

In vitro studies on pulmonary- and immunotoxic effects of industrially relevant multiwalled carbon nanotubes

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Carbon nanotubes (CNTs) are among the most produced nanomaterials and their exceptional properties are exploited in many fields of technology and medicine with possible applications ranging from nanocomposites to the imaging and treatment of diseases. As a consequence, the industrial production of these novel nanomaterials will increase dramatically in the near future, raising concerns about their possible hazardous effects on the environment and living organisms. Indeed, several studies on mice and rats indicate that CNT might have a toxic potential to human health. Moreover, there is public concern that carbon nanotubes might elicit harmful responses similar to those of other toxic fibrous particles such as asbestos.

A major entry site for nanomaterials to the body is represented by the lung, thus underlining the relevance of toxicity studies on pulmonary cells. Once deposited, nanoparticles may rapidly be spread and distributed throughout the body via the circulation and lymphatic system. Our intricate immune system with numerous cell types constantly patrolling the whole body is central to the detection and elimination of foreign particles and pathogens. However, there is little knowledge on how cells of the immune system interact and cope with these novel nanomaterials.

Here, we investigated possible adverse effects of three industrially relevant multiwalled CNTs (MWNTs) on human lung cells (epithelial alveolar A549) and immune cells (Jurkat T lymphocytes). Cell viability, oxidative stress, cytoskeletal organization and cytokine production were analyzed by colorimetric or fluorimetric assays, flow cytometry, immunocytochemistry and enzyme-linked immunosorbance assay (ELISA). In addition, we compared the effects of MWNTs to those of crocidolite, a particularly potent form of asbestos.

We found that after treatment with MWNTs, both cell types showed signs of oxidative stress such as the induction of reactive oxygen species (ROS) or a decrease in the mitochondrial membrane potential (MMP). However, these MWNTs did not cause major cell death of A549 lung epithelial cells or Jurkat T lymphocytes. Measuring the production of IL-2 protein in T cells, we found that MWNTs did not activate resting T cells nor did they have major immune modulatory effects on PMA/PHA-activated cells. Asbestos slightly decreased cell viability of Jurkat cells and more strongly of A549 cells but did not induce ROS or a shift in the MMP suggesting that at least some mechanisms underlying MWNT-toxicity might be different from those of asbestos.

Our study indicates that pristine, currently large-scale produced MWNTs have low immunogenic and immunomodulatory effects on human T lymphocytes *in vitro*, however, they affect the basic functionality of cultured A549 lung epithelial cells. Further studies will be required to determine the basis of these cell type specific reactions.

In vitro studies on pulmonary and immunomodulatory effects of industrially produced multiwalled carbon nanotubes



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Introduction

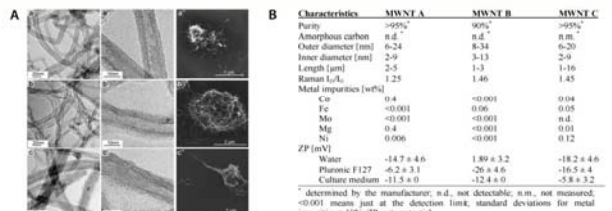
Due to their exceptional properties and their wide range of applications, carbon nanotubes (CNTs) are nowadays produced in large quantities and thus the potential for a widespread exposure to these novel materials is likely to increase. Such exogenous materials may be inhaled and distributed throughout the body by the circulation and lymphatic system. Here we investigated in vitro toxicity of three large-scale produced multiwalled CNTs (MWNTs) and crocidolite asbestos on human lung cells (epithelial alveolar A549) and immune cells (Jurkat T lymphocytes).

Conclusions

In this study we show that despite the induction of reactive oxygen species three large-scale produced MWNTs are not acutely toxic to Jurkat cells and only slightly increase cell death in A549 cells at high concentrations. MWNTs do not affect the immune competence of Jurkat cells. However, the basic functionality of A549 cells appears to be affected by MWNTs but this might be a secondary effect resulting from a reduced adhesion of A549 cells in the presence of MWNTs. However, this hypothesis remains to be tested in further experiments.

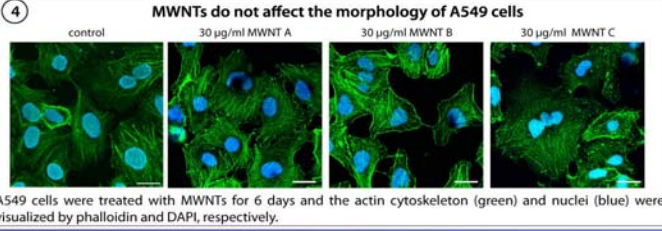
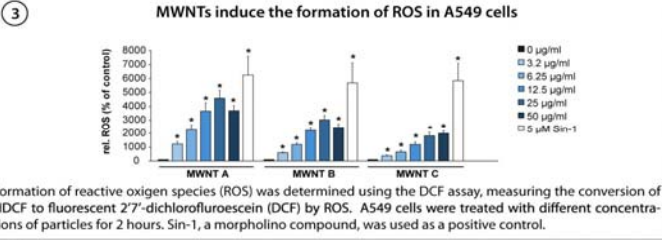
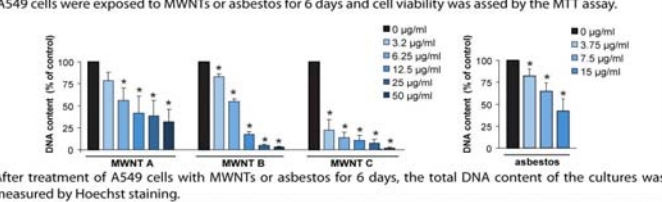
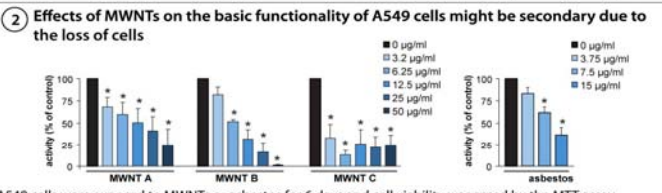
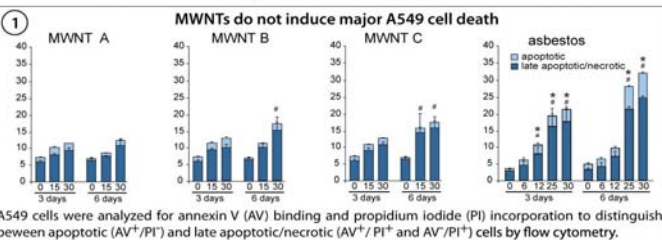
Materials

Materials MWNT were commercially available from Bayer Technologies Services (MWNT A), Cheap Tubes Inc. (MWNT B) and Nanocyl SA (MWNT C). They were dispersed in ultrapure water containing 160 ppm Pluronic F127 to a final stock solution of 200 µg/ml. **Test materials** MWNT effects were compared to those of toxic, fibrous crocidolite asbestos. **Cell culture** Jurkat A3, human leucemic T cells (ATCC, CRL-2570) and A549 lung epithelial cells (CCL-185), were exposed to different concentrations of MWNT and test materials for the indicated times.



A TEM, HRTEM and SEM images of MWNT A (a-a'), MWNT B (b-b') and MWNT C (c-c'). TEM analysis was performed on MWNT powder whereas SEM micrographs present agglomerates present in aqueous suspensions containing 160ppm Pluronic F127. B Summary of the characteristics of MWNTs used in this study.

Results A549



Results Jurkat

