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Toxicity Assay of Multi Walled Carbon Nanotube

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Abstract:
Synthesis of MWCNT by CVD method and in-vitro toxicity assay on breast cancer cell line (MCF-7) was studied. The data reported herein demonstrates the formation of MWCNT of 20 nm widths and induces apoptosis in MCF-7 cells (human breast cancer cells) at the 300 µg/ml and 600 µg/ml concentrations in dose dependent manner.

Keywords: Multi walled carbon nanotube, CVD, MCF-7 cancer cell line, apoptosis, toxicity.

INTRODUCTION

Recent advances in the development of nanomaterials for use in industrial and biomedical applications have yielded several types of nanoparticles less than 100 nm in size. Both engineered nanoscale materials developed in the laboratory, and naturally occurring particulates falling within the 1–100 nm size range, are included in the term nanoparticles [1]. These vary in composition, physicochemical characteristics and biological toxicity [2, 3]. Carbon nanotubes (single walled, multiwalled) [4–10], fullerenes [11–14], carbon nanotube derived structures (horns, loops, peapods), ultrafine particles in commercial products such as aerosols and sunscreens (i.e. SiO_2, TiO_2) [15], or in the work environment (asbestos, beryllium etc) [16–20], diesel and air pollution particles [20, 21], can cause toxicity. The effect and mode of action of these nanoparticles in the environment and within living systems cannot yet be predicted with any certainty because none of these are exactly the same [22]. Each new type of ultrafine nanoparticle has unique physicochemical properties (surface charge, surface properties in terms of biologic reactivity, shape and size, deformability, durability, tendency to aggregate, hydrophobicity), which will determine how this particle will interact with the environment and biological systems [22, 23].

Since their discovery in 1991 [24], carbon nanotubes (CNTs) have been synthesized by numerous methods such as laser vaporization, arc discharge, pyrolysis and plasma-enhanced or thermal chemical vapor deposition (CVD). Among the large variety of these methods, the CVD method has many advantages over others.

CNTs can be synthesized with high purity, high yield selective growth and vertical alignment [25]. Soda-lime glass is commonly used as a substrate. Using the plasma-enhanced CVD method, the growth of CNTs was achieved at 666°C by Ren and his coworkers [26]. However, the growth temperature of thermal CVD is normally as high as 700-1000°C. In this review, we report a low temperature growth (500-750°C) of CNTs by thermal CVD using turpentine oil as carbon precursor and nickel nanoparticles as nucleation sites for the growth of CNTs.

In general, the potential for bio-reactivity of a nanoparticle increases as the particle size decreases due to two inherent factors: (1) The smaller the particle the greater the surface area per unit mass [23, 27], and (2) the particle surface characteristics [28, 29]. The new drug delivery system seeks the application of nanoparticles especially in cancer therapy to minimize the toxicity to the healthy cells ferrying large dose to the cancerous cells. The focus is on strategies that suppress tumor growth by activating the apoptotic program in the cell [30]. The extent of apoptosis could contribute to cell loss in tumors and promote tumor regression. MCF-7 cell line has become a prominent model system for the study of breast cancer as it relates to the susceptibility of the cells to apoptosis. In this study, the effects of the MWCNT on cultured MCF-7 human breast cancer cells were investigated, due to their reported potential biological applications [31].

MATERIALS AND METHODS

CNT Synthesis by CVD method was done as per the following procedure.

MWCNT Synthesis

NiO nanoparticle (0.1gm) and graphite (0.02 gm) was mixed using mortar and pestle and put in a cleaned quartz boat. This was placed at the centre of pyrolysing furnace for 15 minutes at 400°C, for the conversion of NiO to Ni and CO in an inert atmosphere of nitrogen gas.

$$NiO + C = Ni + CO \uparrow$$

The gas was passed through quartz tube at the rate of 4 bubbles/second for 30 minutes. Turpentine oil (2 ml) was taken as precursor in quartz boat and placed in the centre of the tube in the vaporizing furnace. Vaporizing furnace and pyrolysing furnace temperature was set at 250°C and 750°C respectively. After 45 minutes, both the furnaces were switched off. The black powder residue was scratched out from the boat of pyrolysing furnace for further studies. Purification was done adding chromic acid (20%) to the powder with mild heating in water bath. This was followed by repeated centrifugation with distilled water to remove the acid and other contaminants. The black powder was dried in the oven and stored in vacuum desiccators. The yield obtained was 36%.

