Development of *In Situ* Crosslinked Electrospun Gelatin Scaffolds

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Statement of Purpose: Tissue engineering scaffolds that incorporate bioactive factors can provide greater control of cell behavior for improved regeneration. As a result, many researchers have worked on developing techniques to incorporate bioactivity into synthetic polymer scaffolds. Electrospinning is a popular scaffold fabrication technique that produces fiber meshes and allows for high tunability of mesh microarchitecture. This tunability is advantageous as fiber morphology has been shown to strongly influence scaffold mechanical properties, degradation rate, and cell response. Synthetic polymer scaffolds generally have poor cell affinity because they rely on indirect cellular recognition via protein adsorption to the material. Electrospun gelatin has been investigated for its potential to impart greater control of cell behavior on the scaffold due to its cell binding sites. Gelatin scaffolds must be crosslinked to avoid dissolution upon implantation, but current crosslinking strategies do not retain adequate fiber morphology after immersion in water. There is a need for improved crosslinking methods that do not alter fiber morphology. Here, we propose a method to *in situ* crosslink electrospun gelatin fibers with isocyanate crosslinkers by utilizing a double barrel syringe which could result in improved fiber morphology retention by eliminating post-processing steps. This technique would provide a method to predict and reproduce fiber morphology and thus scaffold properties of bioactive meshes. The methodology has potential for use with a broad range of natural polymers (elastin, fibrinogen) and diisocyanate crosslinkers (containing degradable sites, growth factors, or fluorophores) in tissue engineering applications.

Methods: ***Materials:*** Double barrel syringes with barrel ratios of 1:1 and 10:1 and attachable mixing heads with different dimensions (6.3 mm ID x 150.0 mm length, "large"; 3.1 mm ID x 53.5 mm length, "small") were obtained from Nordson EFD. Bovine-derived gelatin and hexamethylene diisocyante (HDI) were each dissolved in 2,2-trifluoroethanol (TFE). The concentration of the gelatin solution was determined such that the final concentration at the end of the mixing head would equal 10 wt%. The concentration of HDI was determined such that the crosslink density would equal 0.5X, 0.75X, or 1.0X molar ratio of amine/isocyanate. ***Electrospinning:*** Double barrel syringes were loaded with gelatin/TFE solution in one barrel and HDI/TFE solution in the other. For the 10:1 ratio syringe, gelatin was loaded into the larger barrel. A metal 18 gauge blunted needle was attached to the end of the mixing head and the syringe was placed into a syringe pump. Solutions were pumped at a rate of 1.0 mL/hr. The needle tip was set 12 cm from a grounded 15 cm square copper plate that was covered with a PET film to facilitate removal of the fiber mesh. A voltage of 10 kV was applied at the needle tip and fibers were collected for 2 hours or until the solution clogged. ***Fiber mesh characterization:*** Fiber morphology before and after 24 hour incubation in water was characterized with scanning electron microscopy.

Results: A 0.5X crosslink density was used for initial studies because this ratio resulted in the longest working time before gelling. When gelatin and HDI were mixed in a single barrel syringe, electrospinning was limited to ~5 min due to rapid gelling in the syringe. As a result, the double barrel syringe was determined necessary to limit the mixing time of gelatin and HDI. First, a double barrel syringe with a 1:1 volume ratio and a large mixing head were used. It was necessary to almost double the concentration of gelatin compared to the desired final concentration due to this syringe volume ratio. This setup resulted in clogging in the mixing head after ~15 min which was hypothesized to be result of the high viscosity of the gelatin solution and rapid gelling in the mixing head. Clogging was eliminated when a 10:1 syringe and small mixing head were used; however, the 0.5X crosslink density resulted in poor retention of fiber morphology after soak in water. Higher crosslink densities of 0.75X and 1.0X were investigated for their potentially improved fiber morphology retention. Utilization of the 10:1 syringe and small mixing head resulted in successful electrospinning over 2 hours for all crosslink densities. Fiber morphology before immersion in water was retained with changing crosslink density, and fiber morphology retention after immersion in water improved with increasing crosslink density, **Figure 1**.

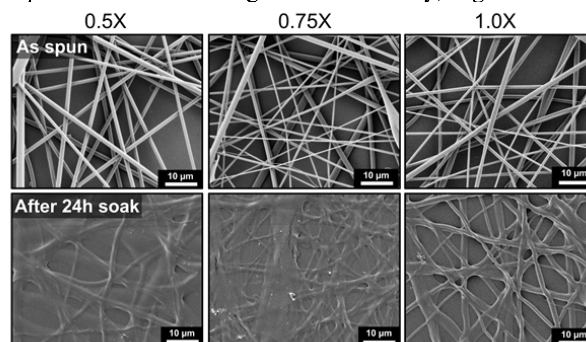

Figure 1: *In situ* crosslinked gelatin fibers of varying crosslink density before and after immersion in water

Conclusions: We have demonstrated a method to fabricate *in situ* crosslinked electrospun gelatin fibers using a double barrel syringe with attached mixing head. Fiber morphology retention after immersion in water improved with increasing crosslink density. This method has potential for fabrication of tissue engineering scaffolds with tunable bioactivity and predictable properties. Current work is focused on investigating higher crosslink density for improved fiber morphology retention, characterizing mechanical properties, and evaluating cell viability.

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