, cells can be better provided with oxygen and nutrients, and are submitted, within the scaffolds, to mechanical stimuli that can help in the maintenance of differentiation or functions.

These biohybrid constructs are thus interesting tools to perform toxicology studies that are more relevant than classical 2D in vitro culture and therefore offer alternatives for animal experiment, to evaluate drugs or chemicals. In the present lecture, we will provide several examples regarding the use in Europe of bioartificial livers or other biohybrid organs in the field on predictive toxicology. Specific attention will be paid to organ on chip and human on chip approaches.
Membrane systems and devices for the biofabrication of microtissues

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One of the most important goals in neuronal tissue engineering is to provide systems and devices that can reproduce tissue-like microenvironments in vitro that can promote and direct neuronal regeneration. The challenge is to create 3D neuronal models for neurorepair and/or for the study of neurobiological events, pharmacological screening, and as investigational platform for neurodegenerative disease. Bioinspired and cell integrated membrane bioreactors are appealing as investigational platforms because their experimental parameters can be tuned and controlled according to the needs of the cellular construct that has to be created, thus providing a neuronal interface quite similar to the in vivo situation. A novel membrane bioreactor was created to provide a 3D well-controlled microenvironment for neuronal outgrowth. The bioreactor consisted of poly-L-lactic acid highly aligned microtubule array membranes assembled in parallel within a chamber that establish an intraluminal and an extraluminal compartment whose communication occurs through the pores of the membrane walls. The high performing device built up in this study modulated and enhanced neuronal outgrowth, thanks to a synergistic action of the membrane properties and the uniform dynamic bioreactor microenvironment. The bioreactor not only supported neuronal survival and differentiation but also successfully allowed a long-term maintenance of specific differentiated features. Neuronal phenotypic identification was demonstrated by investigating the expression and pattern distribution of neuronal specific markers. Neuronal cells in bioreactor exhibited both the neuronal cytoskeletal marker β III-tubulin and the punctate pre-synaptic vesicle protein synaptophysin along neuronal processes and soma. Another neuronal indicator of axonal growth, GAP-43, was also quite visible; it is involved in neurite outgrowth and neuronal plasticity, which expression is related to axonal elongation and synaptogenesis of developing and regenerating neurons. The membrane bioreactor besides enhancing acquisition of neuronal phenotype guided the neurons into a defined aligned orientation according to the direction of the microtubes. Overall, our bioreactor accomplished two main achievements: it promotes long-term growth and differentiation of neuronal cells, and orients neurite alignment generating a neuronal tissue-like construct.

References:

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Building a new treatment strategy for severe heart failure using Cell sheet technology

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Despite recent progress in the treatment of heart failure, heart failure is still life threatening disorder in worldwide. In this clinical situation we have developed cell sheet technology experimentally and introduced to the treatment of severely damaged myocardium in clinical setting as translational research.

In a series of pre-clinical experiments, we proved that myoblast sheets could heal the impaired heart mainly by cytokine paracrine effect. We implanted myoblast sheet to 51 heart failure patients and some patients showed LV reverse remodeling. Myoblast sheet was approved by the government as “Heart Sheet” in the treatment for ischemic cardiomyopathy.

To improve effectiveness of cell sheet we have developed human iPS cell derived cardiomyocyte sheet and obtained Proof of Concept with evidence of synchronous movement with recipient myocardium. And also we have established large culture system and checked safety of GMP grade iPS cell derived cardiomyocyte sheets for clinical trial by the development in new method for removal of immature iPS cells.

Cell sheet technology may open new era in the treatment of severely damaged myocardium.
**91-SY-3 Development of cell sheet therapy for esophageal regeneration**

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Esophageal stricture remains one of the major problems associated with large endoscopic submucosal dissections (ESD) for superficial esophageal neoplasms. Steroids have been used in order to avoid stricture during the recent years, but the treatments have inescapable adverse effects and are not yet spread as the standard treatment.

In our previous safety and feasibility study, tissue-engineered epithelial cell sheets, produced by culturing the patients' own oral mucosal epithelial cells on temperature responsive culture dishes, were transplanted onto the ulcer surface following ESD in 10 patients. The result of the study indicate that autologous epithelial cell sheets were reproducibly produced, transplanted safely, and promoted early re-epithelialization. However, the novel approach had several shortcomings. One of the crucial challenges was the cell sheet transplantation technique – to transport and transplant the sheets properly into the esophageal lumen.

Encouraged by the clinical results and the challenge regarding delivery, we developed a new endoscopic device using a 3D printer. To prevent losing or damaging the cell sheet during delivery through the oral cavity and pharynx, an applied vacuum can draw the sheet in and protect it by the device’s walls. Once at the position of the ulcer surface, pressure increase can expand a membrane with attached cell sheet, thus facilitating rapid, simple, and accurate transplantation. Based on this prototype delivery device, we continued to develop dedicated device as a medical device. Additionally, this dedicated delivery device acquired CE marking.

Furthermore, we conducted clinical study to evaluate the safety and efficacy of endoscopic transplantation of autologous epithelial cell sheets that had been transported by air over a distance of 1200km between Tokyo and Nagasaki. The safety of the entire process including cell sheet manufacturing, transportation, ESD and cell sheet transplantation was assessed. There were no significant complications at any stage of the process.

Thus, we planned clinical trials for autologous fabricated epithelial cell sheets after large-sized ESD for superficial esophageal neoplasms in Japan and Europe. In Japan, multicenter clinical III phase trial of esophageal regeneration for superficial esophageal squamous cell neoplasms using autologous epithelial cell sheets and dedicated devices as human cell tissue products-based therapy be in progress already.

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**91-SY-2 Development of anti-fibrotic cell sheets engineered from mesenchymal stem cells for liver fibrosis**

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**Backgrounds & Aims:** Advanced liver fibrosis results in liver cirrhosis, however, there are currently no approved anti-fibrotic therapies for liver disease. We previously reported that orthotopic transplantation of cell sheets derived from human MSCs ameliorated carbon tetrachloride (CCl4)-induced acute liver injury. These cell sheets were manufactured from MSCs in the presence of hexachlorophene, a Wnt/β-catenin signal inhibitor, on thermoresponsive polymer-coated culture dishes. Furthermore, we identified IC-2 as a suitable compound to commit differentiation of MSCs into hepatic lineage among our synthesized chemical library. To explore the anti-fibrotic effect of IC-2-treated MSC sheets (IC-2 sheets), IC-2 sheets and MSC sheets were transplanted into CCl4-induced chronic liver injury mice model.

**Methods:** To induce chronic liver injury, CCl4 was orally administered twice a week to 7 to 9-week-old BALB/c-nu/nu male mice at a 0.6 ml/kg dose for 4 weeks, and 1.2 ml/kg dose for 6 to 7 weeks. Cell sheets were fabricated as follows: UC7T-13 bone marrow-derived MSCs were plated onto 60mm PIPAAm-grafted culture dishes at the cell density of 9.0×10^5 cells/cm^2, and cells were treated with 15 µM IC-2 for a week. Cell sheets were transplanted onto liver surface of mice with chronic liver injury.

**Results:** The anti-fibrotic effect of IC-2 sheets was superior to MSC sheets without IC-2 treatment, judging from Azan staining, Sirius red staining, Type I collagen expression and liver hydroxyproline content. Hepatic stellate cell activation was also suppressed in mice transplanted with IC-2 sheets according with remarkable decrease of α-smooth muscle actin (αSMA) expression. The mice receiving IC-2 sheets exhibited higher matrix metalloproteinases (MMPs) activities of MMP-1 and MMP-14. These MMPs were upregulated in MSCs by the treatment of IC-2. Finally, MMP-knock-down experiments clarified that MMP-14 proved to be especially effective for improving liver fibrosis.

