Poly(ethylene glycol) diacrylate (PEGDA) multiluminal scaffold for spinal cord repair functionalized with layer-by-layer controlled protein release

Dena Shahriari[1], Daniel Lynam[2], Yacov Koffler[3], Mark Tuszynski[3], Jeffery Sakamoto[1]
[1]Univ.of.Mich.- Ann Arbor, MI. [2]Mich.State.Univ.- E. Lansing, MI. [3]Univ.of.California- La Jolla, CA

Statement of Problem: Approximately 1.2 million Americans have spinal cord injuries (SCI). While physical rehabilitation can improve functional recovery, obtaining full recovery requires alternative therapies. One approach is to develop multifunctional nerve growth scaffolds (NGS) that provide physical guidance cues to guide axons toward distal targets while simultaneously eluting growth factors to stimulate growth. Leveraging previous experience with multi-luminal non-degradable agarose scaffolds employing transfected cells to deliver brain-derived neurotrophic factor (BDNF)[1,2], we are now progressing toward the next generation NGS. First, degradable poly(ethylene glycol) diacrylate (PEGDA) replaces agarose because it can be engineered to degrade over a range of time from weeks to months. As it will be shown, PEGDA is also compatible with our fiber templating fabrication process to enable >60% lumen volume scaffolds with 166 micron channels and ~20 micron thick walls. Second, the complexities associated with cellular drug delivery provide the impetus to develop acellular technologies. Our group uses acellular layer-by-layer (LbL) polyelectrolyte technology[3,4] to intercalate proteins between pH responsive, molecular-scale polymer layers. As the pH rises to physiological pH, bioactive proteins are released. Owing to its high internal surface area, PEGDA hydrogel augments LbL dose response commensurate with the clinically relevant requirement. Third, to improve integration and reduce inflammatory response, PEGDA scaffolds were conjugated with RGD peptides to reduce inflammation[5]. This paper will discuss the materials and materials processing technology to fabricate multifunctional PEGDA scaffolds as well as rats' T3 full-transection *in vivo* efficacy testing.

Methods: PEGDA scaffolds were fabricated using the hexagonally-ordered arrays of polymer fibers previously reported[1]. PEGDA monomer (10 wt%) (8000 MW; Alfa Aesar; Ward Hills, Ma), photoinitiator (Sigma; St. Louis, Mo), and RGD (American Peptide Company; Sunnyvale, Ca) were dissolved in deionized water. PEGDA was functionalized with LbL by immersion in the nominal sequence: polyacrylic acid, lysozyme (a BDNF analog), polyethylene glycol at pH = 3.5[3,4]. Release profiles were acquired using assays or ELISA. Fisher 344 adult female rats were used for T3 full-transection *in vivo* studies. The animals were perfused after 4 weeks and stained for neurofilaments to highlight axonal regeneration.

Results: Dose response eluted from the LbL-coated PEGDA hydrogels reached a cumulative release of 880 µg per mL of hydrogel in 14 days with a steady and linear release profile from day 6 to 14 (Fig. 1). PEGDA-RGD modified scaffolds with linear micro-channels were successfully fabricated demonstrating that the optical fiber templating is compatible with PEG-based hydrogels. *In vivo* histology indicated that the immunoresponse was significantly reduced compared to agarose scaffolds. A high density of axons entered the microchannels and were guided towards the distal end (Fig. 2.).

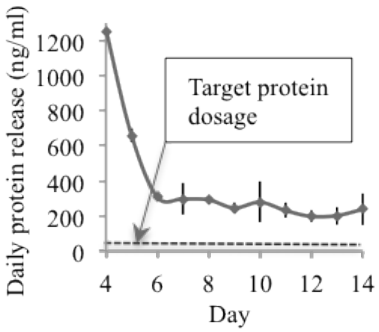

Fig. 1. Daily LbL protein release from PEGDA. Data are normalized to estimate release from a PEGDA LbL-coated scaffold.

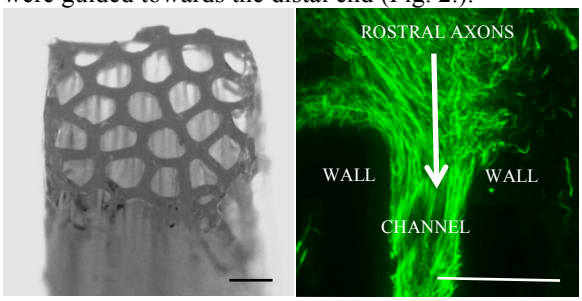

Fig. 2. (Left) A templated scaffold. (Right) Neurofilament staining of axons adjacent to the rostral scaffold end. Scale bars are 200 µm.

Conclusion: Progress towards clinically-relevant NGS has been made. The integration of degradable hydrogels (PEGDA-RGD) with improved compatibility compared to previously used agarose hydrogel scaffolds was demonstrated. The LbL process is also compatible with PEGDA–RGD hydrogel, capable of protein release above required therapeutic dosage. *In vivo* testing indicated that the PEGDA scaffolds show promise toward establishing therapies for spinal cord injuries.

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