Electrospun Poly(ε-caprolactone)-Silk Fibroin Scaffold for Vascular Tissue Engineering

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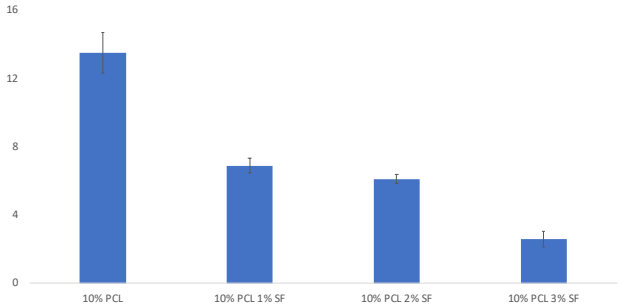
Statement of Purpose: Due to the prevalence of cardiovascular diseases, there is a large need for small diameter vascular grafts that cannot be fulfilled using autologous vessels. Although medium to large diameter synthetic vessels are in use, no suitable small diameter vascular graft has been developed due to the unique dynamic environment that exists in small vessels. To achieve long term patency, a successful tissue engineered vascular graft (TEVG) would need to closely match the mechanical properties of native tissue, be non-thrombotic and non-immunogenic, and elicit the proper healing response and undergo remodeling to incorporate into the native vasculature. Electrospinning is a versatile technique used to generate scaffolds composed of micro or nanoscale polymer fibers, mimicking the fibrous structure of native extracellular matrix. One of the most used synthetic polymers is poly(ε-caprolactone) (PCL) due to its biocompatibility, mechanical properties, and biodegradation. However, the main drawback of PCL is its hydrophobicity, which limits cell adhesion and infiltration. On the other hand, silk fibroin (SF) is a natural polymer purified from the cocoons of the domesticated silkworm *Bombyx mori*. It too possesses good mechanical properties and biocompatibility. The degradation and mechanical properties of SF can be tuned by modulating the formation of β-sheets, which renders it insoluble in water. Importantly, SF can improve the hydrophilicity of the scaffold, cell adhesion, and proliferation. In this study an electrospun PCL-SF scaffold was created and evaluated using primary human endothelial cells (ECs) and vascular smooth muscle cells (VSMCs). The effect of the PCL-SF scaffold on mesenchymal stem cell (MSC) differentiation into ECs and VSMCs was also evaluated.

Methods: Silk cocoons were degummed by boiling in 0.02 M Na_2CO_3 for 30 min followed by several rinses in DI water and dried. Dried SF was then dissolved 9.3 M LiBr to make a 20 w/v% solution. The resulting solution was then dialyzed against ultrapure water using 3500 MW cutoff dialysis cassette. The water was changed several times over the course of 48 hr. The purified SF solution was then centrifuged and lyophilized. 10 w/w% PCL and 0-3 w/w% SF were dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol with gentle stirring overnight. For electrospinning, the PCL-SF solution was dispensed at 0.8 mL hr^{-1} with a 22G blunt tip needle and fibers were collected on a 2.5 cm diameter mandrel at 1000 rpm. The electrical voltage and needle tip to collector distance were varied to in investigate changes in morphology and mechanical properties. Mechanical testing was carried out on a mechanical tester with 100 N load cell (MTS Systems, USA). Fiber morphology was analyzed with a scanning electron microscope (FEI Quanta 450, USA). Human ECs, VSMCs, and MSCs were seeded onto the scaffolds and cultured for 14 days. Cell proliferation was assessed with MTT assay. MSC differentiation into ECs and VSMCs was directed using VEGF and TGFβ-1, respectively. Differentiation was evaluated with immunocytochemistry using EC and VSMC specific antibodies and observed on a confocal microscope.



(Figure 1: Elastic modulus of electrospun PCL-SF scaffolds.)

Results: Bead-free fibers were obtained for 10% PCL, 10/1 PCL-SF, 10/2 PCL-SF, and 10/3 PCL-SF scaffolds. While a higher SF content lowered the minimum voltage required to get bead-free fibers, no clear trend was observed for fiber diameter. Likewise, no clear trend between voltage and fiber diameter was observed within a given polymer mix. The PCL-SF scaffolds had elastic moduli similar to reported values for native vessels. The 10% PCL scaffold had the highest elastic modulus and ultimate tensile strength with both properties decreasing with increasing SF content. Increasing voltage appeared to reduce the elastic modulus in the 10/1 PCL-SF scaffold, a trend that was not seen with the other blends. *In vitro* studies showed that the PCL-SF scaffolds supported the proliferation of ECs and VSMCs as well as the growth factor directed differentiation of MSCs. Together these results show the PCL-SF electrospun scaffolds may be a promising material for a TEVG.