Directing Neuron and Glial Response Utilizing Surface Chemistry, Topography and Electrical Stimulation

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Statement of Purpose: Repair of traumatic injuries in the nervous system requires a coordinated migration and growth of glial and neural axons. However, due to a poor innate healing response, intervention is often necessary, to guide neurons across a lesion site. Micro-channeled scaffolds have demonstrated effectiveness as a platform to bridge the gap and allow guided axon regeneration. Improving the performance of these scaffolds relies on utilizing environmental cues to guide cell behavior, including surface chemistry and topography, which affect migration and cellular morphology (Davidenko 2016). In addition, electrical stimulation, during culture, can encourage glial alignment and axonal growth (Koppes 2014). The goal of this study was to investigate the factors which direct the behavior of neuronal and glial behavior on FDA approved scaffold materials, and combine these cues with electrical stimulation, to encourage faster regeneration across nerve gaps.

Methods: Porous and non-porous films of (poly-ε-caprolactone (PCL) and poly(lactic-co-glycolic acid) (PLGA)) were cast using a Dr. Blade with 70 or 0 vol% of NaCl, respectively. Once dry, the salt was removed in water for 1 hour. Films were placed in inserts (CellCrown), with tissue culture polystyrene (TCPS) serving as a positive control. Primary rat Schwann cells or dorsal root ganglion (DRG, mouse) were seeded on surfaces either uncoated or coated: fibronectin (Sigma), laminin (ThermoFisher), or poly-D-lysine (PDL, MP Biomedicals),. After 24 hours, cell morphology and attachment (Quant-iT™ PicoGreen® assay) was assessed. Electrical stimulation was applied by culturing cells within circuits, sputter coated onto TCPS petri dishes. Platinum wires, attached with silver paste (PELCO® Colloidal Silver Paste), were connected to a power supply. Cells were cultured for a specified time (2-24 hours) before applying an electrical field (50 – 100 mV/mm) for 10-60 minutes; morphology was assessed 24 hours after stimulation. Morphology was evaluated via fluorescent staining; Schwann cells: actin (AlexaFluor), and DRGs: TUJI (Promega) and p75 (Neuromics).

Results: On scaffold materials, cell attachment was affected by both surface chemistry and topography. Chemistry dominated Schwann cell attachment on smooth, non-porous, surfaces. Fibronectin encouraged the highest attachment, and greatest amount of cell spreading, on all substrates, followed by laminin. The significant differences were due to the type of adhesion ligand displayed, which varied with each protein, Figure 1(a-b).

Surface topography, introduced with the addition of porosity in the polymer films, reduced the effect of surface chemistry. Most likely, cell binding was through non-specific reactions rather than adhesion ligands, as it was on smooth surfaces. With topography, cell attachment dropped significantly, regardless of the polymer. In addition, topography was found to direct axonal growth of DRGs, Figure 1(c-d).

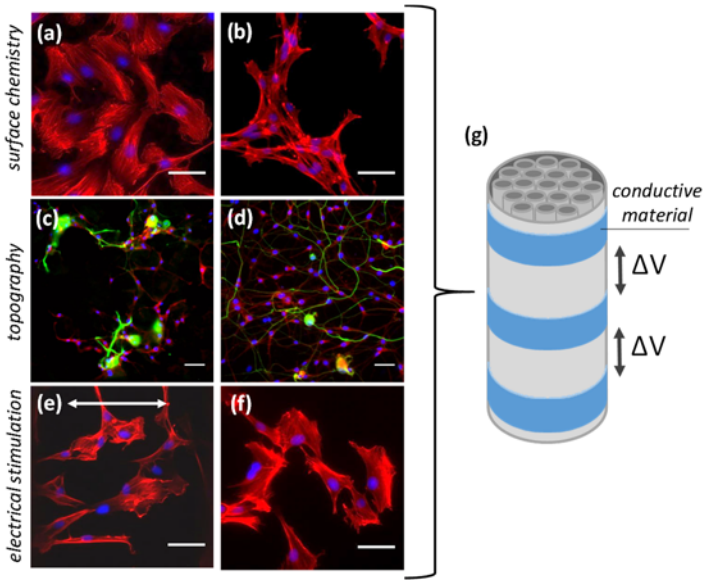

Figure 1. Directing cell response via (a-b) surface chemistry, (c-d) topography, (e-f) electrical stimulation and integrating all cues into (g) an implantable device. (a-b, e-f) Schwann cell morphology (red: actin, blue: nucleus): (a) PCL plus fibronectin, (b) no coating, (e) electrical stimulation (50mV/mm, arrow shows direction of field), and (f) no stimulation. DRG growth on (c) porous PCL and (d) non porous PCL (red: Schwann cells, green: neurons, blue: nucleus). Scale bar: 50 µm.

In response to electrical stimulation, Schwann cells were observed to align within the electrical field, Figure 1(e-f). The ability of cells to respond to stimulation was dependent on cell density, and the time at which cells were stimulated. Lower density, less than $1x10^4/cm^2$, and stimulation within 4 hours, post seeding, produced glial alignment, which might be used to direct axon growth.

Conclusions: Working with FDA approved polymers, cell attachment was affected by the coating protein, which altered the adhesion ligands available. On 3D surfaces, topography dominated attachment, most likely through non-specific binding. Both topography and electrical stimulation acted to guide Schwann cell and axonal growth. By integrating surface chemistry, topography and electrical stimulation into an existing scaffold platform, future work will focus on enhancing regeneration of traumatic nerve injury.

References:

(Davidenko N. J Mater Sci: Mater Med. 2016; 27: 148.)
(Koppes AN. Tissue Eng, Part A. 2014; 20: 494-506.)