Optimization of Fiber Alignment Within Polycaprolactone Nerve Conduits

Danielle Minteer[1,2], BS; Yen-Chih Lin Ph.D.[2], Sarah Pixley, Ph.D.[3], Kacey Marra, Ph.D.[1,2];
[1]Department of Bioengineering, University of Pittsburgh, Pittsburgh, PA 15213
[2]Department of Plastic Surgery, University of Pittsburgh, Pittsburgh, PA 15213
[3]Department of Cancer and Cell Biology, University of Cincinnati, Cincinnati, OH 45267

Statement of Purpose: The purpose of this study was to optimize crosslinking agent and concentration to hold filaments inside polycaprolactone (PCL) nerve conduits for peripheral nerve regeneration. Factors considered include homogeneity of surgical suture alignment inside the PCL guide as well as degradation at body temperature over a period of fourteen days. The surgical sutures used mimic the role carbon nanotubes will eventually serve inside the nerve guide. Peripheral nerve damage results from trauma, infection, or surgical procedures, and, while collagen nerve guides are commercially available, the current solutions do not provide adequate mechanical support required by the growing axon nor provide promotion of axonal growth. Tissue-engineered nerve conduits have become the standard for peripheral nerve regeneration across gaps of less than three centimeters[1]. Carbon nanotubes (CNTs), in thread form, are popular biomaterials in tissue engineering due to the biocompatible, electrical conductivity, and mechanically strong, yet flexible, characteristics[2]. CNTs are of particular interest in peripheral nerve regeneration because of the neurons' ability to grasp onto the carbon matrix and extend along the CNT[3]. Previous research performed in our laboratory has shown PCL nerve conduits successfully aid peripheral nerve regeneration across 1.5 cm gaps *in vivo* with proper growth factors[4].

Methods: PCL nerve guides were created following a previously established method[4] with 6-0 surgical sutures (Ethicon Inc., Somerville, NJ) inside. Various concentrations of genipin (Wako Pure Chemicals Industries Ltd., Chuo-ku Osaka) were combined with a 10% gelatin (Sigma-Aldrich, St. Louis, MO) solution, kept at 37 ºC, and injected into the PCL guides. Concentrations of genipin were 0, 5, 10, 15, and 20 mM. Gelatin + genipin solutions were allowed to gel and scanning electron microscopy (SEM) photos were taken. A second batch of PCL guides with 6-0 sutures testing a wider range of genipin concentrations (0, 10, 20, 30 mM) were used for degradation analysis. After gelation of the guides occurred, the guides were placed in PBS at 37 ºC. Small amounts of the PBS solution in which the guides were placed were extracted daily, for fourteen days, and tested for the amount of amine groups, which indicates the amount of residue from the gelatin in the solution.

Results: SEM imaging indicated PCL nerve guides injected with 10 mM genipin + 10% gelatin solution yielded the most homogeneous alignment of 6-0 surgical sutures when compared to other concentrations of genipin crosslinker (Fig 1). Degradation of 10% gelatin in PCL guides was most optimal with 10 mM concentrations of genipin (Fig 2). After 24 hours (day 2 in Fig 2), a significant difference in degradation was observed between the PCL guides filled with 0 and 10 mM concentrations of genipin.

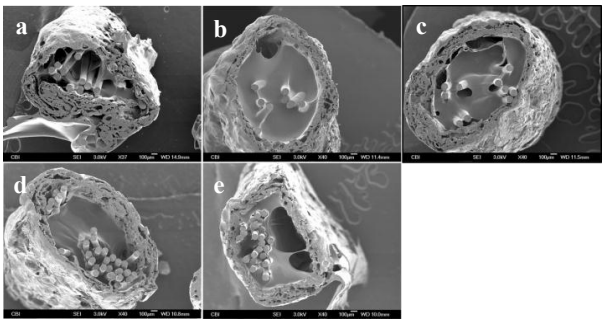

Fig. 1. SEM images taken in the Center for Biological Imaging at the University of Pittsburgh; PCL nerve guides at 40X containing 6-0 surgical sutures in a 10% gelatin and a) 0 b) 5 c) 10 d) 15 and e) 20 mM genipin solution. Scale bar represents 100 µm; WD = 11.5 mm.

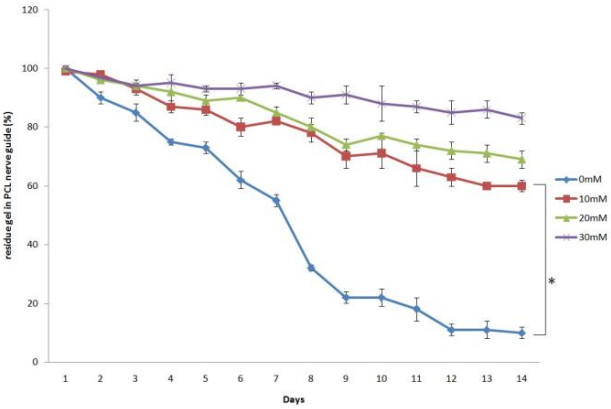

Fig 2. Degradation rates of 10% gelatin inside of PCL nerve guides over 14 days. * indicates significance at the $p < 0.05$ level between groups.

Conclusions: 10 mM concentration of genipin crosslinker provides the most homogenous alignment of 6-0 surgical sutures in combination with 10% gelatin inside PCL nerve guides when compared to genipin concentrations of 0, 5, 15, and 20 mM. The 10 mM concentration of genipin displayed optimal degradation rates inside PCL nerve guides after 14 days when compared to other concentrations of 0, 20, and 30 mM. The 6-0 sutures modeled CNT threads to be placed inside PCL nerve guides and used for peripheral nerve regeneration.

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