Assessment of the risks of graphene nanomaterials for the marine environment and human health

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In the last few years, the market for graphene and its derivatives is in exponential increase and it is expected that large quantities of graphene-based wastes will end up in the environment. In this work we applied alternative toxicity testing methods to assess the potential risks for the marine environment and human health of two graphene derivatives: graphene oxide (GO) and reduced graphene oxide (rGO) nanoplatelets. For this, mussel (*Mytilus galloprovincialis*) hemocytes and human alveolar type-I-like lung cells (TT1 cells) were used as they are sensitive to nanomaterial exposure. Cytotoxicity of GO and rGO (with and without polyvinylpyrrolidone (PVP) as stabilising agent) was screened at a wide range of concentrations (0.001 to 100 mg/L). GO, GO-PVP and rGO-PVP showed low and dose-dependent cytotoxicity to mussel hemocytes and human TT1 cells, being rGO-PVP slightly more toxic than GO-PVP. PVP was not cytotoxic but increased bioavailability of nanoplatelets. GO and rGO-PVP increased ROS production and caused a significant decrease in plasma membrane (PM) integrity in both cell types. In TT1 cells, inflammatory responses were significantly increased after exposure to both GO and rGO-PVP. As seen by TEM, GO and rGO-PVP produced invaginations and perforations of the PM and were found in the cytosol and in endolysosomal vesicles of mussel hemocytes (Fig. 1). GO, GO-PVP and rGO-PVP were internalised into TT1 cells via endocytotic mechanisms (Fig. 1). In conclusion, studied graphene derivatives are not highly cytotoxic for mussel and human cells but they trigger important cellular mechanisms leading to toxic responses. Chemical reduction of GO increased its bioreactivity possibly due to the restoration of its surface electronic structure. Finally, *in vitro* assays provide valuable and sensitive tools to assess the potential risks of graphene nanomaterials for the marine environment and human health.

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Figures

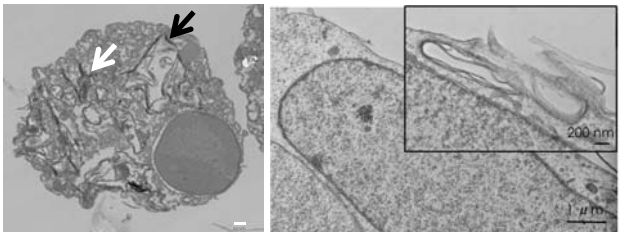

Figure 1: TEM. In hemocytes (left) GO and rGO-PVP were found in the cytosol (white arrows) and in endolysosomal vesicles (black arrows). In TT1 cells (right), nanoplatelets are internalised via endocytotic mechanisms.