**Conclusion:** IC-2 sheets possess potent fibrolytic activity by induction of MMP-1 and MMP-14 as well as suppression of hepatic stellate cell activation. Since there are currently no approved anti-fibrotic therapies for liver cirrhosis, development of novel anti-fibrotic therapy based on cell sheet technology will be of a potent therapy for liver fibrosis.
**91-SY-4**

**Transplantation of Autologous induced Pluripotent Stem Cell-Derived Retinal Pigment Epithelium Cell Sheets for Exudative Age Related Macular Degeneration: A Pilot Clinical Study**

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We have started a clinical research using induced pluripotent stem cells (iPSCs) for age-related macular degeneration (AMD) and we have carried out transplantation of the first patient’s iPSC-derived retinal pigment epithelial (iPSC-RPE) cell sheets successfully. iPSC-RPE cell sheets have the clinically necessary quality, such as expression of typical RPE markers, tight junction formation, polarized secretion of growth factors and phagocytic ability. Furthermore, autologous primate iPSC-RPE cell sheets showed no immune rejection or tumor formation after transplantation into the subretinal space of the cynomologus monkeys, while allogeneic transplantation showed immune rejection. In the clinical research, patients with active wet type AMD after repeated existing treatment such as anti-VEGF drug injection into the eye are enrolled. The primary endpoint is safety of the treatment. We have followed the first patient for more than three years without adverse events arising from the transplanted cell sheet. The patient have kept visual acuity without additional anti-VEGF injection.

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**92-SY-1**

**Novel Biofunctional Inks for Stem Cell-Based Cartilage Tissue Printing**

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Bioink that is able to mimic tissue microenvironment and guide cell differentiation is critical for the successful tissue/organ engineering with 3D printing technology. We synthesized two new materials for cartilage bioprinting, [poly-L-lactic acid/polyethylene glycol/poly-L-lactic acid] (PLLA-PEG) and [poly-D,L-lactic acid/polyethylene glycol/poly-D,L-lactic acid] (PDLLA-PEG), to form hydrogels that are biodegradable, biocompatible, and possess high, physiologically relevant mechanical strength (~1,500 to 1,800 kPa). We then examined the chondrogenic activity of human bone marrow stem cells (hBMSCs) encapsulated in these hydrogels at physiologically relevant cell densities. To achieve one-step point-of-care tissue engineering, transforming growth factor-β3 (TGF β3) was directly pre-loaded into the PDLLA hydrogel, or PDLLA with the addition of hyaluronic acid (PDLLA/HA). We found that the inclusion of HA within PDLLA resulted in a controlled release of pre-loaded TGF β3 and led to a robust cartilage formation without exogenous TGF β3 added to the culture medium. Using these optimized hydrogels, we also developed a protocol to suppress chondrocyte hypertrophy to enhance the quality of the engineered cartilage. Specifically, culturing hBMSC-laden hydrogel for 14 days in the presence of TGF β3, followed by 7 days culture in TGF β3-free medium, with supplementation of a Wnt/β-catenin inhibitor, XAV939, from day 10-21, resulted in significantly reduced hypertrophy phenotype. Currently, we are testing these 3D printed, live cell-encapsulated functionalized cartilage constructs for the repair of cartilage injury in preclinical animal models.
**92-SY-1** Generation of Functional Tissues through Cell Printing

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Bioprinting technology has evolved as a promising approach for delivering various tissue elements, including cells, biomaterials and bioactive factors, for a wide range of applications. This innovative tool enables delivery of live cells ranging from single cell types to multi-cellular tissue constructs with structural and spatial orientation. The advantage of bioprinting over other delivery methods includes the ability to print single cells to small scale tissue organoids to clinically relevant tissue constructs with precision and reproducibility. This session will discuss the desired parameters to deliver and maintain viable cells, as well as some of the present challenges encountered for use in cell-tissue applications.

**92-SY-2** Multi-Scale Biofabrication Approaches for Osteo-Chondral Tissue Regeneration

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Biofabrication technologies provide the opportunity to further replicate native tissues at different length scales through the convergence of different fabrication approaches. We have adopted this for osteo-chondral defect repair. Specifically, we developed cell-laden hydrogel (pre-cursor) bioinks and used extrusion-based bioprinting processes to enable their controlled deposition, also addressing the zonal (layered) nature of the cartilage tissue. Moreover, melt electrowriting (MEW) that enables the fabrication of (sub)-micrometer scale fibers was used to generate fiber structures that were combined with hydrogels and resulted in mechanically stable tissue constructs. Importantly, the controlled deposition of these reinforcing fibers also allows for the generation hierarchical reinforcing structures, further mimicking the native tissue biomechanics. As this two-step process still limited the freedom of design regarding the use of multiple materials and cell types, these techniques were converged into a single-step process. Mechanically stable constructs with the spatial distributions of different cell could be generated without compromising cell viability and (chondrogenic) differentiation. For the bone compartment, a low-temperature setting printable calcium phosphate paste, consisting of\(\alpha\)-tricalciumphosphate (\(\alpha\)-TCP), nano-hydroxyapatite and biodegradable, crosslinkable poloxamer was used and combined with the MEW and bioprinting processes. This resulted in a compressive modulus and ultimate strength in the range of cancellous bone, as well as efficient embedding of the fibers in the bone phase upon setting of the cement. This works illustrates essential steps for the fabrication of more biomimetic tissue equivalents, and highlights the opportunities provided by these emerging technologies.

Selected references:

Malda et al. Adv Mat 2013
Visser et al. Nature Comm 2015
Castilho et al. Adv Healthc Mat 2017
de Ruijter et al. Small 2018
de Ruijter et al. Adv Healthc Mat 2018
The efficacy of a scaffold-free Bio 3D conduit developed from dermal fibroblasts on peripheral nerve regeneration in a canine ulnar nerve injury model

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Background
Autologous nerve grafting is widely accepted as the gold standard treatment for segmental nerve defects when direct repair cannot be achieved. However, to overcome its inevitable disadvantages, alternative methods such as the tubulization technique have been developed. Several studies have investigated the ideal nerve conduits modified with supportive cells, scaffolds, growth factors, and vascularity. Previously, we confirmed that biological scaffold-free conduits fabricated from human dermal fibroblasts promote nerve regeneration in a rat sciatic nerve injury model.¹ The purpose of this study is to evaluate biological scaffold-free conduits that are entirely composed of each animal's own dermal fibroblasts using a large animal model.

Materials and Methods
Six male beagle dogs were used in this study. Eight weeks before surgery, dermal fibroblasts were harvested from their groin skin and grown in culture. Bio 3D conduits were assembled from proliferating dermal fibroblasts using a Bio 3D printer. The ulnar nerve in their forearm was exposed under general anesthesia and sharply cut to create a 5 mm interstump gap, which was bridged by the prepared 8-mm Bio 3D conduit. Ten weeks after surgery, nerve regeneration was investigated.

Results
Electrophysiological studies revealed that compound muscle action potentials (CMAPs) of the hypothenar muscles and motor nerve conduction velocity (MNCV) were detected in all animals. Macroscopic observation showed regenerated ulnar nerves. Histological and morphometric studies confirmed the existence of many myelinated axons through the Bio 3D conduit. A small amount of hypothenar muscle atrophy was confirmed.

Conclusions
Hypothenar muscles were re-innervated by regenerated nerve fibers through the Bio 3D conduit. The scaffold-free Bio 3D conduit fabricated from dermal fibroblasts is effective for nerve regeneration in a canine ulnar nerve injury model. This bioprinting technology would be useful for segmental nerve defects in clinical settings.

