Biological reactivity assessment of graphene oxides

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1. Introduction

Materials, especially advanced materials, are the backbone and source of prosperity of an industrial society (Materials 2030 Manifesto). Graphene Family Materials (GFMs) are considered an advanced material with a wide range of present and potential future applications. GFMs represent a group of carbon-based materials that share the same sheet-like structure as graphene, a single layer of carbon atoms arranged in a hexagonal lattice. GFMs exhibit unique properties such as high strength, conductivity, and flexibility, making them promising for a wide range of applications in electronics, biomedicine, energy sector and for photovoltaics, photocatalysis, biosensors, and functional coatings. The versatility and potential of GFMs have attracted great interest from researchers and industries worldwide, paving the way for innovative advancements in various technologies. Successful commercialization of GFMs requires proper toxicological evaluation to ensure their safe and sustainable use. Although various methods are available to adjust synthesis parameters to refine the morphology and chemistry of the final product and to produce safe by design (SSbD) materials, there is still a lack of practical characterization methods to evaluate the safety and sustainability of GFMs when fine-tuning the synthesis parameters. Within ACCORDs EU Horizon project, we are developing and testing correlative approaches for the physico-chemical characterization of GFMs. Among tests supporting a feedback loop to assure adherence to SSbD criteria during laboratory scale production are acetylcholinesterase (AChE) adsorption and inhibition test and simple cytotoxicity assays. Here we present the initial biological evaluation of 2D graphene oxide (GO) materials synthesized within the Accords EU Horizon project.

2. Initial assessment of biological reactivity

The two GO materials studied have been synthesized using the improved Hummers' method, in which oxidation is guaranteed by the use of $KMnO_4$ as an oxidizing agent (Marcano et al., 2010). In the two cases, different quantities of $KMnO_4$ were used during the synthesis, guaranteeing a different level of oxidation: higher in the case of the GO_2 material. This difference may be one of the parameters to consider when evaluating the functional properties.

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An initial assessment of the biological reactivity of GO was performed using an acetylcholinesterase (AChE) adsorption and inhibition assay, and by measuring the cytotoxicity of GO with A549 human lung adenocarcinoma cells.

Interactions between the enzyme acetylcholinesterase (AChE) and nanomaterials are a reliable high-throughput biological assay that provides information on the adsorption potential of materials by measuring the activity of AChE after adsorption on a material of interest that can potentially interfere with enzyme functionality. The more reactive the surface, the more intense the adsorption and inhibition of enzyme activity. Adsorption of two different graphene oxides (GOs) to AChE and its inhibition were tested using the Ellman's method adapted for microtiter plates as described in Mesaric et al. (2013). The AChE adsorption assay examines how AChE binds to GO by incubating them together, separating GO-enzyme complexes and measuring AChE activity using the Ellman's method. The AChE inhibition assay examines the effects of GO on AChE activity. AChE is incubated with GO and then AChE activity is measured. Together, these assays provide a comprehensive understanding of the interactions between enzyme and nanomaterials, including both adsorption and inhibition effects. The AChE assay is very sensitive for detecting effects of nanomaterials on enzyme function or structure, even at low concentrations of the nanomaterial tested.

The cytotoxicity of GO on A549 cells was tested using the Neutral Red Uptake (NRU) assay, which distinguishes viable cells with functional lysosomes from non-viable cells with unstable lysosomes. Cytotoxicity was measured after a 24-hour exposure to different GOs, according to the protocol described in Kononenko & Drobne (2019).

