Towards Single-Walled Carbon Nanotubes as an Integrated Component of Conductive Biomaterials: The Effect of Production Contaminants on in vitro Cell Viability and Metabolic Activity

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Introduction: Single-walled carbon nanotubes (SWNT) have been the focus of considerable attention as a material with extraordinary mechanical, electrical, and thermal properties[1]. Due of these properties SWNT have been proposed for use in a number of materials and biointerface applications such as nanoelectronics, biosensors, materials and tissue engineering applications including, neural, bone and dental tissue engineering[2, 3]. As an implantable composite biomaterial, SWNT will come into direct surface contact with surrounding tissues, in the case of degradable tissue engineering scaffolds; SWNT will be completely integrated within host tissue. As such the boundary between host tissue and construct will become less clear and compatibility issues will become more acute.

Little is known about the effects SWNT have on mammalian cells and tissues [4]. This investigation describes the effect of SWNT on 3T3 mouse fibroblast viability and metabolic activity. Three different SWNT preparations were investigated to determine the effect of production contaminants and hydrophobic/hydrophilic interactions of SWNT on cell viability and metabolic activity. As purchased (AP-NT) were purified by acid treatment (PUR-NT), AP-NT were also chemically modified by functionalizing glucosamine (GANT) to improve hydrophobic-hydrophilic interactions[5].

Experimental: AP-NT (AP grade) were purchased from CarboLex Inc (Lexington, KY), produced by arc discharge method using nickel as the catalyst and yttrium as catalyst support. Various SWNT preparations were analyzed: as purchased (AP-NT), purified (PUR-NT) and functionalized with glucosamine (GA-NT), over a concentration range from 0.001-1.0% (wt/vol). SWNT interactions were separated into two groups, 1. direct application of SWNT in cell culture media, and 2. the effect of soluble components by dialyzing media against SWNT (Dialysis tubing MWCO: 12000-14000). Treatments were applied to confluent 3T3 fibroblast cell cultures. Cell viability was determined using the Trypan Blue dye exclusion assay and metabolism assessed by Alamar Blue assay to determine the redox potential of cell/SWNT composites on a per cell basis. Structural and histological analysis was carried by SEM, TEM and phase contrast light microscopy. Additional controls of oxidized and annealed carbon (graphene 99.9%) were assessed to separate the effects of SWNT production contaminants from pure carbon.

Due to a layering effect of NT preparations on 3T3 cells at higher concentrations, potential mass transfer limitations were assessed. SWNT preparation were allowed to settle BD inserts for three days and the inserts were then placed in wells with 1.5ml PBS. Glucose content of the solutions in the wells was measured over time for 72hrs using an Accu-Check glucose analyzer. Total protein content in the wells with inserts was measured at the endpoint (72hrs) using Bradford's total protein assay. Results obtained were statistically analyzed using 1-way and 2-way ANOVA with a significance level $p<0.05$.

Results: TEM and mass analysis shows a reduction in carbon and catalyst residues as SWNT samples were purified and functionalized. FTIR confirmed the presence of carboxylic acid groups (purified by nitric acid reflux) and amide bonds (glucosamine functionalized). Inoculation of SWNT directly onto 3T3 cell cultures showed a negative dose dependent relationship for all preparations. As SWNT concentrations increased, cell viability decreased with AP-NT having the lowest viability and GA-NT having the highest. All the SWNT preparations showed significantly lower viability compared to control values. Significant variation from control values in metabolic activity was dependant on SWNT preparation. No significant differences were found between PUR-NT, GA-NT, and controls, with AP-NT having a higher activity at lower concentration and less activity at higher concentrations. Results from conditioned media for the effects of the soluble components present with SWNT show that GA-NT conditioned media has the highest cell viability. No significant mass transfer limitations were detected with the direct inoculations with the exception of protein transfer through PUR-NT at higher concentrations. At concentration range 0.25-1% (wt/vol) of PUR-NT preparation, a significant decrease in protein transfer was observed.