References
The development of cell based therapies, including tissue engineering (TE) for tendon regeneration requires biomechanically-stimulating culture environments as tendon tissue functionality is known to be highly dependent on mechanical loading. That can be achieved modulating the scaffold architecture, properties and composition. Nevertheless, the incorporation of magnetic nanoparticles (MNPs) within 3D constructs constitutes a novel and attractive strategy towards the development of magnetically-responsive system that may eventually combine therapeutic and diagnostic functionalities. An additional advantage is that cells naturally respond to magnetic forces, and consequently, the application of a magnetic field may enhance stem cells biological performance, and ultimately stimulate cell proliferation and/or differentiation. This work reports on recent studies concerning the development of specific scaffolds architectures based on various polymers, doped with MNPs and fabricated using different technologies enabling responsive systems for culturing stem cells, stimulating their tenogenic differentiation. Moreover, we hypothesized that ex-vivo application of pulsed electromagnetic field therapy (PEMF) applied in combination with magnetic responsive materials, may also enable to modulate the inflammatory response and consequently promote a better tissue regeneration.
Multifactorial approaches towards tenogenic phenotype maintenance, trans-differentiation and differentiation

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INTRODUCTION: Current cell-based therapies require removal of cells from their optimal in vivo tissue context and propagation in vitro to attain suitable numbers. However, bereft of their optimal tissue niche, cells lose their phenotype and with it their function and therapeutic potential. Biophysical signals, such as surface topography and substrate stiffness, and biochemical signals, such as collagen I, have been shown to maintain permanently differentiated cell phenotype and to precisely regulate stem cell lineage commitment. Furthermore, standard in vitro culture conditions for tenocytes result in delayed and reduced extracellular matrix (ECM) deposition, impeding the development of scaffold-free approaches. Deposition of ECM in vitro can be enhanced by macromolecular crowding (MMC), a biophysical phenomenon that regulates the intra- and extra-cellular milieu of multicellular organisms, which has demonstrated accelerated ECM deposition in human tenocytes and BMSCs. Herein, we developed and characterised substrates of various stiffness with controlled nanotopographical features and assessed these substrates in culture in combination with MMC on various cell sources to determine their suitability for the in vitro fabrication of tendon-like tissue.

RESULTS: Cellular morphology of the various cell types was analysed after 3, 7 and 14 days of culture on anisotropic substrates of varying stiffness with MMC. The nanotopographical features on the substrate surface induced cellular alignment in all cell types, which was not affected by MMC or substrate stiffness, and it is also seen that the ECM deposited follows the alignment of the cell cytoskeleton. Immunocytochemistry was used to assess the composition of the ECM deposited by the different cell sources. It is seen that, when MMC is used, cells show increased deposition of collagen type I, one of the main components in tendon ECM. Other collagen types relevant to native tendon composition were also analysed, including types III, V and VI, and their deposition was shown to be modulated by the use of MMC. FACS analysis in BMSCs indicated a significant reduction in surface marker expression as a function of MMC but not surface topography or stiffness, while gene analysis showed MMC had a greater influence on phenotypic markers in all cell types compared to topography or stiffness.
Hippo pathway is the main axis regulating organ size and geometry, with the overexpression of its downstream effector YAP leading to tissue overgrowth and organomegaly. YAP acts as a mechanosensor by perceiving dynamic modifications of substrate mechanics and nanostructure, with its transcriptional co-activator function being prompted by extracellular matrix (ECM) stiffening and nanotopography modification. Interestingly, YAP is needed for completion of cardiogenesis during murine foetal development but is repressed in adult cardiomyocytes. Our group and others recently demonstrated that YAP expression is not detectable in adult mouse heart tissue, while it is re-expressed at the myocardial infarction border zone, possibly as a pro-survival response to tissue remodelling. The mechanisms underlying YAP depletion during heart development and its reactivation after ischemic insult are unclear. We recently provided evidence that the indirect DNA binding activity of YAP through TEAD transcription factor results in the strengthening of cell-matrix interaction and in the enhancement of cell mechanics. This effect is due to the formation of YAP-TEAD complex on genes involved both in focal adhesion and ECM remodelling (1). In this study, we confirmed the restriction of the mechanosensor in human adult cardiac tissue and adopted differential analysis of YAP DNA targets and interactome to unveil YAP function across critical phases of cardiac cell development. While confirming the role of YAP in pluripotent stem cell development, we identified a number of novel cardiac-specific YAP-TEAD DNA targets in cardiomyocytes and delineated the molecular mechanisms by which the function of the protein is progressively restricted to allow cell maturation. By dissecting the multiphasic activation of YAP, we highlight the role of the Hippo pathway in controlling cardiomyocyte mechanics and function and the transition to the adult state by repressing YAP activity in contractile cells.


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YAP-Dependent Extracellular Matrix Remodeling directly influences Cell Mechanics

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The extracellular matrix (ECM) is a macromolecular complex whose physical and biochemical parameters are the result of active cellular decisions to regulate not only the inert deposition of its components, but also to modulate the cellular tension applied to the environment and needed to induce the assembly of the ECM constituents.

We recently proved that Hippo pathway effector YAP can control cell mechanics by exerting its co-transcriptional regulation over key components of focal adhesions (FAs), including relevant integrin subunits [1]. YAP loss results in a significant reduction in cell tension and consequent inability to acquire a defined shape and size.

Here we demonstrate that cell mechanics is at least partially regulated by YAP inducing the transcriptional activation of genes involved in several stages of ECM arrangement: matrix composition, deposition, fibrillogenesis and degradation.

In fact, numerous ECM genes including structural components, matrisome-associated secreted factors and crosslinking agents were identified as YAP targets in different cell lines. Moreover, YAP is also able to influence collagen secretion and incorporation in the matrix, while determining ECM remodeling by altering the levels of metalloproteases and cellular actin-membrane machinery required for the degradation process.

As a result, the alteration of YAP levels, as obtained by CRISPR/Cas9 or overexpression, has a significant impact on the contraction of 3D collagen gel pads, a measure of the force exerted by the cell on the surrounding ECM.

To quantify the impact of YAP-dependent ECM remodeling on cell mechanics, we adopted decellularized matrices obtained from cell lines expressing physiological or pathological levels of YAP. By this means, we clarified that cell mechanics can be recovered in soft YAP mutant cells when they are put in contact with ECM produced by pathological YAP.

Moreover, we demonstrate that YAP target CTGF can phenocopy YAP-induced cell stiffening in a process which appears to be independent of its paralog protein TAZ and RhoA/ROCK pathway activation. We therefore characterize YAP as a key regulator of ECM remodeling being able to partially determine cell tension independently of its intracellular activity.

1. Nardone G, Oliver-De Cruz J, et al. YAP regulates cell mechanics by controlling focal adhesion assembly. Nature Communications 8, 15321, 2017

Tracking therapeutic shockwaves and their impact on regeneration

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Extracorporeal shockwave treatments have been shown to accelerate tissue regeneration in diverse clinical situations, ranging from soft to nerve and bone tissue. The underlying mechanisms of these mechanically-induced beneficial effects are not yet fully understood. We used surgically placed PVDF hydrophones to measure the time-dependent pressure fields during shockwave treatment in various pre-clinical models ranging from rats to pigs, both in vivo and post mortem. A statistical analysis of single shot parameters over the duration of a treatment yielded insight into the tissue transmission of shockwaves providing clinically relevant data as our findings show a significant dependence on the type of shockwave generating technology.

