

Analysis of Neurite Outgrowth on Electrospun Fiber Scaffolds in the Presence of Blebbistatin and Paclitaxel

A.R. D'Amato^{1,2}, N.J. Schaub^{1,2}, C.D.L. Johnson^{1,2}, J. Cardenas^{1,2}, R.J. Gilbert^{1,2,3}

Rensselaer Polytechnic Institute

Troy, NY 12180-3590

damata@rpi.edu

Abstract - In an attempt to increase the rate of neurite extension from E9 chick embryo dorsal root ganglion (DRG) on electrospun fiber scaffolds we experimented with two drugs that have been used previously to affect neuronal growth. Blebbistatin and paclitaxel were added to DRG cultured on electrospun fibers in an attempt to increase the rate of neurite extension along fibrous scaffolds. Blebbistatin increased the length of neurites extending from the DRG explant in comparison to the drug-free control group. In contrast, paclitaxel completely eliminated all neurite extension from the DRG explant when compared to the control. A third treatment group which combined both drugs also resulted in no neurite extension from the DRG explant as was found in all paclitaxel containing treatment groups.

Keywords-Neurite, Blebbistatin, Paclitaxel, Biomaterials, DRG, Electrospun fibers

I. INTRODUCTION

Approximately five million people suffer from neurodegenerative diseases in America and this number is expected to increase to 12 million by the year 2045 [1]. Biomaterial strategies to increase neural regeneration for individuals with neurodegenerative diseases or for individuals sustaining central nervous system (CNS) injury are being investigated routinely. One biomaterial strategy, the creation and use of electrospun fiber scaffolds is used broadly within experimental models of peripheral nerve and CNS injury [2].

Blebbistatin is a non-muscle myosin II inhibitor that weakens the interaction between extending neurites and the substrates to

which they are attached [3]. Alternatively, paclitaxel, is a microtubule(MT)-binding molecule that stabilizes MT dynamics. Thus, both drugs are capable of increasing the rate of axonal regeneration [4].

In an attempt to promote nerve regeneration in the presence of an electrospun fiber scaffold we experimented with these two drugs. There is no study that assesses neurite outgrowth in the presence of the aforementioned drugs after culturing a neural explant on an electrospun fiber scaffold. Thus the goal of this study is to elucidate the influence that these drugs have on increasing the rate of neurite extension along aligned electrospun fiber scaffolds.

II. MATERIALS AND METHODS

A. Electrospun Fiber Fabrication

Our electrospinning apparatus used for fiber fabrication was described in detail previously [2]. Briefly, 12% (w/w) solutions of poly-L-lactic acid (PLLA) containing 0.240 g of PLLA in 2.0 g of 1,1,1,6,6,6-hexafluoro-2-propanol (HFP) were electrospun onto glass coverslips coated with a thin film of PLLA to create aligned electrospun fiber scaffolds. Fiber diameter and alignment were assessed using previously published procedures [2]. Prior to cell culture, fiber scaffolds were sterilized in a solution containing 70% (v/v) ethanol in deionized water.

B. Dorsal Root Ganglion (DRG) Isolation and Culture

DRG were isolated from the lumbar and thoracic regions of day 9 chick embryos (E9) as previously described [2]. Isolated DRG were placed into a 100 μ L drop of neurobasal media supplemented with B-27 serum-free supplement and allowed to attach overnight in a tissue culture incubator. For samples in which the DRG attached, nerve growth factor (NGF) was added along with more neurobasal media to reach a final media volume of 1 mL and NGF concentration of 50 ng mL⁻¹. DRG isolation and culture was performed in biological triplicate (n=3).

¹Department of Biomedical Engineering, Rensselaer Polytechnic Institute, Troy, NY 12180-3590, USA

²Center for Biotechnology and Interdisciplinary Studies, Rensselaer Polytechnic Institute, 110 8th Street, Troy, NY, 12180-3590

³Rensselaer Nanotechnology Center, Rensselaer Polytechnic Institute, 110 8th Street, Troy, NY, 12180-3590

C. Blebbistatin or Paclitaxel Treatment

Blebbistatin or paclitaxel was added to DRG culture medium to obtain the following drug concentrations: 10 μ M, 25 μ M, or 50 μ M for blebbistatin, or 10 nM, 25 nM, or 50 nM for paclitaxel. Another experimental group consisted of blebbistatin and paclitaxel in combination at concentrations of 25 μ M and 25 nM respectively. The negative control group for this study consisted of DRG cultured on electrospun fibers in drug free media.

