

# Comparative Proteomics and Biological Effects of Functionalized Carbon Nanotubes in Intestinal Cell Co-culture

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## Abstract

“Carbon nanotubes (CNTs) possess unique electrical, mechanical, and thermal properties, with potential applications in the electronics, catalysts, polymer composites, aerospace, and other industries. CNTs are also being developed for a broad range of applications in biomedicine, including oral drug delivery. Functionalized, water dispersible CNTs (fCNTs) can be expected to enter the digestive tract and exert biological effects on its barrier epithelial cells. To characterize these effects, we developed an in vitro model of the large intestinal tract using a co-culture of Caco-2 (75%) and HT29-MTX (25%, mucus secreting) cells, and exposed these cells to functionalized single-walled (SWNT) and multi-walled (MWNT) carbon nanotubes at realistic concentrations (500 pg/mL and 10 µg/mL; 48 h). Protein expression was analyzed using our recently developed label-free quantitative mass spectrometry (LFQMS) platform, IdentiQuantXL™, while typical toxicological endpoint assays were used to characterize various cellular responses. LFQMS identified 5,007 unique protein database entries, from which 4,200 proteins were considered qualified for quantitation. These proteins represented 1,978 protein groups (containing isoforms, splice-variants, etc). Differences in expression were calculated by ANOVA ( $P < 0.001$ ) and post hoc Holm Sidak comparisons ( $P < 0.05$ ). fCNT significantly altered protein expression in a moderate number of proteins, the extent and type of which were fCNT specific. Only 13 proteins were universally altered by all exposures (except 500 pg/mL COOH-SWNT which had no effect), and these represent a broad range of cellular functions. Bioinformatic analysis using the Gene Ontology Database and Ingenuity Pathway Analysis revealed statistically significant protein associations with a broad range of functional networks and signaling/metabolic pathways. Again, little overlap between fCNT was observed. None of the exposures was associated with overt toxicity or proinflammatory response. The results suggest that significant biological effects result from fCNT exposure, responses that are specific to CNT-type and dose, but occurring in the absence of toxicity or irritation. Supported by NIEHS RC2ES018810.”