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Oral Abstracts

Session: Additive Manufacturing of Polymer Melts for Scaffold-Based Tissue Engineering

Date and Time: Wednesday, September 9, 2015, 10:30 AM - 12:00 PM

Patterned and Photo-cross-linked Fibrous Scaffolds Via Melt Electrospinning Writing

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Melt electrospinning writing (MEW) enables the design and fabrication of micrometer-thin fibrous scaffolds with highly controllable architectures and patterns.1 So far, MEW has been mainly applied using a low melting point thermoplastic, poly(ε-caprolactone). However, scaffolds prepared from such thermoplastics exhibit a large reduction in modulus upon absorption of water in application. It was reasoned that cross-linking would result in a hydrated scaffold that retains a high modulus. The objective of this study was to manufacture and characterize a scaffold using a photo-cross-linkable polymer (poly(L-lactide-co-ε-caprolactone-co-acryloyl carbonate), P(LLA-CL-AC)) using the MEW process and assess its modulus following hydration. The P(LLA-CL-LA) was successfully melt electrospun in a direct writing mode without inducing crosslinking, as confirmed from sol measurements. The average fiber diameter of the scaffold was 30 μm and the fibrous layers of the scaffold were oriented at 90°. The MEW scaffolds were efficiently cross-linked with UV light. The uncross-linked scaffolds possessed lower modulus (66.3 MPa) compared to cross-linked groups (92 MPa). Interestingly, the uncross-linked scaffolds exhibited a marked reduction in modulus following hydration, to 6.3 ± 0.5 MPa, while the modulus of the hydrated cross-linked scaffolds was significantly higher, at 23.2 ± 3 MPa. This work demonstrates for the first time the melt electrospinning writing of a photo-cross-linkable polymer. This scaffold preparation approach is of potential interest for ligament or tendon tissue engineering applications. Future studies will examine the fatigue properties of the scaffolds, the change in their mechanical properties following *in vitro* degradation, and cellular response within a dynamically loaded bioreactor.

Melt Electrospinning Writing of Suspended Fiber Arrays

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Introduction: Additive manufacturing is extending design capabilities, allowing complex shape fabrication. Challenges currently facing fused deposition modelling include: 1) achieving small filament diameters and 2) filaments spanning voids, without underlying support structure. Melt electrospinning-writing (MEW) solves both challenges: generating well defined arrays of 3 μm diameter fibers that were suspended across voids, at least 6 mm wide. This allows the fabrication of defined, suspended fiber arrays as scaffolds.

Methods: MEW was performed with poly(caprolactone) (PCL) on a custom-built direct writing machine. Nozzle to collector distance was 6 mm, with a high voltage of 5 kV and − 1.5 kV applied to the nozzle and collector respectively.

Results/Discussion: Fiber diameters were consistent and much smaller than for melt extrusion; ranging from 3–15 μm (variations of less than 10% for each diameter investigated). Initially, a frame of PCL fibers were generated with repeated deposition (5–25 times) at a spacing between 150 μm-6 mm. Suspended fibers were then deposited across the void between the PCL supports with interfiber distance of 100 μm. Repetition of this format creates the 3D spacing required to build defined, orientated scaffolds that could be handled *in vitro* while mechanically protecting the suspended fiber array.

Conclusions: Defined, fiber arrays were suspended across millimeter gaps using MEW. The highly controlled alignment and spacing in all axes will assist in the development of 3D constructs for investigating numerous aspects of cell-substrate interactions, as well as in regenerative medicine as tissue engineered scaffolds for inducing repair and regeneration after traumatic nervous system injuries.

Gradient Scaffolds for Tissue Engineering and Regenerative Medicine

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As our tissues have been developed following biophysical, mechanical, and biological gradients, the aim of this study was to engineer 3D scaffolds displaying these kind of gradients to actively influence stem cell activity for skeletal regeneration. We implemented different gradients in the design of scaffolds fabricated by additive manufacturing: (i) structural, through the variation of fiber pattern; (ii) physico-chemical, through the deposition of different biomaterials; and (iii) biological through polymer-brushes mediated binding of growth factors.