D. Immunocytochemistry

DRG were cultured for 60 hours after the addition of NGF and drug. After culture, DRG were fixed by adding 1 mL of an 8% paraformaldehyde solution to the 1 mL of culture media for 30 minutes. After fixation, cultures were washed three times in PBS then blocked in a solution of PBS, 10% normal goat serum, and 0.1% Triton X-100 overnight. Blocking solution was removed, and samples were incubated with mouse anti-RT97 polyclonal antibody (1:500 dilution) for one hour. Samples were then washed again with PBS and incubated with Alexafluor 594 goat anti mouse secondary antibody (1:1000 dilution) for one hour. A 10 μ g mL⁻¹ solution of DAPI in PBS was then added to samples and incubated for fifteen minutes. The DAPI solution was removed, and cells were washed with PBS three times prior to imaging. Samples were imaged using an Axiovert 200 M microscope equipped with an AxioCam fluorescence camera both purchased from Zeiss.

E. Measurement of Neurite Outgrowth

Images of DRG were analyzed using NIH ImageJ software. For each DRG the ten longest neurites from each side will be measured and averaged to determine the average neurite outgrowth length for each sample. Since each DRG has two sides and three DRG were imaged for each experimental group, six length measurements will be gathered for each condition.

III. RESULTS

All DRG cultured in the presence of paclitaxel (10nM, 25nM, and 50nM) did not extend neurites (Fig. 1C). All DRG that were cultured in the presence of solely Blebbistatin in the media (10 μ M, 25 μ M, and 50 μ M) extended neurites a greater distance than the control group (Fig. 1B/1A). This is preliminary data and no quantification or statistical analysis has been performed yet.

DRG cultured in the presence of both blebbistatin (25 μ M) and paclitaxel (25nM) did not extend neurites (Fig. 1D). This indicates that the activity of paclitaxel surpassed the activity of blebbistatin although blebbistatin was at a 1000 fold higher concentrations.

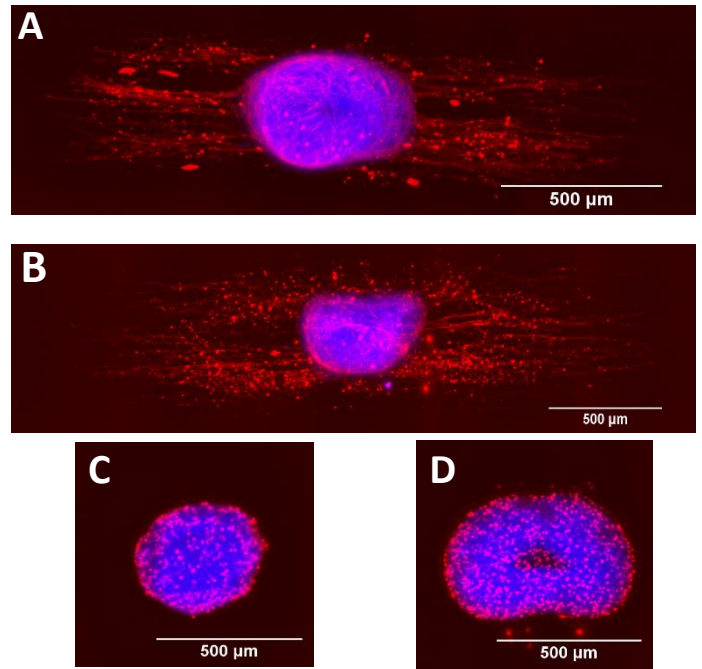


Figure 1. A) DRG culture in drug-free media. B) DRG cultured in media containing 25 μ M Blebbistatin. C) DRG cultured in media containing 25 nM Paclitaxel. D) DRG cultured in media containing 25 μ M Blebbistatin and 25 nM Paclitaxel.

IV. FUTURE WORK

Quantification of neurite outgrowth will be done to determine if there are statistical differences between the blebbistatin groups as well as between the blebbistatin and control groups. We will also experiment with lower concentrations of each drug to determine if there is a specific concentration of each drug that will result in increased neurite extension.

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