Additionally, optical phase contrast imaging was used to visualize different shockwaves in various in vitro test setups in validation of computational models to calculate the propagation of shockwaves in test waterbaths. Resultant shockwave pressure fields were subsequently measured directly within the test samples. Cell culture tests carried out in these waterbath setups identified extracellular ATP as a trigger of the biological effects of shock wave treatment and an increase in cell proliferation was observed. Purinergic signaling-induced Erk1/2 activation was found to be essential for this proliferative effect, which was further confirmed in an ischemic flap wound healing model in rats where shockwave treatment induced proliferation and increased wound healing in an Erk1/2-dependent fashion.
**Shape-memory cell culture platforms for dynamic mechanobiology**

Koichiro Uto, Cole A DeForest, Deok-Ho Kim, Mitsuhiro Ebara

Coordinated extracellular matrix spatiotemporal reorganization helps regulate cellular differentiation, maturation, and function in vivo, and is therefore vital for the correct formation, maintenance, and healing of complex anatomic structures. In order to evaluate the potential for cultured cells to respond to dynamic changes in their in vitro microenvironment, as they do in vivo, the single cell or collective behavior of fibroblasts and primary cardiac muscle cells cultured on nanofabricated substrates with controllable anisotropic topographies were studied. A thermally induced shape memory polymer (SMP), poly(epsilon-caprolactone) (PCL) was employed to assess the effects of dynamic change of surface topography on the alignment of fibroblasts and a 90° transition in substrate pattern orientation on the contractile direction and structural organization of cardiomyocyte sheets. In fibroblast system, we were able to dynamically manipulate not only cell orientation mode but also orientation direction of adhered cell in a programmed way. On the other hand, cardiomyocyte sheets cultured on SMPs exhibited anisotropic contractions before shape transition. 48 hours after heat-induced shape transition, the direction of cardiomyocyte contraction reoriented significantly and exhibited a bimodal distribution, with peaks at ~ 45 and -45 degrees. Immunocytochemical analysis highlighted the significant structural changes that the cells underwent in response to the shift in underlying topography. The presented results demonstrate that initial anisotropic nanotopographic cues do not permanently determine the organizational fate or contractile properties of cardiomyocytes in culture. Instead, cardiomyocytes are capable of reorganizing their nucleus, cytoskeletal, and focal adhesion structures and attempt to realign with the altered substrate directional cues. Given the importance of surface cues in regulating primary and stem cell development, investigation of such tunable nanotopographies may have important implications for advancing cellular maturation and performance in vitro, as well as improving our understanding of cellular development in response to dynamic biophysical cues.
**Injectable Mesenchymal Stem Cells Loading Hydrogel for Spinal Cord Regeneration**

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The loss of neuronal function after spinal cord injury (SCI) is a permanent impair which strongly affect patient life, thus the regeneration of neural axons after traumatic injuries still remains a big challenge in medicine. Recently, the use of mesenchymal stem cells (MSCs) locally injected at injured site has significantly improved functional outcome after nerve injury by reducing glial cyst formation (1) and enhancing nerve regeneration (2). The therapeutic potential of MSCs has been demonstrated in many applications thanks to their paracrine support, differentiation and immune-modulation (3). However, local injection of cells suffers from low degree of cells survival and limited cells retention at injured site.

In this work, a hydrogel-based strategy for improving MSCs viability and engraftment efficacy is presented as a promising therapy for spinal cord regeneration. A novel injectable hydrogel based on chitosan (CS) was developed allowing cell encapsulation in a highly hydrated and mechanically supportive 3D environment.

Complete physicol-chemical characterization of CS-based hydrogel was performed and MSCs were loaded into the hydrogel in mild condition and prior to injection. MSCs viability within CS-based hydrogel was confirmed through colorimetric assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide - MTT. Sigma Aldrich) and Live and Dead staining (Sigma Aldrich). The release of MSC-microvesicles from the MSC-loaded into the CS-based hydrogel was assessed by dynamic light scattering (DLS) to confirm the maintenance of MSC-trophic factors release after encapsulation. Furthermore, the therapeutic effect of MSC-loaded into CS-based hydrogels was assessed in vitro using cell line and in vivo on mice.

**References**

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Mechanisms of Angiogenesis in Bioprinted Endothelial/Stem Cell Cocultures for Vascularized Tissue-Engineered Constructs

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Bioprinting enables precise control over spatial distribution of cells/materials and has been leveraged to replicate vessel development via deposition of endothelial cells (ECs) and mesenchymal stem cells (MSCs).1,2 Accordingly, bioprinting hold great promise in the fabrication of vascularized scaffold, a central challenge in tissue engineering.3 Yet, studies reported contrasting findings on the role of MSCs, which supported arteriogenesis in some cases while exerting an antiangiogenic action in others.1,3 Therefore, in this study we investigated how ECs/MSCs cooperatively drive the formation of new vasculature within bioprinted scaffolds. Cells were cultured in gelatin methacrylate scaffolds according to different ratios (1:0, 3:1, 1:1, 1:3, and 0:1) for 2 weeks. Scaffolds were fabricated via extrusion-based bioprinting (EnvisionTEC®) and evaluated for cell proliferation (PicoGreen), gene expression against VEGF, PDGF, and TGF-β1-related signaling pathways, and immunohistochemistry.

Cell viability was maintained over 14 days in all groups, with 2-fold change in DNA content. EC/MSC 3:1 group displayed upregulation of VEGF, PDGF, and TGF-β1 at day 7 compared to homotypic ECs (or MSCs) cultures. Interestingly, we observed a physiologically relevant pattern with initial upregulation of angiogenesis in ECs (VEGF, PDGF) followed by enhanced arteriogenesis in MSCs (PDGF, TGF-β1). These findings were corroborated in an animal model, where we observed enhanced neovascularization in EC/MSC-laden scaffolds compared to EC/MSC homotypic constructs, as shown by histology and lectin-based imaging of vascular structures.

The results obtained in this study allowed to uncover specific mechanisms governing angiogenesis/arteriogenesis in EC/MSC cocultures that served as guiding criteria for bioprinting prevascularized scaffolds, resulting ultimately in a robust neovascularization in vivo.

References:

Acknowledgements
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Mimicking N-cadherin Interactions via Engineered Hydrogels Confers Growth Factor Sensitivity and Enhances Paracrine Activity of Mesenchymal Stromal Cells

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Introduction:
Injectable hydrogels delivered in a minimally invasive manner are attractive materials for local cell and drug delivery. However, in most cases their nanoscopic structure prevents any physical interactions between encapsulated cells. We recently reported that increased N-cadherin based cell-cell interactions in macroporous – but not nanoscopic – hydrogels enhances the paracrine activity of mesenchymal stromal cells (MSCs) [1]. In the current study, we hypothesized that functionalizing hydrogels with N-cadherin mimicking peptide fragments (HAVDI) could result in a nanoporous biomaterial that retains injectability and simultaneously harnesses the benefits of cell-cell interactions.

Materials and Methods:
A mixture of low and high molecular weight ultrapure alginate (Pronova) was functionally modified with RGD and HAVDI peptides using carbodiimide chemistry. Peptide attachment was confirmed by 1H NMR. Bone marrow MSCs were encapsulated in alginate with constant RGD, but varying HAVDI ratios. Expression of growth factor receptors, and intracellular signaling molecules β-catenin and Akt was quantified using qPCR. Phosphorylation of β-catenin, Akt, AMPK, and GSK3ß was investigated by Western Blot analysis. Paracrine activity was analyzed by membrane based cytokine arrays (BioCat), and conditioned media from encapsulated MSCs was used to study migration and differentiation behavior of myoblasts.

Results:
Expression of growth factor receptors on MSCs correlated positively with increasing 3D N-cadherin engagement (high HAVDI ratio) and were dependent on mechanical stabilization via actin. Growth factor stimulation of MSCs in hydrogels with high HAVDI ratio led to GSK3ß phosphorylation and inactivation mediated via the PI3K/Akt pathway and resulted in enhanced β-catenin nuclear translocation. This activation of the canonical Wnt signaling pathway consequently enhanced cytokine secretion by MSCs which exerted potent paracrine effects on myoblasts, modulating key functions such as migration and differentiation.

