Development of Co-Electrospun Scaffolds with Tunable Mechanical Properties and Bioactivity

C. M. Radzicki, R. M. Nezarati, E. Cosgriff-Hernandez
Biomedical Engineering, Texas A&M University, College Station, Texas

Statement of Purpose: Electrospinning is a popular scaffold fabrication technique that produces fiber meshes and utilizes various types of polymers to tune mesh properties. By varying the polymer selection, scaffolds that result in different mechanical properties and cell responses can be produced. Synthetic polymers are commonly electrospun for their tunable mechanical properties; however, these polymers generally have poor cell affinity. Natural polymers, such as gelatin, have the potential to allow greater control of cell behavior on the scaffold due to improved cell affinity but generally have poor mechanical properties. Here, we propose a method to co-electrospin gelatin fibers with a synthetic poly(carbonate urethane) (PCU). Electrospun gelatin scaffolds must also be crosslinked to avoid dissolution upon implantation. We have developed a method to crosslink gelatin during the spinning process with isocyanate for improved fiber morphology retention and controlled degradation. Overall, this co-spinning technique with *in situ* crosslinking could provide a method that combines the bioactivity of the gelatin with the tunable mechanical properties of the PCU.

Methods: Double barrel syringes with a barrel ratio of 1:1 and attachable mixing heads (3.1 mm ID x 53.5 mm length) were obtained from Nordson EFD. Bovine-derived gelatin, 1,4-diazabicyclo[2,2,2]octane (DABCO) and hexamethylene diisocyanate (HDI) were each dissolved in 2,2-trifluoroethanol (TFE). The concentration of the gelatin solution was determined such that the final concentration when mixed in the double barrel syringe would equal 10wt%. The concentration of the DABCO was determined such that the concentration would equal 5wt% of total solids. The concentration of HDI was determined such that the crosslinker density would equal a 10X ratio of isocyanate/amine. Poly(carbonate urethane) (PCU) was dissolved in N-N'-dimethylacetamide (DMAc) at a concentration of 18wt%.

Electrospinning: Double barrel syringes were loaded with the gelatin/TFE and 5wt% DABCO solution in one barrel and HDI/TFE solution in the other. A metal 18 gauge blunted needle was attached to the end of the mixing head and placed into a syringe pump. The needle tip was set 12cm from a rotating mandrel. The double barrel syringe solutions were pumped at a rate of 1.0 mL/hr. A voltage of 10kV was applied to the needle tip while a -5kV was applied to the rotating mandrel. Simultaneously, a glass syringe was loaded with PCU/DMAc solution. Plastic tubing connected the syringe to a blunted 20 gauge needle. The solution was pumped at a rate of 0.5 mL/hr through the tubing to the needle which was suspended 50cm above the mandrel and perpendicular to the gelatin. A voltage of 18kV was applied to the needle tip.

Fiber mesh characterization: Fiber morphology was characterized with scanning electron microscopy (SEM) and fluorescence microscopy using rhodamine B. Mechanical properties were characterized using a uni-axial tensile tester. Electrospun, crosslinked gelatin mechanical properties were tested with a 2.2lb load cell in a water bath to mimic physiological conditions as well as dry in ambient conditions. The PCU meshes were tested with a 1000N load cell.

Results: The mechanical properties of the PCU and gelatin scaffolds were first determined separately. The dry gelatin meshes had a much higher modulus and ultimate tensile strength but a lower percent elongation compared to the wet meshes as seen in Table 1. Compared to the gelatin meshes, the PCU meshes had a higher modulus, ultimate tensile strength, and percent elongation. Co-electrospun meshes were fabricated and examined with a scanning electron microscope. Due to difficulty distinguishing between the different fibers using scanning electron microscopy (Figure 1), fluorescence microscopy was utilized. Fluorescent fiber morphology was characterized separately on gelatin and PCU meshes that were electrospun with rhodamine B. Fiber characterization was clear and distinct using magnification up to 40X.

Table 1: Mechanical properties of 10X gelatin and PCU meshes

Polymer		Modulus (MPa)	Ultimate Tensile Strength (MPa)	Ultimate Elongation (%)
10X Gelatin	Dry	21.2*	0.7*	15*
	Wet	0.7*	0.3*	185*
PCU	Avg	9.9±0.4†	33.1±4.3†	395±37†

*average of n=2;†n=3

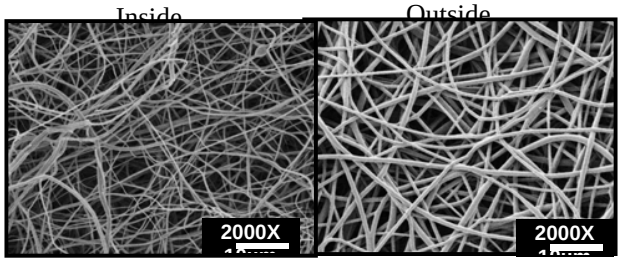

Figure 1: Scanning electron microscopy of co-electrospun meshes

Conclusions: We have demonstrated a method to fabricate co-electrospun gelatin that was crosslinked in situ and PCU. By fabricating a mesh composed of crosslinked gelatin and PCU, we aim to incorporate the strength of the PCU with the bioactivity of the gelatin. Future work involves differentiating fiber populations using fluorescence microscopy and characterizing mechanical properties of co-electrospun meshes. Additionally, cell viability, adhesion, and proliferation will be evaluated on co-electrospun meshes compared to gelatin controls. These meshes could provide improved control over cell behavior and mechanical properties tissue engineering scaffolds.