Decellularized Equine Sciatic Nerve Hydrogel for Peripheral Nerve Repair

Samuel T. Lopresti[1,2], Travis A. Prest[1,2], Christopher J. Medberry[1,2], Matthew J. Martin[3], Stephen F. Badylak[1,2,4], Jonathan Cheetham[5], Bryan N. Brown[1,2,*]

1. Department of Bioengineering, University of Pittsburgh, 2. McGowan Institute for Regenerative Medicine, University of Pittsburgh, 3. Department of Biomedical Engineering, Cornell University, 4. Department of Surgery, University of Pittsburgh, 5. Department of Clinical Sciences, Cornell University

Statement of Purpose: Peripheral nerve impairment, causing loss of motor and sensory function in the upper extremities, is a common result from trauma injury, affecting more than 360,000 people. Many alternatives to traditional nerve auto-grafting, including synthetic and biological scaffolds, have been created for peripheral nerve repair. A recently described protocol for the decellularization of nervous tissues was used to maintain increased structural and functional components of native tissues as compared to other methods. The degradation products of decellularized equine sciatic nerve were shown to have neurogenic properties and to be able to form a hydrogel that could support nerve growth.

Materials & Methods: Equine sciatic nerve was dissected to remove the outer sheath. The nerve was then decellularized through a series of agitated washes: water, trypsin/EDTA, sucrose, sodium deoxycholate, peracetic acid/ethanol, 1X PBS, water, 1X PBS. The tissue was frozen then lyophilized. Tissue was milled into fine particulates. ECM degradation products were created by solubilizing 20 mg/mL of ECM for 48 hr in 2mg/mL pepsin / 0.1N HCl with constant stirring. Enzymatic digestion was neutralized by raising the pH of the solution to 7.4 and diluting in PBS to the desired concentration. The neural cell line N1E-115, a commonly used cell line to examine neurite extension, was used to identify neurotrophic potential of enzymatic degradation products in two-dimensional culture. The effects of both nerve and urinary bladder matrix (UBM) ECM were upon N1E-115 neurite extension were assessed as an indicator of neurotrophic potential of the bioactive molecules present in the ECMs. N1E-115 cells in DMEM with 2.5% FBS/1% pen/ strep were seeded at a density of 8,500 cells/well in a 96 well plate, which allowed neurite extension in response to ECM digests to be quantified. After 16 hours, neutralized ECM digests were added to the media at concentrations of 1µg/mL, 10µg/mL, 50µg/mL, and 100µg/mL and cells were incubated for 48h. N1E-115 cells were fixed with 50:50 methanol:acetone for 5 minutes at room temperature. Attached cells were stained for F-actin filaments using Alexa Fluor phalloidin 488, and nuclei were counterstained with DAPI. Six images at 20X magnification were taken per well. The number of cells with neurite extensions and the length of neurite extensions were counted using Neurite Tracer NeuronJ, an ImageJ (NIH) add-on. ECM hydrogels were formed as previously described. Briefly, gelation was induced by adjusting the pH of the pepsin digest to 7.4 using 0.1M NaOH and PBS. Neutralization was accomplished by the addition of one-tenth the digest volume of 0.1 N NaOH, one-ninth the digest volume of 10X PBS, and then diluting to the desired final ECM concentration. Concentrations of 20, 15, and 10 mg/mL were examined for their ability to form a hydrogel. Dilutions were performed on ice and ECM pre-gel was cast into a stainless steel ring within a 6 well cell culture cluster. The gel solution was placed in a non-humidified 37°C and allowed to gel for 1h. Resultant hydrogels were then investigated using SEM as described above. N1E-115 cells, were cultured in DMEM with 10% fetal bovine serum (FBS) /1% pen/strep in T-75 flasks. N1E-115 cells in DMEM with 2.5% FBS/1%pen strep were seeded at a concentration of 8,500 cells on the surface of a 12mg/mL nerve or UBM ECM gel in a 96 well plate. Wells seeded with cells in a media containing pepsin digest instead of ECM digest were used as controls. Following 18-24hrs in culture with ECM, 2µM calcein-AM and 2µM ethidium homodimer-1 was added to each well to evaluate cytotoxicity. Membrane-permeable calcein-AM, but not ethidium homodimer-1, is hydrolyzed in live cells that fluoresce in green and dead cells that bind and activate ethidium homodimer-1, but not calcein-AM, fluoresce in red.

Results After 24 and 48 hours of incubation within the equine sciatic nerve hydrogel, the viabilities of the neurite cells were in excess of 95%. For the neurite outgrowth assay, the average length of neurites exposed to nerve ECM degradation products was 386.92 ± 218.41 for the 50 ug/mL case. The pepsin control for that case had an average length of 308.50 ± 176.24. The SEM of the hydrogel showed cross-linking of the collagen fibers that were present in the ECM degradation products.

Conclusions Equine sciatic nerve hydrogels provide an adequate substrate for neuronal growth. The degradation products of the hydrogel matrix are not cytotoxic to nerve cells. Also, based on the results of the neurite outgrowth assay, the ECM degradation products do not inhibit neurogenicity. The SEM shows that a proper hydrogel matrix is formed to provide an adequate 3D environment for neurons to differentiate and proliferate.

References

Medberry, CJ et al. *Hydrogels derived from central nervous system extracellular matrix*. Biomat. 2012 Nov 15. S0142-9612(12)01213-6.

Crapo, PM et al. *Biologic scaffolds composed of central nervous system extracellular matrix*. Biomat. 2012 May; 33(13):3539-47.

Abstract #494

©2013 Society For Biomaterials