Conclusions:
Injectable hydrogels functionalized with N-cadherin mimicking peptides strongly enhance the paracrine activity of encapsulated MSCs and hold promise for improving the outcome of cell therapies targeting musculoskeletal tissue regeneration.

References:
1. Qazi TH, Mooney DJ, Duda GN, Geissler S. Biomaterials that promote cell-cell interactions enhance the paracrine function of MSCs. Biomaterials (2017) 140, 103-114.
**95-SY-5**

**HUMANIZE MICE AS AN ACCURATE PRECLINICAL TOOL TO STUDY MESENCHYMAL STEM CELLS (MSC)-T CELL INTERACTION DURING WOUND HEALING CELL THERAPY**

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Development of accurate preclinical in vivo model systems for human transplantation and cell therapy is important for understanding complex biological processes involved in wound healing and mechanisms underlying allograft rejection. Because of ethical limitations inherent in clinical trials and inadequacies of in vitro cell culture systems to accurately model wound healing and allograft rejection, small animal models are valuable research tools (1,2). However, not all observations made in animals recapitulate successfully the human situation (3). Humanized mice, animals bearing different elements from the human immune system, arise as a powerful weapon to predict the effect of a treatment in the host immune system. In this study, we evaluated the effect of a new MSC-functionalized device for treating full-thickness skin wounds, in the immune system of Hu-PBMC mice.

Through these study, we aim to predict the effect of our MSC functionalized device in the immune system of patients enrolled in future clinical trials. Different immune mediators were analyzed at the injury site and systemically, in blood, for predicting possible toxicity and acute rejection in future potential patients. The treatment with our MSC functionalized device did not induce necrosis, tumors, and ulcers around the treated areas confirming the absence of toxicity or additional signs of Graft versus host disease and rejection. Our results indicated that the MSC exerted their immunomodulatory effect by interaction with T lymphocytes through IDO and COX2 expression, triggering the T regulators phenotype presence, absence of activated T lymphocytes or macrophages and the expression of cytokines involved in anti-inflammatory and tissue regeneration processes, such as IL-10 or TGFb1 in the mice model. This is in line with previous results obtained by other authors in vitro and in vivo, stating that the crosstalk between exogenously added MSC and recipient immune cells, specifically T cells, plays a key role in the success of MSC-mediated tissue regeneration (4). These findings validate the use of the huPBMC model as a powerful tool for predicting the effect and demonstrate the safety of tissue engineered devices in vivo before starting a clinical trial.

**References**


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**95-SY-6**

**Microvesicles of adipose derived mesenchymal stem cell improve the survival of transplanted fat grafts**

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**Background:** Autologous fat grafting for soft tissue augmentation and reconstruction is a promising surgical technique in the field of plastic surgery. However, it is limited by the unpredictable and often low survival of the autologous fat tissue, due to the insufficient revascularization of such grafts. Previous studies have demonstrated that adipose tissue mesenchymal stem cell-derived microvesicles (ADSC-MVs) can induce angiogenesis, the present study hypothesized that co-transplantation of fat with ADSC-EVs during grafting could improve the survival of transplanted fat grafts. **Objective:** To investigate the effect of adipose tissue mesenchymal stem cell-derived microvesicles (ADSC-MVs) on the survival rate of fat transplantation. **Methods:** Microvesicles were isolated from the supernatant of cultured human adipose tissue mesenchymal stem cells, and characterized by Transmission Electron Microscope and confocal microscopy. Human fat tissue grafts, with (MV Group) or without (Control Group) ADSC-MVs, were subcutaneously injected into nude mice. A total of 4 weeks following transplantation, the mice were sacrificed and the grafts were harvested. Wet weight of fat grafts was measured for macroscopic aspects. After CD31 and HE staining, blood vessel density, viable adipocytes and fibrous proliferation were counted respectively for histological evaluation. **Results:** The grafts from the MV group had a higher survival rate and an increased number of vessels compared with grafts from the control group, as demonstrated by tissue volume, weight and histological analyses. **Conclusions:** ADSC-MVs can improve the survival rate of transplanted fat grafts, indicating a wide clinical application in the future.
**95-SY-7** Enhanced therapeutic properties of allogeneic adipose-derived mesenchymal stromal cells cultured in a serum-free medium

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Mesenchymal stromal/stem cells (MSCs) show therapeutic potentials against various diseases such as immune and neurological disorders for which no effective therapies exist. To develop safe and effective off-the-shelf MSC therapies, it is essential to understand cell properties and mechanisms of action. We have initiated a Phase 1/2 study in patients with liver cirrhosis since July 2017 in which ADR-001 composed of allogeneic adipose-derived MSCs (ADMSCs) is investigated.

One of the key features of ADR-001 is that ADMSCs are cultured in a serum free medium (R-SF) which is prepared in-house and in compliance with the Japanese Standards for Biological Ingredients. In this study, ADMSC cultured in the R-SF medium were compared with the one cultured in a conventional cell culture medium supplemented with fetal bovine serum (MEM-S). Parameters such as proliferation, cell morphology, cell surface markers and secreted factors were examined. Among these parameters, we focused on secreted factors and procoagulant activity that were thought to be relevant to the therapeutic potency and safety of ADMSCs, respectively. We found that R-SF/ADMSC secreted more HGF than MEM-S/ADMSC. Moreover, R-SF/ADMSC showed less procoagulant activity both in vitro and in vivo. The results suggest that R-SF/ADMSC are more potent and safe compared with MEM-S/ADMSC. They also suggest that the properties of MSC can be modified by selecting cell culture supplements which enables to develop MSC therapies optimized for each target disease.

**95-SY-8** Substrate Mediated Redox Signaling in Human Mesenchymal Stem Cells for Functional Tissue Repair

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Cell-substrate biointerface plays a pivotal role in regenerative medicine, especially in the development of advanced culture platforms to drive novel materials-driven stem cell responses. Using fibronectin-conjugated polyacrylamide (FN-PAA) gels with bio-mimicking stiffness (E ~1-20 kPa), we showed that the therapeutic performance of human mesenchymal stem cells (MSCs) derived secretome can be mechano-regulated. Compared to MSCs cultured on conventional FN-coated “hard” (E ~ GPa rage) coverslips, MSCs mechano-senses the soft substrate by displaying smaller spreading area and the focal adhesion are less developed under serum deplete condition. Specifically, it was also observed that MSCs respond to the compliant FN-PAA hydrogel by increasing the level of endogenous reactive oxygen species (ROS) as well as a panel of redox sensitive secreted cytokine, *inter alia*, IL-6, IL-8, MCP-1, VEGF, at the transcript and protein level. N-acetyl-cysteine (NAC) assay further confirmed that substrate mediated redox status in MSCs is implicated and serves as a critical upstream molecular event modulate the secretome profile. Importantly, the enhancement in the therapeutic efficacy of the mechano-regulated secretome was demonstrated using a series of *in vitro* assays, as well as an *in vivo* full thickness mouse excisional wound model. Taken together, our novel findings revealed a “soft approach” to target the redox signaling network of MSCs, which could be exploited as a cell-free therapeutic intervention to foster functional tissue repair.
**Overview of tissue engineering and regenerative medicine of the vocal fold**

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Tissue engineering and regenerative medicine has received extensive attention in the field of laryngology. The reasons include limitation of laryngeal transplantation for patients with total laryngectomy and the limitation of phonosurgery for the cases with vocal fold scar and atrophy. Vocal fold scar and atrophy cause stiffened vocal folds which lead to permanent deterioration of the vocal fold vibration with severe dysphonia. Although enormous efforts have been paid to resolve this problem using phonosurgical strategies, no consistent treatment has been developed.