Characterization

Microscopical analysis was done by TEM [transmission electron microscopes, Morgagni 268D, Fei Electron Optics] and SEM [scanning electron microscope,

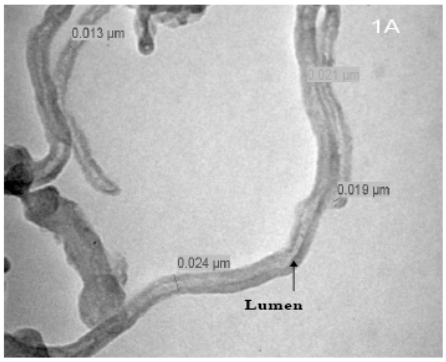
Leo 435 VP]. Samples were suspended in Tween 20 by sonicating for 30 minutes before microscopical examination.

In-vitro cell toxicity assay

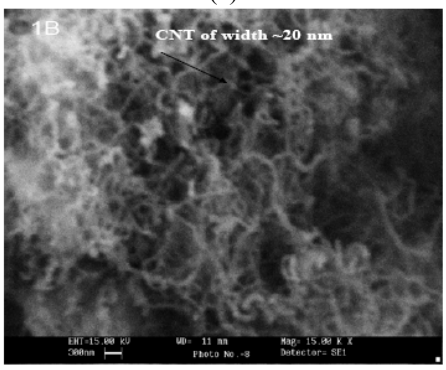
MCF-7 cells were seeded in a 6 well plate containing 2 sterile glass cover slips in each wells, at a density of 10.000 cells per well and incubated for 2 days at 37ºC and 5% CO_2 in RPMI medium with 10% fetal calf serum (FCS). Different concentrations (300 µg/ml and 600 µg/ml in Tween 20) of MWCNT solutions were exposed to cells for 48 hours. The cells with media were kept as negative control. Propidium iodide (PI, Sigma), 50µg/ml was added to each well to detect apoptosis. The coverslips were washed with PBS taken out and the morphology of the cell nuclei was observed using Olympus (D71) microscope and shown in figures 2a, 2b and 2c.

RESULTS AND DISCUSSION

As observed as in figures 1a& 1b, the average diameter of MWCNT was about 20 nm which is desirable for biological applications [31]. The lumen of the tube is clearly observed as shown by arrow in Figure 1a.The walls appear to be relatively thick indicating the formation of MWCNT [32].



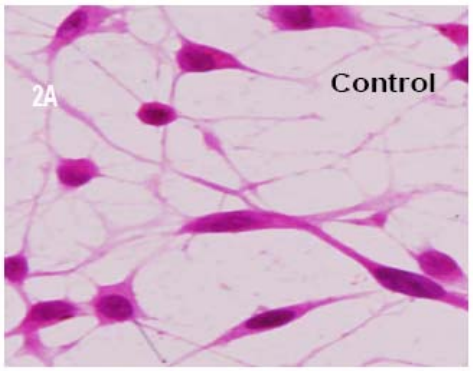
(a)



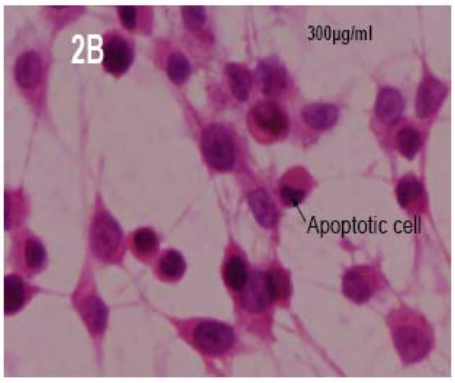
(b)

Figure 1. TEM micrograph of purified MWCNT (a) and TEM micrograph of carbon nanotubes grown at 750°C (b)

