Effect of Surface Modulus and Extracellular Matrix (ECM) Adhesion Proteins on PC12 Cell Proliferation and Neurite Outgrowth

Shailly H. Jariwala[1], Eddie Bevilacqua[2], Julie M. Hasenwinkel[1]

[1]Department of Biomedical and Chemical Engineering, Syracuse University, Syracuse, NY 13244

[2]Department of Forest and Natural Resources Management, State University of New York, College of Environmental Science and Forestry, Syracuse, New York 13210, USA

Statement of Purpose: It is crucial to establish axonal regeneration following a spinal cord injury (SCI). Lately, hydrogels have been used as substrates to study how neurite outgrowth is affected by varying mechanical properties of substrates [1, 2]. Extracellular matrix (ECM) plays an important role in cell migration, adhesion, and differentiation, and thus it is important to look at the role of ECM proteins in nerve regeneration[3]. Polyacrylamide (pAA) hydrogels are being investigated in this study as a model substrate material for looking at neurite outgrowth in vitro since they are optically clear, their mechanical properties are easily tunable and their macroporous structure allows for penetration of media to provide a physiological environment. The goal of this study is to compare neurite outgrowth of pheochromocytoma (PC12) cells on pAA substrates of varying modulus coated with different ECM adhesion proteins.

Methods: Micromechanical Testing of pAA Hydrogels: The four compositions of pAA gels were prepared by varying the concentration of acrylamide (3, 5, 8 and 10 % wt/vol) while the crosslinker (bisacrylamide) concentration was kept constant at 0.1% wt/vol. pAA hydrogels were prepared according to Wang and Pelham[4]. The micromechanical testing was done using a custom built mesoindentation system for gels with and without proteins[5]. The indenter probe was 174 µm in radius with a spherical geometry. The indenter probe was positioned 100 µm above the sample surface and driven into the sample until a predefined load was reached (8 µN).

Cell Culture: PC12 cells (ATCC, VA), a rat adrenal pheochromocytoma cell line, were induced by nerve growth factor (NGF, 50ng/ml) into a neuronal phenotype. Four compositions of polyacrylamide with tissue culture polystyrene (TCPS) wells used as controls were coated with ECM adhesion proteins collagen, laminin and fibronectin for cell culture experiments at concentrations of 50µg/ml, 10µg/ml and 50µg/ml, respectively. Proteins were adsorbed on the surface using polylysine and also covalently bound by photo activation of sulfo-Sanpah crosslinker (Pierce Biotechnology, IL) [4]. Since moderate cell adhesion was observed with proteins adsorbed at the surface using polylysine, cell adhesion studies were performed on 8% gel substrate by covalently binding the proteins at the gel surface using sulfo-Sanpah and varying the concentration of proteins bound at the surface of 8% pAA gel from 10ug/ml to 100ug/ml.

Results: The Young's modulus increases linearly with acrylamide content in pAA hydrogels. The Young's moduli for all four compositions are as follows: 3% gel (E= 0.26± 0.01 kPa), 5% gel (E= 3.75±1.31kPa), 8% gel (E=13.4± 1.89 kPa) and 10% gel (11.52±1.58 kPa). From the one way ANOVA results we saw that the moduli are significantly different ($p<0.001$) as a function of acrylamide concentration. The varying surface modulus as well as surface chemistry affects the neurite outgrowth in PC12 cells. The neurite lengths are significantly higher ($p<0.001$) for the 8 % (E=13.4± 1.89 kPa) gel substrates but only with the proteins fibronectin and laminin, for surface adsorbed proteins as shown in Figure 1. Better cell adhesion was seen upon covalently binding collagen onto substrates as compared to its adsorption on the surface of gels as shown in Figure 2. Cell adhesion was poor for substrates which had laminin and fibronectin covalently bound at the surface of the gels. Neurite lengths were observed to be optimized on substrates with covalently bound surface collagen.

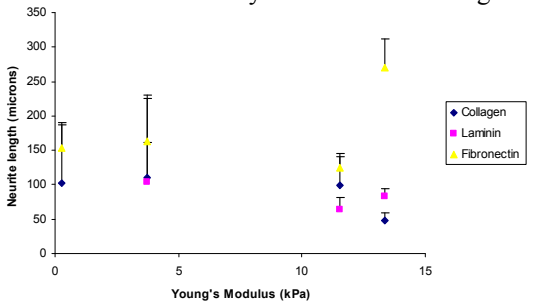
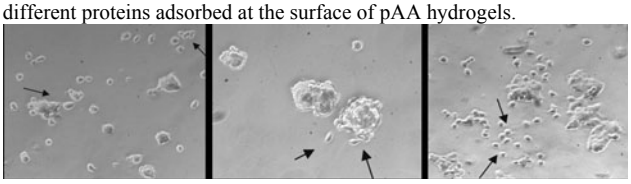


Figure 1. Neurite length, at day 7, as a function of modulus for the different proteins adsorbed at the surface of pAA hydrogels.



a. Laminin (50ug/ml) b. Fibronectin (50ug/ml) c. Collagen (50ug/ml)

Figure 2. Day 5 light micrographs of PC12 cells on 8% pAA gel substrates with a. laminin b. fibronectin and c. collagen covalently bound at the gel surface. Cell adhesion and neurite outgrowth was observed to be higher on collagen coated pAA gels as compared to fibronectin and laminin. Arrows indicate sprouting of processes.

Conclusions: The Young's modulus of the hydrogels increases linearly with acrylamide content as seen in previous studies [1, 2]. Neurite lengths for 8% gels are significantly higher than 3, 5 and 10% gels. For 8% gel composition where proteins were adsorbed at the surface, the fibronectin coating showed significantly higher neurite lengths, followed by laminin; but no significant neurite outgrowth was observed on collagen coated substrate. Cell adhesion and neurite outgrowth of PC12 cells was observed to be better on 8% gels having collagen covalently bound at their surface as compared to adsorbed collagen, but was low for covalently bound laminin and fibronectin. These results suggest that PC12 cell neurite outgrowth is optimized with covalently bound collagen on the 8% gel. Future studies will use this surface treatment to further explore modulus effects.

References: 1. Leach JB et al. J. Neural Eng. 2007; 4:26-34 2.Flanagan LA et al. Dev. Neurosci. 2002; 13:2411-15 3. Attiah DG et al. J. Mat. Sci: Mat. in Med. 2003; 14:1005-1009 4. Wang YL and Pelham JR. Methods in Enzymology 1998;298:489-96 5. Saxena et al. JBMR A 2008, In press.