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Supplemental Data

Development and characterization of polyethylene glycol-carbon nanotube hydrogel composite

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Methods: Cell Viability on 2D PEG-CNT Composite Hydrogels

7.5% PEG hydrogels (100 µl volume) without CNTs were prepared as described earlier between two hydrophobic-treated glass plates separated 1 mm apart using silicon spacers. The hydrogels were then swollen in PBS (1X, pH 7.4) for 24 hrs, and CNTs were "stamped" from a glass plate onto the hydrogel. Briefly, a thin layer of CNT powder was spread on a glass plate and then the swollen hydrogel was firmly pressed to the plate. The gels were rinsed with PBS to remove loosely attached CNTs from the surface and sterilized under ultraviolet light (UV, 302 nm) in a tissue culture hood for 2 hrs. The CNT-stamped hydrogels were then placed into a 24-well plate for further experiments. The PEG-CNT hydrogels were used as is or coated with 0.05% w/v BSA, 10% w/v (100 µg/mL) laminin, or a mixture of the two by soaking the hydrogel in the protein solution for 2 hrs at room temperature. The hydrogels were rinsed once with PBS to

remove excess proteins and a 50 µl of cell suspension ($1.0x10^6$ cells/mL) in supplemented RPMI medium (10% FBS, 1% pen/strep) was added on the surface of the hydrogels. The hydrogels were then placed in the incubator at 37^oC and 5% CO_2 for 30 min to allow for initial cell attachment onto the hydrogels only. The hydrogels were then fully submerged in supplemented medium and the cells were cultured for 24 hrs prior to imaging. Live/dead staining was used to assess cell viability. The cells were stained with acrydine orange (AO), which is a membrane-permeable dye that stains all cells, and DAPI, which is a membrane-impermeable dye that stains the nuclei of dead cells. Briefly, both dyes were added simultaneously at a concentration of 0.1 µg/mL for 10 min. The cells were then imaged immediately without rinsing to avoid dislodging of dead cells.

Results

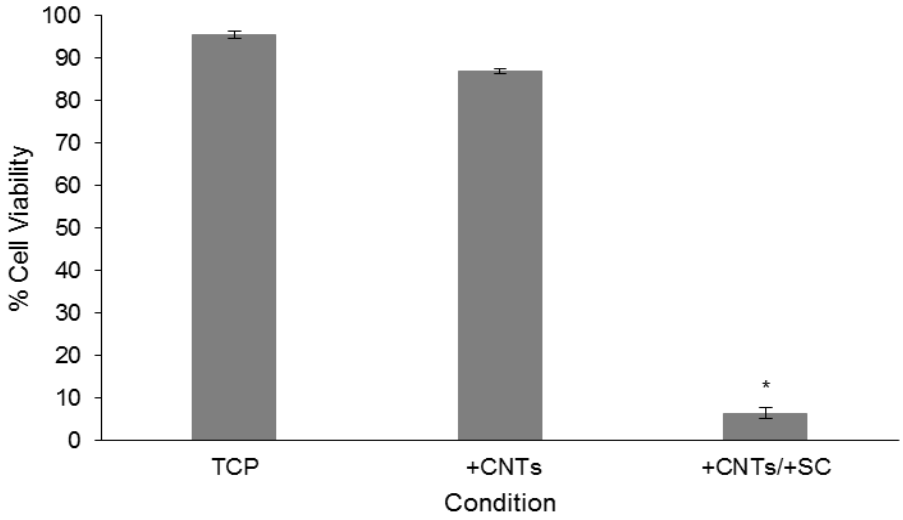

Figure 1S. PC12 cell viability within PEG-CNT composite hydrogels after 2 hours. PC12 cells were encapsulated within hydrogel samples to determine the effect of 1% w/v SC on cell viability. Previously, we have shown successful dispersion of CNTs with an added 1% sodium cholate surfactant (Figure 2). Percent cell viability was determined by counting the cells via ImageJ software after the hydrogels (+CNTs and +CNTs/+SC) were incubated for 2 hours. Significant differences are designated by an asterisk compared to the +CNTs condition ($n = 3$; $p<0.05$).

