Nanoparticle and Nanofiber-based Sundew (*Drosera*) Scaffolds for Tissue Engineering

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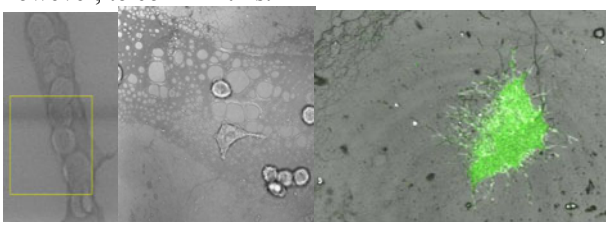
Statement of Purpose: We have discovered using Atomic Force Microscopy (AFM) that the Sundew (*Drosera*) secretes nanoparticles and nanofiber-based scaffolds for capturing insects. The scaffold has unique high elasticity and forms a complex network of nanofibers. The scaffold forms a dense network at both the micro and nano-scale, which is ideal for the attachment of many types of cells. Further examination led to the hypothesis that the scaffold could be used for the attachment of cells in tissue engineering applications. The goal of this study was to determine if the Sundew derived scaffold was capable of providing a suitable substrate for the attachment of PC12 neuronal cells and if the scaffold could be used for directional growth.

Methods: The Sundew species (*Drosera binata* and *Drosera capensis)* were purchased from the Carnivorous Plant Nursery, Derwood, MD and maintained in the lab. Adhesive was harvested by the centrifugation of leaves in an Eppendorf® 5702R for 5 minutes at 3000 g at a controlled temperature of 4^{o}C. After collecting the adhesive, petri dishes with a cut-out for glass coverslips, were coated with the adhesive and allowed to dry under a bio-safety cabinet for 24 hours. The coated samples were then stained with Alcian Blue pH 2.5 (Richard Allen Scientific®) for 30 minutes to confirm the areas coated by the adhesive. After staining, the petri dishes were UV sterilized for 15 minutes and incubated with PC12 cells in DMEM for 24 hours. At this time the media was removed, and the cells were stained with a live/dead stain containing calcein and ethidium bromide. Living cells were stained green and dead cells were stained red. To determine if PC12 cells were able to differentiate, a similar experiment was conducted on cells incubated for 48 hours. All samples were imaged using an Olympus Fluoview 1000 confocal microscope to visualize the fluorescence. The first 100 cells were counted for viability and were scored as either live or dead. For all experiments, a positive control containing poly-L-lysine (Electron Microscopy Sciences®), and a negative control of bare glass were examined. AFM imaging using an Agilent 6000 ILM/AFM was conducted on all samples to ensure that the stained scaffold maintained its nanostructure throughout the experiment.

Results: For all experiments, the positive poly-L-lysine control had a high percentage of attached cells, and the cells were 99% viable as determined by scans with the confocal scope. Initially PC12 cells appeared to be attached to the negative control samples, but after careful washing of the samples with PBS to remove and staining solution, no cells were found to attach to the bare glass surface. This negated the need for live/dead staining, since no cells were observed over the entire surface. The samples coated with mucin did allow the PC12 cells to attach, and the pattern of attachment seemed to mimic the pattern of the stained mucin. Similar to the poly-L-lysine control, the attached cells were 99% viable and appeared to begin to take on the elongate shape of neuronal cells. The number of attached cells was greater on the positive control, compared to the mucin sample; however, this was expected due to the non-uniform coating of the mucin samples. Figure 1A and B demonstrates the pattern of PC12 cell attachment onto the stained scaffold. Figure 1C shows a calcein stained PC12 cell attached to the Sundew scaffold. It appeared from calcein staining that PC12 cells were able to adhere to the substrate, and that the cells may grow in the direction of the scaffold. Further examination of this principle will need to be conducted, however, to confirm this.



(A) **(B)** **(C)**

Figure 1. Low-Resolution and Confocal images of attached PC12 cells to the Alcian Blue stained Sundew scaffold. A) Low-resolution DIC image of PC12 cells tightly aligned to the Sundew scaffold. B) Confocal laser scanning image of PC12 cells attached to the scaffold, differentiation of cells and extension of neurite-like structure can be distinguished. C) Calcein stained PC12 cell, showing differentiation and neurite-like structure on the scaffold.

Conclusions: This study demonstrated that PC12 cells were capable of attaching to the Sundew scaffold and that the attached cells were viable. The percentage of attached cells was not as great as the positive control, but this was due to an inability, at present, to uniformly coat a surface with mucin. Since PC12 cells are more difficult to attach than other cell types, such as osteoblasts and fibroblasts, we expect that the mucin scaffold will have great potential in the field of tissue engineering. This naturally occurring scaffold could be applied to material surfaces to increase the ability of cells to attach, and could lead to many advances in biomaterial research.