Electrospinning of a Composite Vascular Graft

Rebecca C Rowe, Ping-Cheng Wu, Kenton Gregory.
Oregon Medical Laser Center, Portland, OR.

Statement of Purpose: The goal of this study was to determine the feasibility of electrospinning collagen onto decellularized porcine elastin to produce a composite vascular graft. Electrospinning is a method of fabricating biomaterials that mimics native fibrillar protein sizes of 50-300 nm[(1)] that should encourage cell attachment and infiltration. A rotating mandrel was used to produce tubular conduits of electrospun proteins[(2)].

Methods: Electrospinning solutions were made using Type I calfskin Collagen (Elastin Products Company), 1,1,1,3,3,3-Hexafluoro-2-propanol (HFP, ≥ 99.8%, Sigma) and 2,2,2-Trifluoroethanol (TFE, Sigma). The decellularized elastin was obtained by treating porcine carotid arteries with 80% ethanol followed by a hot alkali digestion (0.25 M NaOH for 70 minutes at 60ºC), and two 30-minute HEPES buffer rinses.

An 8.3 wt % collagen in HFP solution was mixed on a LabQuake rotator for 20 minutes at 4°C and then loaded into a glass syringe (Popper, 2mL) with a SS blunt tip needle (Popper, 18G). The syringe was placed on a syringe pump (Kd Scientific) set at a flow rate of 2.0 mL/hr and the rotating mandrel placed a gap distance of 14cm from the syringe tip. A high voltage power supply (Glassman FC30P4) was set to 18kV. The mandrel rotation speed was constant (4050 RPM). The electrospinning procedure was repeated using either 0.75 mL 8.3wt % collagen in HFP or 0.75 mL 8.0wt % collagen in TFE. The collected fibers were imaged using confocal microscopy (Figure 1).

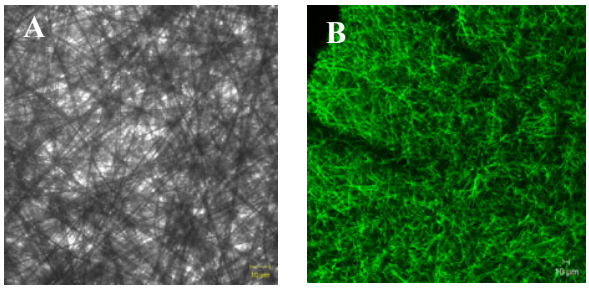

Figure 1. Electrospun Collagen matrices imaged using different Confocal Microscopy Techniques; DIC (A) and Fluorescence (B, stained with 2.5µg/mL Oregon Green).

Burst pressure testing was performed on both the porcine elastin and the elastin composite grafts. All conduits were rehydrated in PBS prior to testing. The ends of the scaffolds were fixed in position under zero longitudinal load and saline was infused at a rate of 100 mL/min to failure. The pressure and diameter were continuously monitored by an inline pressure transducer (Transpac IV, Abbott Labs, N. Chicago, IL) input into a data acquisition program developed with Labview. The measure of significance was a two-tailed Student's t-test.

Results/Discussion:
The electrospun layers of collagen produced were approximately 50 µm thick (Figure 2).

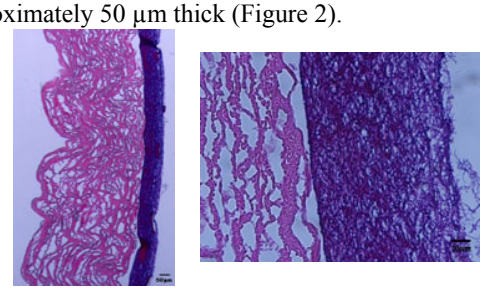

Figure 2. Histology sections (H&E staining) of the elastin-collagen composite graft at 5X(A) and 40X(B) magnification(elastin: pink, electrospun collagen: purple).

The burst pressures of the grafts for freeze dried elastin (298±60 mmHg; n=5), elastin with 8.3 wt% collagen in HFP (257±54 mmHg; n=5) and 8.0 wt% collagen in TFE (360±62 mmHg; n=6) all crosslinked with glutaraldehyde are shown in Figure 3. Hydrated elastin was used as a control (162±36 mmHg; n=10).

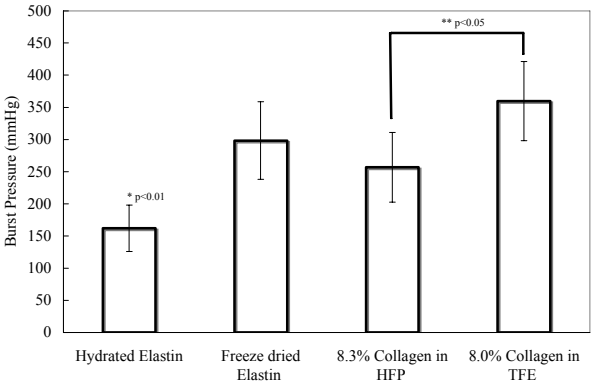

Figure 3. Burst pressure testing to compare Elastin controls (hydrated and freeze dried) and Elastin composites with an outer layer of electrospun collagen (8.3wt% in HFP and 8.0% in TFE).

Conclusions:
A reproducible collagen layer can be electrospun onto the outer surface of an elastin conduit to produce a composite graft. The electrospinning solvent had a significant effect on the burst strength of the composite material. Further research to improve the mechanical strength of the graft is warranted to increase the margin of safety for *in vivo* studies.

References:

1. Matthews JA. Biomacro. 2002 Mar-Apr;3(2):232-8.
2. Ayres C. Biomat. 2006 Nov;27(32):5524-34.