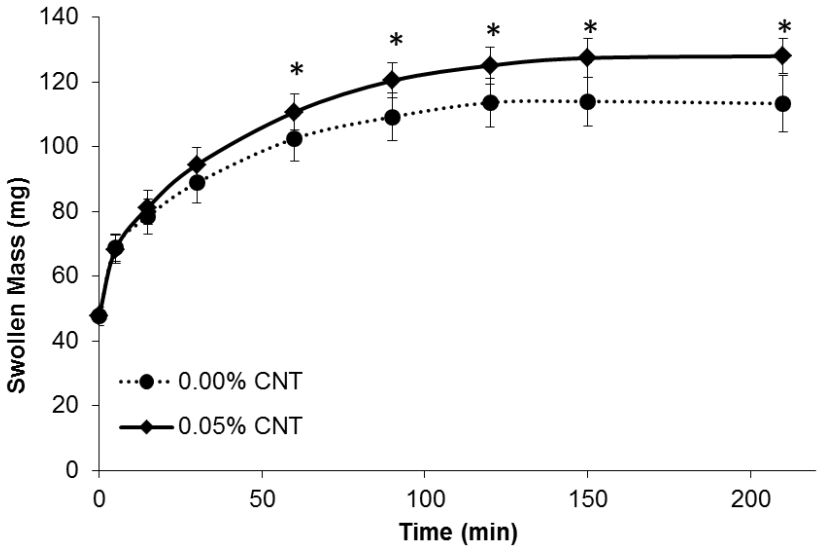

Figure 2S: Hydrogel swelling as a funciton of time for a hydrogel without CNTs (0.00% w/v) and with 0.05% w/v CNTs. Asterisks designate significant difference from the 0.00% w/v condition.

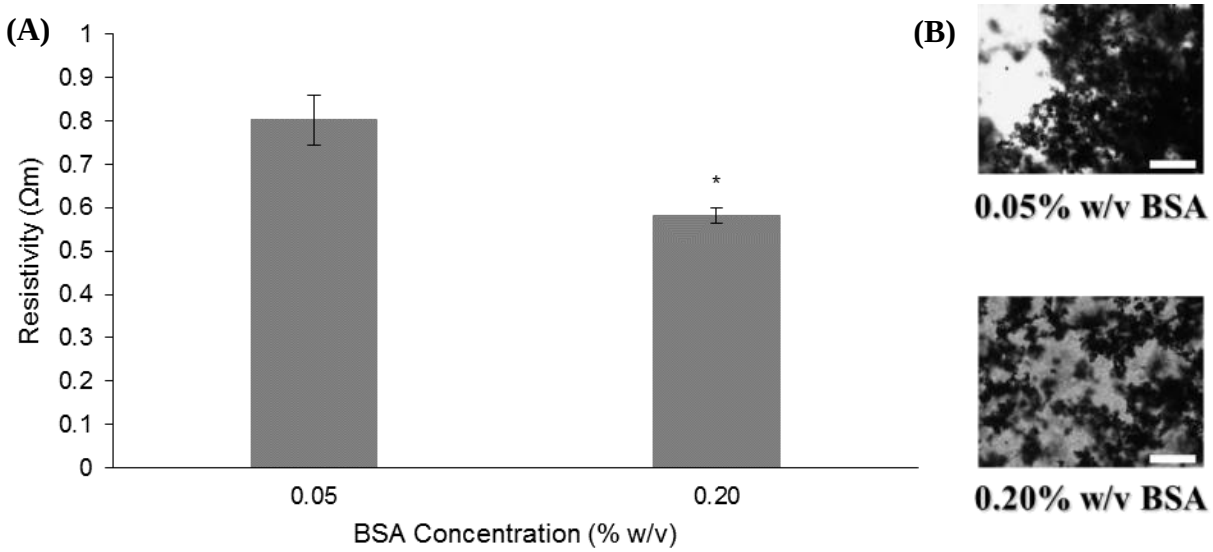

Figure 3S. Resistivity characterization of PEG-CNT composite hydrogels as a function of CNT dispersion (i.e. BSA concentration). Resistivity measurements of composite hydrogels with 0.20% w/v CNTs were taken at two different BSA concentrations: 0.05% w/v and 0.20% w/v **(A)**. It was determined that as the concentration of CNTs increased to 0.20% w/v with added 0.05% w/v BSA, larger CNT aggregates were present throughout the hydrogel. To effectively produce a homogenous dispersion of CNTs within the PEG hydrogel, the BSA concentration was increased proportional to the amount of present CNTs: 0.20% w/v **(B)**. Significant difference in comparison to the original 0.05% w/v BSA concentration is designated by an asterisk ($n = 3$, $p<0.05$).

