Graphene oxide lateral dimensions can mediate different molecular response of human immune cells.

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Abstract

In the last few years, there has been enormous interest in graphene oxide (GO) for its wide variety of applications [1, 2]. However, for any medical application, the immune system-impact of GO still remain to be fully understood [3]. Here we focused on the molecular effects of two GOs, different for lateral size dimensions, on human primary immune cell populations: peripheral blood mononuclear cells (PBMCs). GOs were fully characterized, then we performed a wide range of standard assays looking at cell viability, cell activation and multiple cytokines secretion. We characterized the molecular impact of GOs on 84 genes immune-response-related. Additionally, a whole genome analysis was conducted on T cells and monocytes as representative of the innate and adaptive immune responses. In **Fig. 1** TEM and AFM characterization of GO-Small (140 nm) and GO-Large (4μm). We did not detect any toxicity in GO PBMCs treated samples. The 84 gene expression analysis evidenced a clear dimension-dependent impact of GOs on cell activation (**Fig. 2**). In particular, GO-Small modulated 16 genes (Fold Regulation >4) compared to only 5 of GO-Large (in red in **Fig.2 C).** Action confirmed also by cytokine analysis (**Fig. 2 D**). Further evidences were given by microarray analysis on T and monocytes cell lines. GO-Small impact the immune cell activation, underlined by the over expression of many pathways such as leukocyte chemotaxis pathway (**Fig.3**), genes such as CXCL10 and its receptor CXCR3 (**Fig 3, red box**). These genes are commonly activated during acute inflammatory processes as those associated with immune-mediated tumor rejection and pathogen clearance [4].

Moreover, we found a strong action on cell metabolism with a down-regulation on energetic pathways such as oxidative-phosphorylation pathway in both cell types (data not shown). Our work represents a comprehensive molecular-characterization of different sized GOs on immune cells giving crucial information for the chemical and physical design of graphene for biomedical applications i.e. as a new possible drug delivery systems and nanoimmunotherapy tools.

References

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Figures

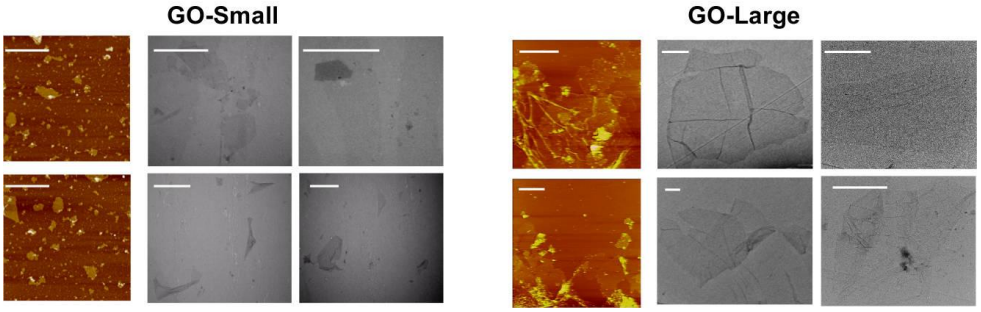

Figure 1. Characterizations of GO-Small and GO-Large. Atomic force microscopy (AFM) and transmission elettronic microscopy (TEM) images of GO-Small and GO-Large respectively. All scale bars are 1μm.

