05-3041

Proceedings of the ASME 2007 Summer Bioengineering Conference (SBC2007)
June 20-24, Keystone Resort & Conference Center, Keystone, Colorado, USA

SBC2007- 176153

NEURITE ELONGATION AND BRANCHING ON DNA CROSSLINKED POLYACRYLAMIDE HYDROGELS

Frank X. Jiang (1), Yangzhou Du (2), Uday Chippada (1), Lulu Li (1), Bonnie L. Firestein (2), Bernard Yurke (3), Noshir A. Langrana (1)

(1) Department of Biomedical Engineering
Rutgers University
Piscataway, NJ

(2) Department of Cell Biology and Neuroscience
Rutgers University
Piscataway, NJ

(3) Bell Laboratories
Alcatel-Lucent
Murray Hill, NJ

INTRODUCTION

Mechanical cues have been found to play an important role in the cell decision making process, as manifested in survival, adhesion, growth, proliferation, differentiation and functioning. Due to their resemblance to the natural tissues, polyacrylamide hydrogels have been used in the studies of mechanobiology and cell-substrate interactions for various cell types including fibroblasts, hepatocytes and neurons [1]. As the initial investigation, we have successfully cultured rat spinal cord neurons on bis-crosslinked gels with large range of stiffnesses [2].

With the introduction of a DNA crosslinking mechanism, a new class of hydrogels has been developed with tunable mechanical properties including stiffness and viscosity. It also gives rise to the new possibilities of altering mechanical properties in a controllable fashion [2], and providing dynamic stimuli. In this study, rat spinal cord cells were subjected to these DNA crosslinked polyacrylamide hydrogels ('DNA gels', hereafter) with varying stiffness due to changing crosslink density or different length in crosslinker DNA. The results from this work serves as another examination of effect of mechanical stiffness on cells and also lay down reference points for further study of cellular response to mechanically dynamic substrates.

MATERIALS AND METHODS

DNA gels were prepared in micro centrifuge tubes (600μl) as detailed previously [1]. Briefly, Acrydite^{TM} modified SA1 and SA2 (Table 1) were respectively co-polymerized with acrylamide monomer (10%) to form viscous fluid containing polymer long chain with SA1 or SA2 as side chains. After mixing the two, upon the addition of proper amount of L2 crosslinker DNA a hydrogel was formed.

30μL of these hydrogels were heated up to 60°C till degelation, and was transferred onto a circular cover glass (12mm) on a heater after a drop of optical glue (Adhesive #72, Norland, New Brunswick, NJ) was dipped in the center. We let gels spread on the glass freely, and removed them from the heater to allow gel reform. The gels were exposed to UV for 5 mins then re-hydrated and exposed to UV again for 30 mins to complete cure of the glue and hence gel immobilization.

Poly-D-lysine was attached to gel surface for cell adhesion with the help of a bifunctional crosslinker Sulfo SANPAH (Pierce, Rockford, IL). Spinal cord cell culture was the same as reported previously [2]. Briefly, spinal cords were dissected from E16 rat embryos and cells were dissociated and plated on DNA gel surface in serum containing medium, which was changed to neural basal medium with β-mercaptoethanol. On DIV (days *in vitro*) 6, cells were fixed and incubated with blocking solution containing primary antibodies against MAP2 for neuronal cell body and dendrite, Tau-1 (Chemicon, Temecula, CA) for axons and GFAP (Abcam, Cambridge, MA) for mature astrocytes. Next, after incubation with proper secondary antibodies, stained cells were visualized with immuno-fluorescence. DAPI nuclear staining was also performed to identify all intact cells.

RESULTS AND DISCUSSION

Mechanical testing on DNA gels was carried out following the same approach as described in [2] and the crosslinking density induced mechanical stiffness changes in DNA gels of two designs (Table 1) is demonstrated in Fig. 1. DNA gels with shorter crosslinker (20 vs. 40 bases) display a relatively larger range of stiffness (60 vs. 10 kPa).

As the first step, we examined the specificity of Tau-1 staining of spinal cord neurons owing to the previous report that it stains both axons and dendrite of hippocampal neurons in *in intro* studies. We found that there was little overlap between MAP2 and Tau-1 stain indicating the specificity of Tau-1 stain on axons, though Tau-1 was also found expressed in soma (Fig. 2).

