Visual mapping of computational shear stresses implies mechanical control of cell proliferation and differentiation in bone tissue engineering cultures

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glycol) hydrogels for *in vivo* optical-sensing and diabetic therapy. The real-time optical readout of encapsulated heat-shock-protein-coupled fluorescent reporter cells made it possible to measure the nanotoxicity of cadmium-based quantum dots *in vivo*. Using optogenetic cells producing glucagon-like peptide-1, we also performed light-controlled therapy and demonstrated improved glucose homeostasis in diabetic mice. In addition, we developed *in situ* supramolecularly assembled and modularly modified hyaluronate hydrogels for genetically engineered mesenchymal stem cell (eMSC) cancer therapy. The long-term expression of mutant interleukin-12 (IL-12M) by eMSCs within the supramolecular hydrogels resulted in effective inhibition of tumor growth with a significantly enhanced survival rate. Finally, we developed silicone hydrogels for a smart contact lens composed of biosensors, drug delivery systems, and power sources for the treatment of diabetes as a model disease. All these systems will be discussed in detail for further clinical applications.

Controlling the Astrocytic Response to Brain Injury using Self-assembling Peptide Hydrogels

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After traumatic brain injury, the acute phase of astrocytosis is an essential physiological response. It is responsible for demarcating the lesion site, preventing secondary degeneration and arresting growth through the production of pro-inflammatory molecules [1]. This response is detrimental if persistent, as chemical and physical barriers provided by the 'reactive' astrocytes prevent functional recovery. Therefore, attenuation of reactive astrocytosis post-insult is an important biological challenge to promote central nervous system repair and reconstruction.

We have implanted an Fmoc-based self-assembling peptide (SAP), Fmoc-DIKVAV, presenting the laminin sequence IKVAV, into the caudate putamen. This hydrogel self-assembles under physiological conditions, forming an injectable hydrogel with a nanofibrous network [2]. The sequence DIKVAV is presented on the outer surface of the nanofibres, resulting in the high-density presentation of a biologically relevant sequence, whilst also providing morphology reminiscent of the native extra cellular matrix. We functionalised this hydrogel with an anti-inflammatory molecule and show that concomitantly providing physical and biochemical cues into a lesion post-insult significantly reduced astrocyte numbers in the parenchyma at 7 and 21 days. We will further develop this technology to control the persistence of 'reactive' astrocytes after injury, and hence, encourage functional regeneration across the lesion site.

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2. Rodriguez, A.L., *et al.,* Tuning the amino acid sequence of minimalist peptides to present biological signals via charge neutralised self assembly. Soft Matter, 2013. 9(15): p. 3915–3919.

Session: Quantitative 3D Micro- and Nano-CT Imaging in Tissue Engineering: Emerging Technologies and Recent Advancements

Date and Time: Friday, September 11, 2015, 9:15 AM - 10:45 AM

NanoCT and Contrast-Enhanced NanoCT as First Line Screening and Quality Control Tools for Robust Production of Tissue Engineered Products: an Overview

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Advanced 3D imaging is one of the enabling technologies that is of increasing importance in the field of tissue engineering (TE) to assess and guarantee high product quality, and to provide better knowledge on the mechanisms behind tissue formation and regeneration. Indeed, TE constructs (such as scaffolds with cells and/or growth factors) are 3D structures with complex spatial heterogeneity, for which traditional 2D imaging techniques are insufficient for comprehensive characterization or to assess their quality. In this overview, we show the potential value of nanofocus computed tomography (nanoCT) and contrast-enhanced nanoCT (CE-nanoCT) as first line screening and quality control tools throughout the entire production process of TE constructs.

