Effect of Microtopography and Surface Modulus on Pheochromocytoma (PC12) cell Neurite Outgrowth

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Statement of Purpose: It is crucial to establish axonal regeneration following a spinal cord injury (SCI). Functional recovery post-injury is dependent on successful guidance of regenerating axons to their appropriate targets along with formation of functional synapses. Lately, hydrogels have been used as substrate materials to study how neurite outgrowth is affected by varying topographical properties [1, 2].The effect of microtopography in guiding neurite outgrowth has been evaluated by micropatterning of polyacrylamide hydrogels. The goal of this study is to compare neurite outgrowth of PC12 cells on patterned versus non-patterned pAAm substrates of varying modulus.

Methods: The three compositions of pAAm gels were prepared by varying the concentration of acrylamide (Acros Organic,NJ) as 5, 8 and 10 % wt/vol, while the crosslinker (bisacrylamide, Fisher Scientific,NJ) concentration used was 0.1% wt/vol. The non-patterned pAAm hydrogels were prepared according to Wang and Pelham[3]. The pAAm gels were micropatterned using a master pattern of parallel ridges of dimensions 15 µm x 15 µm x 5 µm (ridge width x groove width x ridge height), etched on a silicon wafer. About 100 µl of the pre-polymer solution was directly pipetted onto the silicon wafer and covered with an activated coverslip. Upon complete polymerization, the coverslip bound gel was gently peeled off of the silicon wafer. Three compositions of patterned and non-patterned pAAm gels were coated with 50 µg/ml of rat tail type-I collagen and this functionalization was done by photo activation of sulfo-SANPAH crosslinker (Pierce Biotechnology, IL) [3]. The micromechanical testing was done using a custom built mesoindentation system for non-patterned gels with collagen [4]. Briefly, the indenter probe was positioned approximately 100 µm above the sample surface and driven into the sample until a predefined load was reached (8 µN), after which the indenter probe was retracted [4]. PC12 cells (ATCC, VA) were seeded at a density of 5000 cells/well and induced by nerve growth factor (NGF, 50ng/ml) into a neuronal phenotype. Neurite lengths were measured each day for a period of 7 days using ImagePro and were reported as mean length ± standard deviation (n=3, five image fields per sample). The overall direction of neurite was measured as an absolute value of the angle formed between the line parallel to the microchannel wall (or image horizontal axis for non-patterned gels) and the line connecting the point at which the neurite emerges from the cell soma to few micrometers of the growing tip of the neurite.

Results: Figure 1 shows the comparison of light micrographs from patterned and non-patterned hydrogels, with PC12 cells cultured on them. Figure 2(a-c) is the graph showing the orientation of neurites where the data is divided into sectors of 10° from 0° to 90°, 0° being along the direction of the pattern. About 85% of the neurites on the patterned substrates were aligned within 30° of the micropattern as shown graphically. The graph in Figure 2(d) depicts the neurite lengths varying with respect to the modulus for the patterned and non-patterned substrates.

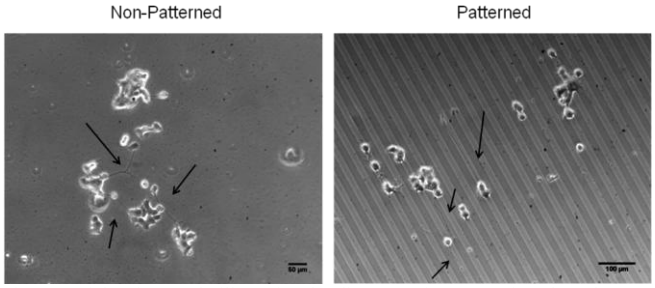
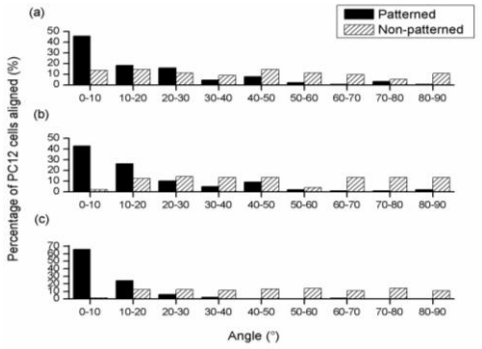


Figure 1. Light micrographs illustrating the alignment of PC12 neurites along the micropatterns compared to their random orientation on non-patterned substrates.



(d)

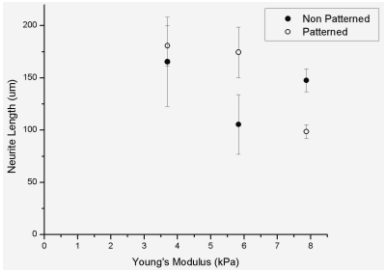


Figure 2. Analysis of neurite alignment on patterned versus non-patterned substrates. The data above are grouped in 10°sectors for patterned and unpatterned gels varying in acrylamide content: (a) 5% wt/vol, (b) 8% wt/vol and (c) 10% wt/vol, (d) Graph shows neurite length as a function of modulus for micropatterned and non-patterned gels. Error bars indicate mean ± one standard deviation.

Conclusions: On the 5% gels, over 80% of the neurites, and approximately 79% and 96% of neurites on the 8% and 10% gels respectively, were aligned within 30° of the micropattern as illustrated in Figure 2. Neurites grown on the non-patterned substrates were randomly oriented and no particular bias in alignment was observed. The 5% ($p = 0.011$) and 8% ($p = 0.019$) patterned gels had significantly longer neurite lengths as compared to 10% patterned gels. These results suggest that PC12 cell neurite outgrowth can be guided using microtopography and further studies should explore the formation of functional synapses and cell signaling.

References: 1) Mahoney MJ. *Biomaterials*. 2005; 7:771-8. 2) Recknor JB, Sakaguchi DS, Mallapragada SK. *Biomaterials*. 2006; 22:4098-108. 3) Wang YL and Pelham JR. *Methods in Enzymology* 1998; 298:489-96. 4) Saxena T. *JBMR A.* 2008, *In press.*