Impact of graphene - related materials on primary glial cells

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Graphene (G) has the potential to make a very significant impact on society, with new interesting and beneficial applications for both individuals and industrial organizations [1]. The emerging interest toward applying this nanomaterial within the central nervous system (CNS) prompted us to focus our attention on the toxicity and biocompatibility of G nanostructures in contact with primary neural cells. We are currently pursuing two lines of research, focusing on G-based material interactions with primary neurons and glial cells. In detail, we describe here the interaction between G-based materials and primary cortical astrocytes, which are key players in maintaining brain homeostasis, by exploring (i) the molecular mechanisms of graphene flake internalization, (ii) the inflammatory responses triggered by exposure to G flakes, and (ii) the effects of G exposure on cell morphology and physiology.
Primary astrocytes were exposed to pristine graphene (GR) and graphene oxide (GO) flakes at various concentration (1 and 10 µg/ml) for up to 7 days. Cell viability and cell death, investigated by flow cytometry, displayed no statistically significant difference between the various G-treated samples and the respective controls. Fluorescence light microscopy and electron microscopy (SEM and TEM) were used to investigate the physical interaction of the flakes with astrocytes, from the early interaction with the cell membrane to internalization, while electrophysiology was applied to investigate glial function upon exposure to flakes.
A larger amount of internalized flakes were observed compared to neuronal cells, which were mostly internalized in vesicular compartments. The endo-lysosomal pathway resulted the preferential route of G-flakes intracellular trafficking (Figure 1). Although no cell mortality was induced, morphological changes associated with G-exposure were noticed by both light microscopy and SEM. Astrocytes kept under control conditions showed a characteristic polygonal flat shape; on the other hand, astrocytes treated with GR and GO showed a small rounded appearance with long cellular processes (Figure 2). We reported a statistically significant decrease of the cell area both in presence of GR and GO.The idea behind this morphological transformation is that the interaction with nanomaterials may induce cytoskeletal reassembling of microfilaments, intermediate filaments, and/or microtubules [2], which could correlate with the increase in K^+ current observed in the same samples. Upon application of the voltage-step protocol, typical outward K^+ currents were evoked under all experimental conditions at potentials more positive than -40 mV. Quantitative analysis showed an increase of current density in the presence of GO at +120 mV (Figure 3). Ongoing experiments are focalized on (i) investigating dose-response effects of the above phenomena on astrocytes exposed to lower G concentrations (ii) exploring shape-dependent Cl^- current expression [3].
In conclusion, our results show that exposure to G flakes did not affect primary cortical astrocyte growth, survival and functionality, making G a promising material for biomedical interfaces and applications. Interestingly, we noticed a reduction in the cell area of glial cells once exposed to G-flakes, suggesting a physical interaction of the G with the cytoskeleton of proliferative cells. Additionally, current investigation aims at elucidating the long-term effects of G exposure and its influence on microglia, the main immuno-competent cells in the CNS.

References

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[2] Safavi-Abassi S., Glia, **36(1)**, (2001), 102-15.
[3] Lascola and Graig, JNeurosci, **16(8)**, (1996), 2532-2545

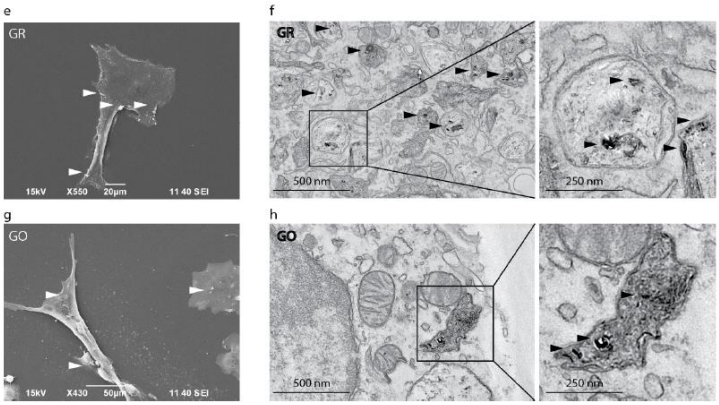

Figure 1. GR and GO flakes interaction with astrocyte cell membrane and intracellular location

