Carbon Nanopipe Dispersions in aqueous solutions and their effect on cell viability

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ABSTRACT

The development of carbon nanomaterials for biomedical applications appears attractive due to their nanoscale properties although their biological profile is still in doubt. Herewith, we report that the amorphous vs crystalline character of carbon nanomaterials produced using chemical vapour deposition (CVD) in anodic alumina templates can have a marked influence on their aqueous solubility and their effect on cell viability. We prepared 250nm diameter carbon nanopipes (CNP) which were removed from the templates as loose tubes. Methods were applied to optimize the length of these pipes. Nanopipes were compared with multiwall carbon nanotubes (MWNT) for their aqueous dispersion using spectrophotometry. Carbon nanopipes and MWNT were investigated for their effect on cell viability on HeLa cells using lactate dehydrogenase (LDH) assay. Results showed that CNP remained in fine dispersion in water for at least 48h whereas MWNT precipitated during the first 2h. Surfactants had minimal effect on carbon nanopipes but variable effect on MWNT dispersion. Nanopipes remained in dispersion in cell culture media during their co-incubation for the cell viability studies. LDH assay revealed that carbon nanopipes showed substantially less cytotoxicity on HeLa cells when compared to MWNT indicating that they are a promising material for biomedical applications.

Keywords: nanopipes, nanotubes, carbon, dispersion, cytotoxicity

1 INTRODUCTION

Nanomedicine is defined as the application of nanotechnology in biomedical areas for diagnostic and therapeutic purposes. One of nanomedicine's main sub-field is targeted drug delivery. In recent years, a series of nanomaterials have been investigated for their potential to improve drug delivery and/or targeting. Such systems should minimize drug degradation and loss, avoid deleterious side effects and increase the availability of the drug at the disease site (1). One class of novel drug carriers and delivery devices are based on carbon nanomaterials. Carbon nanotubes with fullerene structures show promise as potential nanoscale carriers of several small drugs and macromolecules (2). In our group we aim to investigate larger amorphous carbon nanopipes produced via CVD in carefully prepared AAO templates. These nanopipes generally have a larger diameter than nanotubes and as such can serve as carriers for large macromolecules (3). These nanopipes could be used as drug delivery systems for proteins and peptides for local or mucosal drug delivery. In this study we investigate the dispersion properties of CNP in aqueous buffers and compare them with MWNT. Then we investigate the effects of CNP and MWNT on HeLa cells viability at a range of concentrations.

2 CARBON NANOPIPES AQUEOUS DISPERSION AND CELL TOXICITY

2.1 Materials

The nanopipe arrays used in our study were produced using an established CVD method in commercially available AAO templates (Whatman Anodisc 13mm diameter, nominal pore size 200nm) (4 ,5, 6). The template consists of a 60 μm thick film of alumina covered with a well-ordered, high density array of open pores produced by electrochemical etching of aluminium metal foil. Amorphous carbon was deposited on the template by flowing ethylene gas (30% in helium) for six hours at a rate of 160 sccm at a temperature of 675ºC. SEM (scanning electron microscopy) was used to characterise the size and density of the resulting carbon nanopipe array. Pore diameter was measured to be 160 nm. The carbon covered templates were crushed into small fragments and soaked with 2M sodium hydroxide (NaOH). A mild sonication (Jencons waterbath) was introduced for 30 minutes to separate the CNPs from the membrane and dissolve the embedded AAO matrix using the NaOH. Brief high power sonication was used to segment the 60 μm long CNP into shorter lengths. The resulting dark suspension was then centrifuged at 13,000 rpm so that the CNP formed a compact sediment. The supernatant was removed using a pipette and replaced with an equal volume of deionised water followed by resuspension. This procedure was repeated three times following by two washes in 99.7% ethanol.

MWNT (Sigma-Aldrich) were also sonicated before testing.

HeLa cells were grown in a cell culture flask with complete growth cell medium at an air-fluid interface at 37ºC in a 90% humidified incubator with 9.9% CO_2 Cells were trypsinised and passaged to 96-well plates for cell viability assays.

