Evaluating Nanosafety of Nanomaterials by In-vitro Cytotoxicity Tests on Fibroblast Cells

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Abstract
In the recent years, the use of nanomaterials has increased exponentially. In spite of the extensive research in this field, some areas are not completely understood, one of them being nanosafety. Because of their size and shapes, these nanomaterials have the ability to be easily absorbed in the biological and the ecological systems and interfere with them by hampering smooth functioning. In this study, MTT Assay was used to evaluate the nanosafety of carbon based nanomaterials (Carbon Nanowire, Graphene and Carbon Nanotubes). All of the nanomaterials exhibited various levels of cytotoxicity. The level of cytotoxicity was dependent on the concentration, the size and shape of the nanomaterial. Smaller particle sizes exhibited higher cytotoxicities, which may be useful for the students, scientists, engineers and other participants who involve in these nanomaterials.

1. Introduction
Nanotechnology holds the promise to change the world. It is presently at the forefront of almost every field of research be it – electronics, structures, construction, medicine, paints and coatings, storage, fuel cells, energy saving, military etc [1]. At the nanometer scale, certain materials exhibit new properties which are otherwise not exhibited at the macro scale. Carbon nanowires are a one-dimensional array of carbon atoms which are threaded through carbon nanotube [2, 3]. Carbon nanotubes are allotropes of carbon in the shape of a tube having nano scale diameter [4]. Graphene is a single-atom-thick sheet of sp2-bonded carbon atoms in a closely packed honeycomb two-dimensional [5]. It has a unique form. Most of the nanomaterials have a high aspect ratio which makes them sharp needle like structures. These nanomaterials due to their small scale and needle-like morphology can enter the biological cells easily and once they enter they can cause complete destruction of the cell by necrosis. The possibility of nanomaterials having an adverse impact on human health and the environment has been a cause of concern [1, 6]. These nanomaterials have numerous applications in the technological world, most of which would involve occupational or public contact with biological systems. It is hence essential to understand the nature of the material and timely take preventive steps.

MTT that is a type of colorimetric assay in which (3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide is used as the reacting agent. In MTT assay the yellow tetrazolium salt (MTT) is reduced, in the present of metabolically active cells, to form insoluble purple formazan crystals [7,8].

2. Experiment, Results and Discussion,

The MTT assay was carried out on carbon nanowire (100 ply), graphene nanoflake (Figure 1), carbon nanotubes (multiwall carbon nanotubes, 10-20nm diameter, 1-2µm length, Figure 2).

Figure 1 – SEM image of graphene powder/flakes

Figure 2 – SEM image of multiwall carbon nanotubes
The nanomaterials were soaked in the DMEM solution for 1-10 days in an incubator at 37°C and this nano-rich solution was separated and stored. The solution was then diluted to concentrations from 1:1 to 1:32. 200µl of the solution added to the fibroblast cells in a 96 well plate. The fibroblast cells are shown in figure 3. The cells in each plate were the same. They were incubated for 3 days after which 20µl of MTT was added to each well. The yellow MTT is reduced to purple formazan in the mitochondria of the living cells. The percentage of living cells can be estimated by the level measuring the absorbance of the colored solution. A spectrophotometer at 590nm was used to measure the absorbance. The results have been discussed further.

![Figure 3 – Microscopic image of cultured fibroblast cells.](image)

![Figure 4 - Graph showing the effect of dilution on the cytotoxicity of carbon wires, graphene and carbon nanotubes.](image)

From the graph in figure 4, it can be clearly seen the level of cytotoxicity is the highest in graphene followed by carbon nanotubes and then carbon nanowires. Graphene powder is very fine and has higher level of hydrophilicity as compared to the other two. Besides that it is much smaller in size as compared to carbon nanotubes. Due to this it can easily penetrate the cells and cause complete necrosis at higher concentrations.

The effect of dilution on cytotoxicity of carbon nanowire is minimal. The reason for this effect was because of the single dimensional threaded structure of the nanowire, which is made by tightly twisting nanotubes together into a single unit. These nanotubes are not free to disperse into the medium (DMEM) and hence the total amount of medium in contact with the external surface of the nanowire remains constant. All the other cytotoxicities are concentration dependent. With a decrease in the concentration the cytotoxicity of the nanomaterials also decreases.

3. Conclusions

Carbon nanowire has the least cytotoxicity which makes it safest material out of the three. They are known to have excellent mechanical properties and fairly good electrical properties, which make them useful in bio sensors, implant strengthening, high performance composites etc. Many applications also use graphene and carbon nanotubes and due to their exceptional properties their applications are bound to increase in the near future. Rules and regulations must be in place to prevent occupational or public toxicity due to these nanomaterials. A separate standards body must be formed which foresees the safe and ethical use of nanomaterials and nanotechnology.

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References