Concerning scaffold selection, using nanoCT combined with empirical modelling, we highlighted the important influence of the scaffold material and structure on its bone forming capacity, allowing to select and reverse engineer scaffolds with optimized properties. For bioreactor-driven TE construct development, we showed that CE-nanoCT can be used as a 'whole-construct' imaging technique allowing to quantify *in vitro* formed neo-tissue (cells and extracellular matrix) in large 3D TE constructs. With respect to *in vivo* tissue formation, we have shown that CE-nanoCT allows 3D multi-tissue imaging (using one imaging modality for visualizing multiple tissues) as 'virtual histology' of skeletal tissues (bone, cartilage and bone marrow including fat tissue and blood vessels). For all these steps in the production process, nanoCT and CE-nanoCT also enabled to get a better insight in the mechanisms driving tissue formation both *in vitro* and *in vivo*.

Current Research Trends in Micro-CT Evaluation of Biomaterials for Tissue Engineering

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Currently one of the most incisive needs in the industry and in research is the development of non-destructive methods to analyze biomaterial microstructures. Furthermore, the only 2D analysis, provided for example by histology, is totally inadequate in the case of samples which are too fragile to be cut (for example powders) or in the case of connectivity or tortuosity quantification of different material phases. The intrinsic ability to provide a huge amount of data allows the evaluation of biomaterials in two different ways: by studying the 3D structure or by studying the regenerated tissue morphology and thus their efficacy in the event of implantation. Tissues damaged by injury or disease could be replaced using constructs based on biocompatible materials, cells and growth factors. Scaffold design, porosity and early colonization are key components for implant success. Thus, the need to display the architecture of materials and the 3D cellular distribution is a key aspect. In addition, 3D models could be used as a basis for the creation of prototypes (e.g. STL or PLY files, useful in the additive manufacturing technique) or for the creation of 3D meshes useful in the FE analysis. Given the non-destructive nature of the technique, another important aspect is the opportunity to follow the evolution of a microstructure under controlled environmental conditions (e.g. load, temperature and/or corrosive environment). This aspect is sometimes called 4D imaging (3D + time) extending the analysis to dynamic parameters or validating numerical predictions of structural and microstructural evolution (mathematical modeling).

Visual Mapping of Computational Shear Stresses Implies Mechanical Control of Cell Proliferation and Differentiation in Bone Tissue Engineering Cultures

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The advantages of longitudinal monitoring techniques are getting more attention in various tissue engineering approaches. They

provide consecutive information about one and the same sample over time and as such may decrease sample numbers tremendously. These techniques also allow taking the actual environmental status of a tissue into account for predicting future development. Micro-computed tomography has been previously shown to be suitable to monitor mineralized extracellular matrix deposition in bone tissue engineering cultures. In this study, shear stresses (SS) acting on human mesenchymal stromal cells (hMSC) seeded on silk fibroin scaffolds in a flow perfusion bioreactor were calculated by computational fluid dynamics. Two different flow rates were investigated, mimicking expected loads on cells during early bone repair (0.001 m/s) and during bone remodeling (0.061 m/s), respectively. The three-dimensional (3D) distribution of these stresses was then visually mapped to the distribution of the mineralized extracellular matrix deposited by the cells. SS values from 0.55–24 mPa were shown to promote osteogenic differentiation of hMSCs, whereas SS between 0.06 and 0.39 mPa were found to induce cell proliferation. Histological and biochemical analyses have confirmed these findings. In the future, these results may allow predicting the behavior of hMSC in 3D tissue culture. The non-destructive nature of this technique may even allow tight control and adaptation of the mechanical load during culture by taking the present status of the tissue into account.

Quantification of Mechanical Stimuli and Bone Formation in Fracture Healing Using *In Vivo* Time-Lapsed Imaging

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During bone regeneration, mechanical loading is believed to be responsible for provoking bone formation, however previous investigations into tissue level loading have been limited to cross-sectional studies and relied upon idealized models for mechanics. By applying *in vivo* time-lapse micro-computed tomography (microCT) in concert with imaged based micro-finite element (microFE) analysis we have overcome these limitations and have identified an association between tissue loading and bone formation during fracture healing.