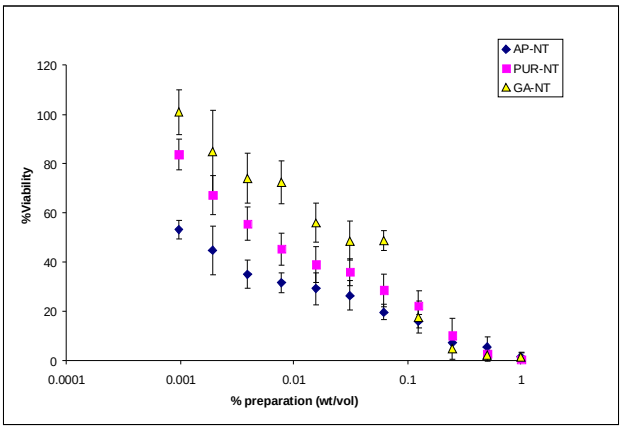

Figure 1: Cells in GA-NT preparation have the highest viability followed by PUR-NT, followed by AP-NT. All the preparation and concentrations show a statistically significant difference ($p<0.01$) with the exception of concentrations higher than 0.125%.

SEM at higher magnifications gave further insight of cell-NT interactions. AP-NT cultures, see Figure 2a, spherical bodies ranging from 5 to 15 microns in size were observed. These spherical bodies were hypothesized to be detached cells covered with nanotubes that may, in some cases, have coalesced to form the larger spherical bodies. These spherical bodies were not observed in GA-NT cultures, irrespective of GA-NT concentration. Figure 2b, shows 3T3's adhered throughout the 3-D matrix in GA-NT cultures. SEM images shown are from cultures at 0.0625% NT concentration (wt/vol). At this concentration there was a significant difference in the cell viability between the preparations. Cell viability in AP-NT culture was 19.65% and 48.79% in GA-NT cultures (Control value was set at 100%).

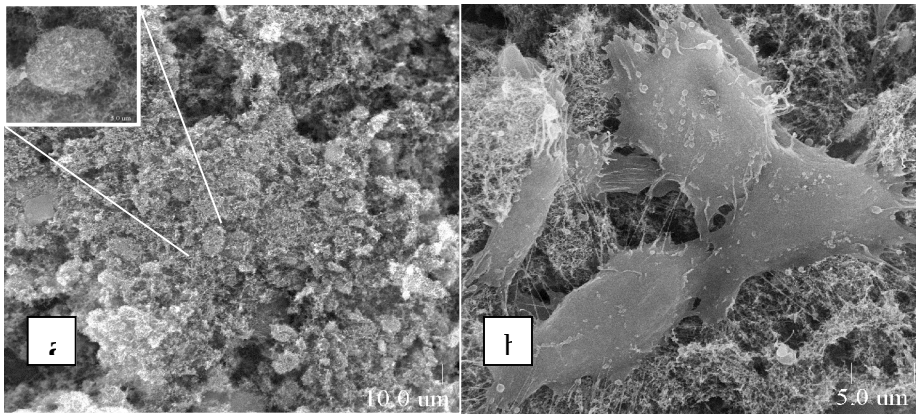

Figure 2: SEM images were taken of AP-NT and GA-NT cultures at 0.0625% NT concentration (wt/vol) a) SEM Image showing spherical bodies in AP-NT cultures hypothesized to be detached cells covered with nanotubes. b) No spherical bodies were observed in GA-NT cultures. 3T3 cells appeared to adhere throughout the 3-D matrix.

Discussion: SWNT composites provide a unique opportunity to develop biocompatible 3D matrices with programmable electrical, mechanical, and thermal properties. By modulating these properties it maybe possible to enhance tissue regeneration to speed patient recovery. In these investigations AP-NT resulted in reduced cell viability across all concentration ranges. Purification (PUR-NT) and chemical modification (GA-NT) of SWNT improved cell viability, compared to AP-NT, emphasizing that SWNT purity is vital for biological applications.

Our results have shown that the preparative method also has distinct effects on cell behavior, indicating the purification and functionalization can have profound effects on cellular function. To further understand the sole effect of SWNT, SWNT from different production, purification and chemical modification methods need to be explored. It is clear that in order to take full advantage of SWNT properties a through understanding of both chemical and physical effects of SWNT on cell populations is required. Our results suggest that the negative effects observed in AP-NT cultures can be countered by purification and chemical modification, where the optimum modification is likely to be application specific.

References:

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