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Bio-Functionalized Silk Nanofibers for Peripheral Nerve Regeneration

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Abstract : A severe injury to a peripheral nerve leads to its degeneration and the loss of sensory and motor function. To this day, there still lacks a more effective alternative to the autograft which has long been considered the gold standard for nerve repair. In order to overcome the numerous drawbacks of the autograft, tissue engineered biomaterials may be effective alternatives. Silk fibroin is a favorable biomaterial due to its many advantageous properties such as its biocompatibility, its biodegradability, and its robust mechanical properties. In this study, bio-mimicking multi-channeled nerve guidance conduits made of aligned nanofibers achieved by electrospinning were functionalized with signaling biomolecules and were tested in vitro and in vivo for nerve regeneration support. Silk fibroin (SF) extracted directly from silkworm cocoons was put in solution at a concentration of 10wt%. Poly(ethylene oxide) (PEO) was added to the resulting SF solution to increase solution viscosity and the following three electrospinning solutions were made: (1) SF/PEO solution, (2) SF/PEO solution with nerve growth factor and ciliary neurotrophic factor, and (3) SF/PEO solution with nerve growth factor and neurotrophin-3. Each of these solutions was electrospun into a multi-layer architecture to obtain mechanically optimized aligned nanofibrous mats. For in vitro studies, aligned fibers were treated to induce β-sheet formation and thoroughly rinsed to eliminate presence of PEO. Each material was tested using rat embryo neuron cultures to evaluate neurite extension and the interaction with bio-functionalized or non-functionalized aligned fibers. For in vivo studies, the mats were rolled into 5mm long multi-, micro-channeled conduits then treated and thoroughly rinsed. The conduits were each subsequently implanted between a severed rat sciatic nerve. The effectiveness of nerve repair over a period of 8 months was extensively evaluated by cross-referencing electrophysiological, histological, and movement analysis results to comprehensively evaluate the progression of nerve repair. In vitro results show a more favorable interaction between growing neurons and bio-functionalized silk fibers compared to pure silk fibers. Neurites can also be seen having extended unidirectionally along the alignment of the nanofibers which confirms a guidance factor for the electrospun material. The in vivo study has produced positive results for the regeneration of the sciatic nerve over the length of the study, showing contrasts between the bio-functionalized material and the non-functionalized material along with comparisons to the experimental control. Nerve regeneration has been evaluated not only by histological analysis, but also by electrophysiological assessment and motion analysis of two separate natural movements. By studying these three components in parallel, the most comprehensive evaluation of nerve repair for the conduit designs can be made which can, therefore, more accurately depict their overall effectiveness. This work was supported by La Région Picardie and FEDER.

Keywords : electrospinning, nerve guidance conduit, peripheral nerve regeneration, silk fibroin
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