Copyright © 2007 by ASME

Cells were first subjected to DNA gels of two different designs (Table 1, at 100% crosslinking) with average stiffness of 10 and 60 kPa (Fig. 1). No significant difference was found in cell number of neurons and astrocytes as well as total cell number (Fig. 3). Though the average neurite length did not display significant difference, the branching number was relatively higher on stiffer gels (Fig. 3), which is seemingly different than one of the previous reports [3]. However, the range of stiffness under study and the cell type might contribute to this distinction.

As a next step, we investigated the cell response to DNA gels with distinctive crosslinking densities (50% and 100%) of the same design (B, Table 1), and resulting mechanical stiffness of 5 and 60 kPa respectively. The results are summarized in Fig. 4. Cells are more spreaded on gels of higher crosslink density based on difference in neuronal cell spreading area. This is in accordance with previous reports that cell spreading reduced on softer gels [4]. The neurons also show some level of polarity (aspect ratio>1) in morphology but this polarity is not sensitive to stiffness (Fig. 4). Interestingly, neuronal branch number and neurite length were not significantly affected, while significantly longer axons were seen on fully crosslinked gels, suggesting the potential of promoting axonal regeneration. The average neuronal branching number is about 2.8 indicating large portion of cell population as bipolar neurons. Additionally, there was no significant difference in neuronal cell number, mature astrocyte number and total cell number on two different gels (data not shown).

The observations confirmed again that neuronal cells respond to mechanical properties of the hydrogel substrate, and suggest that the sensitivity to stiffness might be affected by the nature the biomaterials. This work demonstrated the versatility in manipulating physical properties of DNA gels and will continue to be carried out to provide further information for the dynamic studies.

ACKNOWLEDGEMENTS

This work was performed towards partial fulfillment of the PhD dissertation of the first author. The authors would like to acknowledge the funding from New Jersey Commission on Spinal Cord Research (Grant # 05-3041-SCR-E-0). We also thank Dr. Baogang Li for assistance in cell culture, and Dr. David C. Lin for help in DNA design.

REFERENCES

1. Beningo, KA, Lo, CM, and Wang, YL, 2002, "Flexible polyacrylamide substrata for the analysis of mechanical interactions at cell-substratum adhesions," Methods Cell Biol, 69, pp. 325-339.
2. Jiang, FX, Du, Y, Firestein, BL, Shreiber, DI, Rosenson-Schloss, RS, Yurke, B, Chippada, U, Li, L, Langrana, N.A., 2006 "Neural Cell Engineering using a Polyacrylamide Hydrogel: a preliminary study". ASME Summer Conference. Amelia Island, Florida, USA.
3. Lin, DC, Yurke, B, Langrana, NA, 2004, "Mechanical properties of a reversible, DNA-crosslinked polyacrylamide hydrogel," J Biomech Eng, 12, pp. 104-110.
4. Flanagan, LA, Ju, YE, Marg, B, Osterfield, M, Janmey, PA, 2002, "Neurite branching on deformable substrates," Neuroreport. 13, pp. 2411-2415.
5. Georges, PC, Miller, WJ, Meaney, D, Sawyer, ES, and Janmey, PA, 2006, "Matrices with compliance comparable to that of brain tissue select neuronal over glial growth in mixed cortical cultures," Biophys J, 90, pp. 3012-8.

Table 1. Two different designs of DNA crosslinked hydrogels with length of DNA strand and initial concentration shown. SA1 and SA2 are DNA strands that are incorporated into polymer long chains, and L2 is crosslinker DNA.

Design	SA1	SA2	L2	Initial DNA conc. (mM)	Stiffness (100% crosslinking)
A	20	20	40	3	10kPa
B	10	10	20	3	60kPa

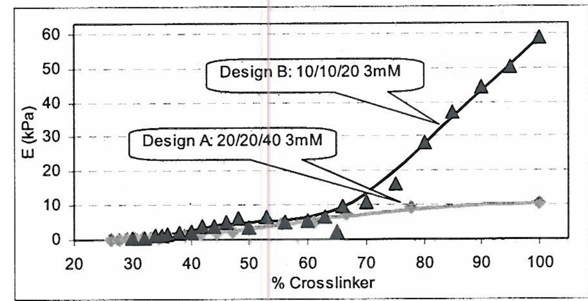

Figure 1. Changes of mechanical stiffness of DNA crosslinked polyacrylamide hydrogels with crosslinking density at room temperature.

