Identifying Localized Shear Forces and Nutrient Gradients in 3D Flow Perfusion MSC Cultures

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Statement of Purpose: Every year, over a million people in the US require some kind of bone graft in order to help in the healing of bone loss either from disease or injury. These grafts usually come in the way of bone taken from other parts of a patient's body or decellularized bone taken from cadavers. These methods are problematic with limited supplies, threat of infection, and donor site morbidity. Bone tissue engineering seeks to create new bone grafts in order to help fulfill this need. The ultimate goal of our project is the creation of preosteoblastic cell/biodegradable scaffold constructs for bone regeneration that exhibit enhanced osteoinductive properties providing a superior alternative to the current therapies in patients with large defects.

Methods: Flow perfusion bioreactors have been proposed as ideal vehicles for the culture of cell seeded scaffold constructs for bone tissue engineering, due to their ability to provide improved nutrient transport and expose the cultured cells to shear stress that has been shown to stimulate osteoblastic differentiation. In order to better understand the mechanosensation and nutrient transport, we are investigating the distribution of fluid shear forces in polymer scaffolds for bone tissue engineering using micro-computed tomography, analyzing the effects of scaffold architecture and surface chemistry on the shear forces and cellular/tissue developments and also identifying oxygen and nutrient concentration gradients at different stages of the culture. For this study, we are using three types of 85% porous poly(L-lactic acid) (PLLA) polymeric scaffolds: spunbonded, solvent-cast/particulate leached, and 3D printed. The scaffolds are dynamically seeded with one million adult rat mesenchymal stem cells (MSCs), obtained directly from the bone marrow of adult Wistar rats. After seeding, the constructs are cultured for 16 days with samples being taken at 0, 4, 8, 12, and 16 days. During the culture period, we are measuring the levels of oxygen, glucose, and lactate, and validating the outcome of these measurements with the results obtained from the fluid dynamic simulations. These samples are then imaged using micro-computed tomography.

Results: In previous studies, we successfully characterized the velocity flow field and stress distributions within the scaffolds by using lattice Boltzmann method fluid dynamic simulations. We found that scaffolds made of the same material and having similar porosity and surface-area-to-solid-volume ratios did not exhibit differences in average shear stress or shear stress distributions. We expanded upon our initial goal by incorporating oxygen and glucose readings during culture, and by incorporating the data into computational fluid dynamic (CFD) simulations to obtain the nutrient gradients throughout the scaffold. By taking nutrient readings at different time points during a 21 day culture, we are able to monitor the metabolic state of the cells in the porous scaffolds. For the macroscopic nutrient levels, we used a convection-dispersion model to represent the consumption of oxygen as it is perfused through the scaffolds, which assumes the solute, oxygen, is flowing into porous medium, and is being consumed by a bed of uniformly distributed particles which represent the cells. As for the microscopic CFD models, we were able to create a three dimensional view of the localized oxygen and glucose levels within the scaffold. This was accomplished by creating a model that would continuously inject nutrient particles into the computation area, and simulate the consumption using the cellular uptake rate and collision probabilities. In addition to these findings, we were able to locate and differentiate cells attached to scaffold fibers, and also differentiate mineralized tissue, cells, and soft tissue from the scaffold itself, as shown in **Figure 1**, by utilizing a multi-masking technique in combination with micro-CT.

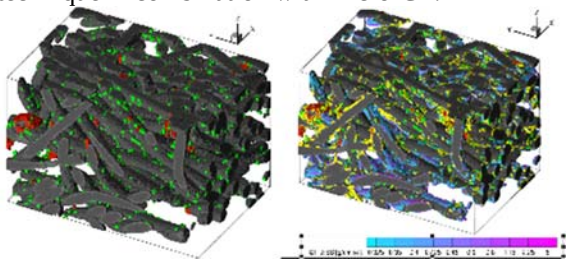

Figure 1. 3D reconstruction of a piece of a 16 day cultured scaffold. PLA fibers in gray, cells outside of ECM in green, soft tissue in yellow, mineralized tissue in red, and fluid shear levels in blue and purple

Conclusions: We have successfully modeled the oxygen gradient within our scaffolds with and without the consideration of molecular diffusion. There is a linear oxygen concentration drop throughout the scaffold, which is directly related to the scaffold cellularity. Furthermore, the system reaches steady state in a matter of minutes. Furthermore, we were able to take this data and develop a model for determining the localized nutrition levels within the scaffolds. By coupling this with the 3D printed scaffolds, we can predict the micro-environment before we even run the bioreactor experiments. In regards to the micro-CT imaging, the multi-masking technique shows great potential for being able to analyze a 3D tissue construct's structure without the need for more advanced equipment, contrasting agents, or physically destroying the sample. Further work must be done by increasing the sample size, segmenting out cells from deposited ECM, and incorporating the nutrient models into our 3D reconstructions.

References:

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