Vocal fold scar is featured by histological alteration of the superficial lamina propria (SLP) with loss of hyaluronic acid (HA) and excessive deposition of thick collagen bundles. These changes make the vocal fold rigid and stiff. Vocal fold atrophy occurs with ageing, and the SLP becomes thin occasionally with fibrosis. It is essential to regenerate the SLP in treatment of vocal fold scar and atrophy. Several approaches using cell therapy, growth factors, and bioscaffolds have been researched and partially clinically applied to human patients. Recent development of tissue engineering has provided several types of regenerative scaffold including collagen/gelatin-based, HA-based scaffolds as well as nano-tube materials. Three dimensional printer enables precise scaffold for each organ and tissue. Decellularization technology is expected to provide whole laryngeal scaffold for laryngectomized case. Recellularization is possible using several types of cells such as stem cells or progenitor cells. To date, more attention has been paid to mesenchymal stem cells (MSC), because they are autologous, they have multi-potency to differentiate into many kinds of mature cells beyond germ layers, and they have paracrine effects by production of growth factors and cytokines. Growth factors are potent regenerative proteins that stimulate cell growth and also change the phenotype of the cells towards regeneration. Basic fibroblast growth factor (bFGF) and hepatocyte growth factor (HGF) are expected to resolve vocal fold scar by increasing HA and dissolving collagen deposits. They are clinically applied to patients with vocal fold scar and atrophy with improved vocal outcomes. More researches are necessary to develop consistent methods to regenerate vocal fold mucosa with optimal vibratory function.

**Developmental tissue engineering principles to generate vocal fold mucosa**

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The vocal fold lamina propria extracellular matrix is critical to vocal quality, but its poor healing ability and limitations of surgical repair have motivated cell based strategies to engineer living tissue replacement. Paucity of human vocal fold fibroblasts has necessitated the pursuit of surrogate cells that can be exploited alone, in combination with scaffolds and/or growth factors for optimal therapeutic application. The goal of this works is to identify and define vocal fold lamina propria pre-natal chemical and mechanical cues to regulate guided differentiation of stem cells. In this short presentation we will report our recent findings related to dynamic gene expression signatures and lineages in the murine vocal fold as it progresses through embryonic to postnatal development, in addition to our understanding of vocal fold mucosal growth and alterations in tissue elasticity in utero. Continued identification and optimization of synergistic factor combinations and a deeper understanding of their effects on stem cell function will inform scaffold- and bioreactor-based strategies and accelerate efforts to regenerate vocal fold lamina propria. The innovative use of developmental biology as a motivation in our research strategy will serve as a platform for exponentially reforming key tissue engineering specifications in laryngology.
Bioorthogonal Synthesis of Hydrogel Matrices for the Engineering of a Vocal Fold Tissue Model

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There is a critical need for a physiologically relevant in vitro model of the human vocal fold that can be used to investigate vocal fold development, health, and disease, and more importantly, to facilitate the discovery and testing of new treatment options. To this end, we are developing vocal fold-mimetic synthetic extracellular matrices (ECM) displaying a layered and gradient structure with tissue-like anisotropy. The synthetic environment will provide the resident cells with guidance cues for the establishment of appropriate tissue structures. Using cytocompatible polymer or peptide building blocks and employing an ultrafast cycloaddition reaction between s-tetrazine and strained trans-cyclooctene (TCO), we demonstrated the first example of interfacial bioorthogonal crosslinking: the use of tetrazine ligation, to create hydrogel materials through a diffusion-controlled reaction at the gel-liquid interface. The interfacial bioorthogonal crosslinking method has been used as a general tool for the creation of a biomimetic 3D environment with well-defined heterogeneity to modulate cell behaviors spatially. We have also successfully applied tetrazine ligation to the synthesis of stably crosslinked hydrogel microfibers that mimic the structure and functions of fibrous proteins found in the native ECM. Using a hydrophilic, poly(ethylene glycol) (PEG)-based bis-tetrazine monomer and a hydrophobic trifunctional TCO crosslinker, we devised an interfacial polymerization strategy for in situ fiber production. As the polymerization proceeded, mechanically robust hydrogel microfibers were continuously pulled out of the immiscible oil-water interface. These hydrogel microfibers promoted cell attachment, migration and neotissue formation under in vitro cell culture conditions. Further elaboration of the interfacial bioorthogonal processes gave rise a synthetic matrix consisting of a bottom fibrous layer, a top basement membrane-like layer and a middle gel layer with a gradient of crosslinking density and biochemical signals. Coculture of mesenchymal stem cells and stem cell-derived vocal fold epithelial cells in the vocal fold mimetic ECM is in progress. Overall, the combination of tissue-mimetic synthetic matrix and pluripotent stem cells offers an exciting opportunity for the engineering of reliable and viable vocal fold tissue models.

Computational Modeling of Vocal Fold Biomaterials Design and Evaluation

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Patients with recalcitrant chronic voice disorders often suffer from fibrotic or atrophic changes in their vocal fold mucosae, severely hampering their communication functions. Tissue engineering-based strategies have been harnessed for defected vocal fold replacement or regeneration through the delivery of cells, bioactive molecules and/or supporting scaffolds. Conventional engineering processes of biomaterials are, however, expensive and laborious that heavily relies on in vitro and in vivo models. To mitigate high costs and accelerate clinical translation, in silico computational models can be used to explore a much wider parameter space and a much longer time scale than what would be very costly or sometimes impossible with animal and human models. In fact, the Food and Drug Administration in the United States had recently planned to incorporate in silico computational modeling and simulation as part of the regulatory approval process of drugs and medical products including tissue engineered materials.

Our team has recently developed agent-based computational models based on published data of vocal fold hyaluronan-based hydrogel studies. The model numerically simulates the bioactivity of vocal fold fibroblasts as a function of various scaffold properties including elastic modulus (Pa), crosslinking density (mmol/ml), swelling ratio (w/w), mass loss (%) and pore size (µm). Powered by our high-performance computing scheme, a 30-minute long biological event in real world would take only 6.2 seconds to simulate in the model. Morris screening and Sobol variance-based sensitivity analyses were also performed. Initial pore size and swelling ratio of the hydrogel were key material parameters influencing vocal fold fibroblast proliferation and collagen production for the first nine days of simulated period. Additional laboratory and computational work is now undergone to enhance the fidelity and versatility of our computational model. Our ultimate goal is to develop a user friendly software package to guide the rational design of vocal fold scaffold biomaterials.
Collagen is the main protein content in the human vocal fold lamina propria (LP). Its type, thickness, and orientation play an important role in regulating the biological and mechanical properties of the LP. Hahn’s group observed a clear alignment of collagen from the LP histology and quantified the angular deviation of the collagen orientation to be between 24.9° to 36.8°. In this study, type-I collagen fibers were aligned inside a biocompatible chitosan-collagen (Ch-Col) hydrogel using 3D bioprinting to form a biomimetic collagen environment. The viscoelastic properties of the bioink and the degree of the fiber alignment due to different nozzle sizes were also investigated.

Chitosan powder (MW: 50-190 kDa, Sigma, MO) and type-I bovine tendon collagen (Advanced BioMatrix, CA) were dissolved in 0.2 mol/L acetic acid separately. Ch-Col hydrogel (3% chitosan, 0.45% collagen) was prepared by mixing 6% chitosan and 9 mg/ml collagen solution in a ratio of 1:1. The pH of the final hydrogel precursor was adjusted to 7.2 using 40% β-Glycerophosphate disodium (Sigma, MO). A torsional rheometer (TA Instruments, DE) was used to characterize the viscoelastic properties of the hydrogel precursor. Human vocal fold fibroblasts (hVFFs) were encapsulated inside Ch-Col hydrogel and transferred to an extrusion-based bioprinter (GeSiM, Germany). Conical syringe tips with inner diameter from 210-1600 μm were used for printing. Confocal microscopy (Zeiss, Germany) was used to examine cell growth. Nonlinear laser scanning microscope (NLSM) was employed to image unlabeled fibrillar collagen in the hydrogel by intrinsic second harmonic generation.