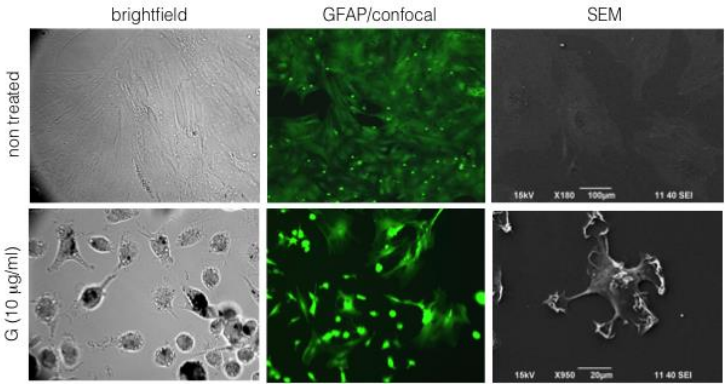

Figure 2. Morphological investigation of primary astrocytes exposed to GR and GO flakes

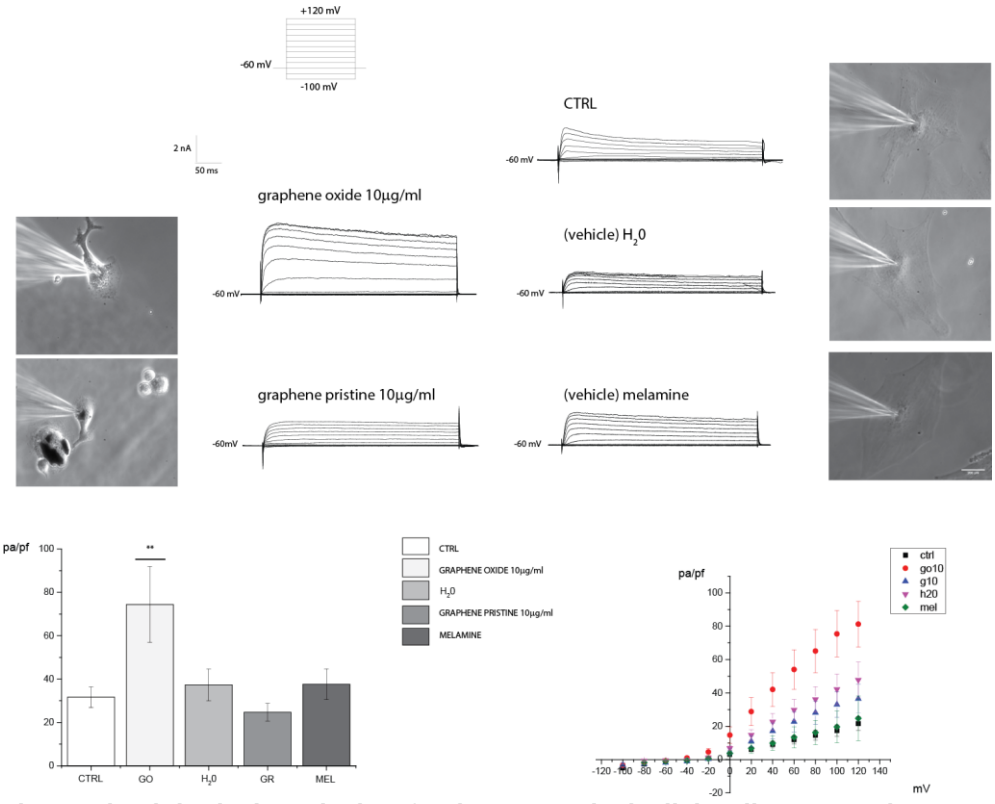

Figure 3. Electrophysiological analysis of primary cortical glial cells exposed to GR and GO flakes