Activation of Binding, Adhesion and Proliferation of Epithelial Cells by Pristine Graphene in Human Colon ex vivo

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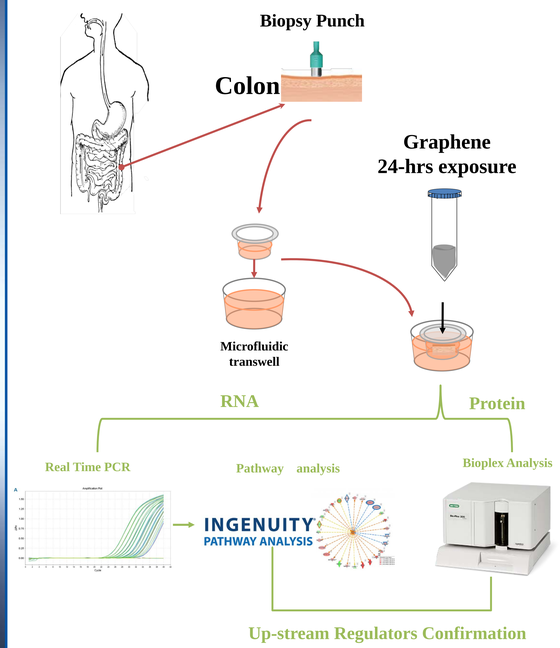
Abstract

Risk Assessment of emerging technologies that use pristine graphene in potential bio-applications require a thorough understating of the toxicity of this nanomaterial. Toxicology results obtained using single cell *in vitro* models generally do not mirror actual human responses to nanomaterial exposure. The aim of this study was to use intact human colonic mucosal tissue to evaluate the response to pristine graphene exposure. Biopsy punches of human colon tissues from healthy individuals were used to assess the biological response after exposure to graphene at three different concentrations (1,10 and 100 µg/ml). RNA and proteins were extracted at 2 and 24 hrs post-exposure and specific genes were assessed by mRNA expression level or at intestinal cytokine abundance using Real-time PCR and the Bioplex Multiplex Immunoassay System, respectively. The results showed that pristine graphene activated many cell surface binding genes within the first 2 hrs of exposure and upregulated essential genes for eukaryotic cells proliferation such as *PCNA* (Proliferating cell nuclear antigen). The Ingenuity Pathway Analysis of the real time PCR data revealed that STAT3 and VEGF signaling pathways were upregulated. Both of these pathways are related to cell proliferation and growth. Further analysis of predicted up-stream regulators suggested that mucosal immunity may be perturbed upon exposure to pristine graphene. Moreover, proinflammatory cytokines IFNγ, IL-8, IL-17, IL-6, IL-9, MIP-1α and Eotaxin were significantly increased after exposure to graphene at 10µg/mL and at the 24 hrs timepoint. The gene expression data along with the intestinal immune response indicated that pristine graphene may activate the STAT3 – IL23 – IL17 response axis and lead to inflammation. The findings of this study are of significant interest to regulatory agencies to consider when developing efficacious product safety strategies of graphene and other nanomaterials when evaluating their future bio-applications. This research provides alternative methods for toxicity assessment and is relevant to FDA Forum 2021 topic area "Product Development and Manufacturing."

Approach



Results

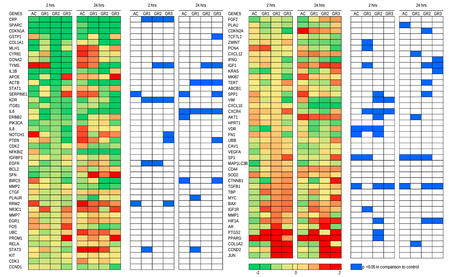


Figure 1. Heatmap analysis for gene that were differentially expressed after exposure of colon tissue to graphene. Tissues was exposed to graphene at 1 (GR1), 10 (GR2) and 100 (GR3) µg/ml and to activated carbon (AC) at 100µg/ml. Sampling was done for 2 and 24 hrs. Colors toward green shows genes that were downregulated in relation to control (water only). Colors toward red shows upregulated genes in relation to control (water only). Blue cells mark treatments that has significant gene expression (p<0.05) in comparison to control (water only).

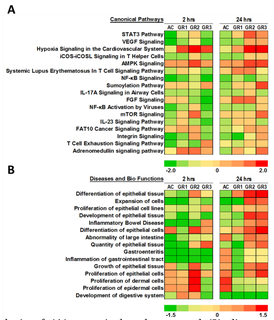


Figure 2. Heat map analysis of (A) canonical pathways and (2) diseases and biofunction affected by graphene exposure to colon tissue. Tissue was exposed to graphene at 1 (GR1), 10 (GR2) and 100 (GR3) µg/ml and to activated carbon (AC) at 100µg/ml. Sampling was done for 2 and 24 hrs. Colors toward green shows functions with low z-scores. Colors toward red shows functions or pathways with higher z-scores.