Storage (G') and loss (G'') moduli were 1001±144 Pa and 59.4±3.9 Pa respectively at 37°C. The hydrogel exhibited shear-thinning behavior and maintained high fidelity post-printing. From confocal images, fibroblast-shaped ECM content secreted by hVFFs were observed inside the gels starting from Day 3 of culture, which suggested that collagen fibers provided support for cells to spread. The angular deviation of collagen orientation from samples printed by 27G (210 μm) and 14G (1600 μm) syringe tips were 22.7° and 42.5° respectively. The NLSM images showed that syringe tips with smaller inner diameter yielded a higher degree of collagen alignment.

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Carbon nanotubes (CNTs) have been investigated for different applications in the human body [1]. However, they have been never incorporated in hydrogels for increasing cell migration in the scarred vocal folds. The similarities between CNTs’ porous structure and that of the fibrous proteins suggest that CNT-based composite hydrogels could be used as injectable biomaterials for damaged vocal folds. Adding CNTs to hydrogels affects their physical properties, specifically their swelling behavior and pore size. In this research, the effect of CNT concentration on the swelling rate and pore size of the hydrogel was studied.

Chitosan-glycol/glyoxal hydrogel was selected as the matrix for CNTs due to its biocompatibility. Composite hydrogels were prepared using various concentrations of CNT (US Research Nanomaterials). Hydrogel groups with CNT concentrations of 250, 500 and 750 μg/ml, along with a control sample (CNT-free) were immersed in phosphate-buffered saline (PBS) 1x for different time periods (0, 1, 4, 7, 14, 30 days). At each time point, the PBS was removed, and the samples’ weight was measured. Afterward, the samples were frozen and lyophilized for 24 hours, and the average pore size was measured via scanning electron microscopy (SEM).

The swelling ratio is defined as the percentage of increase in sample weight due to PBS absorption. Our findings suggest that using CNTs in chitosan-glycol/glyoxal hydrogel enhance the swelling ratio. On day 30, the swelling ratio for samples with CNT concentrations of 0, 250, 500 and 750 μg/ml were 24%, 27.5%, 28%, and 29%, respectively. Since the functionalized CNTs are hydrophilic, samples with a higher concentration of CNT absorb more PBS, and thus, have a higher swelling rate. SEM images confirmed that the pore size in the samples with higher CNT concentration was increased. Greater pore size may increase the ability of the injectable biomaterial to recruit cells from its surrounding native environment. CNT-based composite hydrogels could enhance cells recruitment in the biomaterial, which will be the objective of future studies.

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References
Functional Engineered Microvessels in Degradable Synthetic Elastomeric Scaffolds

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Current strategies to engineer microvasculature in full-thickness tissue constructs often rely on the use of natural gels and/or matrix additives to promote vasculogenesis in vitro and neovascularization in vivo, despite the impractical features of these materials. In the current work, perfusion co-culture on a degradable polyurethane scaffold with optimized mechanical and surface properties for wound healing (D-PHI) was used to generate micro-vascularized gingival tissue constructs. It was hypothesized that human umbilical vein endothelial cells (HUVECs) and human gingival fibroblasts (HGF) co-cultured on D-PHI under medium perfusion at a flow rate optimized for tissue production would produce constructs with a functional microvascular network, i.e. non-leaky interconnected microvessels. Cells were co-seeded onto D-PHI scaffolds, placed in a custom bioreactor, and subjected to medium flow at 0.05 mL/min for 14 days. Constructs were then either fixed for histological analysis or implanted subcutaneously into athymic mice. Surgical placement of a dorsal window at 2 weeks allowed for in vivo live imaging of the construct using confocal microscopy. HUVECs within the construct were stained using AF594-conjugated anti-human CD31 antibody administered topically. The fluorescent blood-pooling agent FITC-dextran (2000 kDa) was injected intravenously prior to imaging. At 2 weeks, some HUVEC-associated vessels were perfused with host blood (FITC-dextran and hCD31 positive); other areas in the construct showed non-perfused HUVEC vessels, while still others showed host vessels that had integrated into the construct. Prior to implantation, constructs had an average vessel lumen density of 0.51 ± 0.3/mm². After 2 weeks in vivo, lumen density had increased to 10.95 ± 8.33/mm². This was significantly greater than on non-vascularized controls (density of 0.51 ± 0.3/mm² after 2 weeks in vivo, histological analysis, n=5, p<0.05). In conclusion, microvessels generated via perfusion co-culture on D-PHI are part of a functional network that anastomose with the host vasculature system as soon as 14 days after implantation. The results validate the use of perfusion co-culture for generating pre-vascularized tissue engineered constructs on a 100% synthetic scaffold. Ref: 1. Landau S et al. Biomaterials. 122, 72-82, 1993. 2. Laschke, MW et al. Biotechnol. Adv. 34, 112-121, 2016. 3. Battistion, KG et al. Acta Biomater. 24, 35-32, 2015. Acknowledgements: NSERC Discovery, NSERC CGS D.

Effective vascularization and robust bone formation in osteogenic grafts requires VEGF dose control

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Spontaneous vascularization of large-size bone grafts based on bone marrow-derived mesenchymal stem cells (BMSC) is insufficient and requires therapeutic stimulation to ensure progenitor survival and bone formation. We found that, while Vascular Endothelial Growth Factor-A (VEGF) sustained over-expression by genetically modified human BMSC effectively improved vascularization of osteogenic grafts, it also caused excessive bone resorption. Recently, we found that short-term delivery of recombinant VEGF protein prevented osteoclast recruitment while ensuring increased vascularization. Here we investigated the role of VEGF dose on the coupling of angiogenesis and bone formation, in order to define a VEGF therapeutic window for vascularized bone grafts. Recombinant VEGF was engineered with a transglutaminase substrate sequence (TG-VEGF) to allow covalent cross-linking into fibrin hydrogels. Osteogenic constructs were prepared with human BMSC and hydroxyapatite granules in a fibrin hydrogel containing different TG-VEGF concentrations (0.1, 1, 10 and 100 μg/ml). Control grafts were generated with BMSC only or retrovirally transduced, VEGF-expressing BMSC. Histological analysis was performed at 1, 4 and 8 weeks after ectopic implantation in nude mice. All TG-VEGF doses increased vessels density up to 5-fold already after 1 week and vascularization persisted at all later time points. Interestingly, vascularization reached up to 30% of the construct area in grafts with 0.1 μg/ml of TG-VEGF, whereas constructs with higher VEGF doses were significantly less invaded by blood vessels. After 4 and 8 weeks, bone tissue development was enabled by 0.1 μg/ml of TG-VEGF as efficiently as with naïve BMSC alone. Grafts with 0.1 μg/ml of TG-VEGF also contained significantly more mature bone tissue than all other conditions. Higher VEGF doses progressively impaired bone formation, in correlation with increased osteoclast recruitment and decreased progenitor differentiation, marked by human bone sialoprotein (BSP). These data suggest that VEGF effects on promoting vascularization and bone resorption are dose-dependent and that a therapeutic window exists that enables both rapid vascularization and efficient bone formation. This could provide a clinically applicable strategy, which allows no genetic modification, homogeneous and tunable factor doses and limited and controllable duration of factor delivery.
Basic fibroblast growth factor (bFGF) is an important protein to establish efficient vascular networks during regeneration processes. Its long-term controlled release from engineered scaffolds to induce effective angiogenesis is of particular interest in tissue engineering because it has disadvantages, such as a short half-life and rapid diffusion away from the injured site, hampering its applications. Based on the fact that CBD-bFGF (CF, a fused protein where a collagen-binding domain (CBD) was fused into the native bFGF) could bind specifically to collagen scaffolds and heparin (HP) has high specific affinity to bFGF and can help bFGF to maintain its bioactivity, we developed a collagen/heparin bi-affinity multilayer delivery system (Col(Col/CF/HP/CF)ₙ). Col(Col/CF/HP/CF)ₙ was fabricated by alternate deposition of positively charged collagen (Col), CF, negatively charged HP and CF on collagen scaffold via electrostatic or specific interaction. Relative to the control group, the bi-affinity multilayer delivery system resulted in an obvious increase in the loading amount of CBD-bFGF, and the increment was regulated by the number of multilayers. The in vitro release profiles indicated that Col(Col/CF/HP/CF)₁₅ could provide a drug release period lasting more than 35 days. Dorsal subcutaneous angiogenesis model of SD rats was utilized to evaluate the efficiency of our bi-affinity multilayer delivery system where the Col(Col/CF/HP/CF)₁₅ group elicited the highest density and the largest average diameter (~70 μm) of newly formed blood vessels both at 3rd and 5th weeks. Our work suggested that the bi-affinity multilayer delivery system could act as a versatile approach for bFGF delivery and further improve its therapeutic efficacy for injured tissues.

