Fluid Shear Calculation and non-Destructive Histology in Cell/Scaffold Constructs Cultured under Flow Perfusion

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Statement of Purpose: Flow-induced shear stresses have been found to be stimulatory in pre-osteoblastic cells seeded in 3D porous scaffolds and cultured under flow perfusion. However, due to the complex internal structure of porous scaffolds, analytical estimation of the local shear forces is impractical. The primary goal of this work is to investigate the shear stress distributions within Poly(l-lactic acid) scaffolds of different architectures (salt leached and non-wovens) via micro-computed tomography and numerical simulations**.** The surface stress distributions within porous scaffolds were characterized in empty scaffolds and cell/scaffold constructs that have been cultured for 16 days in a flow perfusion bioreactor using the Lattice-Boltzmann method. High resolution micro-computed tomography is used to obtain their 3D structure of the scaffolds. Especially for scaffolds containing cells and extracellular matrix a new method has been developed that allows the spatial identification of cells, hard ECM, and soft ECM on non-woven scaffolds overcoming the challenge of similar X-ray attenuation between the scaffold, soft tissue, and cells.

Methods: Porous Poly(L-lactic acid) (PLLA) foam scaffolds were prepared using solvent casting/particulate leaching method. Nonwoven fiber mesh scaffolds were constructed using PLLA micro-fibers produced by spunbonding. In spunbonding, a hot polymer melt is extruded from a heated die and then fed through a high speed air venturi to attenuate the polymer strand to a fine diameter fiber[1]. Using young Wistar rats adult MSCs were extracted with well-established methods. Before seeding, scaffolds were pre-wetted with ethanol to reduce surface tension and allow the scaffolds to ubiquitously be penetrated by cell suspensions and media during seeding and culturing. The scaffolds were seeded with 500,000 cells per scaffold using oscillatory flow perfusion seeding and cultured for 4, 8, and 16 days under continuous flow perfusion using a flowrate of 0.5 and 1 ml/min. Cellularity was established using the picogreen® assay. Alkaline phosphatase activity and mineralization was also measured using established protocols. The PLLA scaffolds were analyzed via high resolution μCT by an Xradia XCT 400 at a spatial resolution of 1 μm. Intensity image slices were obtained at optimum settings of 200 μA intensity and 40 kV energy. Acquired 2D X-ray images were filtered for noise reduction and assembled into 3D reconstructions of the scaffolds using custom Matlab® code. The Lattice-Boltzmann numerical method was used to simulate the fluid flow and characterize the local flow fields at 1 μm. A custom-written, in-house code was developed for this work. Periodic boundary conditions were applied in all three directions, in order to approximate an infinite domain.[2] The no-slip boundary condition was applied at the wall faces using the "bounce-back" technique.

Results: The calculation of the shear stress implemented in the LBM code is as follows: $\sigma = \mu\,(1/2)(\nabla U + \nabla U^T)$ where σ is the shear stress tensor, and U is the local velocity vector. Figure 1 is a representative surface stress map overlaid onto a salt-leached polymer scaffold imaged via μCT. The color map represents the local surface stresses experienced in the scaffold at typical culturing conditions. The more porous areas within the scaffold experience higher flow rates of the cell culture media and therefore those parts of the scaffold correspond to the higher surface stresses that would be experienced by cells. Scaffolds with similar surface area to volume ratios were found using numerical simulations to have very similar surface shear stress distributions when exposed to the same macroscopic fluid flow perfusion rates (Figure 2). Interestingly, when mesenchymal stem cells were seeded on these scaffolds and cultured under the same perfusion fluid flow rate in bioreactors using osteogenic media followed similar osteoblastic differentiation time profiles.

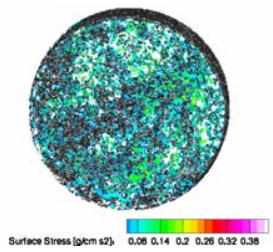

Figure 1. *μCT scaffold image, overlaid with local fluid shear stress values*

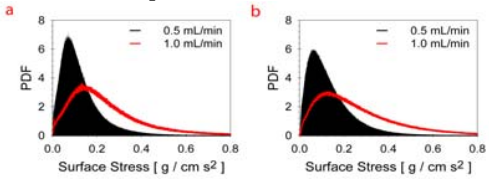

Figure 2*. Surface stress distributions in (a) porous foams and (b) fiber mesh scaffolds obtained from LBM calculations.*

By utilizing this information, along with the differences between the cells and tissue[3], it is possible to create images such as shown in Figure 3, where all four materials (scaffold, cells, soft and hard tissues) are segmented based on contrast-free information from μCT. This not only allows performing truly 3D "virtual histology", but also, due to its nondestructive nature, the technique allows to preserve the sample for future use.

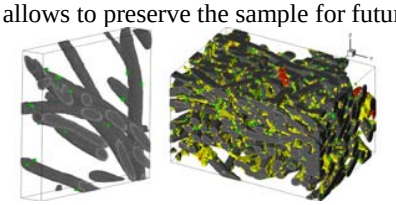

Figure 3. 3D reconstructions from μCT. LEFT: Scaffold seeded with cells RIGHT: Scaffold on culture day 16. Gray: Scaffold, Yellow: Soft Tissue, Green: Cells, Red: Mineralization.

Conclusions: Our results make possible to monitor cell growth and production of ECM over time, while at the same time obtaining shear rate data using μCT images.

References:

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2. Voronov, R, *J Biomechanics* 2010; 43, 1279-1286
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