Modeling Oxygen Transport in Modular Tissue Engineering

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Statement of Purpose: A major obstacle in tissue engineering is overcoming hypoxia in thick, three-dimensional engineered tissues which is caused by the diffusional limitations of oxygen and lack of internal vasculature to facilitate mass transfer[1,2]. Modular tissue engineering, as developed by our group, is a bio-mimetic strategy that forms scalable, vascularized and uniform three-dimensional constructs. The modules are sub-mm collagen cylinders embedded with functional cells and coated with endothelial cells. Randomly packing modules together results in a perfusable construct, where interstitial spaces among the modules form interconnected channels lined with endothelial cells. Previous studies have demonstrated the ability to maintain "large" constructs (0.038-0.075 cm^3) with high cell densities, indicating that the interconnected channels between packed modules provides sufficient mass transport to prevent hypoxic conditions. Design constraints for the modular system based on shear stress, pressure drop and oxygen depletion over the length of a modular construct were also developed[2]. The goal of this project is to demonstrate that internal mass transfer within modules can be modeled by conventional chemical engineering equations and to use these equations and experimental analysis to define ideal fabricating conditions for modular systems.

Methods: Theoretical analysis of the mass transport phenomena was performed using Thiele modulus and first-order effectiveness factor calculations for cylindrical geometries, modified to describe the modules,

$$\text{Thiele modulus} \qquad \phi^2 = \frac{\rho_{cell}(OUR)(D_m/4)^2}{C^* D_{eff\ (collagen)}} \qquad (1)$$

$$\text{Effectiveness Factor} \qquad \eta_1 = \frac{2}{\phi}\frac{I_1(\phi)}{I_0(\phi)} \qquad (2)$$

Where the oxygen uptake rate (OUR), concentration of oxygen in bulk fluid (C*) and the diffusivity constants of oxygen (D_{eff}) in collagen were estimated from literature to be 1.34 nmol O_2/cell/hr, 0.13 mol/m^3 and 2.99 x 10^{-5} cm^2/sec, respectively. Seeding cell density (ρ_{cell}) and module diameter (D_m) were the independent parameters. I_0 and I_1 are the modified Bessel functions of the zeroth and first order, respectively.

Modules were made by gelling a HepG2-collagen suspension in polyethylene tubing, which was then cut into 2mm sections using an automated cutter. The modules were separated from the tubing by gentle vortexing and were coated with HUVEC-C, a human umbilical vein endothelial cell line. HUVEC-C cells were allowed to contract the modules for three days. On day 3 and day 7 post-fabrication, modules were tested for cell function and survival. Cell numbers within modules were quantified by GAPDH Western Blot. Albumin secretion was measured using a human albumin ELISA kit and Alamar Blue reduction was used as a marker for overall metabolic activity. Cell density and tubing diameter fabrication conditions were selected based on theoretical analysis.

Results: For the purpose of this study, a minimum effectiveness factor of 0.9 was chosen to describe modules with negligible mass transfer resistance. From this constraint, Equations 1 and 2 were used to determine a critical cell density, above which the modules would be expected to experience diffusional limitations, Figure 1.

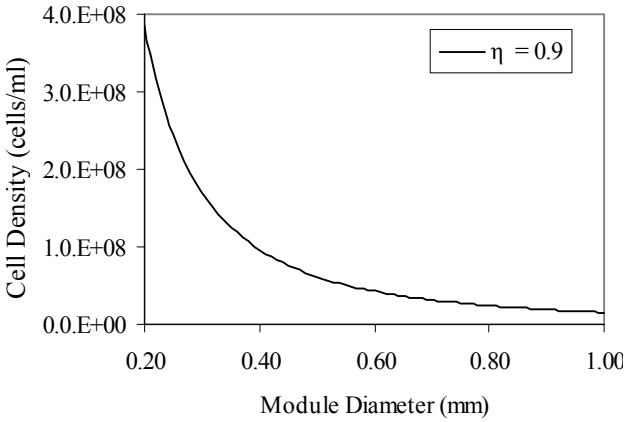

Figure 1. Critical cell density for modules. The cell density at which modules become diffusion limited, based on theoretical mass transfer calculations.

Modules are initially 0.76 mm in diameter, 2 mm in length and have a cell density of $2x10^6$ cells/ml. After gel contraction by endothelial cells, the module dimensions decreased to approximately 0.4 mm x 0.76 mm and cell density increased to $1.9x10^7$ cells/ml (neglecting proliferation). Both cell densities are below the critical cell density of $2.6x10^7$ and $2x10^8$cells/ml for module diameters of 760 μm and 400μm, respectively.

From experimental observation, the structural integrity of modules is maintained to a maximum cell seeding density of $2x10^7$ cells/ml. For module diameters up to approximately 1mm, this maximum cell density acts as the limiting constraint in module fabrication. However, upon module contraction, cell densities increase and this may create a diffusion limited environment.

Preliminary experimental results suggest that modules with theoretical effectiveness factors above 0.9 do not experience mass transfer limitations, as expected.

Conclusions: Theoretical analysis of internal mass transport phenomena in modules supports previous assumptions that the internal mass transfer resistance is negligible at the fabrication conditions currently used in modular tissue engineering applications (0.762mm, $2x10^6$ cells/ml). Further experimental analysis is required to refine the theoretical models and demonstrate their validity in describing the modular system.

References: 1. Fidkowski C et. al., *Tissue Engineering*, 11(1/2), 302-309, 2005. **2.** McGuigan AP and Sefton MV, *Tissue Engineering*, 13(5), 1079-1089, 2007.