Capillary Flow Networks in Collagen-Based Scaffolds for Microvascularized Tissue-Engineered Products

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Statement of Purpose: Nutrient mass transfer limitations pose a serious problem for current tissue-engineered (TE) products. Several strategies including porous matrices, hydrogels, and angiogenic factor delivery[1], have been employed to address the nutrient limitations. However these strategies have a limitation that mass transfer primarily occurs by passive diffusion until the product is vascularized *in vivo* through angiogenesis. Synthetic biodegradable polymers have been used to fabricate microvascular networks[2]. The primary goal of this research is to design and develop capillary flow networks with optimal transport characteristics to be integrated with natural biodegradable scaffolds for skin tissue engineering (Figure 1). The built-in endothelialized flow networks would efficiently deliver nutrients and remove waste products from the TE product with minimal frictional losses for better host integration, and form a basis for generating a scalable design that can be used for tissue engineering of three dimensionally complex tissues.

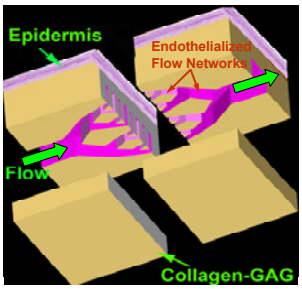

Figure 1. A Model of TE Skin with Built-in Vasculature

Methods: Collagen-GAG membranes were fabricated by vacuum filtering a mixture of collagen and GAG homogeneously suspended in 0.01 M acetic acid solution[3]. Micron dimension flow networks optimized for maximum mass transfer efficiency and minimum pressure losses were fabricated on Silicon wafers using standard photolithography techniques. These microvascular networks were cast onto the collagen-GAG biopolymeric matrices using a novel collagen soft lithography technique developed in our lab. Four process parameters were optimized to obtain well-resolved and stable features: acetic acid (AA) solution concentration, surface dissolution time, applied pressure and glutaraldehyde concentration. Scanning Electron Microscopy (SEM) was used to image the collagen-GAG networks. We also assessed the growth and viability of Bovine Aortic Endothelial Cells (BAEC's) within the flow network channels. Further, the pressure drop-flow rate relationship was verified as a function of network generations and porosity in a polydimethyl siloxane (PDMS) analog of our designs. The mechanical strength of our collagen-GAG constructs were tested using a Universal Material Testing apparatus (Series 5560, Instron, Norwood, MA).

Results / Discussion: Results from the collagen microfabrication process are summarized in Figure 2, an SEM image of a typical collagen-GAG membrane cast with flow network designs. The process conditions used were 1M AA solution, 2 hours of surface dissolution time, 3500 Pa of pressure and 2% w/w glutaraldehyde solution. We have obtained feature resolutions of up to 50 microns. Figure 3 shows an SEM image of viable BAEC's attached to the collagen-GAG matrix. The small pore size (~2 microns) promotes cell adhesion and establishment of cell-cell junctions. Our crosslinked wet samples showed an average modulus of elasticity of 13±2.4 MPa and tensile strength of 8.4±1.6 MPa, indicating they have adequate mechanical strength for integration with the host tissue. A typical pressure drop versus flow rate curve in the network shows that there are two regions with Poiseuille flow behavior (Figure 4). The experimental frictional resistance at lower flow rates (41 mmHg/ml/min) compares well with the theoretically predicted values (27 mmHg/ml/min).

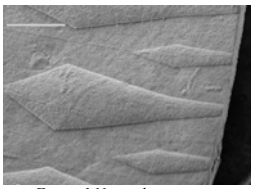
Figure 1 Networks cast on a collagen-GAG matrix

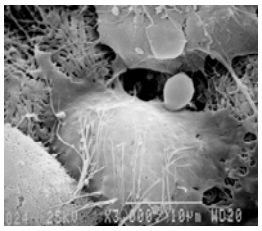
Figure 3. Adhesion of BAEC's to the matrix

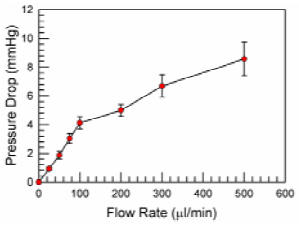

Figure 4: Pressure Drop Vs. Flow Rate

Conclusions: By engineering a built-in microvasculature *in vitro* before implantation, TE products can overcome mass transfer limitations. To this effect, we have developed a new technique to transfer rationally designed flow networks onto a natural biopolymeric collagen-GAG scaffold. This technique is amenable for adaptation in other scaffold materials, and can be used for making TE skin with built-in flow channels. Further, we have demonstrated endothelial cell attachment and viability in our scaffolds.

References:

1. Murphy WL. Biomaterials. 2000; 21(24): 2521-27.
2. Fidkowski C. Tissue Eng. 2005; 11(1-2): 302-9.
3. Yannas IV. J Biomed Mater Res. 1980; 14(2):107-32.