The fabricated scaffolds showed to support early differentiation of adult bone-marrow derived stem cells into the osteogenic and chondrogenic lineages. Osteogenesis was favored by an increase in pore size and pore rhomboidal shape at a structural level, by a hydrophilic and softer polymer composition at a physico-chemical level, and by the binding of BMP-2 at a biological level. Conversely chondrogenesis was favored by a decrease in pore size and pore rhomboidal shape at a structural level, by a more hydrophilic and stiffer polymer composition at a physico-chemical level, and by the binding of TGF-β3 at a biological level.

Ultimately, we present here the creation of a new library of active scaffolds able to steer stem cell activity by engineering cell-material interactions in three-dimensions.

Microfabrication and Perfusion Culture of New Tissue Elements Possessing Hollow Structures with High Mechanical Strength towards Scaling-up to Implantable Liver

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Addressing the issue of insufficient mass transfer in scaling-up to implantable liver tissue equivalent, we designed a new three-dimensional (3D) microscaffold with integrated channel-like structures supporting greater medium distribution. Fabricated from biodegradable poly-ε-caprolactone (PCL), the module of cylindrical microstructure is produced via selective laser sintering (SLS) aiming at sufficient mechanical strength and higher design accuracy with 60% porosity. The modules (1100 μm in diameter; 1500 μm in

stem cells injected into the penis could improve and restore erectile function in a rat model of neurovascular erectile dysfunction (NVED). Three different cell types were used in this study; human endothelial cells (ECs), adipose derived stem cells (ASCs) and amniotic fluid derived stem cells (AFSCs). Each cell type was characterized using flow cytometry assay using cell specific markers; ECs expressed CD31 and CD34, while human AFSCs and ADSCs expressed CD73, CD90, CD105, CD146 but did not express CD31 and C34. A nude rat model of NVED was established by crush injury of the bilateral cavernous nerves and ligation of the bilateral internal pudendal bundle. Animals were divided into five groups (G): G1 was treated with ECs, G2 with ASCs, G3 with AFSCs, G4 with normal saline and G5 was an age match group. Approximately 2.5 million cells in 0.2 ml serum free medium per animal were injected intracavernously into penile tissue. At 12 weeks post-injection erectile function analysis showed that the ratio of intracavernous pressure and mean artery pressure was increased in the cell therapy groups. Among the cell treated groups, the endothelial cell injection group showed the most improvement in intracavernous pressure. These results show that cell-based therapy may be an effective treatment modality for patients with trauma induced ED.

Cell-based Therapies for Urogenital Tract Disorders

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The genitourinary system is constantly exposed to different adverse conditions, which can lead to structural and functional damage. Over the past decade, the field of tissue engineering and regenerative medicine has made a significant progress in developing cell-based therapies for genitourinary tissue applications. One approach to augment tissue function is through cell therapy. A wide variety of cell sources, ranging from somatic to stem cells, have been investigated. Another method commonly employed is tissue engineering to restore damaged tissues. This approach uses cells and biomaterials to build tissue constructs that results in functional tissues when introduced *in vivo*.

The genitourinary tract system is one of the more studied areas in the field, as many technologies have already been translated into patients. It is becoming increasingly clear that the field has the potential to treat various urological disorders that are known to be a challenge with conventional treatment methods. Clinical experiences with regenerative therapies for genitourinary disorders, such as tissue engineered bladder, urethra and vagina, have demonstrated that successfully developed technologies can profoundly affect and improve the lives of patients who do not have many options. The progress of tissue regeneration in the genitourinary system will be discussed.

Tissue Engineering a Cell-based Therapy for Pelvic Organ Prolapse (POP) using Autologous Human Endometrial MSC and Novel Scaffolds

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POP is the herniation of the pelvic organs into the vagina due to childbirth injury, causing incontinence and sexual dysfunction. 19% of women are treated for POP by surgery with or without polypropylene mesh. Surgical failure and complication rates approach 30%. We propose to use autologous MSC, purified from the endometrium (eMSC) delivered in a composite mesh scaffold as a new approach for treating POP. EMSC are isolated from biopsies obtained without anaesthesia from pre-menopausal and estrogen-treated post-menopausal women by magnetic bead (W5C5/SUSD2+) cell sorting. We are developing protocols for eMSC culture expansion under cGMP conditions.

We fabricated a polyamide and gelatin (PA+G) composite scaffold for delivering human DiO-labelled eMSC into a fascial defect in a nude rat model. We showed that eMSC promoted early neovascularisation and a rapid influx of M1 macrophages which changed to a M2 wound healing phenotype. Longer term, fewer macrophages and minimal fibrosis surrounded the PA filaments compared to non-cell-seeded scaffolds. The eMSC/PA+G tissue complexes were less stiff and more distensible compared with PA+G alone, indicating improved biocompatibility.

