05-3041

Proceedings of BIO2006
2006 Summer Bioengineering Conference
June 21-25, Amelia Island Plantation, Amelia Island, Florida, USA

BIO2006-157302

NEURAL CELL ENGINEERING USING A POLYACRYLAMIDE HYDROGEL: A PRELIMINARY STUDY

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INTRODUCTION

It is known that physical stimuli influence axonal generation. It is thus of interest to design polymeric hydrogels capable of providing such physical clues. Previously, based on an established protocol, polyacrylamide (PAM) hydrogels have been used for studying cell mechanobiology [1] of various cell types. However, to date there are few studies on utility of these hydrogels for neural cell engineering.

Our group [2] has shown that, with the use of DNA-crosslinks incorporating molecular actuators driven by DNA hybridization, PAM hydrogels can be created whose mechanical properties can be changed through the application of DNA strands. We are currently exploring the use of these hydrogels for guided axonal regeneration. As a preliminary study, bis-crosslinked PAM hydrogels have been prepared in house and obtained from commercial pre-made gels, functionalized and conjugated with proteins, on which we have successfully grown neural cells. Here we report on the experiment of culturing neurons on the commercial pre-made PAM hydrogels, with neuronal behavior being examined. We hope that this success will carry over to DNA crosslinked PAM gels.

MATERIALS AND METHODS

A sheet of 10% polyacrylamide hydrogel (PAGEr Precast Gels, Cambrex, Rockland, ME) were first rinsed in sterile water overnight and washed extensively before experiments. Gels discs were obtained by cutting the sheet with a ¼" hole punch, and then transferred to wells of a TC-treated 96-well plate after placing a drop of optical glue (Norland, New Brunswick, NJ) in the center of each well. The plate was immediately subject to UV exposure in a UV crosslinker (Spectronics, Westbury, NY) for 15mins to ensure attachment to the wells, which is followed by daily rinsing for three weeks.

In order to activate the gels for cell attachment, a bifunctional photoactivable crosslinker Sulfo SANPAH (Pierce, Rockford, IL) in 200mM HEPES buffer was applied allowing attachment of type I collagen (USB, Cleveland, OH) to the gel surface.

Rat spinal cord cells were obtained freshly from dissection of E16 rat embryos and counted, which contain both glial cells and neurons. They were then plated on functionalized and un-functionalized hydrogels as well as poly-D-lysine coated wells at a cell density of $40K/cm^2$ in serum-containing medium, which is composed of minimum essential medium (MEM), 10% horse serum and 0.6% glucose supplemented by antibiotics (penicillin/streptavidin). The plate was kept in a humidified CO_2 incubator at 37°C. One day after cell plating, the medium was changed to neural basal medium with B-27 supplement and antibiotics together with β-mercaptoethanol (1μL per 500mL medium) to specifically promote neuronal growth.

Three days after cell plating, observations were made to examine cell growth and on Day 5 cells were fixed using paraformaldehyde (4% in PBS), followed by incubating with primary antibodies against MAP2 (microtubule-associated protein 2), a neuron specific marker (diluted 1:200), and Cy3-conjugated anti-mouse secondary antibodies (1:200) (Jackson ImmonoResearch, West Grove, PA). Labeled neurons were visualized by immunofluorescence (Olympus IX50 microscope with a Cooke Sensicam charge-coupled device cooled camera, fluorescence, imaging system, and Image Pro software).

RESULTS AND DISCUSSION

Figure 1 shows phase contrast images of neural cells that were adhered to and grew on the surface of both functionalized and un-functionalized PAM hydrogels (Fig. 1A) with image at higher magnification shown in Fig. 1B and corresponding fluorescent image in Fig. 1C. Cells on both types of gels did not show appreciable

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difference in adhesion strength, and further immunostaining confirmed that rat spinal cord neurons grew on both gels (Fig. 1C) displaying typical morphology as exhibited by those in the control (Fig. 2). Only a fraction of the total population of cells growing on the gels was neurons, which agrees with the fact that only about 10% CNS cells are neurons (Figure 1). We also found that it was hard to focus on neurite (axon or dendrite), because either cells were not growing on a flat surface, or they extended into the gels with images distorted by diffraction from gels. Based on our observations, neural cells on the gels had a greater tendency to form cluster-like structures as shown in Fig. 1B and 1C (note the bright area) than those in control. Fiber-like structures connecting the clusters also appeared which could be bundles of neurites considering their thickness and visibility in staining.

