An Investigation of Cellular Toxicity of Carbon Nanomaterials in African American Breast Cancer Cells

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ABSTRACT

Functionalized carbon nanotubes (CNTs) and nanofibers (CNFs) are attractive not only because they are theoretically easier to disperse in organic solvents and water, but also because there is some evidence that these modifications may enhance solubility and/or decrease cytotoxicity in biomedical applications. The cellular toxicity of CNTs and CNFs was tested in vitro on breast cancer cells. A Cell survival assay was evaluated with different concentrations of unmodified and modified CNTs and CNFs.

Keywords: cytotoxicity; carbon nanotubes; carbon nanofibers; solubility

1 INTRODUCTION

Carbon based nanomaterials (eg, carbon nanotubes and nanofibers) have become the subject of intense research over the past 10 years. In the life sciences, carbon nanotubes in particular are being touted for a range of applications, including biosensing, drug delivery, electrochemical detection of biological species, and tissue scaffolding. However, understanding the cytotoxicity of these materials is essential before their potential can be realized [1-2]. The carbon based nanomaterials of particular interest to this study are carbon nanofibers (CNFs) and multi-walled carbon nanotubes (MWCNTs).

The structure of a carbon nanotube can be visualized as a two-dimensional sheet of graphene which has been wrapped into perfect cylinders. There are two types of carbon nanotubes: single walled carbon nanotubes (SWNTs), consisting of a single graphene layer, and multi-walled carbon nanotubes (MWCNTs), consisting of graphene layers stacked one inside the other. MWCNTs have been shown to have less toxic effects on cells than SWCNTs [3].

Carbon nanofibers are more closely related to graphite, which form cup or cone structures that stack to form fibers. Though CNFs do not contain the physical properties of CNTs, they have the advantage of being inexpensive, easy to produce, and can be synthesized with predetermined properties [4].

Pure CNMs are significantly unreactive and insoluble. Research has shown that increasing solubility by the addition of functional groups to carbon nanomaterials decreases cytotoxicity in some cells while increasing cytotoxicity in others [5,6]. This study evaluates the effects of concentration, size, and chemical modification on the cytotoxic effects of carbon nanomaterials.

2 MATERIALS AND METHODS

MWCNTs were purchased from Materials and Electrochemical Research (MER) Corporation. MWCNTs were synthesized by catalytic chemical vapor deposition (CVD) with 50 nm diameter and approximately 30 micron length. The purity of as received MWCNT is greater than 90%, with less than 0.1% metal (Fe) content. The amine modified tubes were acquired from Nanocyl, inc. These tubes have an average diameter of 10 nm. The CNFs (Pyrograph III™, grade PR-19-HT) were acquired from Applied Sciences Incorporated. They have an average diameter 100 nm.

2.1 Oxidation of MWCNTs and CNFs

A 10mg portion of MWCNTs and CNFs was added to a 40mL mixture of sulfuric acid/nitric acid (3:1 by volume, respectively). The mixture of MWCNTs was sonicated for 3h at 40^{o}C and the mixture of CNFs was sonicated for 5h at 40^{o}C. Both mixtures were then diluted 1:5, by volume, with distilled water for 24h. The modified MWCNTs and CNFs were then filtered and allowed to air dry for 24h. The modified MWCNTs and CNFs were characterized by FTIR (Fig. 1).

2.2 Solubility/Dispersion

Solubility data was analyzed for modified and unmodified carbon nanomaterials between 2μg/mL and 10μg/mL using distilled water and dimethyl sulfoxide as solvents. CNMs were sonicated and the observed solubility/dispersion was recorded on a scale of 0 to 4 (4=soluble; 3-0=dispersion with 3 being the most dispersed).