We are developing an autologous, large animal, pre-clinical model of vaginal surgery using sheep. We have developed an objective clinical measurement that shows a subset of parous ewes have similar vaginal tissue laxity as women, suggesting subclinical POP. Pilot data showed vaginally implanted lentiviral-mCherry-labelled-autologous eMSC on PA+G constructs survived 21 days. Our tissue engineering approach using autologous eMSC delivered on polyamide/gelatin scaffolds may be an alternative approach for treating POP with potential to improve surgical outcomes.

Off-the Shelf Collagen-fibrin based Scaffolds for Bladder Augmentation

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In the management of congenital and acquired bladder pathologies augmentation of the bladder is commonly performed using vascularized digestive tract patches. This can be linked to severe complications, such as metabolic disturbances and even cancer. Therefore tissue engineering and laminated collagen-fibrin hybrid grafts is a promising alternative. We compared the mechanical, functional and cellular behavior of different collagen-fibrin based scaffolds used for augmentation in 5 groups of 4 nude rats each. In the first acellular scaffolds were applied. In the second human smooth muscle cells (hSMC) were added within the scaffold. In the third group hSMC's have been cultured within the scaffold for 4 weeks, followed by decellularization and DNA removal. In the fourth a low dose of IGF-1 was covalently bound to fibrin and in the fifth group a high dose of IGF-1 was used. After a transverse hemi-cystectomy the bladder was augmented with the respective collagen-fibrin based patches. Four weeks post hemi-cystectomy all animals showed the same bladder volumes than before surgery, voiding capacity was normal and host cells had populated all the grafts. The cellular, the decellularized and the high dose IGF-1 scaffolds showed faster regeneration as compared to the acellular and low dose IGF-1 scaffolds. Apart from the cellular scaffold all the remaining can be manufactured as an off-the-shelf products and may become beneficial in clinical use.

Session: Fibrin and Fibroin – What "O" Difference

Date and Time: Wednesday, September 9, 2015, 4:00 PM - 5:30 PM

Silk: From Textiles to Tissue Regeneration

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Silks, and in particular silks from silkworms cocoons, have been used for many years as suture filaments, more recently acquiring novel attention for other applications in medicine and namely in tissue engineering. The "silks success" should be attributed to the material versatile properties that can be tuned according to the requirements via tailored processing and post-treatment methods that, in turn, affect molecular structure, supramolecular conformation and final properties.

By adjusting biopolymer architecture and chemical composition, the formation of the extra cellular matrix (ECM) can be triggered such as to guide cells to the generation of functional tissues.

Crystallinity and morphologies at different scale level can induce different blood responses in terms of platelet adhesion and activation, modulate the interaction with the inflammatory system, and control the stem cell fate.

Materials source, advanced processing strategies and selective chemical modifications can control chemistry and thus silk's function.

Silk fibroin can be used as polymer model to debate the relationship between material structure, physical and biological properties.

The lecture will explore the history, uses and potential future of silk fibroin as elective materials for tissue engineering applications, starting from basic ''primitive'' bidimensional substrates till the design and fabrication of microniches populated 3D structures able to guide the cell behavior and the regenerative process.

Generation of Highly Aligned Skeletal Muscle-Like Tissue through Strain-Induced Patterning of Fibrin

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The natural hydrogel fibrin is considered a suitable scaffold material for skeletal muscle engineering since its mechanical properties can be adjusted to match those of native skeletal muscle tissue. In addition, fibrin responds to uniaxial mechanical stimulation with fibril alignment along the axis of strain, allowing for guided cellular patterning. To exploit this feature in a biomimetic skeletal muscle engineering approach, we have developed a novel bioreactor system (MagneTissue) for the rapid generation of highly aligned skeletal muscle-like tissue constructs. With this system, murine myoblasts embedded in ring-shaped fibrin scaffolds are subjected to static mechanical strain via magnetic force transmission, leading to cellular alignment concomitant with the patterning of the scaffold material into highly organized fibrin fibrils. Within 9 days, a parallel array of myotubes with a more mature phenotype in terms of sarcomeric patterning, width and length is obtained using a daily stimulation protocol of 10% static strain for 6 hours and 3% for 18 hours. Moreover, static mechanical stimulation leads to enhanced myogenic determination/differentiation on the gene expression level, demonstrated by the upregulation of MyoD and Myogenin as well as the contractile structural marker TnnT1. The MagneTissue bioreactor system provides a versatile platform for engineering tissues whose functionality requires parallel cellular patterning, such as skeletal muscle - with the advantage that strain protocols can be individually adjusted. In future work, this will allow implementing mechanical stimulation with different strain regimes in the maturation process of tissue engineered skeletal muscle constructs and elucidating the role of mechanotransduction in myogenesis.