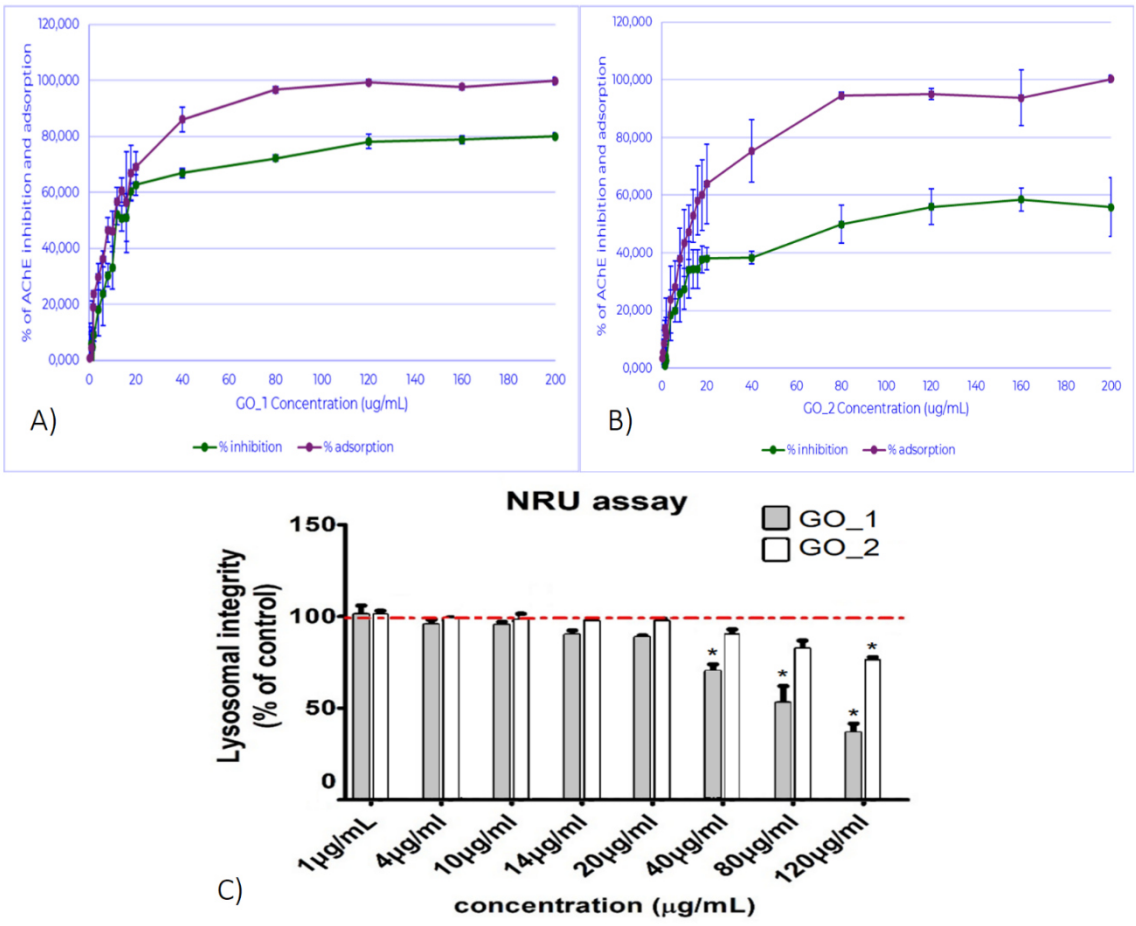

Figure 1: Results of the AChE adsorption and inhibition test (A, B) and evaluation of the cytotoxicity of different graphene oxides (C).

The results of AChE adsorption and inhibition test showed higher reactivity of sample GO_1, which caused about 60% inhibition and 70% adsorption at a concentration of 20 µg/mL (Fig. 1A), while GO_2 caused about 40% inhibition and 60% adsorption (Fig. 1B). The cytotoxicity of GO_1 was also higher compared to GO_2 (Fig. 1C).

3. Conclusions

Here we present the first assessment of the biological reactivity of two types of GOs. The results showed that GO_1 was more reactive than GO_2. GO_1 caused a higher degree of AChE inhibition and adsorption, and it was also more cytotoxic to A549 human lung adenocarcinoma cells. These findings suggest that GO_1 is more biologically reactive compared to GO_2. A possible explanation could be the different oxidation level of the two materials. The presence of functional groups on the surface (higher in GO_2) can play a role in determining reactivity. Further research is needed to fully understand the hazards that GOs pose to humans and the environment, and to develop GO materials for safe and sustainable applications.

4. Acknowledgment

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5. References

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