2.2 Experimental Methods

Prior to spectrophotometry measurements, the MWNTs and CNPs stock suspensions in ethanol were mildly sonicated to ensure good dispersion. 0.1ml of stock suspension was added with 0.9ml of aqueous solutions (i.e. deionised water, different percentages of Tween 20 or Dimethyl sulfoxide; DMSO in deionised water). The measurements were then carried out using an Ultrospec 4000 UV/visible spectrophotometer with the absorbance wavelength set to 600nm (scattering wavelength). Toxicity for both carbonaceous nanomaterials was tested using LDH (Lactate Dehydrogenase) assay (Promega). LDH is a stable cytosolic enzyme which is released upon cell membrane lysis. Both CNP and MWNT were added in a series of concentrations in Optimem medium (Invitrogen) and incubated with the cells for 4 h at 37°C. Subsequently, the media containing nanotubes/pipes were removed followed washing with PBS (phosphate buffered saline). PBS was replaced by the complete growth cell medium into the well plates following by 20 hours incubation inside the incubator. Assay protocol was followed according to the manufacturer.

2.3 Results

Dispersion of carbon materials in water was investigated to assess the extent of sedimentation over time in the presence or absence of surfactants. Tween20 and DMSO were chosen as surfactants as it has been shown that they minimally compromise cell viability. Figure 1 (upper panel) shows the results of the CNP dispersion studies in water with and without surfactants. Absorbance measurements at 600nm showed that CNPs remained well dispersed in water for at least 48h. Addition of surfactants does not affect the dispersion of the amorphous carbon material. Figure 1 (lower panel) shows the dispersion of MWNTs. In this study MWNT were sonicated prior to dispersion experiments to match the treatment of the CNPs. Sonication altered the length of both the MWNT and the CNP to ca 5µm (as determined by electron microscopy; data not shown). However despite this, aqueous dispersion of MWNT showed poor stability overtime. MWNTs started to aggregate and precipitate at 30 min after their addition in water. Addition of surfactants affected MWNT stability. Tween20 had an effect of prolonging MWNT. Increasing the concentration of the surfactant gave a small but significant increase on their dispersion. DMSO had less effect on MWNT dispersion stability compared to Tween20.

Figure 2 shows the effect of carbon nanomaterials on cell viability. At all concentrations tested CNPs induced significantly less LDH release compared to MWNTs. CNPs remained in dispersion in Optimem during the experiment (data not shown). MWNTs dispersion however was affected in the experimental cell medium.

2.4 Discussion

In the current study two different forms of carbon nanotubes were investigated for their potential biomedical applications in drug delivery. Aqueous solubility or dispersion is one of the first properties to be tested in a novel material. In this study amorphous CNPs with an outer diameter of 250nm were tested for their aqueous dispersion in the presence and absence of surfactants.

