2D-hexagonal boron nitride and lung exposure: Exploring cellular interaction and potential health effects in bronchial and alveolar airway epithelial cell models

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Introduction and Study Design

2D materials are extensively exploited for numerous industrial and biomedical applications that includes energy storage, superconductors, catalysts, drug delivery, and therapeutics. Inhalation is one of the most pertinent routes of exposure to nanoscale materials in occupational settings. There are evidences that carbon-based 2D materials elicit immunomodulatory effects in the lungs that can sensitize and aggravate lung diseases such as asthma. However, biological interactions and safety of non-carbonaceous 2D materials (e.g., hBN) has not been well studied. Here, an investigation of how 2D-hBN interacts with bronchial (Calu-3) and alveolar (A549) epithelial cells under air–liquid interface-based exposure was conducted.

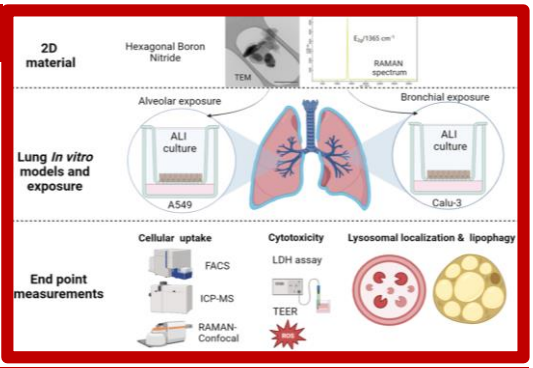

Figure 1: Study design. *In vitro* exposure and safety assessment of hBN in lungs using air-liquid interface (ALI) models of alveolar (A549) and bronchial (Calu-3) epithelial cells. Schematic was prepared using BioRender.

Uptake and Subcellular Localization

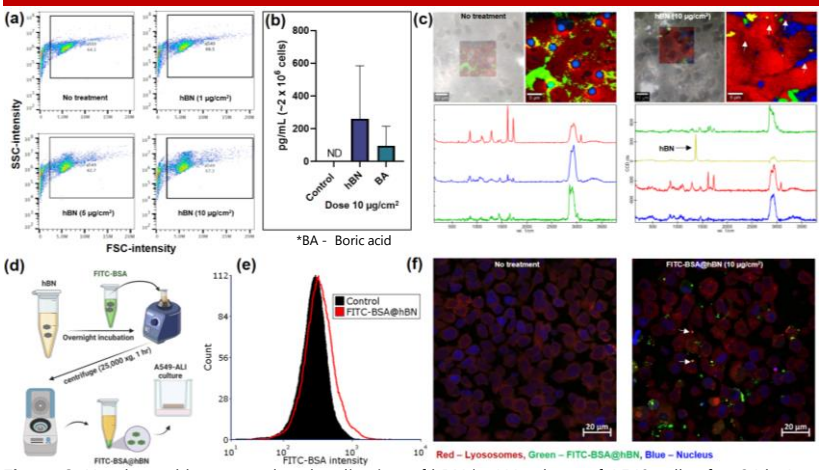

Figure 2: Uptake and lysosomal co-localization of hBN in ALI culture of A549 cells after 24 h. (a-c) Uptake of hBN determined and validated using flow cytometer (shown as increase in side-scatter intensity) (a), ICP-OES (B content) (b), and confocal-Raman scanning microscopy (c). (d-f) FITC-BSA labelling on hBN for intracellular tracking (d), cellular uptake (e), and lysosomal localization of FITC-BSA@hBN as shown in confocal micrographs (f). Data - Mean ± SD (n=3).