A femoral defect of 1.24[SD=0.13] mm was created in five female mice (C57BL/6); the femur was first stabilized with an external fixator (MouseExFix, RISystem, Switzerland). Weekly scans were performed using microCT imaging (vivaCT 40, Scanco Medical, Switzerland) over a period of 6 weeks, resulting in a series of time-lapsed images. We determined sites of mineralization by registering and overlaying images from the second and third week. Combining this with microFE (Parosol) simulations based upon images of the second week, we separated strains in volumes where mineralization occurred, from volumes where no change occurred.

To assess the efficacy of strain as a predictor of mineralization, receiver operating characteristic analysis was used. The optimum strain level correctly predicted 60[SD=9] % of the mineralization which occurred, and the final state for 86[SD=4] % of the entire volume.

We have for the first time, quantitatively demonstrated that an association exists between local tissue strain and bone formation during fracture healing. This could be used to determine the optimal stiffness for biomaterials intended to promote bone healing.

A Staining and Tensioning Method for Micro X-ray CT Scanning of Sutured Tendons

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Suturing has been recommended to re-join tendons and permit tissue regeneration for over a century. However, 25% of flexor tendon patients achieve unsatisfactory postoperative mobility. Traditional *in vivo*, *ex vivo* and clinical studies have failed to determine an ideal suture arrangement for tendon repair. We aim to employ in silico analysis in approaching a conclusion. Tissue acellularity and impaired remodelling may be preceded by stress concentrations and stress shielding respectively. We therefore employed Micro X-ray CT and finite element analysis (FEA) to observe the stress patterns in tendon following suture withdrawal. To ensure characteristic deformation during suture withdrawal, the contrast agents must assert minimal change to tendon and suture mechanical behaviour. Quill barbed suture and porcine flexor digitorum profundus tendon were stained using potassium iodide solutions (0.2%KI, 0.1%I) for 24 hours. This stained their surfaces only, preserving the mechanical interaction between suture and tissue. 10 mm of suture was passed through the tendon centre. Using a tension rig, Micro X-ray CT imaging was permitted whilst samples were submerged in phosphate-buffered saline, and following a 2 mm suture withdrawal a subsequent scan was performed. Using the unloaded reconstructed volume data, a 2 mm suture withdrawal was simulated by FEA. Tendon Constitutive behaviour was described using the anisotropic, hyperelastic Holzapfel model in Abaqus. There was no significant difference in failure load between unstained and surface-stained tendons during suture pull-out, and the surface staining enabled reliable data reconstruction. FEA results and reconstructed volumes showed good agreement, thus validating the method.

Optimization of Contrast Enhanced Ct for Neo-tissue Quantification using a Design of Experiment Approach

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To progress the fields of tissue engineering and regenerative medicine, development of quantitative methods for non-invasive 3D characterization of engineered constructs (i.e. scaffolds with cells and/or growth factors) becomes essential. In order to enable the use of X-ray based imaging techniques for quantitative analysis of soft tissue fractions in TE constructs the use of contrast-enhanced nanofocus computed tomography (CE-nanoCT) was optimized.

A fractional factorial 'design of experiments' approach was used to elucidate the influence of the staining time and concentration of two contrast agents (Hexabrix® and phosphotungstic acid - PTA) and the neo-tissue volume on the image contrast and dataset quality. Additionally, the neo-tissue shrinkage that was induced by PTA staining was quantified to determine the operating window within which this contrast agent can be accurately applied.

For Hexabrix® the staining concentration was the main parameter influencing image contrast and dataset quality. A concentration of 60% and a staining time of 30 minutes were sufficient to allow accurate and fully automated quantification of the neo-tissue formed.

Using PTA the staining concentration had a significant influence on the image contrast while both staining concentration and neo-tissue volume had an influence on the dataset quality. The use of high concentrations of PTA did however introduce significant shrinkage of the neo-tissue indicating that, despite sub-optimal image contrast, low concentrations of this staining agent should be used to enable quantitative analysis. As the staining time did not have any significant influence, this can be kept as low as possible.

Metabolic Tracking of Muscle Precursor Cells for Skeletal Muscle Tissue Engineering using PET/CT

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