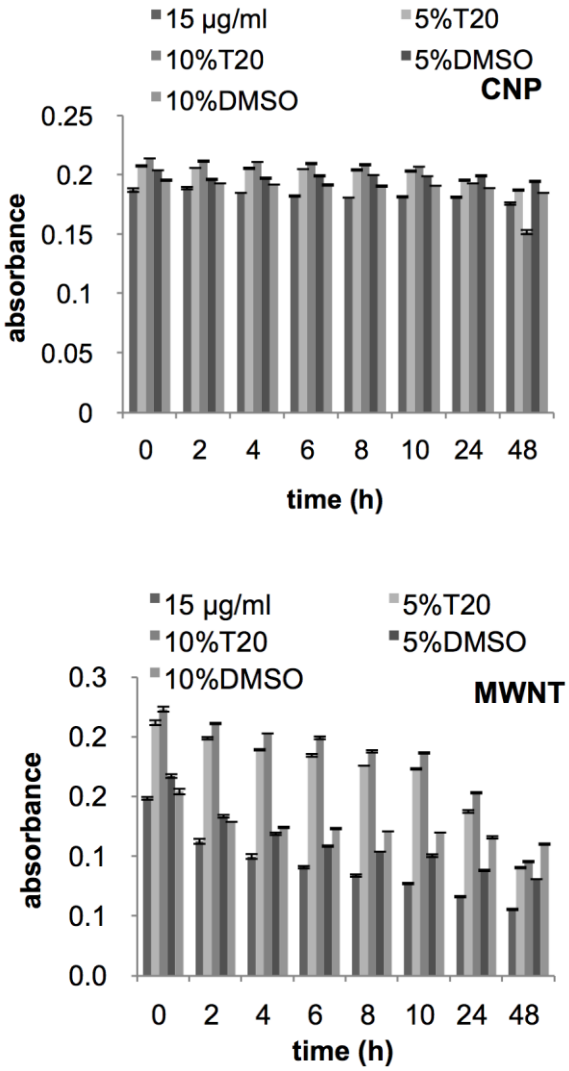

Figure 1. Upper panel and lower panel represent dispersion of CNPs and MWNTs respectively, at 15µg/ml with or without Tween 20 (T20) and/or DMSO surfactants both at 5 and 10% w/v.

The results show that amorphous carbon tubes show superior aqueous dispersion compared to MWNTs. This may be due to the amorphous structure and the chemical characteristics (-OH or -O- groups) of their surface. In contrast pristine nanotubes showed less stable aqueous dispersions. This property is important for the design of

delivery systems based on carbon materials. If delivery systems are designed for intravenous delivery they should avoid aggregation in aqueous, high salt, high protein concentration conditions such as the ones found in blood. Carbon material aggregates may lead to embolism or undesirable accumulation in lung and/or liver. Likewise if delivery systems are designed for mucosal administration they should be well dispersed to allow release of the content or the conjugated therapeutic. The second important parameter to be considered in the design of delivery systems is the effect on cell viability. In this study we tested the effect of the carbon nanotubes and nanopipes in HeLa cells, which are widely used for such investigations. In these experiments incubation time was chosen to be 4 h as this was an optimum period for MWNT dispersion.

CNPs proved to have less effect on cell viability compared to MWNT at all concentrations tested. At 11.75 µg/ml MWNTs induced substantial LDH release and cell death. In contrast CNP incubation with the cells showed minimum LDH release which represents an acceptable cytotoxic level.

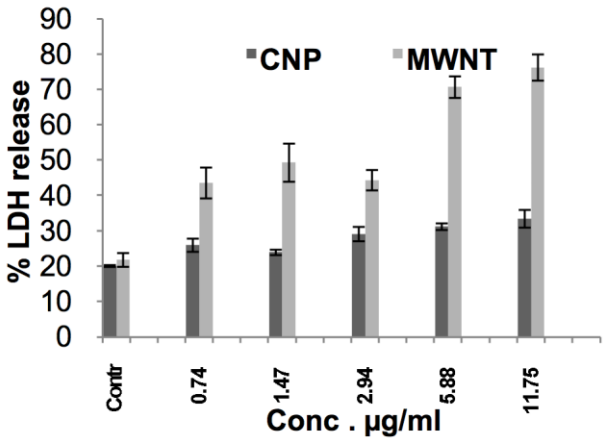

Figure 2. LDH release from HeLa Cells after incubation for 4h with carbon nanopipes and multi-walled nanotubes at increasing concentration in Optimem.

These results indicate that amorphous carbon nanopipes show substantial dispersion advantages combined with low cytotoxicity in HeLa cell. Further studies are required to better understand the effects of these materials on cell viability and function. Experiments involving a series of cell lines (including epithelial and macrophages) are required before *in vivo* administration in animal models. In addition, a series of suitable cytotoxicity and apoptosis assays are necessary to elucidate the biological properties of these novel materials

3 CONCLUSIONS

Carbon nanopipes are novel carbon nanomaterials that show sustained aqueous dispersion and an acceptably low impact on cell viability. They merit further investigation for biomedical applications.

4 ACKNOWLEDGEMENTS

All authors acknowledge support from the EPSRC, and M Thanou to The Royal Society, UK for funding. M Whitby acknowledges the support of the EPSRC through a doctoral training award. RGB Research Ltd is gratefully acknowledged for providing additional laboratory facilities.

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