Controlling Dispersion of Axonal Regeneration using a Multichannel Collagen Nerve Conduit

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Introduction

A variety of synthetic and natural materials has been used in peripheral nerve repair, but these studies have yielded limited success. The nerve tissue matrix is composed of basal lamina tubes around the axon-Schwann cell unit and longitudinally oriented collagen fibrils. Previous nerve regeneration studies have been performed predominantly on single channel conduits. However, axons regenerating across single channel tubes may disperse resulting in inappropriate axonal re- innervation. We fabricated a series of collagen-based multichannel nerve conduits and characterized the architectural, material and mechanical properties of these conduits. An in vivo sciatic nerve repair study was performed with the multichannel collagen conduits. The outcomes of nerve regeneration were evaluated by nerve morphometry, functional recovery and target motor neuron re-innervation.

Materials and Methods

1-, 2-, 4- and 7-channel collagen conduits were fabricated by crosslinking with EDC/NHS. The anti-collagenase digestion ability and the morphological change of the conduits in PBS at 37°C were studied. Shrink temperature and ninhydrin studies were performed to show the conduit crosslinking efficiency. The conduit mechanical properties were characterized by tensile, compressive, and 3-point bending tests. The axonal growth of rat embryonic whole dorsal root ganglia (DRG’s) on EDC/NHS crosslinked collagen was studied.

An in vivo study was performed using 72 female Lewis rats. The fabricated multichannel conduits and commercial (Integra®) single channel conduits were implanted to bridge a 10-mm rat sciatic nerve defect. The nerve repair was evaluated 4 months after implantation. Quantitative results of regeneration were analyzed with compound muscle action potential (CMAP) recordings and quantitative nerve morphometry. Qualitative measures of regeneration included simultaneous retrograde axonal tracing and ankle motion analysis. For simultaneous tracing, fast blue and diaminido yellow were applied to the tibial and peroneal nerve branches respectively.

Results

In vitro analysis using collagenase degradation assay, ninhydrin and shrink temperature study (DSC) showed that the optimal concentrations of EDC and NHS to crosslink multichannel collagen conduits are 30mM and 10mM respectively. These concentrations resulted in the lowest degradation rate and highest thermal stability. Swelling studies showed that the crosslinked collagen conduits maintained their initial morphology over the study period in 37°C PBS. Mechanical studies showed the elastic nature of the collagen conduits and that the stiffness of the conduits increased as the number of channels increased. The DRGs maintained their cell viability on collagen films with EDC/NHS crosslinking. In vivo analysis showed that the CMAP amplitude and the number of regenerated axons were significantly better after 4-channel and 1-channel fabricated nerve tube repair compared with 2- and 7-channel fabricated and commercial (Integra®) single-channel nerve tube repair. Simultaneous retrograde tracing demonstrated that 4-channel conduits resulted in less dispersion compared with 1-channel conduits with a significantly smaller percentage of double projecting motoneurons to the tibial and peroneal nerve branches and peroneal nerve branches.

Discussion and Conclusions

This study shows that multichannel collagen conduits with optimal architectural and physico-chemical properties can be fabricated by crosslinking with EDC/NHS. Quantitative results of in vivo regeneration were comparable for 4-channel and 1-channel collagen conduits; however, the 4-channel conduits resulted in less dispersion.

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Disclosures

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