Quantification of mechanical stimuli and bone formation in fracture healing using In vivo time-lapsed imaging

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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 engineered scaffolds. 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 0.35–24 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.

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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, R1System, 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 = 9] % 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 tendons 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.

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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 nano-focus 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 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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