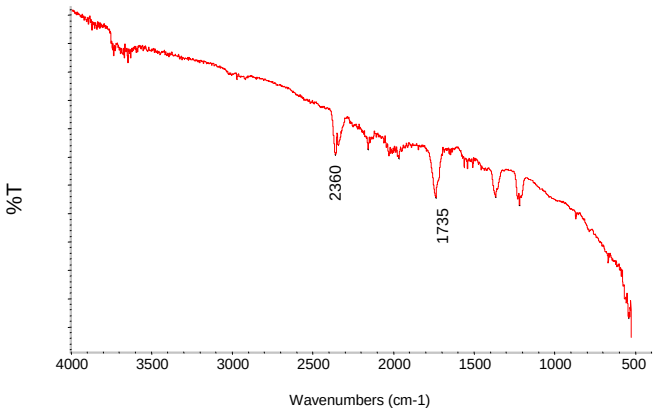

Figure 1: IR of M-CNT-COOH

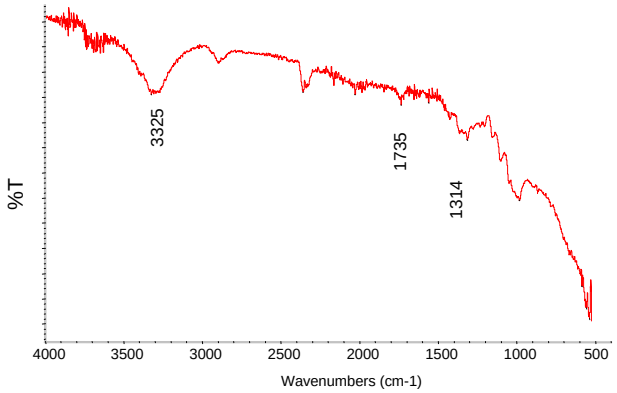

Figure 2: IR of M-CNF-COOH

2.3 Cytotoxicity Assay

African American breast cancer cell line MDA-MB468 was grown to ~80% confluency at 37°C. Cells were plated in 96-well culture plates at approximately 5000 cells/well. The cells were exposed to modified and unmodified MWCNTs and CNFs in concentrations ranging from 0.002µg/mL to 0.2µg/mL. The treated cells were then assayed for viability using a MTT solution (2mg MTT/mL PBS) after 48h [7]. Yellow MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide is reduced by metabolically active cells resulting in purple formazan which can be quantified spectrophotometrically. Cells were treated with MTT for 2h. DMSO was then added to each well and the optical density, which is proportional to living cells, was analyzed on a plate reader using Soft Max program (Molecular Devices Corportation, CA). This assay was done in triplicate.

3 RESULTS AND DISCUSSION

Solubility and dispersion are key factors when treating cells. Solubility refers to a substance completely dissolving in a solute; however, dispersion refers to a system of fine particles evenly distributed in a medium. Untreated CNMs can be dispersed for a relatively short period using the aid of mechanical energy (Fig.3).

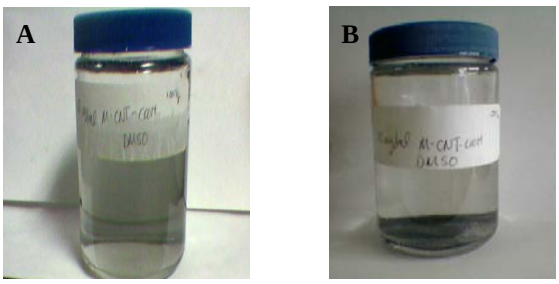

Figure 3: (A) Sonicated sample of M-CNT-COOH in DMSO. (B) M-CNT-COOH 10 days after sonication.

Modifications increase the hydrophilic character of CNMs and thus aid in dispersion [8]. Solubility data showed a general trend suggesting that CNMs used in this study were more soluble when using DMSO as the solvent, and these nanomaterials remained dispersed for a longer period compared to the unmodified nanotubes (Fig 4). In addition, modification rendered CNMs more soluble (Fig 5).