We are aware of only one previous report on designing PAM gels with different mechanical properties for neural cell attachment [3]. In our study, we deployed this hydrogel for rat spinal cord neuron's growth. Some of our observations deviated from that of [3]. Specifically, we found cells adhered to the gel surface with only collagen (type I) coating when non-specific binding was blocked. Surprisingly, the cells also grew on gels without functionalization or protein conjugation. Additionally, though Flanagan et al. [3] reported that glial cells did not grow or proliferate on the gels, those cells in our experiment seemed to do otherwise (Figure 1A, 1B). Precautions were taken in choosing the appropriate cell plating density, since cells plated at a high density were seen to easily detach from functionalized gel surface implying stronger cell-cell interaction than cell-substrate attachment. However, at a low density, these cells did not survive on either gel substrate or polystyrene plates (control). We also grew neural cells on the gels made in the wells of a 96-well plate based on the modification of protocols in Beningo et al. [1].

The results from this study can be directly applied to the preparation of DNA crosslinked PAM hydrogels that could be engineered to promote neurite elongation and branching. Research into the use of these gels for stem cell differentiation is ongoing.

SUMMARY

Our experiments demonstrated the biocompatibility of polyacrylamide gels and their ability to support neuronal adhesion and growth. The results presented provide complementary information to the few studies conducted on neuronal behavior on this type of hydrogel substrate. They are also important in tailoring DNA crosslinked hydrogels for neural cell engineering.

ACKNOWLEDGEMENTS

This study is funded by New Jersey Commission on Spinal Cord Research (NJCSCR) under Grant No. 05-3041-SCR-E-0. We would like to thank Dr. Lin for his help in this project.

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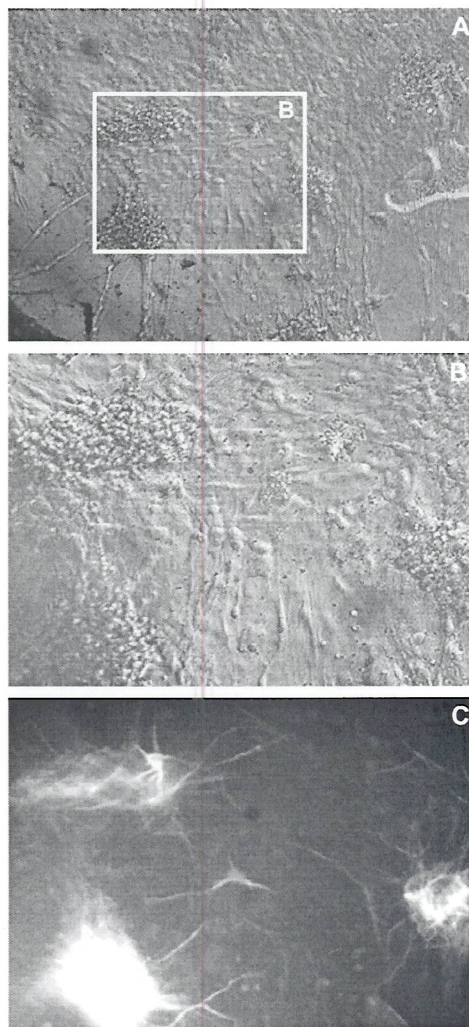

Figure 1. Neural cells grew on polyacrylamide hydrogels (A, 10×) and among all the cells shown in B of higher magnification (20×), only a small portion of them were neurons as seen in corresponding fluorescent image C.

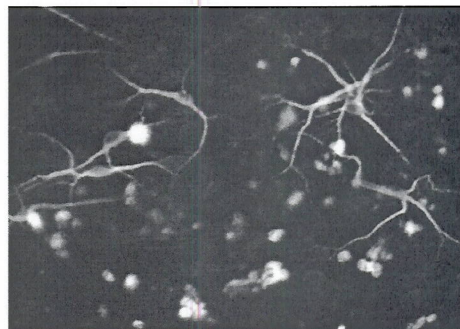

Figure 2. Fluorescent image of rat spinal cord neuron (E16) on poly-D-lysine treated polystyrene plates

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