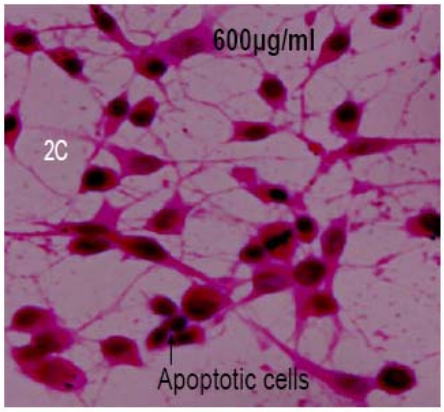
The synthesis of MWCNT by CVD method used is efficient and gives high yield. To investigate the potential effects of MWCNT on proliferation and survival of MCF-7 cells, the cells were exposed to two different concentrations of MWCNT solution (300 µg and 600 µg/ml of medium) for 48 h. Figures in 2a, 2b and 2c show MWCNT induces apoptosis in dose dependent manner. MWCNT treated MCF-7 cells (Fig. 2b and 2c) when compared with the untreated group (Fig. 2a) gives the idea of the effect. MCF-7 cells with lower DNA staining relative to diploid analogs were considered apoptotic.



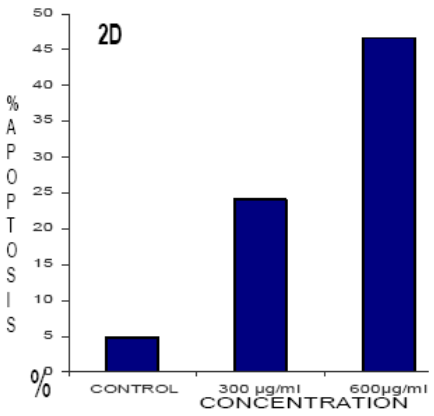
(a)



(b)



(c)



(d)

Figure 2. (a) MCF-7 cells without MWCNT treatment. Cells show normal proliferation. (b) MCF-7 cells exposed to 300 µg/ml. Few apoptotic cells were observed. (c) MCF-7 cells exposed to 600 µg/ml MWCNT. Many apoptotic cells were observed. (d) Apoptotic levels in MWCNT treated cells after 48 h. Treatment of 600 µg/ml significantly increased the level of apoptosis by in MCF-7 cells when compared to untreated controls, as judged by apoptotic morphology by nuclear staining assay described in the Experimental Procedures. Increased levels were observed till 72 hours of SPD treatment. Results were presented as the means ±SD of 6 independent experiments.

300 µg/ml and 600 µg/ml show 24.13% and 46.66% apoptotic cells respectively. Thus by doubling the concentration of nanotubes, two fold increase in apoptosis is noted. As seen in figures (2a and 2c) the nuclei of many cells appear condensed and dark. The morphological hallmarks of the apoptotic process include loss of cell volume, hyperactivity of the plasma membrane, and condensation of peripheral heterochromatin, followed by cleavage of the nucleus and cytoplasm into multiple membrane-enclosed bodies containing chromatin fragments [33-36].

From these images (figures 2a, b, c and d) we can say that in comparison to untreated cells MWCNT has induced apoptosis in dose dependent manner. It has been reported in previous studies (37, 38), that the mechanism of induction of apoptosis is present and functional in MCF 7 cells and can be initiated by external stimuli. The data reported herein appear to demonstrate that MWCNT induces apoptosis in the MCF-7 cells at the higher concentration, exhibiting the morphological changes.

CONSLUSION

Due to the immense application possibilities of CNT in diverse fields including therapeutics, it is imperative to investigate the environmental effects of this nanomaterial. Considering the futuristic use of this nanomaterial in medicine, its effect on breast cancer cell line, MCF-7 was studied. The low temperature CVD method used for synthesis resulted in the formation of fine MWCNT. These nanotubes exhibited apoptosis in MCF-7 cell line, which indicates possible toxicity to mammalian cells.

On the other hand, the stimulation of apoptotic cell death as observed in this study can be used beneficially for the control of tumor growth. Thus, the carbon nanotubes need to be tested, and their biocompatibility fine-tuned to be optimized for the biological applications. Both aspects of the results need to be considered following in depth analysis.

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