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TITLE: Cellular Response of Small Airway Epithelial Cells and Human Microvascular Endothelial Cells in a Coculture System following Exposure to MWCNT

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ABSTRACT BODY: Nanotechnology, particularly the use of multiwalled carbon nanotubes (MWCNT), is a rapidly advancing field with implications for advancement in a variety of disciplines such as biomedical, electrical, and thermal research. A major route of exposure to MWCNT in both occupational and environmental contact is inhalation. While many studies showed adverse effects to either the lung epithelium or vascular endothelium upon MWCNT exposure, *in vitro* results did not often correlate with *in vivo* effects. Therefore, a more relevant cellular model to mimic *in vivo* exposure was needed. This study sought to create a coculture system in which both human small airway epithelial cells (SAEC) and human microvascular endothelial cells (HMVEC) were grown in coculture so as to resemble an alveolar-capillary interaction. Exposure of the epithelial layer to MWCNT-induced (at a low dose relevant to *in vivo* lung burden) multiple changes in the

endothelial barrier including an increase in ROS, actin rearrangement, loss of VE-cadherin, and potentially increased permeability. An increase in endothelial angiogenic ability, as well as overall increases in secreted VEGFA, ICAM1, and VCAM1 protein expression, was noted after epithelial exposure. Additionally, alterations to both SAEC and HMVEC mRNA and miRNA levels were noted after MWCNT exposure. This coculture system identified that epithelial exposure to MWCNT-induced multiple changes to the endothelium, potentially through cell signaling mediators, and suggested that the coculture system is an improved *in vitro* method to study the pulmonary toxicity and potential signaling pathways of MWCNT exposure.

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