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# SPINAL CORD NEURONAL CELL PROPERTIES RESPOND DIFFERENTIALLY TO THE STIFFNESS OF DNA CROSSLINKED HYDROGELS

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## INTRODUCTION

Mechanical cues arising from extracellular matrices greatly affect cellular properties, and hence, are of significance in designing biomaterials. Similar to many other cell types, including fibroblasts and hepatocytes, central nervous system (CNS) neurons have been found to exhibit distinct responses to the stiffness of the substrates they reside on [1]. There is an increasing awareness that mechanical properties also play a key role in successful utilization of scaffolds for those tissues whose major functions are not load-bearing, such as the spinal cord. In light of this, there is a growing interest in incorporating mechanical cues in biomaterial design for neural tissue engineering applications, including spinal cord injury.

Using cell culture systems, a bis-crosslinked polyacrylamide hydrogel has been frequently used as a substrate for cellular growth. Introducing DNA crosslinking mechanism into gel formation should provide new opportunities, such as modifying the mechanical properties of the gel with cell present. Here we report that different neuronal cellular properties of spinal cord cells respond differentially to the range of stiffness presented by a DNA crosslinked polyacrylamide hydrogel.

## MATERIALS AND METHODS

The base sequences for the DNA strands used in this structure were designed in a manner similar to that reported by Lin and colleagues [2]. These customized DNA strands, some modified with Acrydite™, were manufactured by Integrated DNA Technologies, Coralville, IA.

DNA gels were prepared according to the protocol previously outlined [2]. Briefly, Acrydite™ modified DNA single strands SA1 and SA2 were co-polymerized with 10% acrylamide monomer, initiated by APS and catalyzed by TEMED to form polymer solutions. The solutions ontained long polymer chains with SA1 or SA2 as side chains. They were then mixed, and a solution containing crosslinker L2 (3 mM in buffer containing 0.01 mM tris, 0.001M EDTA, pH approximately 8.0) was added to the mixture to form gels.

These DNA gels were then immobilized on cover glasses, and functionalized allowing for protein conjugation, as detailed in (4). Spinal cord cell culture, immunocytostaining and DAPI staining were performed in a way similar to that in [3]. The mean primary dendrite length, primary dendrite number, and axonal length were recorded for each neuron and grouped for each gel condition.

## RESULTS AND DISCUSSION

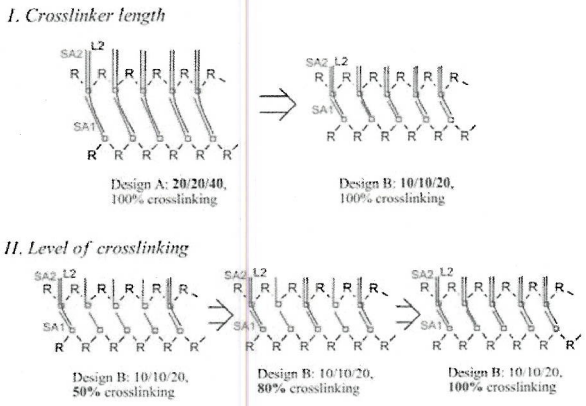



Figure 1. Two schemes of DNA gel comparisons. I. Changes in the length of crosslinkers at full crosslinking; II. Changes in the level of crosslinking (50%, 80% and 100%) with the same crosslinker length. *R* indicates the monomer of vinyl polymers.

Two schemes of comparisons were made for DNA crosslinked gels as shown in Fig. 1. In Scheme I, the effect of crosslinker length on the mechanical properties of the gels was studied, and the cells were plated onto the gels of two different designs at full crosslinking. The crosslinker length in Design A is double that of Design B. In Scheme II, the dependency of mechanical properties on the level of crosslinking for gels with Design B was investigated. Mechanical testing was performed at room temperature.

To assess the effect of substrate stiffness on outgrowth of the primary dendrites (as identified by MAP2+ immunostaining), the number of primary dendrites was counted for each neuron, and the length of each dendrite was measured only for those neurons with clear morphology.

Rat spinal cord neurons exhibit typical multipolar morphology on DNA gels and MAP-2 immunostaining demonstrates neurite outgrowth on DNA gels (Fig. 2). Neurons extended processes (Fig. 2A). There were a variety of morphologies seen in GFAP+ mature astroglia, such as elongated fibroblastic and process bearing cells. Among the intact cells on DNA gels, approximately 60% were MAP2+ neurons and GFAP+ mature astroglia, although the proportion of each cell type varied between 20% and 40%. No significant differences in mean (Fig. 2B), maximal, or total (data not shown) primary dendrite length per neuron were found for cells on DNA gels with stiffness ranging from 5 to 60 kPa. However, there was a significant difference ($p < 0.01$) in primary dendrite number between gels with greatest stiffness (60 kPa) and the other three stiffnesses, and the increase is approximately 10% (Fig. 2C).

The length of axons, identified by Tau-1 immunostaining (Fig. 3A), was measured, and comparison of axonal length per neuron on different DNA gel groups revealed a decrease of approximately 25% from gels with stiffnesses less than 10 kPa to those of tens of kPa (Fig. 3B). It should also be noted that there is no significant difference in neurite outgrowth on DNA gels with stiffness of 5 kPa (Design B, 50% crosslinking) and 10 kPa (Design A, 100% crosslinking) (Fig. 7), suggesting that physical properties other than stiffness do not influence neurite outgrowth behavior.

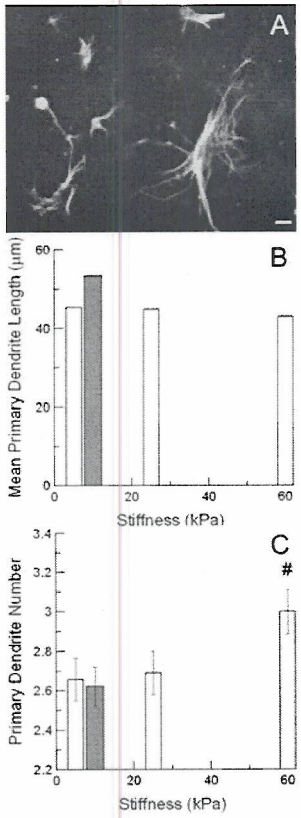
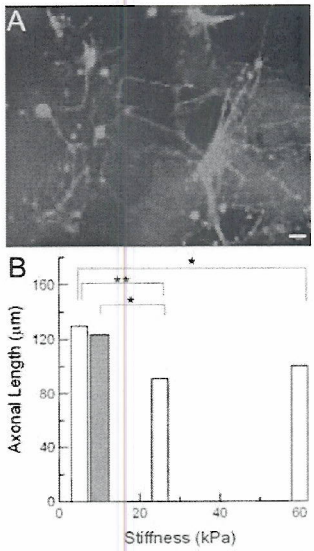



Figure 2. Primary dendrite outgrowth on DNA crosslinked gels on DNA gels of Designs A (white bar) and B (gray bar). (A) Typical image of MAP-2 positive neurons. (B) Changes of mean primary dendrite length with stiffness. (C) Changes of primary dendrite number with stiffness. Error bars represent standard error of mean. $n \geq 50$.

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## SUMMARY

Taken together, these results and our data on bis gels (1) suggest that there is a distinct sensitivity and threshold for specific properties of dendrites and axons, as well as other cellular properties, which could be beneficial for biomaterial design for spinal cord regeneration.

## ACKNOWLEDGEMENTS

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