Deciphering hydrodynamic shear stress in bioreactor configurations used for mechanical stimulus

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Statement of Purpose: Use of biodegradable porous scaffolds in tissue engineering have proved to be an attractive solution for creating functionally replaceable tissue parts, and producing synthetic surrogates to test diseases propagation. To grow tissues outside the body, porous scaffolds are seeded with appropriate cells, and cultured in a bioreactor to provide nutrients and regulate culture conditions to mimic the native environment familiar to the cells. However, challenges associated with *in vitro* tissue regeneration include incomplete understanding of the nutrient distribution and stress applied due to the flow of growth medium. The objectives of this study were to analyze shear stress on scaffold due to media flow, nutrient distribution inside the bioreactor, and validate the model with an experimental setup using Hep G2 cells in freeze dried chitosan-gelatin scaffold. We also used co-axial electrospun polycaprolactone-cellulose acetate (PCL-CA) fibers to validate the model to understand the role of biomaterial mechanical properties in fluid induced deformation.

Methods: The bioreactor designs, flow-through and axial-flow, were evaluated using Computational Fluid Dynamics (CFD) to support 100-mm diameter and 2-mm thick scaffolds. Chitosan-gelatin scaffolds formed by freeze drying and PCL-CA scaffolds by coaxial electrospinning were characterized using scanning electron microscopy (SEM) for pore size and porosity. The elastic properties were determined using Instron 5542 (INSTRON, Canton, MA) for Young's Modulus and a custom-build apparatus for Poisson's ratio values at physiological conditions [1]. CFD simulation was performed to predict shear stress across the scaffold due to media flow [1]. The outlet concentration and nutrient distribution were predicted with CFD using the convection diffusion equation with the assumption of uniform cell distribution throughout the scaffold [2]. To predict exit concentrations, the kinetic parameters for Hep G2 were calculated for three different cells densities, which were either half or double of 1.2×10^{12} cells/m^3, providing analysis for two doublings. Simulations were extended to a broad range of material properties. Bioreactors of appropriate flow configuration were constructed in-house, similar to previous publications [2]. Hep G2 cells were cultured in Eagles's Minimum Essential Medium (EMEM) on chitosan-gelatin scaffolds and were tested for oxygen consumption. The oxygen concentrations and pressure drop across the scaffold were monitored in real-time. Samples were collected at the outlet to determine glucose concentration. Figure 1 shows the cell culturing process used during the study. Cell seeded scaffolds from 5 different locations in the scaffold were analyzed by histology. Scaffolds were also formed by co-axial electrospinning and fibers were tested for the utilization of appropriate permeability equation.

Results: The results showed uniform shear stress across the scaffold in axial flow bioreactor, however, higher shear stress were observed at the beginning of scaffold in flow-through configuration (Figure 2).

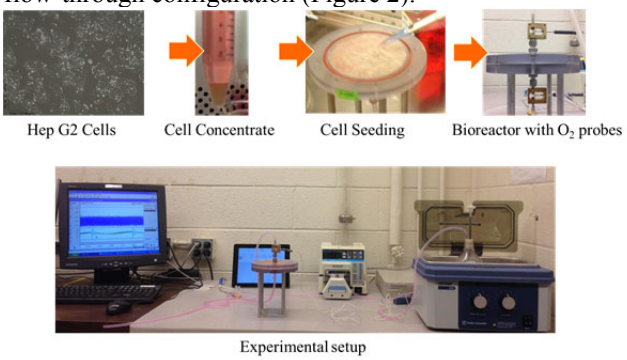

Figure 1. Schematic of the cell culture experimental setup.

The higher shear stress in flow-through configuration could deform soft scaffolds at higher flowrates. On the other hand, axial flow bioreactor had relatively lower deformation at similar flowrates. This allows independent application of mechanical stress on the cells. The outlet oxygen concentration at 1mL/min with a cell density of 1.2×10^{12} cells/m^3 was 0.166±0.015 mol/m^3 while CFD predicted an outlet concentration of 0.155 mol/m^3. The histology study showed uniform cell distribution in sample obtained from 5 different locations in the scaffold. We also extended the analyses to PCL-CA scaffolds where pressure drop measurements agreed with the simulation results. Also, higher elastic modulus of PCL-CA scaffolds experienced significantly less shear stress relative to scaffolds with low elastic modulus.

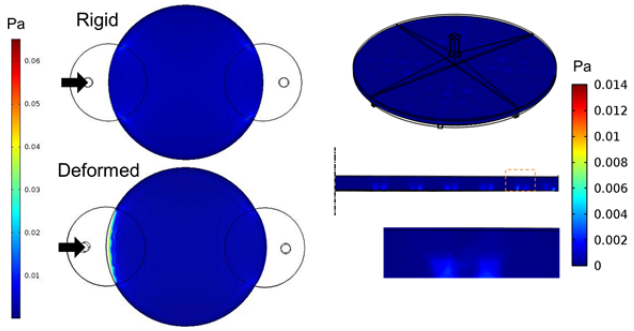

Figure 2. Shear stress profile in flow-through and axial – flow bioreactors.

Conclusion: Axial flow bioreactors showed less shear stress distribution relative to flow-through reactors. Simulation results with and without cells agreed with the experimental results. These insights help monitor *in vitro* tissue regeneration and help understand the effect of mechanical stimulus on 3D cell culture.

References: [1] Podichetty JT, Madihally SV. Modeling Growth Medium Perfusion-Induced Porous Scaffold Deformation. Journal of Biomedical Materials Research Part B: Applied Biomaterials. August 2013 (in press). [2] Devarapalli M, Lawrence BJ, Madihally SV. Modeling Nutrient Consumptions in Large Flow-Through Bioreactors for Tissue Engineering. Biotechnology/ Bioengineering. 103(5):1003-1015, 2009.