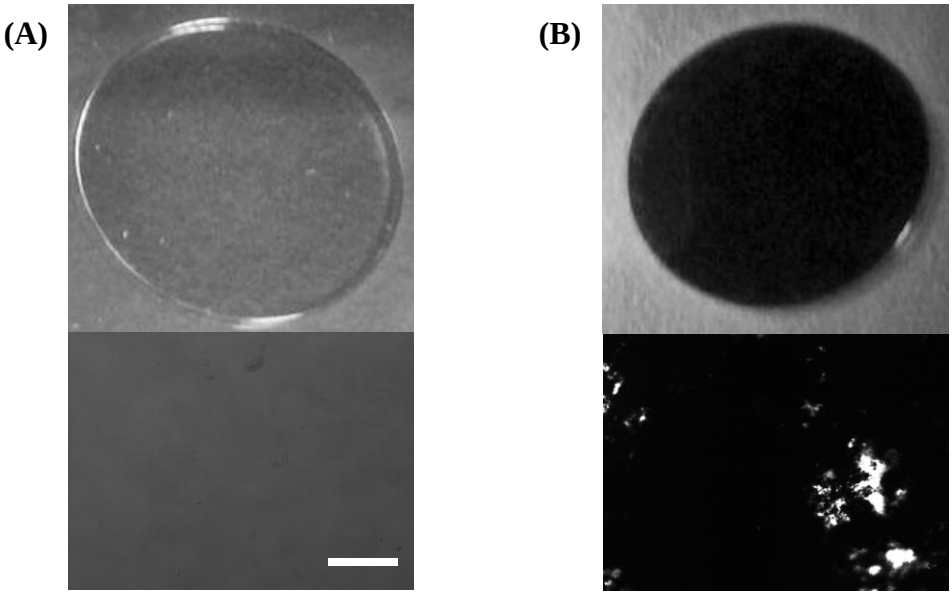

Figure 4S. Macroscopic and microscopic images of PEG hydrogels with and without CNTs. PEG hydrogel in the absence of CNTs is a transparent material **(A)**; however, the dispersion of 0.20% w/v CNTs yielded a significant colorimetric difference **(B)**. The microscope images were taken at 10x magnification and the scale bar is 200 μm.

