Effect of Surface Functionalisation on the Interaction of Carbon Nanotubes with Human Respiratory Alveolar Cells In Vitro

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Introduction

- Nanomaterials (NM) have at least one dimension measuring less than 100nm.
- Carbon nanotubes (CNT) are fibre-shaped NMs comprising layers of graphene.
- CNTs are strong but light; they are in a wide range of products including sports, cosmetics and aeronautical goods.
- Some multi-walled CNTs (MWCNTs) have been compared to asbestos due to their needle-like shape and ability to be synthesised at very high aspect ratio.
- MWCNTs cause lung lesions in mice.
- We hypothesise that aspect ratio and surface functionalisation (charge) will critically affect MWCNT bioreactivity with cells comprising the human alveolus.

Materials

<table>
<thead>
<tr>
<th>Sample Code</th>
<th>Grafted Chemical for Functionalisation</th>
<th>Zeta Potential (mV)</th>
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</thead>
<tbody>
<tr>
<td>AR</td>
<td>as received, not functionalised</td>
<td>-10±1.6</td>
</tr>
<tr>
<td>PEGMA</td>
<td>poly(ethylene glycol) methacrylate</td>
<td>-7.8±15.3</td>
</tr>
<tr>
<td>MAA</td>
<td>methacrylic acid</td>
<td>-17.2±1.3</td>
</tr>
<tr>
<td>APTAC</td>
<td>3-acrylamidopropyl trimethyl ammonium chloride</td>
<td>+19.2</td>
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Overview of the 4 chosen CNTs with the same dimensions (9-15 nm diameter; 0.7-1.4 um length) but functionalised to exhibit different zeta potentials.

Methods

- Immortal human alveolar epithelial type 1-like cell s (TT1), primary human alveolar epithelial type 2 cells (AT2) and primary human alveolar macrophages (AM) were exposed to MWCNTs for 4, 8 and 24h.

Results: Cell Viability and Inflammation

- Fig. 1. MWCNT-induced cytotoxicity (MTT assay) induced by MWCNTs on TT1 and AT2 cells, and AM following 24h exposure. *, p<0.05.
- Fig. 2. Release of IL-6 (ELISA) from TT1 cells and AM following 24h MWCNT exposure. *, p<0.05.
- Fig. 3. Release of IL-8 (ELISA) from TT1 cells and AT2 following 24h MWCNT exposure. *, p<0.05; **, p<0.01.

Discussion & Conclusion

- There was a significant decrease in cell viability in TT1 cells exposed to 25µg/ml APTAC MWCNT and a general trend towards increasing cell death with increasing dose in all pulmonary cell types. APTAC caused the greatest cell death in AT2 cells after 24h exposure, although not significant (Fig 1).
- Although there appeared to be an increase in IL-6 release with increasing dose, this was only significant for AM exposed to AR MWCNT at 50µg/ml with varying significant increases in AT2 cells (Fig 2).
- There was a significant increase in IL-8 by TT1 cells exposed to APTAC MWCNT compared to the non-treated control (Fig 3).
- There was pattern for an increase at low doses, then a decrease at high doses, for IL-8 release by AT2 cells exposed to MWCNTs for 24 hours (Fig 3).
- ROS was significantly elevated in TT1 cells and AM (not shown); the greatest increase in fluorescence intensity in TT1 cells was after 24h exposure to APTAC at 50µg/ml (Fig 4).
- MWCNT physicochemistry, particularly surface charge, affects their interaction with human alveolar respiratory cells.
- Oxidative stress is involved in the responses, notably for positively-charged APTAC; further studies are needed to elucidate the exact mechanism.

References


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Theme 4, Project 4: Health Impacts of Nanoparticles