Decellularized Equine Sciatic Nerve as a Scaffold for Peripheral Nerve Repair

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Statement of Purpose Peripheral nerve injury resulting in motor and sensory impairment of the upper extremities affects more than 360,000 individuals annually in the United States, largely as a consequence of trauma. Many nerve injuries can be managed surgically. However, functional recovery is often less than satisfactory.

A number of biologically derived materials have also been investigated for the fabrication of nerve guidance conduits. The use of decellularized allograft nerve tissues as ideal scaffolds for reconstruction of peripheral nerves due to maintenance of the native tissue architecture and functional molecules in their relative tissue specific constituent proportions have been suggested for the fabrication of nerve guidance conduits. However, not all of the proposed decelluarlization methods have been shown to be effective for removal of sufficient cellular content and maintenance of tissue structure, resulting ineffective recovery in some studies.

The objectives of the present study were: (1) to examine the effectiveness of recently described method for central nervous tissue decellularization when applied to derivation of a peripheral nerve repair scaffold composed of equine sciatic nerve, (2) to determine the ability of such degradation products to be fabricated into a hydrogels suitable for injection based approaches for nerve repair.

Methods: Equine sciatic nerve was harvested and then frozen for at least 16h at -80°C. The epineurium was stripped and the tissue was quartered longitudinally and cut into lengths of < 5cm. The decellularization process consisted of a series of agitated washes: water (type 1), 0.02% trypsin/0.05% EDTA (60 min at 37°C), 3.0% Triton X-100 (60 min), water rinse (type 1, repeated until agitation no longer produced bubbles), 1M sucrose (15 min), 4.0% sodium deoxycholate (60 min), 0.1% peracetic acid/4% ethanol (120 min), 1X PBS (15 min), water (15 min), water (15 min), 1X PBS (15 min). Following treatment samples were frozen (-80^{o} C) and then lyophilized.

Enzymatic degradation products were generated as previously described. Briefly, lyophilized scaffold materials were powdered. The powdered material was solubilized at a concentration of 20 mg/mL in a solution containing 2.0 mg/mL pepsin in 0.01 N HCl at a constant stir rate for 48h. Enzymatic digestion was stopped by raising the pH of the solution to 7.4 using NaOH and diluting the solution to the desired concentration with PBS prior to further testing.

Quantitative and qualitative assessments of DNA content were performed, including DNA extraction followed by PicoGreen analysis and gel electrophoresis and slides stained for H&E and DAPI. Additional slides were stained with luxol fast blue to determine removal of myelin. Scaffold architecture was assessed by electron microscopy, histologic staining, and immunolabeling. Immunolabeling was performed with antibodies specific to ECM components indicative of the neuronal basal lamina (collagen IV and laminin).

Results: No nuclei were visible in hematoxylin and eosin stained sections under light microscopy. Few nuclei were observed when samples were labeled with DAPI. When nuclei were observed, they were present within the inner most bundles of the treated tissues. A luxol fast blue stain was performed to assess the maintenance of myelin, a potentially immunogenic component of the axons. Luxol fast blue indicated that the majority of the myelin was removed when observed both in cross section and longitudinally. Myelin can be used as a surrogate measure of decellularization as it is a component of myelinated axons. Quantitative PicoGreen assay showed that dsDNA content of the decellularized tissue (158.07 ± 34.53 ng/mg) was significantly decreased as compared to native tissues (1043.65 ± 291.20 ng/mg) representing an approximately 85% reduction in DNA content. These values are consistent with those reported for FDA approved, commercially available ECM scaffold materials. Gel electrophoresis of the remaining DNA isolated from the scaffold material demonstrated that all remaining residual DNA had a base pair length of less than 200 kDa.

Under SEM, the decellularized sciatic nerves were characterized by an ultrastrurcture similar to that of native tissue. Hematoxylin and eosin stained samples in cross section were characterized by a diffuse epineurium, a dense and intact perineurium and basal lamina devoid of cells. Immunolabeling confirmed the presence of collagen IV and laminin, two major constituents of the basal lamina, within the decellularized scaffold material. Luxol fast blue stains for myelin presented a significant removal of myelin after decellularization.

ECM digest concentrations of 12 mg/mL were able to form stable gels after incubating at a non-humidified 37°C for 1 hour. SEM micrographs of the hydrogel surface showed dense, moderately organized collagen fibrils.

Conclusions: Based on the tests performed, the decellularization protocol adequately removes cellular content and maintains a significant amount of tissue structure. The decellularization method developed produces a viable biomaterial in the form of an injectable hydrogel derived from peripheral nerve for use in peripheral nerve recovery.