Genipin-crosslinked Chitosan/Poly(lysine) Blends to Promote Oligodendrocyte Progenitor Cells' Attachment and Proliferation *In-vitro*

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Statement of Purpose: One of the many pathophysiological outcomes of spinal cord injuries (SCI) includes demyelination and it involves the unwrapping of the myelin sheath from around the axons due to oligodendrocyte death or axonal degeneration. Axons that have been demyelinated due to oligodendrocyte death will lose the ability to carry out normal saltatory conduction, and even though remyelination takes place after injury by endogenous cells called oligodendrocyte progenitor cells (OPCs), it is incomplete (McDonald JW. J Neurotrauma. 2006;23:345-59). Therefore, to promote adequate remyelination of denuded axons during the acute phase of SCI, a more desirable environment for OPC proliferation and differentiation is required. We propose the design of a chitosan/poly(lysine) (PLL) substrate that promotes OPC attachment and proliferation in-vitro. Chitosan by itself was shown to be a poor substrate for OPC attachment and proliferation; thus the modified chitosan/PLL substrate is hypothesized to be more cell-compatible.

Methods: High MW chitosan (MW~350,000) and PLL (MW of 30,000-70,000) were purchased from Sigma Aldrich. Films of chitosan/PLL blends were fabricated by dissolving specific concentrations of chitosan and PLL in 1 M acetic acid solution; pouring the solution in glass Petri dishes; and letting the solvent fully evaporate at room temperature. The cast films were neutralized in 1 M NaOH for 24 hours, and then washed thoroughly using ethanol. The different blends were: 100% (0.1% w/v) chitosan, 80:20 chitosan:PLL, 60:40, 50:50, 40:60 and 20:80. Chitosan/PLL films were then crosslinked in a genipin solution for 3, 6, 24 and 48 hours to achieve different degrees of crosslinking. An excess genipin concentration of 0.005M was used for all samples. The genipin solution was prepared by dissolving 0.005M genipin in a 10% v/v PBS in ethanol solution. All chitosan/PLL films were crosslinked

Results: Seeding OPCs on chitosan films for 1 week showed slow adhesion of OPCs to chitosan (OPC spreading observed after 1-2 days) and minimal proliferation. Interestingly, after staining for OPC (A2B5) and mature oligodendrocyte (GalC) markers, some OPCs were observed to differentiate into mature oligodendrocytes, even in the presence of basic fibroblast growth factor (bFGF) and platelet-derived growth factor (PDGF) that prevent differentiation in culture. To improve cell-compatibility, chitosan/PLL films were successfully fabricated and crosslinked using genipin (to stabilize the blend). Fourier Transform Infrared Spectroscopy (FTIR) analysis of the uncrosslinked blends illustrated the increase of PLL-associated peaks at 1654 and 1524 cm^{-1} as the concentration of PLL increased (Fig. 1). The difference in spectra was a clear indication that the blending took place and was maintained as long as the samples were stored in anhydrous ethanol. An FTIR analysis done after storing samples in PBS for 2 days showed no difference in spectra between all blends. This was due to leaching out of PLL from the films (results not shown). This observation was confirmed using another experiment that demonstrated the presence of PLL in the storage solution. Light microscopy images of uncrosslinked films showed phase separation occurring at chitosan:PLL ratios of 50:50, 40:60 and 20:80. The 50:50 samples showed globule-like structures on the surface, while the 40:60 and 20:80 films showed channel-like formations (Fig. 1). These different topographies might elicit effects on OPCs' attachment and alignment.

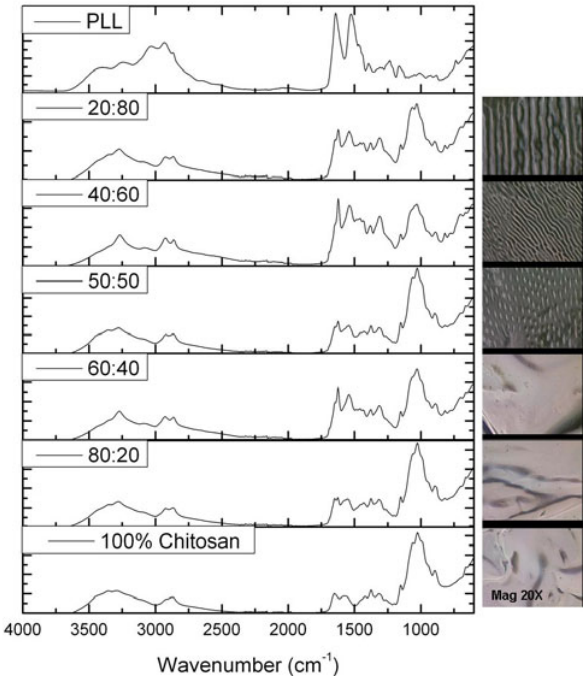

Figure 1. FTIR spectra of the different uncrosslinked chitosan/PLL films. On the right, light microscopy images of films at 20X magnification

Conclusions: To enhance the cell-compatibility of the chitosan substrate, a chitosan/PLL blend was fabricated and crosslinked using genipin. At certain ratios interesting topographical features were acquired, which could alter OPCs' attachment and alignment. Currently, a thorough cell seeding study on all genipin-crosslinked chitosan/PLL samples is underway.

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