Development of a High Throughput Screen to Create an Optimized Biomaterial for Peripheral Nerve Injury

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Statement of Purpose: This study investigates the development of a high throughput screen to optimize the composition of a biomaterial to support the re-growth of injured neuronal and non-neuronal cells for treating large gap peripheral nerve injuries. Current treatments for large gap injuries utilize autografts or guidance channels to bridge the peripheral nerve injury. Failure of axons to re-grow has been linked in part to the lack of non-neural cells migrating into the injury site to support the regenerating axons. Therefore, creating an optimized biomaterial scaffold that is supportive of both neurite outgrowth and non-neural cell migration may yield improved outgrowth and functional recovery[1,2]. We employed a fluorescence multi-channel flatbed scanner to rapidly screen the cellular response to a printed protein array in 2D. To examine the sensitivity of this assay, the seeding density of both neurons and SC was varied by a serial dilution, fixed, immunofluorescently labeled and scanned. Results demonstrate the ability to screen using a high-resolution scanner with the spot intensity increasing with an increase in the number of cells attached, determined by traditional fluorescent microscopy. Single, binary, and tertiary combinations of laminin (LN), fibronectin (FN), and collagen I (Col I) were machine spotted onto glass slides. Cellular response to the protein library was captured via spot intensity of the scanned slide to identify "hits". The hits were selected for further analysis using microscopy to analyze glial and neuronal attachment and morphology. These proteins were chosen based on their ability to influence neurite outgrowth and the response of primary SC and dissociated DRG neurons was characterized (results not shown)[3].

Methods: *Cell Isolation and Culture:* Primary DRG neurons and Schwann Cells (SC) were isolated from P2 neonatal rats (Taconic Farms, Inc.) [4] Neurons were grown in SC media containing 25 ng/mL NGF[5].

Protein Library Printing: Glass microarray slides (Tekdon, USA) with teflon masking were used for protein libraries. 500 nL of poly(d)lysine (PDL) was printed using a robotic fluid printer (Invantis AG, Germany) and matrix proteins (LN, FN, and Col I) were printed on top of PDL coated spots. Each protein combination was repeated on 5 spots. For sensitivity assays, glass slides were deposited with PDL and 50 µg/mL of LN.

Sensitivity Assay: Cells were serially diluted into a silicone gasket allowing 15 concentrations and 4 spots/concentration (500,000-37 cells/mL). Cells were allowed to adhere for 4 h and were rinsed and cultured overnight. Samples were fixed prior to staining with TRITC-Phalloidin (SC), TRITC-anti-βIII-tubulin (neurons), and mounted with Antifade DAPI (Invitrogen).

Imaging and Data Analysis: Slides were scanned using a Typhoon Trio+ (GE Healthcare USA) in fluorescence mode. Images were exported to ImageQuant software (GE Healthcare USA) to generate fluorescence intensity data. Samples were then imaged with an Olympus inverted microscope with a 10x objective and Metamorph software (Molecular Devices). NIH ImageJ was used to quantify cell number to allow correlation with intensity profiles.

Results: A strong correlation is observed between spot intensity measured by a flatbed scanner and number of SC counted by microscopy (Fig 1A). Also, typical morphology is shown for spots with high and low cell numbers. For neuron sensitivity, cell coverage on the surface was fairly uniform and as the concentration of cells increase, the neurite outgrowth covers more of the surface (Fig 1B). This is the first step in demonstrating a degree of sensitivity to changes in total neurite outgrowth.

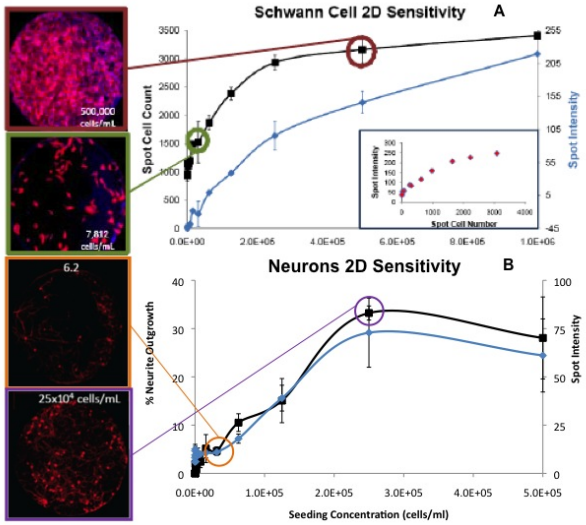

Figure 1: Assay sensitivity in high throughput screening of cells on 2D arrays. A) Plot of seeding density vs. number of cells (black curve) or intensity (blue curve) at a given spot for SC. B) Plot of seeding density vs. number of cells (blue curve) or % neurite outgrowth (black curve) for neurons. Selected inverted fluorescent images showing cellular attachment at varying densities detected by the scanner are shown on left.

Conclusions: This investigation validates the use of fluorescence flatbed scanning for high throughput analysis of biomaterials for neural tissue engineering applications. Correlations between measured fluorescent intensities using the scanner and cell counts using ImageJ software were seen for both neurons and SC, showing the high sensitivity of the system. This system is currently being used to analyze cell-seeded protein libraries to rapidly optimize the scaffold composition to support the regenerating peripheral nerve to create optimal 3D scaffolds can be created for improved guidance channels.

References: (1) Hurtado et al. *Biomaterials* 2006;27(3) (2) Rosner et al. *Exp Neurol* 2005;195(3) Deister et al. *J Biomater Sci Polym Ed* 2007;18(8) (4) Morrissey et al. *J Neurosci* 1991;11(8). (5) Dewitt et al. *J Tiss Eng A*. 2009; 15(10).