Polymeric Nanowire Templates as Scaffolds for Improved Neuronal Cell Functionality

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Statement of Purpose: Growth and maintenance of neural cells on surfaces with unique nanotopography can be beneficial in several tissue engineering applications including central nervous system regeneration, spinal cord injuries, etc[1]. Poly (ε-Caprolactone) (PCL) is biocompatible as well as a biodegradable material that has the potential to be used as a scaffold for a variety of tissue engineering applications. For example, in a damaged spinal column, a PCL scaffold with cultured neural cells could potentially bridge an injury and allow the patient regained motor or sensory ability. Because neural cells are difficult to isolate and culture in vitro, PC12 Cells are used extensively as a neuron model in research relating to stem cell and neural regenerative therapies. In this work, we investigated the effects of PCL nanowire surfaces on neuronal cell viability, adhesion, and differentiation.

Methods: Nanowires were fabricated using a solvent free nanotemplating technique. PCL discs are extruded through nanoporous alumina membranes (200nm) by way of heat application. After PCL extrusion, the membrane is removed by chemically degrading with NaOH. Short-term cell adhesion, morphology, and viability were assessed after 1 and 4 days of culture using Calcien (live cell) stain, MTT assay and scanning electron microscopy (SEM). At day 4, nerve growth factor (NGF) was added to the culture media to differentiate the cells. Cell viability, differentiation and morphology were assessed after 1, 3, and 6 days of differentiation (i.e. after 5, 8, and 11 days of culture) using CMFDA cytoplasm stain and SEM imaging. In addition, neural markers NF-H and TH were stained for using immunofluorescence techniques and imaged using confocal microscopy. Tissue culture poly-styrene and flat PCL (no nano-topography) surfaces were used as controls.

Results: Viability as assessed by the MTT assay showed that after 1 and 4 days of culture, the viability for the cells adhered on the PCL nanowire surfaces was significantly higher than that of the control surfaces. (Fig.1) This implies higher cell coverage on the nanowire surfaces contrasted by low cell coverage on both of the control surfaces.

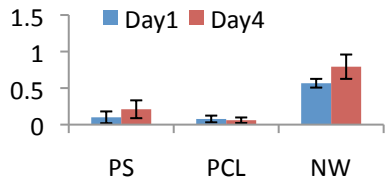

Figure 1: Pre- Differentiation viability was significantly higher on nanowire surfaces than on the control surfaces. PS: Polystyrene, PCL: flat PCL discs, and NW: PCL nanowires surfaces

The nano-topography of the PCL nanowires allowed the PC12 cells to adhere at much higher densities than the control surfaces. This can be seen in fluorescence microscopy images of cells stained with Calcien (Fig.2). The most probable mechanism behind the increased cell coverage was enhanced integrin signaling and adhesion promoted by the nanotopography of the nanowire surfaces.

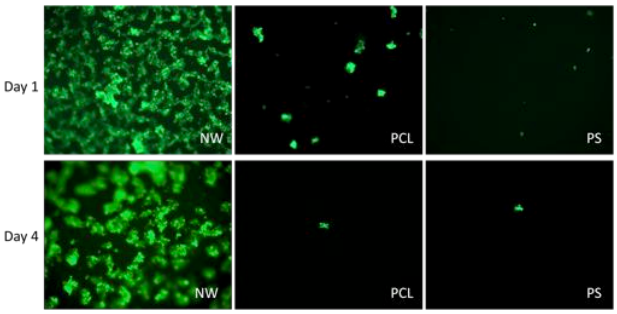

Figure 2: Pre-Differentiation Calcien Stain. All fluorescent images at 10x magnification

After terminally differentiating the PC12 cells by exposing them to NGF, a number of visualization techniques were utilized to assess cell morphology and coverage post-differentiation. CMFDA fluorescent images showed that the nanotopography present in the PCL Nanowires promoted high cell adhesion and extended neurite formation (data not shown). The SEM images reveal extensive grouping and neurite formation on the nanowire surfaces as well as confirm the fluorescent adhesion results (Fig.3). Immuno-fluorescence staining for Tyrosine Hydroxylase and Neurofilament-H was also preformed to confirm neural differentiation (data not shown).

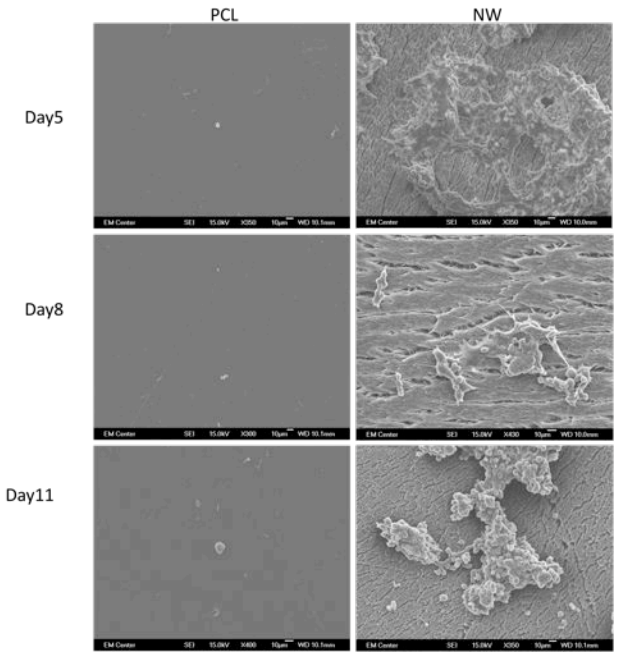

Figure 3: Post-Differentiation SEM images showing increased cell adhesion and increased neurite length.

Conclusion: This work demonstrates that by manipulating nano-topography, PC12 cells can be effectively cultured onto a surface and the material can actually increase growth, differentiation, and adhesion. Opportunities to further this research in neural tissue engineering could ultimately lead to an improved material for bio-scaffolding and stem cell therapies.

References:

[1] *Lijie Zhang, Thomas J. Webster Nanotechnology and nanomaterials: Promises for improved tissue regeneration*

[2] *Porter JR., Henson A., Popat KC., Biodegradable Poly(ε-caprolactone) Nanowires for Bone Tissue Engineering Applications.*