Cytotoxicity and Oxidative Stress

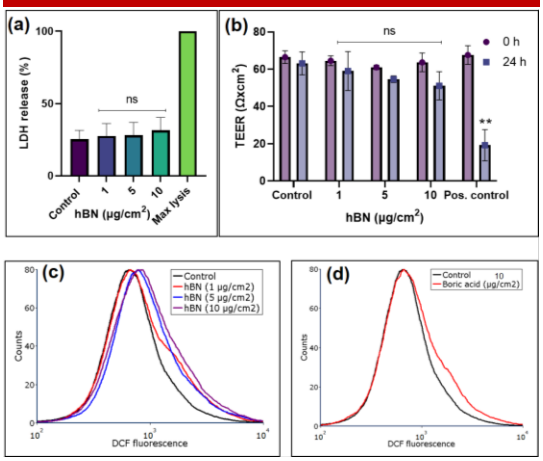

Figure 3: Cytooxicity and oxidative stress in A549 cells after 24 h of hBN exposure. (a-b) No cytotoxicity or loss of barrier integrity was observed in cells as determined by measuring LDH release (a), and TEER (b), respectively. Data: Mean ± SD (n=3). (c-d) Cellular ROS content in hBN (c), boric acid exposed cells (d).

Lipid Accumulation and Lipophagy in A549 and Calu 3 Cells

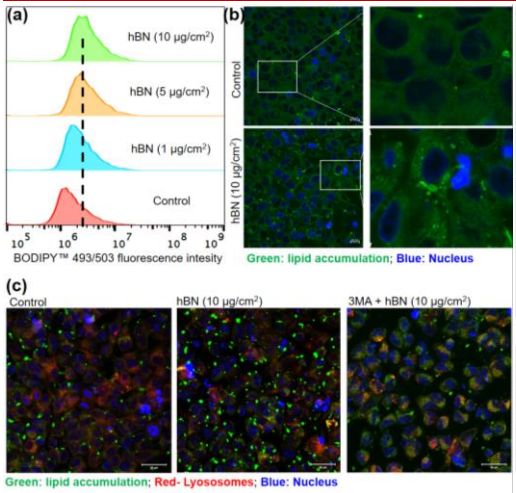

Figure 4: Lipid accumulation and lipophagy in A549 cells after hBN exposure for 24 h. (a-b) A dose-dependent increase in bodipy intensity was detected in the cells using flow cytometry (a), and validated using confocal microscopy (b). (c) Cellular occurance of lipophagy was determined by co-localization of lipid droplets with lysosomes, which was further confirmed by pharmacological inhibition of lipophagy using 3-Methyladenine (3-MA).

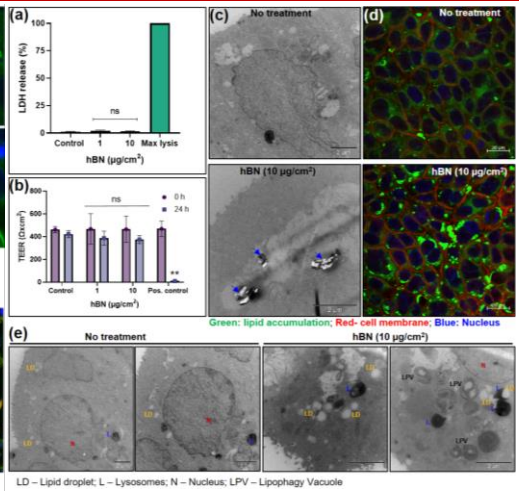

Figure 5: Cytotoxicty, lipid accumulation and lipophagy in Calu-3 cells after hBN exposure for 24 h. (a-b) No cytotoxicity or loss of barrier integrity was observed in cells as determined by measuring LDH release (a), and TEER (b), respectively. Data presented as Mean ± SD (n=3). (c) TEM micrographs indicate cytoplasmic accumulation of hBN (blue arrows). (d) Confocal micrographs showed enhanced accumulation of lipid droplets in hBN exposed cells. (e) TEM micrographs showed an induction of lipophagic vacuoles formation in hBN exposed cells.

Conclusions

hBN exposure in alveolar and bronchial air-liquid interface model resulted in particles uptake in the cells and enhanced lipid accumulation without causing cytotoxicity after 24 h. The excess of lipid accumulation in the lipid droplets induced lipophagy in the cells.

Acknowledgements

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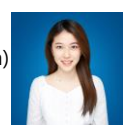

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