Peripheral Nerve Extracellular Matrix Hydrogels for Treating Spinal Cord Injury

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Introduction: Spinal cord injury (SCI) is extremely debilitating and is characterized by cavity formation within the tissue. Using experimental injury models, researchers have shown that grafts of peripheral nerve tissue exhibit vast potential to induce regeneration of spinal axons, in part because of the growth-supportive molecules of the extracellular matrix (ECM). Unfortunately, fresh tissue is often immunogenic upon implantation. Our lab previously developed an optimized method to remove immunogenic cellular components from tissue while preserving the native structure of the ECM [1], and this protocol is now used clinically to prepare ECM grafts for bridging human peripheral nerve defects. In applying this ECM scaffold to repair the injured spinal cord, it is desirable to create an injectable formulation that can applied into the cavity without the need for incising host tissue. To this end, we have created an injectable hydrogel derived from peripheral nerve ECM which exhibits thermal gelation under physiological conditions. Herein we detail the chemical, mechanical, and topographical characterization of this novel material as well as demonstrate in vitro biocompatibility.

Materials and Methods: Peripheral nerves from Sprague Dawley rats were decellularized according to the protocol developed in the Schmidt lab [1]. Acellular nerves were lyophilized, micronized, and digested using a pepsin solution at a pH of 2. After complete digestion, the enzyme was deactivated by neutralizing the pH to 7.4. Thermal gelation was then induced by incubation at 37 °C in a humidified environment. The protein profile of nerve ECM was identified using tandem liquid chromatography-mass spectrometry (LC-MS/MS). Additionally, collagen and glycosaminoglycan content was determined using Sircol and Blyscan assay kits (Biocolor Ltd; UK). An Instron 5542 (Norwood, MA) was used to examine the mechanical properties of this ECM hydrogel, and the topographical features were evaluated via scanning electron microscopy (SEM) after sample dehydration. To examine biocompatibility, primary rat Schwann cells were cultured on nerve ECM substrates in supplemented media, and cell survival was assessed using live/dead staining. Schwann cell metabolic activity in 3D hydrogels was also evaluated using AlamarBlue (Invitrogen).

Results: Protein identification reveals that our decellularization method preserves the relative tissue composition, e.g. the ratio of collagen I to laminin in fresh and acellular nerve is 1.6 and 1.4, respectively. In addition, acellular nerve tissue is shown to retain 83% and 61% of the native tissue's original collagen and glycosaminoglycan content, respectively. SEM images demonstrate that nerve ECM hydrogels are fibrous structures with fiber diameter on the same length scale as that found within native peripheral nerve, roughly 100-150 nm (Figure 1). Additionally, the compressive modulus for nerve ECM hydrogels was found to be 4.5 kPa. Using a live/dead assay, it was found that nerve ECM substrates are biocompatible, supporting 80% survival of primary rat Schwann cells compared to poly-D-lysine positive controls. Cells cultured on these matrices also exhibited an elongated morphology characteristic of Schwann cells whereas cells on dilute (1:10) matrices frequently did not.

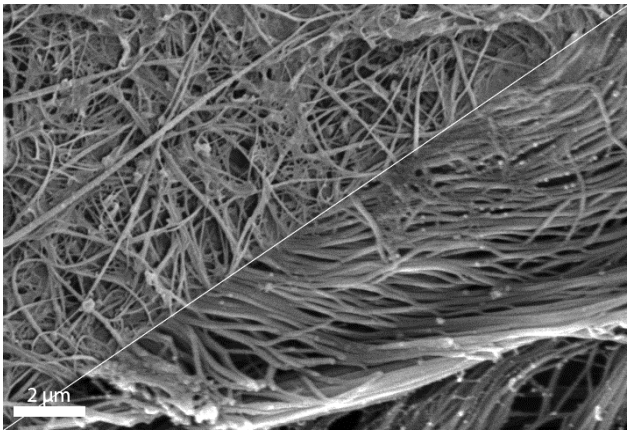


Figure 1: Scanning electron micrographs of peripheral nerve ECM hydrogel (top left) and fresh peripheral nerve (10 µm section, bottom right).

Conclusions: We have developed a protocol to process acellular peripheral nerves into an injectable, in situ gelling hydrogel scaffold. We have shown that this nerve ECM hydrogel approximates the chemical, mechanical, and topographical features of native neural tissue. The collagen and glycosaminoglycan content of peripheral nerve is comparable to that reported for similar tissues including brain and spinal cord [2], supporting the hypothesis that this ECM hydrogel will mimic the native spinal cord to promote tissue regeneration. In addition, the hydrogels exhibit a compressive modulus similar to that reported for native rat neural tissue [3] and optimal for neurite outgrowth [4]. We hypothesize this material will mimic the native tissue environment in vivo to promote tissue repair following SCI, thereby offering a clinically-relevant material to treat debilitating neural injury.

References:

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