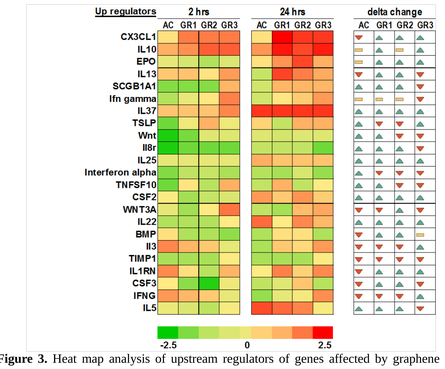


Figure 3. Heat map analysis of upstream regulators of genes affected by graphene exposure to colon tissue. Tissue was exposed to graphene at 1 (GR1), 10 (GR2) and 100 (GR3) µg/ml and to activated carbon (AC) at 100µg/ml. Sampling was done for 2 and 24 hrs. Colors toward green shows functions with low z-scores. Colors toward red shows functions or pathways with higher z-scores. Delta change between 24 and 2 hrs time points is indicated by increase (green arrow), decrease (red arrow) or no change (yellow bars).

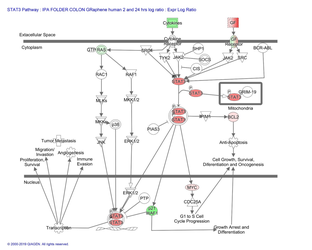


Figure 4. STAT3 pathway analysis after exposure to graphene at 10 µg/ml concentration during the 2 and 24 hrs timepoints.

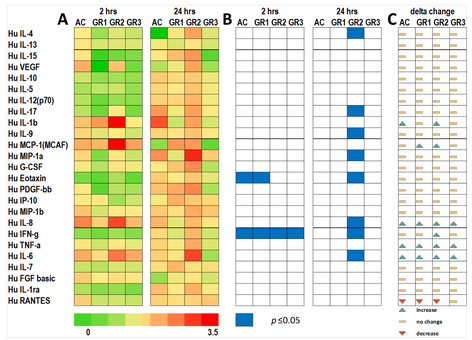


Figure 5. Heatmap analysis for proteins that were differentially expressed after exposure of colon tissue to graphene. Tissues was exposed to graphene at 1 (GR1), 10 (GR2) and 100 (GR3) µg/ml and to activated carbon (AC) at 100µg/ml. Sampling was done for 2 and 24 hrs. (A) Colors toward green shows proteins that were downregulated in relation to control (water only). Colors toward red shows upregulated genes in relation to control (water only). (B) Blue cells mark treatments that have significant proteins levels (p<0.05) in comparison to control (water only). (C) Delta change in protein levels between 24 and 2 hrs time points is indicated by increase (green arrow), decrease (red arrow) or no change (yellow bars).

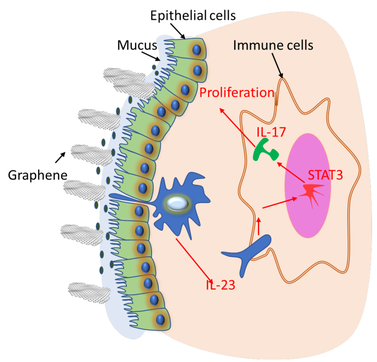


Figure 6. Schematic figure of the interaction of graphene with cells of the human colonic tissue.

Findings

- ✓ New highlights of the pathways affected by graphene upon exposure to the human colon tissue.
- ✓ Graphene can stimulate mRNA expression of gene involved in cell proliferation and growth upon binding/adhering to epithelial tissue.
- ✓ Graphene interaction with epithelial cells is coupled with a dose dependent activation of pro-inflammatory response through many pathways.
- ✓ The correlation between real time PCR data and proteins data showed that IL-23 – IL-17 axis signaling pathway and STAT3 emerged as the principal pathways by which graphene might impact human epithelial tissue.
- ✓ Additional investigations on the subtypes of cells responsible for the observed biological effect will be necessary to fully understand the toxicity and long-term impact of pristine graphene.

Significance

Using graphene as model of carbon-based nanomaterial, *in vitro* and *in vivo* toxicological studies can improve our understanding of graphene-intestinal interaction and its long-term effects. All this research work can help the agency develop efficacious product safety strategies of graphene and other nanomaterials when evaluating their future bio-applications and set the standards for nano-product safety and risk assessment.

Acknowledgement

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