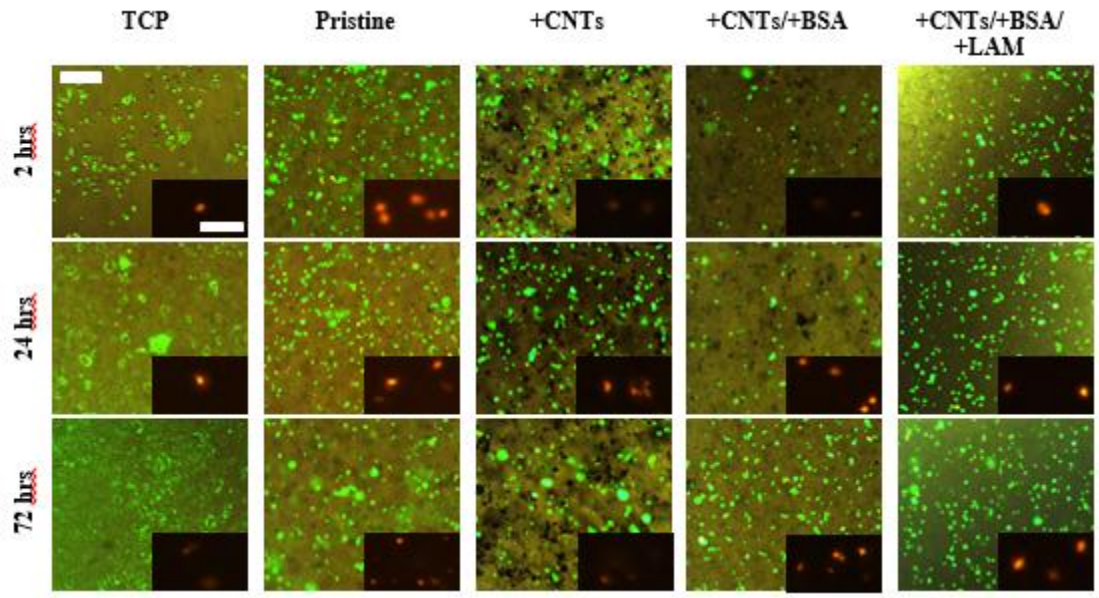

Figure 5S. PC12 cell viability within PEG-CNT composite hydrogels as a function of time. PC12 cells were encapsulated within the various hydrogel samples and stained with DiOC (green) and PI (red) for fluorescence microscopy analysis (top scale bar = 200 μm; bottom scale bar = 50 μm for insert). Images were processed through ImageJ software by counting the number of green and red cells to determine the resultant percent cell viability. We determined a qualitative difference and decrease in cell death with the introduction of CNTs compared to cells encapsulated within hydrogels without CNTs or surfactants (Pristine).

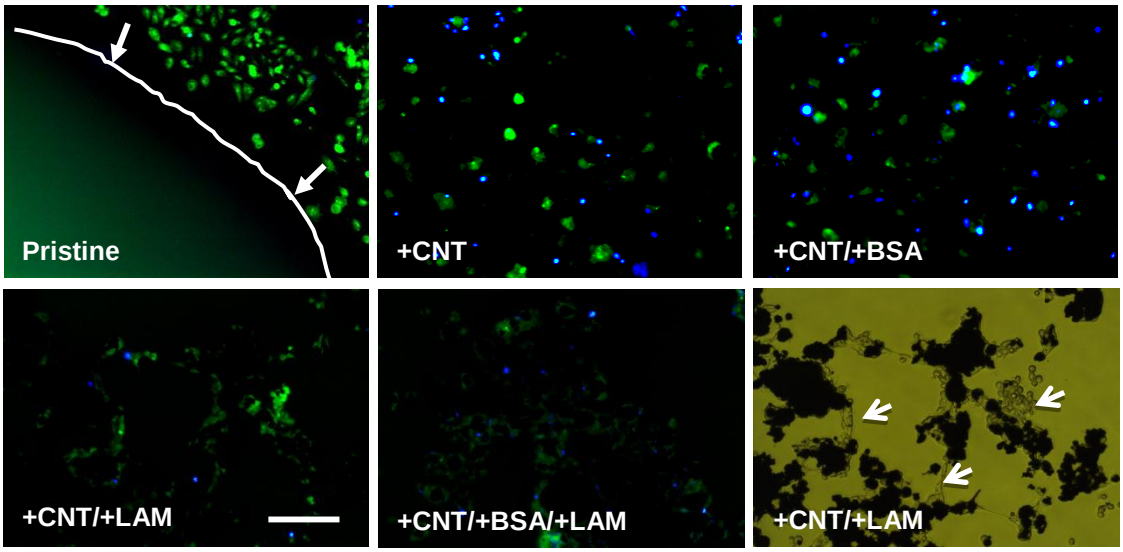

Figure 6S. PC12 cells seeded on 2D PEG-CNT hydrogel composite. Cells were cultured on the hydrogel scaffolds for 24 hrs and stained with acrydine orange (green - all cells) and DAPI (blue - dead cells); scale bar = 200 µm. In the first image (Pristine – no CNTs no surfactants), the white border and arrows indicate the edge of the pristine hydrogel demonstrating that cell were unable to grow on the pristine PEG hydrogel. The green-stained cells on the side of the hydrogel (growing on the tissue culture dish) indicated that the lack of cell growth on the hydrogel was not due to the inherent toxicity of the hydrogel but its inability to support cell attachment. Cells successfully attached to the surface of the CNT coated PEG hydrogel (+CNT), albeit significant cell death was noted. The number of dead cells further increased when the PEG-CNT hydrogel was coated with BSA – a protein that inhibits cell attachment (+CNT/+BSA). Cells attached successfully with minimal cell death on the laminin and BSA-laminin coated PEG-CNT hydrogels (+CNT/+LAM and +CNT/+BSA/+LAM, respectively). Upon closer observation of the phase contrast images of the laminin-coated PEG-CNT hydrogels, we noted that the cells clustered on and around the CNTs (white arrows) and not on the bare hydrogel surface.