Effect of a Young's Modulus Gradient on Neurite Extension in PC12 Cells

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Statement of Purpose: The purpose of this study was to determine if thin polyacrylamide (pAAm) hydrogels with a gradient in Young's modulus (E) could be synthesized via photopolymerization. A further aim was to study the effect of this gradient in Young's modulus and laminin micropatterning on the neurite extension behavior of nerve growth factor (NGF) induced pheochromocytoma (PC12) cells, a rat adrenal gland tumor cell line that demonstrates neuronal characteristics under influence of NGF.

Materials: Thin pAAm hydrogels (8% acrylamide and 0.3% bis-acrylamide (crosslinker) along with 1% photoinitiator Irgacure 2959 were photopolymerized on glass coverslips activated according to Pelham and Wang (1). To obtain the desired gradient in Young's modulus, grayscale filter masks designed using Adobe Photoshop, were placed between the UV lamp and pre-polymer solution during polymerization. The hydrogels were swollen to equilibrium before testing.

Mechanical testing and analysis: Samples were indented using a custom built mesoindenter (2), operated in load-control mode. The tip geometry was spherical (radius 168 µm). Samples were submerged under deionized water during the entire course of testing. Three samples were indented in a straight line across the sample to map the profile of the gradient in modulus. The data was plotted as modulus versus location. Controls without a gradient were indented at 4-5 random locations. The tip, attached to a cantilever, was driven into the sample using a motor until the predefined load (8µN) was reached (loading cycle) and then retracted until it came off the surface (unloading cycle). The sample displacement and cantilever deflection were continuously monitored during the entire test and a resulting load-displacement curve was obtained. A custom written routine in Matlab was then used to filter and analyze the data. Young's modulus was calculated using Hertzian contact mechanics and a correction factor was applied for thin samples to account for substrate effects (3).

Neurite outgrowth assay: PC12 cells were cultured at a density of 5000 cells/well on the hydrogels in a serum free medium with 50 ng/mL supplement of NGF. Laminin (10 µg/mL) was uniformly coated or micropatterned as lanes (15 µm wide) on the hydrogels to facilitate cell adhesion. Micropatterning simplified analysis as the cells were forced to put out processes either up the gradient or down the gradient. Neurite outgrowth images captured on day 6 were measured using Image J. One way ANOVA statistical analysis and Tukey-Kramer post-hoc tests were performed and p-value of < 0.05 was considered to be significant.

Results: Photopolymerized pAAm hydrogels fabricated using a grayscale intensity filter mask to alter the exposure to UV light, resulted in thin, flat hydrogels with a thickness of ~ 270 µm. On performing mechanical characterization, using a custom built mesoindenter, the gradient obtained was 3.67 Pa/µm which decreased slightly to 2.54Pa/µm (Figure 1) following the treatments preceding cell culture experiments. The average neurite length for PC12 cells on controls uniformly coated with laminin that bore no gradient in modulus was 61 ± 15 µm while on samples without a gradient but a laminin micropattern, the average length was 124.3 ± 12.34 µm. It was hypothesized that PC12 cells would preferentially put out longer neurites in the direction of decreasing modulus as neuronal cells prefer softer substrates. As shown in Figure 2, a Young's modulus gradient of 2.54 Pa/µm demonstrated no significant effect ($p >> 0.05$) on the neurite outgrowth behavior of PC12 cells with respect to the outgrowth directionality.

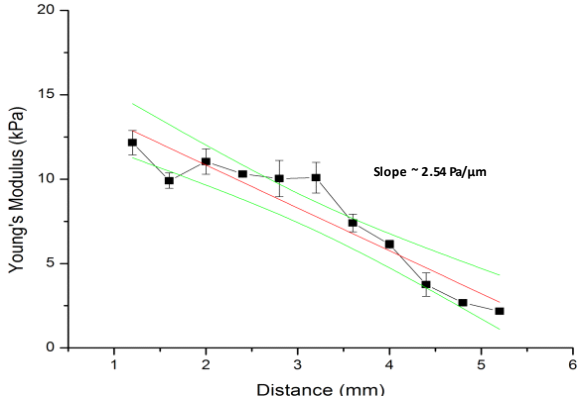

Figure 1. Average Young's modulus profile across the length of the gradient samples from the left to the right end. Sample dimensions were 6 mm x 6 mm. The gradient obtained was ~2.54 Pa/µm.

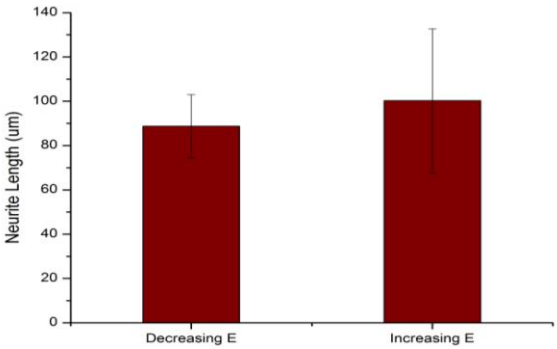

Figure 2. Average length of neurites growing in the direction of decreasing modulus vs. in the direction of increasing modulus on gradient samples. There was no statistically significant difference in the average neurite lengths, indicating that this gradient does not have any effect of the preference of neurite outgrowth in PC12 cells.

Conclusions: pAAm hydrogels with a consistent gradient in Young's modulus (3.67 Pa/µm) were successfully fabricated via photopolymerization technique using simple photomask printed on regular transparencies. Micropatterning of laminin on control samples with no gradient in modulus, induced the NGF treated PC12 cells to give out longer neurites compared to uniformly coated hydrogel substrates. The Young's modulus gradient of 2.54 Pa/µm used in the neurite outgrowth study had no significant effect on the process outgrowth preference for the NGF treated PC12 cells. Future work will focus on testing other gradients in Young's modulus on neurite extension in PC12 cells and primary neurons.

References: 1) Pelham and Wang, Proc. Natl. Acad. Sci., 1997, 13661. 2) Saxena et al. JBMR A., 2008, in print 3) Dimitriadis et al. Biophysical Journal 82; 2002 (2798-2810)