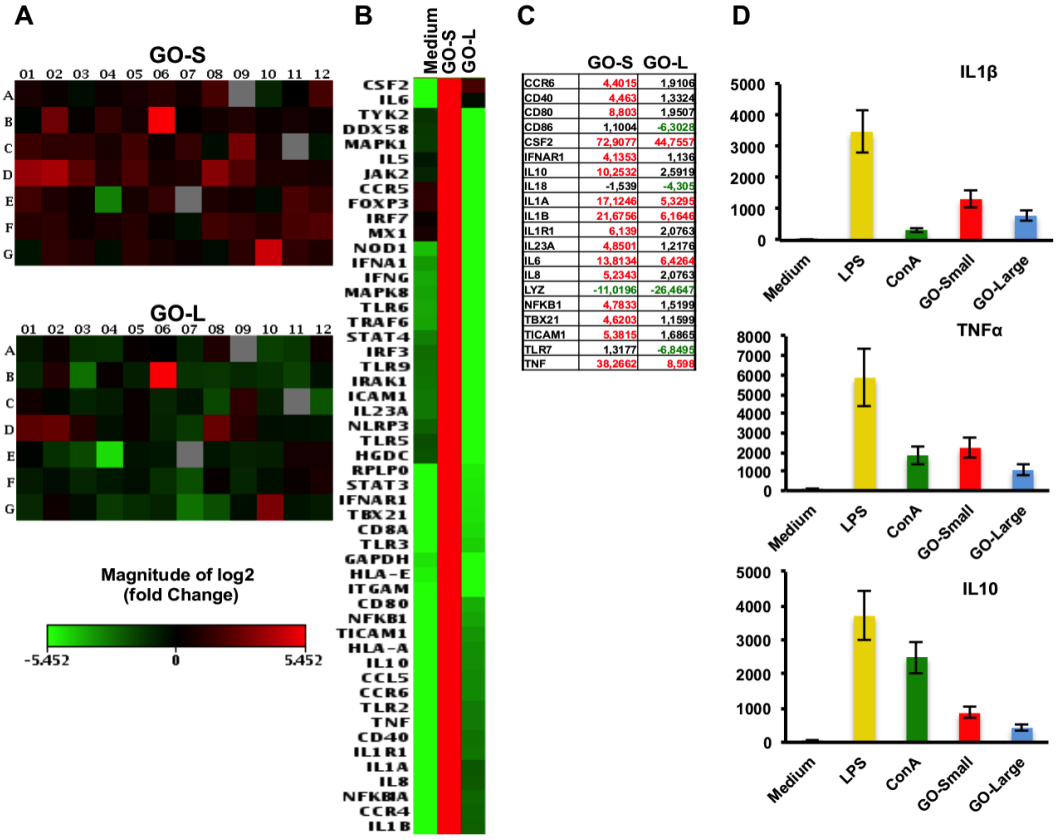

Figure 2. Immune gene expression array. A) Heatmap comparison of 84 genes after exposure to GO-Small or GO-Large. B) Heat map detail showing the immune transcript upregulated by GO-Small in PBMCs. C) Table of modulate genes in GO-Small and GO-Large versus control. Red show genes with a fold change greater than 4, green show genes with a fold regulation less than 4. D) Multiplex cytokine secretion analysis of GO-small and GO-large samples, Interleukin 1β (IL1β), Tumor Necrosis factor α (TNFα) and Interleukin 10 (IL10) are showed.

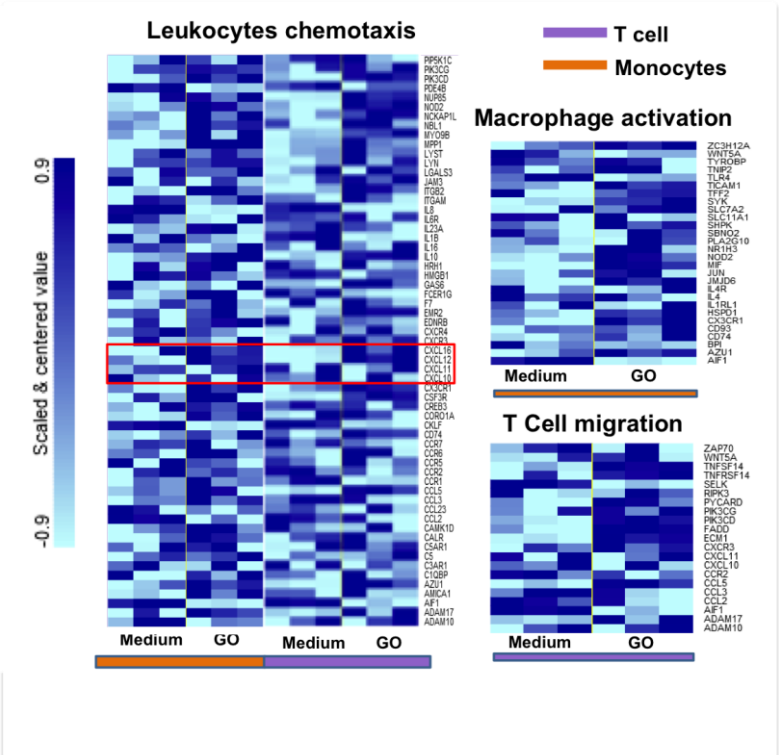

Figure 3. Whole genome expression analysis. Heatmap representation of GO-Small treatment for relevant Gene Ontology categories.