Hydrogel-Electrospun Mesh Composite for Prevention of Anastomosis Leakage

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Statement of Purpose: More than 600,000 surgeries are performed each year to treat colon diseases, a majority of which require an intestinal anastomosis. Current anastomosis techniques have a high failure rate due to leakage resulting from poor healing, as well as obstructions and chronic pain from intestinal adhesions to surrounding tissue. To this end, we have developed a degradable, multi-layered wrap that promotes healing and rapid remodeling of the anastomosis site while resisting adhesion to surrounding tissue. The inner layer consists of a crosslinked gelatin electrospun mesh. Gelatin, a natural polymer, inherently promotes cellular adhesion through its cell binding sites, while the fibrous structure of the mesh promotes cellular integration. We have developed a method to crosslink gelatin during the electrospinning process with isocyanate for improved fiber morphology retention and controlled degradation (targeted 2-3 weeks). The outer layer of the wrap is a degradable poly(ethylene glycol) (PEG)-based hydrogel. PEG hydrogels have an intrinsic resistance to cell adhesion and would thereby prevent adhesion to surrounding tissue. As the degradation rate of PEG-diacrylate (PEGDA) hydrogels is slow, modified PEG-based hydrogels that use thio-β ester linkages to provide controlled hydrolytic degradation can be utilized. Overall, this multi-layered wrap could provide a method to reduce anastomosis failure rate by combining the bioactivity and fibrous structure of electrospun gelatin with the bioinert properties of PEG hydrogels.

Methods: ***Biodegradable hydrogel:*** Briefly, PEGDTT was synthesized by adding d,l-dithiolthreitol (DTT) and triethylamine dropwise to a solution of PEGDA (2 kDa) in DCM. The molar ratio of DTT, PEG and triethylamine was 3:2:0.9. The reaction was then stirred for 24 hours. Next, the solution was precipitated in cold diethyl ether, filtered, dried under ambient conditions then placed under vacuum to remove any excess solvent. Hydrogels were fabricated by making 10 wt% precursor solutions in water of PEGDTT and PEG(6k)DA at various ratios (100:0, 75:25 50:50, 0:100). The solution was pippetted between .5mm spaced plates and exposed to UV light to initiate crosslinking. Hydrogels were swelled in water for 1 hr prior to electrospinning to ensure full hydration.

Electrospinning: 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 HDI was determined such that the crosslinker density would equal a 5X ratio of isocyanate/amine. Double barrel syringes were loaded with 10 wt% gelatin/TFE and 5wt% (of solids) DABCO solution in one barrel and HDI/TFE solution in the other. The double barrel syringe solutions were pumped through a mixing head and an 18 gauge blunted needle at a rate of 1.0 mL/hr and were 12 cm away from the collector. In order to fabricate the multi-layered composite, fibers were collected on a copper plate upon which a 75% PEGDTT hydrogel was secured. A voltage of 10kV was applied to the needle tip while a -0.5kV was applied to the hydrogel collector. ***Fiber characterization:*** Crosslinked gelatin fiber characterization after immersion in water was performed by placing circular punches on glass coverslips in a well plate. Specimens were removed at timepoints up to 1 week, frozen at -80 °C overnight, and lyophilized prior to imaging with SEM. ***Hydrogel degradation:*** PEGDTT hydrogels were punched into discs and incubated in PBS at 37°C for 21 days with weekly solution changes. The swollen masses were recorded at each timepoint. Swelling ratio was calculated and used to characterize network degradation. ***Cellular interactions:*** The multi-layered composites were seeded with human dermal fibroblasts (hDFs, 10,000 cells/cm^2), and incubated for 3 hours (37°C/5% CO_2). Cells were fixed and stained with rhodamine phalloidin and SybrGreen. Cell adhesion and spreading was analyzed using images taken on a fluorescent microscope.

Results: ***Fiber characterization:*** The 5X meshes retained similar fiber diameters to their as-spun counterparts after 1 week of immersion in water, indicating successful crosslinking. ***Hydrogel degradation:*** Increasing amounts of PEGDTT from 75% to 100% resulted in a direct increase of degradation rate, with complete dissolution in 21 and 12 days respectively. 50% PEGDTT hydrogels remained statistically similar to PEGDA hydrogels over the course of 21 days ***Cellular interactions:*** There was a marked increase in hDF adhesion on the gelatin mesh layer of the composite compared to the PEG hydrogel layer (Figure 1).

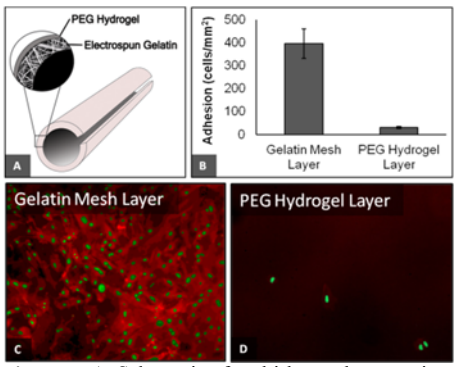

Figure 1: A. Schematic of multi-layered composite. B,C,D. hDF adhesion on gelatin layer and PEG layer of composite.

Conclusions: This study shows the successful control of cellular adhesion to potentially enhance healing while reducing surgical adhesions. Additionally, we have demonstrated a method to tune the degradation rate of PEG-based hydrogels. Current work involves tuning the degradation rate of the gelatin mesh to the desired 2-3 week timeline by varying the crosslinker density. Overall, these multi-layered wraps show strong potential as a tool to reduce anastomosis leakage and improve healing.