Two-layer silk tubular scaffolds for small diameter blood vessel regeneration

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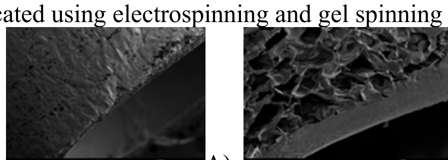
Statement of Purpose: At present, the challenge of vascular tissue engineering is the fabrication of functional grafts for the replacement of small diameter blood vessels (Ø < 6 mm). Currently available substitutes have drawbacks usually correlated to the regeneration of nonfunctional endothelium and a mismatch between the mechanical proprieties of grafts and natural blood vessels [1]. The aim of this work was to overcome these limitations. Silk fibroin was chosen as the biomaterial because of its biocompatibility, excellent mechanical properties and tailorable degradability. A novel combination of two established techniques already studied for the fabrication of silk scaffolds [2,3], electrospinning and gel spinning, were used to design, fabricate and characterize silk tubes for vascular needs.

Materials and Methods: An aqueous solution of silk fibroin was prepared from *Bombyx mori* silkworm cocoons as previously described [3]. This solution (~7% w/v) was electrospun onto a rotating mandrel to fabricate tubular scaffolds. A second layer was produced by gel spinning using a more viscous (~9% w/v) aqueous silk solution. The samples were lyophilized. The morphology of these silk two-layer tubes was analyzed by scanning electron microscopy (EVO MA10, Zeiss, Thornwood, NY). The gel spun layer was treated using different post-processing methods: only lyophilization (LYO), methanol dipping (MeOH) and water vapor annealing (WVA) to obtain scaffolds with different crystallinities by induction of β-sheet formation. Degradation -The degradation behavior of the two-layer silk tubes (N = 6) was studied using Protease XIV from *Streptomyces griseus* (5.3 U/mg) to digest the structures. Samples were incubated at 37°C in a 2.5 mL solution of 0.8 U/mL Protease XIV in PBS at pH 7.4. The percentage weight loss over time was calculated at designated time points (1, 3, 7, 10, 14 days). Mechanical Assessments - Circumferential tensile tests were performed with the two-layer tubes (N = 3) using an Instron 3366 testing frame (Norwood, MA) with pneumatic clamps (Instron 2752-005) and a temperature controlled Biopuls bath (37 ± 0.3°C) filled with 0.1 M PBS. These tests were carried out using custom clamps and a displacement control mode with a crosshead displacement rate of 5 mm/min. From the stress/strain curves the ultimate tensile strength (UTS), elongation to failure and the linear elastic modulus were calculated.

Results: Two-layer silk tubes of 3.2 mm diameter were fabricated using electrospinning and gel spinning (Fig.1).



A) B)

Figure 1. SEM images of a two-layer tube. A) External surface, B) Cross section (Scale bar = 100 µm).

FTIR analysis of crystallinity showed that the β-sheet content was higher in the MeOH tubes and lower in LYO tubes [data not presented]. Two-layer silk tubes showed different degradation behaviors; increased β-sheet content resulted in a decrease in degradation rate (Fig.2) as anticipated.

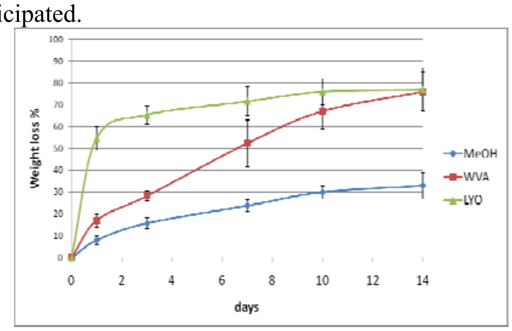


Figure 2. Percentage of weight loss of two-layer tubes treated with different post-processing methods.

Mechanical properties were correlated with the selected post-processing treatment. The elastic modulus was not significantly different between treatments, but ultimate tensile strength and elongation to failure were higher for the MeOH tubes (Fig.3).

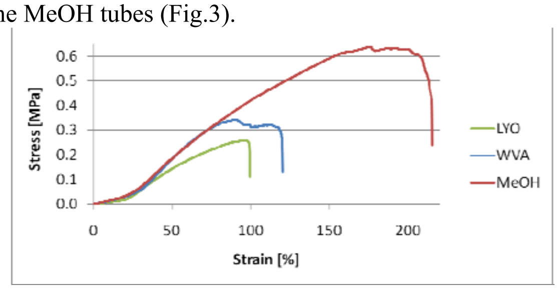


Figure 3. Representative stress/strain curves of two-layer tubes treated with different post-processing methods.

Conclusions: Novel two-layer silk tubes were obtained using a combination of electrospinning and gel spinning with tunable morphological, degradation and mechanical characteristics for blood vessel regeneration studies. The mechanical properties were similar to the native blood vessels. The UTS of silk tubes (Fig 3) was analogous to UTS of human coronary arteries (0.39 MPa) [5] and elongation to failure for silk tubes (Fig 3) is similar to human umbilical cord veins (~180%) [6]. A bioreactor platform [7] was modified for the co-culture of human aortic endothelial cells and human aortic smooth muscle cells, part of the ongoing studies.

References:

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