Astrocyte Extracellular Matrix Incorporation Improves Neurite Growth on Hyaluronic Acid Hydrogels

Russell Thompson[1,2], Lindsey Crawford[1], Shelly Sakiyama-Elbert[2]
[1] Department of Biomedical Engineering, Washington University in St Louis
[2] Department of Biomedical Engineering, University of Texas at Austin

Statement of Purpose: Every year 12,000 Americans suffer a traumatic spinal cord injury (SCI), which often causes some level of paralysis and costs between $1 and 4.5 million over their lifetimes[1]. One major challenge for the treatment of SCI is the limited regenerative capacity of the adult mammalian spinal cord. This lack of regeneration is due, in part, to astrocytes forming a highly organized glial scar that serves to limit the extent of secondary injury. However, the glial scar is both a chemical and physical barrier to axon growth. Interestingly, it has been observed that astrocytes are also present after SCI where axons are able to grow into the lesion site[2]. Taken together, these observations suggest that astrocytes can have both pro-regenerative roles and inhibitory roles after SCI. We have previously derived two major spinal astrocyte subtypes, fibrous (white matter) and protoplasmic (grey matter) astrocytes, from mouse embryonic stem cells (mESCs). We found that neurons extend significantly longer axons on protoplasmic astrocyte-derived substrates than on fibrous astrocyte-derived substrates. Furthermore, we determined that decellularized protoplasmic astrocyte matrices could support neuron growth, suggesting that protoplasmic extracellular matrix (ECM) was sufficient to permit axon extension. This led us to harvest the ECM deposited by both protoplasmic and fibrous astrocytes to test whether astrocyte ECM incorporation improves neurite growth on injectable hyaluronic acid (HA) hydrogels. Since decellularized, xenogenic ECM materials have been successfully transplanted without requiring immuno-suppression[3], we hypothesize that these HA-ECM materials can be transplanted into a rat SCI model and improve histological recovery without requiring immunosuppression (as is needed for live xenogenic cells).

Methods: Both astrocyte subtypes were derived from RW4 mESCs using bone morphogenetic protein 4 (BMP-4) to generate protoplasmic astrocytes and ciliary neurotrophic factor (CNTF) to generate fibrous astrocytes. After 6 days of culture, astrocyte plates were decellularized using a modified Hudson protocol[4], and the ECM was harvested and lyophilized with 50 mM trehalose. For the hydrogels: HA-furan was synthesized from 250 kDa HA (Creative PEGWorks, Chapel Hill, NC), furfurylamine (Sigma, St Louis, MO), and 4-(4,6-Dimethoxy-1,3,4-triazin-4-yl)-4-methylemorpholinium chloride (TCI America, Portland, OR) as previously described[5]. HA-furan was then mixed with various weight ratios of ECM along with PEG-dimaleimide so that a hydrogel formed. The ability of the resulting gels to support neurons was assessed using pure mESC-derived motor neuron cultures[6].

Results: We have successfully harvested ECM from mESC-derived astrocytes. Furthermore, ECM incorporation improved neurite outgrowth on HA hydrogels *in vitro*. We also observed some dose dependency in these studies with neurite growth improving as ECM concentration increased (**Figure** 1). In addition, we observed that the protoplasmic ECM was more potent in supporting axon growth than the fibrous ECM, consistent with our studies on decellularized ECM alone. We transplanted hydrogels conjugated with Alexa Fluor® 555 without ECM incorporation into rats following SCI and could detect the hydrogels for up to 2 weeks following transplant. This demonstrates that these HA gels have sufficient stability for use as a SCI treatment.

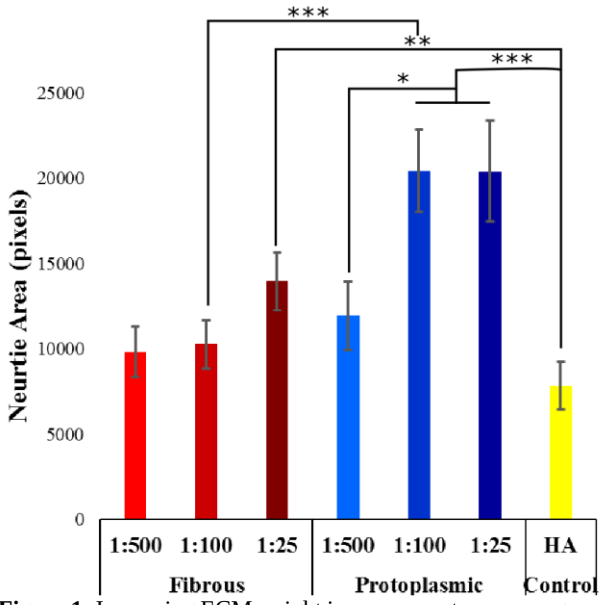

Figure 1: Increasing ECM weight improves motor neuron growth on HA hydrogels after 72 hours in culture. Ratios given are weight of ECM to weight of HA. Error bars: std. error, n=25-45, *: p<0.05, **: p<0.01, ***: p<0.001

Conclusions: These results demonstrate that protoplasmic astrocyte-derived ECM harvested from tissue culture maintains neuronal growth benefit. This suggests that the neurite growth permissive properties of this ECM are intrinsic to the deposited components. Overall, HA-ECM hydrogels could represent a novel approach for improving regeneration following SCI. These gels have many useful properties for translation: the ready capacity of ESCs for expansion, easy storage, and injectability. Future work will focus on the ability of HA-ECM hydrogels to improve neurite infiltration into a SCI lesion area in rats and determining if there are any signs of immune response to the HA-ECM.

References: 1. (Boakye M. *J Neurosurg Spine*, 2012;17:29-37); 2. (Zukor, K, *J Neurosci* 2013, 33:15350-61); 3. (Hudson, T *Tissue Eng* 2004, 10:1641-51); 4. (Hudson, T *Tissue Eng* 2004, 10(9-10):1346-58) 5. (Nimmo, C *Biomacromolecules* 2011, 12(3):824-30) 6. (Dodd LG. Am J Clin Pathol. 1990;93:141-144.)