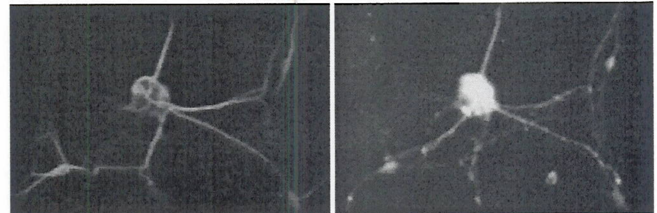

Figure 2. Immunostaining images of rat spinal cord cells on DNA gels. (Left) Red for dendrites and cell body (MAP2) and (Right) Green for axons (Tau-1).

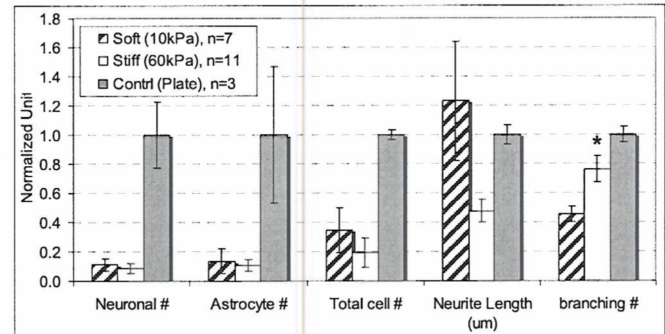

Figure 3. Phenotypical study of rat spinal cord cells on DNA gels with two designs (soft: Design A; Stiff: Design B) and culture plate. All parameters are normalized with respect to the quantity on control culture plate. (*p<0.01)

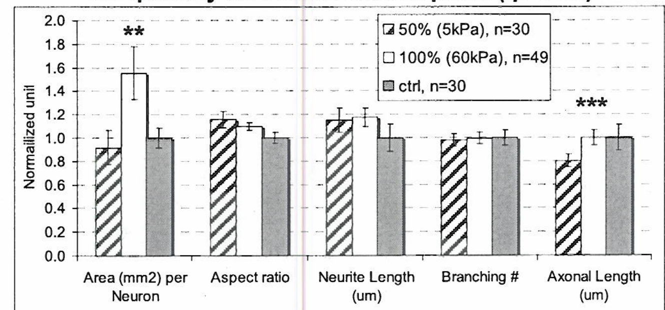

Figure 4. Rat spinal cord cell culture on DNA crosslinked hydrogels with different crosslinking density and culture plate. All parameters have been normalized with respect to the quantity on control culture plate. (p<0.05; ***p<0.02)**

Copyright © 2007 by ASME

[182549]

Study Of Effect Of Substrates Stiffness On Neural Cell Growth Using DNA Crosslinked Hydrogels

F. Jiang[1], Y. Du[2], U. Chippada[3], L. Li[4], B. Firestein[4], D.I. Shreiber[4], R. Schloss[4], Y. Bernard[5] and N.A. Langrana[4]
[1]Rutgers, The State University of New Jersey, Biomedical Engineering, Piscataway, NJ ; [2]Rutgers, The State University of New Jersey, Cell Biology and Neuroscience, NJ ; [3]NJ ; [4]Piscataway, NJ ; [5]Murray Hill, NJ

In this study, we deploy DNA crosslinked hydrogels to probe the effect of substrate stiffness on spinal cord cell growth. The information gained will aid in the design of biomaterials whose properties can be altered for tissue engineering applications. It has been shown by our group and others that spinal cord cell behavior can be affected by substrate compliance. Our pervious studies demonstrated that the mechanical properties of DNA crosslinked hydrogels can be changed in a controlled fashion by modifying crosslink design, such as crosslink length and density.

We first design DNA gels with mechanical stiffness ranging from tens of Pa to over 60kPa by simply varying crosslink density. On the gel substrates with three different mechanical stiffness (5kPa, 30kPa and 60kPa), both neurons and astrocytes show increased survival on stiffer gels as indicated by cell numbers. Additionally, cells are more spread on more rigid gels. Longer axons are also found on the stiffer gels. Next, the the length and sequence design of the crosslink was changed resulting in DNA crosslinked gel substrates with stiffness of 10kPa and 60kPa. Similar trend in the effect of stiffness on cell survival is observed. Interestingly, neurite number is shown to increase as stiffness rises.

With the results from this work, we have gained a better knowledge of cell behavior, including cell survival, adhesion and neurite outgrowth on both DNA and bis crosslinked hydrogels.

This work was under the financial support fromNJ Commission on Spinal Cord Research under Grant No. 05-3041-SCR-E-0.