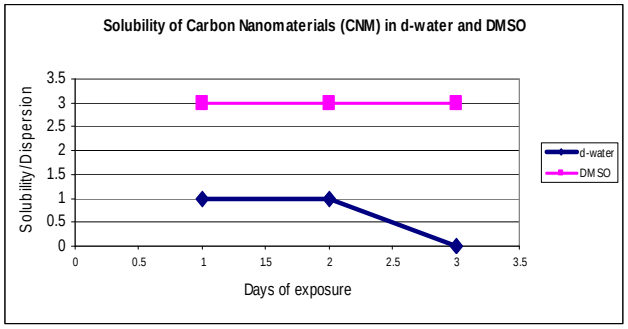

Figure 4: Solubility of CNMs in d-water and DMSO (4=soluble; 3-0=dispersion with 3 being the most dispersed).

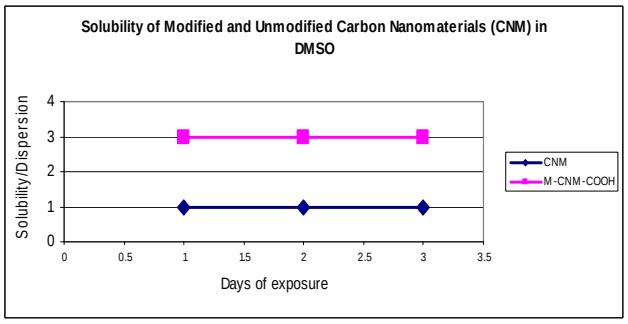

Figure 5: Solubility of modified and unmodified CNMs in DMSO (4=soluble; 3-0=dispersion with 3 being the most dispersed).

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The cytotoxicity of CNMs can be related to their small size and poor solubility. This study measured the effects of concentration, size, and modifications on the cytotoxic effects of CNMs. MTT viability suggested that there was very little difference between 0.002µg/mL and 0.02µg/mL samples, but increasing the concentration to 0.2µg/mL increased cytotoxicity. Compared to the control, both modified and unmodified CNMs resulted in significant toxic effects. Carbon nanotubes modified by the addition of a carboxyl (COOH) showed similar cytotoxicity as unmodified carbon nanotubes, while those modified by the addition of an amine (NH_2) seemed to increase cytotoxicity (Fig.6).

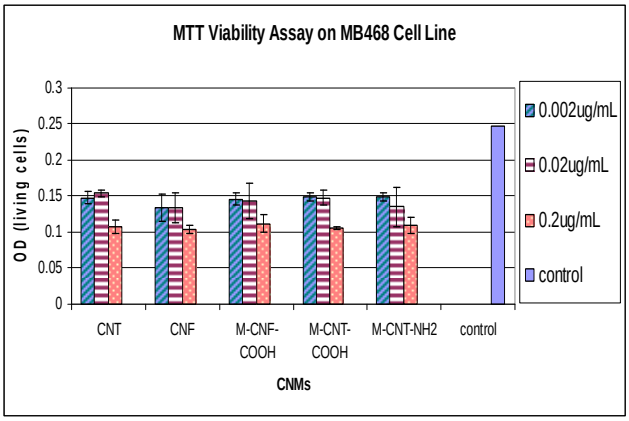

Figure 6: Cytotoxicity of CNMs at varying concentrations.

Though previous data suggests that CNFs are more toxic than CNTs, results from our study indicated that modifying CNFs by the addition of a carboxyl group renders their cytotoxicity comparable to that of modified and unmodified CNTs [6].

In addition, our study demonstrates that the effects of chemical modification on cytotoxicity is specific to the CNM of choice as well as the cell line of choice. Studies involving human dermal fibroblast and cells of the immune system have reported a decrease in cytotoxicity by modifying CNMs with a carboxyl group while studies involving human lung tumor cells showed that this modification leads to an increase in cytotoxicity [5, 9, 6]. In addition recent studies have suggested that CNMs can interfere with the MTT assay giving a false viability reading [10].

Further research involving different cell types, modifications, and viability assays is necessary to better understand the cytotoxic effects of CNMs.

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