Bio-functionalized electro-responsive polymeric nanowire templates facilitating neural stem cell proliferation and differentiation

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Statement of Purpose: Growth and maintenance of neural stem cells (NSC's) on tissue engineering scaffolds with unique nanotopography can lead to a better of understanding of cell-biomaterial interactions and ultimately, can lead to implantable materials seeded with NSC's for use in applications ranging from neuro-degenerative diseases to spinal cord injuries. Our lab has demonstrated that polycaprolactone nanowires (NW) have great potential as a biomaterial in neurological tissue engineering applications[1]. We hypothesized that we could further optimize the NW templates for neurological applications by providing the cells with not only a unique nano-topography, but by making the surface that they interact with electrically conductive. In this work, we investigated the physiological response of neural stem cells exposed to NW templates coated with an electrically conductive polymer Polypyrrole (PPy).

Methods: NW's were fabricated using methods previously described by our lab[1]. PPy coating was achieved by chemical deposition of PPy onto the PCL surfaces. Briefly, the NW were soaked in a pyrrole/para-toluene sulfonate solution followed by addition of 38mM Ferric Chloride to allow polymerization and deposition of the PPy onto the surfaces. X-Ray photoelectron spectroscopy (XPS), Scanning Electron Microscopy (SEM) and resistivity measurements were used to ensure and quantify PPy deposition. C17.2 murine NSC's were used to investigate NSC interactions with the surface. Cell adhesion, viability, and morphology were assessed using a Calcien-AM (live cell) stain and SEM at 1, 2, and 7 days after initial culture. To further characterize the differentiation of the neural stem cells, nervous cell markers NF-H, MAP2, Nestin, APC, and GFAP were immuno- stained at Day7. NF-H and MAP2 are specific to neurons, Nestin is specific to undifferentiated stem cells, APC and GFAP are specific to glial cells. Specifically. oligodendrocytes and astrocytes respectively. Flat polycaprolactone pucks as well as un-coated nanowires were used as a control surfaces.

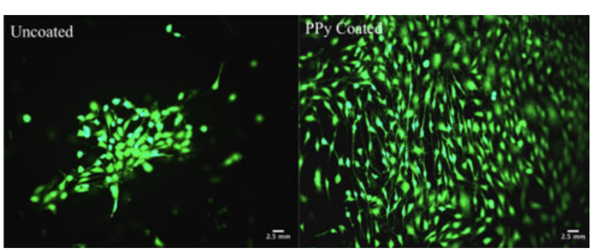

Figure 1: Representative Day 7 Live Stain of Neural Stem Cells on PPy Coated and Un-Coated NW Surfaces

Results: The high resolution XPS scans of nitrogen confirm that PPy was deposited on the PCL nanowire surfaces (data not shown). Nitrogen was chosen because PPy has an amine group that PCL lacks. Furthermore, the resistance measurements confirmed the surface resistance decreased from over 40MΩ down to 3.68kΩ ± 1.43kΩ after PPy coating. Although uncoated NW surfaces did not adversely affect the NSC's viability, live cell data and cell coverage calculations confirm the hypothesis that cell viability and attachment would be higher on PPy coated nanowires at a similar time point (Fig1). The live stain also highlights the increased neurite extensions and consequent cellular communication on the PPy coated samples (Fig1). Finally, neurons, oligodendrocytes, and astrocytes were all identified on PPy coated surfaces by immuno-staining (Fig2). This confirms that the PPy surfaces promote differentiation of the NSC's into all neural lineages.

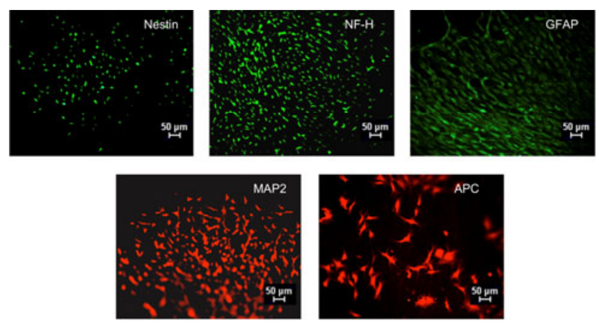

Figure 2: Representative immuno-flourescent images showing Neural Stem Cells differentiated into all neural lineages.

Conclusions: In this work, we have created a technique to fabricate electrically conductive PCL nanowire templates for growth and differentiation of NSCs. Our results confirm that PPy coating reduces the electrical resistivity of the PCL nanowires to a functional level where electrical stimulation has the ability to be applied. Furthermore, we have shown that decreasing the electrical resistivity of the surface results in increased functionality of C17.2 neural stem cells in terms of adhesion, proliferation, and differentiation. Thus, we have shown that by bio-functionalizing the surface characteristics of a scaffold, namely by reducing the resistivity and exposing cells to a unique nanotopography, we can enhance neural stem cell interactions that will ultimately prove to be beneficial in a neural tissue engineering application.

References: [1]Bechara S, Judson A, Popat K. Template synthesized poly(-caprolactone) nanowire surfaces for neural tissue engineering. Biomaterials (2010) 3492-3501