Fibrin or Fibroin: Effect of Hydrogel Formulation on Behavior of Neonatal Cardiomyocytes Cultured within a Bioreactor

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Though extensive studies have investigated the development of engineered myocardium, current methods result in constructs with contractile stresses that are at least one order of magnitude smaller than that of cardiac tissue, necessitating continued improvements in biomaterial design and development. We present a comparison between neonatal cardiomyocyte behavior in fibrin gels or silk fibroin-cardiac extracellular matrix (ECM) hydrogels. The maturation and function of cultured cardiomyocytes was investigated using a dual electromechanical bioreactor that allows for tight control over the timing of the two modes of stimulation. Fibrin constructs cultured under delayed electromechanical stimulation (isovolumic contraction) had the highest twitch forces and an increase in contractility (Troponin I) and calcium handling (SERCA2a) proteins, resulting from upregulation of physiological hypertrophic pathways (Akt).[1] When electrical and mechanical stimulation were completely offset (non-physiological conditions), a reduction in twitch force generation was measured, indicating that the timing of dual electromechanical stimulation is important in generating optimal construct function.[2] On-going work aims to compare these fibrin hydrogel results to cardiomyocyte response in silk fibroin-ECM elastomeric hydrogels formed via horseradish peroxidase-H_2O_2 crosslinking.[3] These highly tunable silk hydrogels present a new class of silk-based materials which support the growth and maturation of cells from neonatal isolations as well as compaction and remodeling by isolated cardiac fibroblasts. Ultimately, we aim to develop a new injectable platform for cardiovascular applications.

1. Morgan KY *et al., Tissue engineering. Part A* **2014**, 20(11–12).
2. Morgan KY *et al., Organogenesis* **2014**, 10(3).
3. Partlow BP *et al., Advanced Functional Materials* **2014**, 24(29).

Silk Fibroin for Peripheral Nerve Regeneration: A Novel Preparation Method Improved Mechanical Characteristics and Supports Regeneration in Rat Sciatic Nerves

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Over the last decade, silk fibroin has been emergently used in tissue engineering. In this study, we describe a novel procedure to produce silk fibroin nerve graft conduits (SF-NGCs): a braided tubular structure of raw Bombyx mori silk was subsequently processed with the ternary solvent CaCl2/H2O/ethanol, formic acid and methanol to improve its mechanical and topographical characteristics.

The combination of the treatments resulted in a fusion of the outer single silk fibers to a closed layer. In contrast to the outer wall, the inner lumen still represented the braided structure of single fibers. Mechanical stability, elasticity and kink characteristics were evaluated and the modifications drastically improved these properties of the SF-NGC. An advanced cell migration assay with NIH/3T3-fibroblasts revealed the impermeability of the SF-NGC for possible invading and scar-forming cells. However, cytocompatibility assays with primary Schwann cells showed its suitability to serve as a substratum for Büngner-band forming Schwann cells.

In vivo, the SF-NGC was implanted to bridge a rat sciatic nerve defect and evaluated histologically (immunofluorescence, axon count) and functionally (electrophysiology, Catwalk analysis). No signs of elicited inflammatory reactions could be detected in the *in vivo* setting and we could demonstrate morphological and functional re-innervation of the distal targets after 12 weeks.

The novel SF-NGC presented here shows promising results for the treatment of peripheral nerve injuries. The novel modification of braided structures to adapt its mechanical and topographical characteristics may support the translation of SF-based scaffolds into the clinical setting, not restricted to the nerve field.

Optimizing Peptide Incorporation Efficiency and Characterizing Changes in the Physical Properties of Fibrin Gels

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Objectives: Recently, the Wu lab has developed a new strategy for light actuated drug delivery-on-demand that uses the photothermal response of biocompatible chromophores to trigger release from thermally-responsive delivery vehicles. However, there is a