Biomimetic Polymer Conduit for Nerve Guidance Following Peripheral Nerve Injury

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Statement of Purpose: Peripheral nerve injury (PNI) occurs from traumatic injury, surgery, or repetitive compression and often leads to loss of function, intractable neuropathic pain, sensory loss, and motor deficits. Neurorhaphy is one surgical repair technique of PNI which involves the direct suturing of discontinued nerve stumps. Although relatively successful at recovering nerve function, neurorhaphy is limited to short nerve gaps as repairs of gaps greater than 5 mm exhibit excessive tension on the nerve, inducing neuronal death. For longer nerve gaps, autografts are considered the gold standard of PNI repair. Autografts currently offer the best results in terms of nerve regeneration, but are also associated with many drawbacks including a second surgical procedure, donor site morbidity, mismatch of donor nerve size, and limited donor nerve length. To avoid these limitations, an electrospun nanofiber polymer scaffold has been developed as a nerve guidance conduit for functional recovery of PNI. A cell-binding peptide and biomimetic anisotropic properties have been incorporated into the design of the nerve guidance conduit. RGD has shown to promote cell differentiation and proliferation and improve healing characteristics in polymeric systems [1]. Uniaxially aligned fibers support and guide regenerating neurites during peripheral nerve repair by increasing axon branching and neurite outgrowth [2].

Methods: Poly(serinol hexamethylene urea) (PSHU) was synthesized using N-Boc-serinol, hexamethylene diisocyanate, and urea. The Boc protecting groups were removed using TFA/DCM to expose free amine groups. RGD was conjugated to the amine groups using EDC/NHS chemistry. Neuronal response was evaluated by seeding PC12 cells onto surfaces coated with either the RGD modified polyurea (PSHU-RGD) or laminin which was used as a negative control. NGF has been shown to induce neuronal differentiation and neurite outgrowth in PC12 cells and used as a positive control when supplemented to the cell cultures. The nerve guidance conduit was produced through electrospinning using a two-electrode setup. Sucrose fibers were drawn and placed between two parallel copper wires which comprised as the collector. PSHU-RGD was blended with polycaprolactone (PCL) in 1,1,1,3,3,3-hexafluoro-2-propanol (HFP) and yielded a flat polymer sheet that was subsequently rolled into a cylindrical tube. The sucrose fibers within the conduit were dissolved by submerging the conduit in water, exposing microchannels.

Results: NMR was used to confirm PSHU production after the initial reaction and showed almost complete removal of the Boc protecting groups after deprotection. FTIR was used to confirm the conjugation of RGD to the PSHU backbone. PC12 cells seeded on PSHU-RGD coated surfaces showed higher levels of attachment, differentiation, and neurite outgrowth compared to PC12 cells seeded on laminin coated surfaces, as shown in Figure 1. Neuronal response of PC12 cells on PSHU-RGD surfaces were similar to that on laminin with NGF, demonstrating the capacity of the polymer to promote neurite outgrowth.

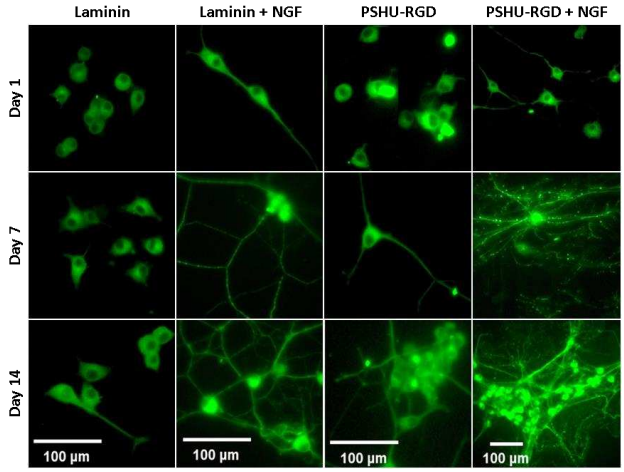

Figure 1. Neuronal response of PC12 cells to laminin and PSHU-RGD with and without NFG. Scale bars apply to respective columns.

Figure 2 shows the electrospun polymer conduit imaged using SEM. The cross-sectional microstructure of the conduit is very similar the structure of nerve bundles in a nerve. The longitudinal section shows uniaxially aligned fibers along the lumen of the microchannels.

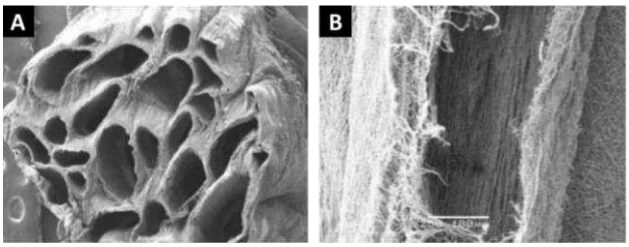

Figure 2. SEM micrographs of (A) cross-section of conduit and (B) longitudinal section.

Conclusions: The potential use of a biomimetic PSHU-RGD/PCL nanofiber scaffold for nerve regeneration has been demonstrated. PC12 cell cultures showed high levels of cell attachment, differentiation, and neurite outgrowth. SEM micrographs show that the microstructure of the nerve guidance conduit is biomimetic as it resembles the structure of the peripheral nerve.

References:

[1] (Zhang P. Biomacromolecules. 2011;12:2667-80.)
[2] (Rangappa N. J Biomed Mater Res A. 2000;51:625-34.)