

Extracellular Matrix Hydrogels Derived from Optimized Acellular Peripheral Nerve

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Statement of Purpose: The extracellular matrix (ECM) plays pivotal roles in cell differentiation, migration, inflammation, and wound healing. This multifunctionality makes the ECM a unique and attractive biomaterial for developing tissue regeneration strategies. Our lab previously developed an optimized method to remove cellular components from tissue while preserving the native structure of the ECM [1]. This optimized acellular protocol is now used clinically to prepare grafts for repairing peripheral nervous system defects in humans. In many injury models, unfortunately, the preformed structure of acellular tissue would necessitate damaging host tissue for graft application. To address this concern, we have created an injectable hydrogel derived from peripheral nerve ECM (pnECM) which undergoes 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.

Methods: Peripheral nerves from Sprague Dawley rats were decellularized according to the optimized acellular (OA) protocol [1]. Acellular nerves were lyophilized, micronized, and digested using a pepsin solution at a pH of 2. After an extended time, digestion was arrested by raising the pH to 7.4. Thermal gelation was induced by incubation at 37 °C in a humidified environment. Protein identification was performed using tandem liquid chromatography-mass spectrometry (LC-MS). Briefly, 10 µg of protein was desalted using brief gel electrophoresis. Bands were excised and subjected to in-gel tryptic digestion before analysis. Additionally, collagen content was determined using Sircol assays (Biocolor Ltd; UK). An Instron 5542 (Norwood, MA) was used for compressive testing, and samples for scanning electron microscopy (SEM) were dehydrated via graded solutions of ethanol and hexamethyldisilazane. To examine biocompatibility, primary rat Schwann cells were cultured on pnECM substrates in supplemented DMEM-F12 media, and cell viability assessed using a live/dead assay.

Results: Protein identification using LC-MS revealed that the ratios of collagen to laminin were 1.4 and 1.6 for OA nerve and fresh nerve, respectively. The collagen content of pnECM hydrogels was found to be between 0.49-0.63 mg collagen/mg dry tissue. This value is comparable to that reported for similar tissues, including brain [2]. Analysis of SEM images demonstrated that the fiber

diameter within pnECM hydrogels is on the same length scale as that found within native peripheral nerve, roughly 100-150 nm (Figure 1). Additionally, compressive modulus for pnECM hydrogels was found to be within the range of rat neural tissue [3]. Using a live/dead assay, it was found that Schwann cells cultured with pnECM substrates demonstrated 80% survival compared to poly-D-lysine controls. Cells cultured on these matrices were also observed to elongate comparable to controls whereas cells on dilute (1:10) matrices frequently did not elongate.

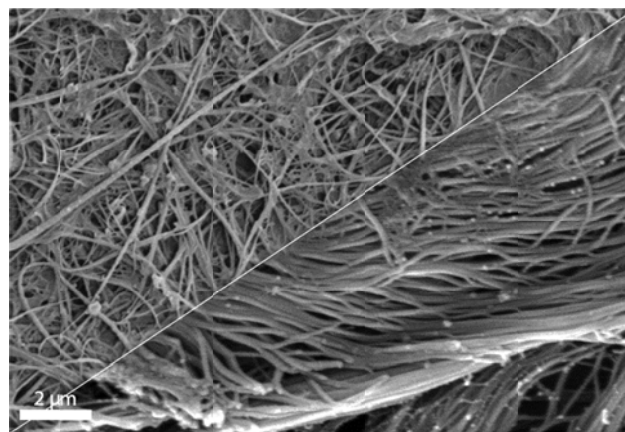


Figure 1: Scanning electron micrographs of peripheral nerve ECM hydrogel (top left) and fresh peripheral nerve (10 µm section, bottom right).

Conclusions: We have developed a protocol to transform optimized acellular peripheral nerves into an injectable, *in situ* gelling hydrogel scaffold. Using this protocol, we have shown that our decellularization protocol preserves the relative chemical composition (e.g., collagen: laminin) of native neural tissue. In addition, the hydrogels exhibit compressive mechanical properties similar to that reported for native rat neural tissue. Analysis of scanning electron micrographs revealed that native topographical cues such as fiber diameter are approximated within these pnECM hydrogels. Conclusively, this novel material closely mimics the native environment of nervous tissue. We hypothesize that the compositional, mechanical, and topographical properties of this material will help promote tissue repair and functional recovery, thereby offering a clinically-relevant material for improving patient outlook following debilitating neural injury.

References:

- [1] Hudson TW et al. *Tissue Eng.* 2004, 10 (1346-58).
- [2] Medberry CJ et al. *Biomaterials.* 2013, 34(1033-40).
- [3] Seidlits SK et al. *Biomaterials.* 2011, 31(3930-3940).