References

Cellular therapies and a Federal transplantation program began in the University Hospital of Lausanne (CHUV) focusing on oxidative stress and aging with special interest of cells from tissue of all ages from fetal to aged skin. In the late 1990's, orientation was on cellular and molecular mechanisms of wound healing and tissue repair in fetal skin of different gestation periods. Research was oriented for cell selection techniques and procedures for potential use of cell sources in the clinic. First clinical trials using progenitor skin cells as novel cell sources and "biological bandages" for pediatric burn patients and chronic wounds in adults began in 2000 with clinical studies published in 2005-2006 (The Lancet, Cell Transplantation & Experimental Gerontology). Further work has advanced parallel to new Regulatory structure adopted in Europe and Switzerland in 2007. Following the new Regulations worldwide, a new program of Transplantation for Musculoskeletal Tissues was organized along with the formal registration and an associated Biobank Program. Extensive cell banks have been produced in the laboratory over the last 25 years consisting of different human bone, cartilage, disc, muscle, tendon and skin sources (fetal, child, young adult, adult, old adult) under GLP and GMP conditions. Biological bandages, formulated with the new Directives and registered clinical cell banks, were used in the clinic particularly for burns and for acute and chronic wounds in humans. The biological bandages were also used as a base for the Project Platform SwissTransMed to associate antimicrobial factors for the treatment of burn patients. This National Platform includes partners in Geneva (UniGe and HUG), Zurich (UniZh and Vet Swiss Zurich), Lausanne (CHUV and EPFL) and Bern (UniBe) and this Platform develops candidate formulations for anti-microbial biological bandages.
**98-SY-3**  
**Tissue perfusion assessment of paediatric burns by Laser Doppler Imaging (LDI)**

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**Background:** The indication for performing autologous skin grafts in cases of burns in children is based on the clinical examination and on the progression of spontaneous healing during the first days. Spontaneous regeneration of the skin after a burn depends on the tissue microcirculation. Laser Doppler Imaging (LDI) is a non-invasive method for assessing tissue perfusion. The objective of this study is to show the usefulness of LDI when having to decide whether or not to perform a skin graft.

**Study Design:** A prospective pilot study involving children with second-degree burns covering a minimum of 10% of the total body surface area (TBSA) was conducted over 14 months from May 2012 to July 2013. LDI images of the burned skin were made under standardized conditions during hydrotherapy over the first ten days, and also, at the same time, of healthy skin in order to obtain a reference value. A clinical assessment determined the degree of severity of the burns of each patient was carried out by the paediatric surgeon. The analysis of the results was performed with the EasyLDI Studio program.

**Results:** This pilot study included seven patients. Our results show a correlation between the numerical perfusion values obtained by LDI and the depth of the burn as determined by the surgeon. In 70% of the cases, the sensitivity of the Laser Doppler imager helps determine during the first few days after the burn whether a skin graft will be necessary.

**Conclusions:** Laser Doppler Imaging (LDI) helps to numerically objectify the residual perfusion of burned tissue.
Development of an in vitro wound infection model to bridge laboratory to the clinic

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Burn wound sepsis is currently the main cause of morbidity and mortality after severe burn injury. Bacterial pathogens such as Pseudomonas aeruginosa, Staphylococcus aureus and Acinetobacter baumannii impair patient recovery and can be fatal. The specialized medical care required after burn injury faces major challenges to prevent bacterial development on burn wounds. The Biological Biodegradable and anti-Bacterial Burn-wound Bandages (BS) platform, granted by Swiss TransMed, was created in order to improve the current biological dressing used to treat burn wounds and to better understand the bacterial pathogenesis in a wound environment.

Our overall objective was to develop an in vitro burn wound infection model in order to improve evaluations of new bandages formulations. In order to address this objective, we focused our work on the following specific aims: i) Characterize the pathogenicity of the major burn wound pathogen P. aeruginosa when growing in human burn wound exudates (BWE), ii) Determine the chemical composition of BWE, and iii) Optimize the formulation of an artificial burn wound exudate medium (ABWEM).

Human BWE, collected within 7 days after burn trauma from 11 burn patients, were used to investigate pathogenesis of bacterial burn wound pathogens and analyze the BWE composition. Growth assay in BWE showed that P. aeruginosa species were the only pathogens able to proliferate compare to A. baumannii or S. aureus. Synthesis of typical virulence factors was strongly enhanced in P. aeruginosa compared to standard laboratory conditions. These results were confirmed by whole genome expression analysis of P. aeruginosa using RNA-Seq approach, which provided a remarkable insight into the expression profiles of bacterial pathways. A detailed chemical composition analysis of BWE was performed before and after P. aeruginosa growth. This enabled us to determine the major metabolic pathways used by this bacterium for its proliferation in BWE. These data are essential for the development of an ABWEM mimicking the burn wound environment and the establishment of an in vitro system to analyze the initial steps of burn wound infections.

Angiogenic effect of anti-microbial dendrimers in combination with Biological Burn-wound bandages

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Skin is the primary body barrier against pathogens. Burn shock destroys this barrier against pathogens and implicates severe pathophysiology conditions. Improvement in fluid resuscitation and respiratory support has allowed to considerably reduce the rate of early death after a burn shock. Nevertheless, nosocomial infections still persist, replacing thus the traumatic injury as the major cause of morbidity and mortality. Indeed, despite topical antimicrobials, early excision and immediate wound closure, the frequency of remaining burn wound infections due to multidrug resistant Pseudomonas aeruginosa has increased, hindering thus further regression in mortality in burn patients. It has been reported that polycationic dendrimers G3KL and G3RL are able to kill multi-drug resistant Pseudomonas aeruginosa. We hypothesized that the combination of these dendrimers with a class of biological bandages made of progenitor skin cells, could positively impact the wound healing process due to the synergy of the cells that secrete growth factors coupled with the antimicrobial effect of the polycationic dendrimers. However, polycations are also known to be used as anti-angiogenic agents in presence of tumors. As neovascularization is pivotal in the healing against all expectations we have shown in this study that polycations G3KL and G3RL dendrimers can actually enhance angiogenesis, as shown by quantitative methods in Tube Formation Assay with UVEC cells cultured on Matrigel, as well as in vivo Chorioallantoic Membrane Assay. It that has been verified that dendrimer concentrations ranging between 50 and 100 μg/mL can suppress bacterial growth and safely be used with the biological bandages without altering cell viability. Hence, the results suggest that the combination of antimicrobial dendrimers with biological bandages could potentially improve healing processes with an enhanced angiogenesis.