Title:

Influence of impurities from manufacturing process on the toxicity profile of boron nitride nanotubes Dataset

Dataset Number: RD-1063-2023-0

Introduction:

The toxicity of boron nitride nanotubes (BNNTs) is confounded by the various manufacturing and purification strategies employed targeted to remove 30-60% of the residuals and impurities. Four BNNTs manufactured by the induction thermal plasma process allowed us to assess the influence of these residuals/impurities on the toxicity profile of BNNTs by producing a gradient of BNNT purity levels through sequential gas purification, and water and solvent washing, followed by filtration. Extensive characterization including Infrared, x-ray spectroscopy, thermogravimetric analysis, size, charge, surface area and density allowed characterization of the alteration in physicochemical properties as the material went through sequential purification. The material from each step was screened using *acellular* and *in vitro* assays that evaluated general toxicity, mechanisms of toxicity and macrophage function.

Methods Collection

- BNNTs were synthesized by hydrogen-assisted BNNT synthesis (HABS) method with hexagonal boron nitride (hBN) powder feedstock. The produced BNNT were called as produced (AP)-BNNT. AP-BNNTs were first purified by a gas-phase process to chemically remove the boron impurity and the resulting material was referred as boron removed (BR)-BNNT. The BR-BNNTs were further purified by sequential washing of solvents and water and referred to as washed 1 (W1) BNNT. In addition to the series of progressively more purified BNNTs (AP to BR to W1), a second washed sample, called washed 2 (W2)-BNNTs was also utilized for the study for toxicity comparison between batches.
- Human peripheral blood monocyte cell line THP-1 cells were cultured in 10% fetal bovine serum, 100 µg/mL Penicillin-Streptomycin, and 50 µM of beta-mercaptoethanol. The cells were differentiated into macrophages by treating them with growth media containing vitamin D3 at 150 nM for 48h and then 5 nM Phorbol 12-myristate 13-acetate (PMA) for 12 h.
- Cytotoxicity and membrane damage was evaluated by exposing differentiated THP-1 cells to containing 0, 1.56, 3.125, 6.25, 12.5, 25, 50 and 100µg/ml of Hexagonal Boron nitride, AP-BNNT, BR-BNNT, W1-BNNT and W2-BNNT for 24 h.
- Acellular reactivity and test materials ability to generate free radical intermediates was evaluated by electron paramagnetic resonance (EPR) spin-trapping by reacting the particulates to H_2O_2 and spin trapping using DMPO (5,5'-dimethylpyrroline N-oxide).
- Nuclear Factor Kappa B (NF-κB) activation due to particulate exposure was evaluated using THP1-Blue™ NF-κB cells (InvivoGen Inc, San Diego, CA) by exposing them to 0, 6.25, 25 and 100 µg/ml of hBN, AP-BNNT, BR-BNNT, W1-BNNT and W2-BNNT for 12 hours.
- For inflammasome activation, the differentiated THP-1 cells were primed to induce the transcription of pro-IL-1β by co-treating them with 10 ng/ml Lipopolysaccharide (LPS) when challenging with 0, 6.25, 25 and 100 µg/ml of the various BNNTs or hBN for 24 h. IL-1β and IL-18, markers for Inflammasome activation were evaluated using ELISA assay.

- Inflammatory protein secretion after exposure to 0, 6.25, 25 and 100 µg/ml of the various BNNT's or hBN for 24 h was determined by measuring 48 proteins in supernatants using a multiplex assay.
- Functional alteration in macrophage after BNNT or hBN was determined by measuring the change in macrophages capacity to uptake bacteria by exposing differentiated cells to 0, 6.25, 25 and 100 µg/ml for 24 h and challenging them with Escherichia coli GFP for 2 h.

Citation

Kodali, V., Kim, K. S., Roberts, J. R., Bowers, L., Wolfarth, M. G., Hubczak, J., Xin, X., Eye, T., Friend, S., Stefaniak, A. B., Leonard, S. S., Jakubinek, M., Erdely, A., Influence of Impurities from Manufacturing Process on the Toxicity Profile of Boron Nitride Nanotubes. Small 2022, 18, 2203259. https://doi.org/10.1002/smll.202203259

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