Graphene interaction with cellular structures and potentiating action on environmental pollutants effects

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Monolayered graphene shows a unique structure constituted by one-atom thick two-dimensional crystal of sp^2 carbon atoms that is responsible of extraordinary physico-chemical properties. As a consequence, graphene has potential applications in so diverse fields as electronics or aeronautics. In addition, graphene can interact with other substances facilitating their transport to the inner of cells what opens the possibility of interesting applications in medicine, and agriculture and livestock farming. The wide spectrum of graphene applications increases also the possibility of release to the environment where it could cause deleterious effects on organisms. Nevertheless, interplay of graphene with environmental toxicants (pollutants) could modulate their toxicity. Taking all this into account, we performed a series of experiments (see references 1, 2 and 3) with the general objective of determining the mechanisms underlying the toxicity of graphene oxide (GO) platelets to different cell lines in vitro, and describing in detail the interaction of graphene platelets with cellular structures. Finally, we wanted to establish if GO could facilitate the entrance into cells of other substances modulating therefore their potential desired or undesired (toxic) effects. Since liver plays a pivotal role in detoxification processes, two cell lines with liver origin were used: HepG2 (human origin) and PLHC-1 (from the fish *Poeciliopsis llucida*). Two different GO were used, one of them with a higher number of carboxyl groups that we have named carboxyl-graphene (CXYG). Suspensions of GO and CXYG obtained in cell culture medium were characterized through a variety of techniques including dynamic light scattering (DLS; to determine frequency size distribution), transmission and scanning electron microscopy (TEM and SEM respectively, to establish the platelet shape), and atomic force microscope (AFM, to determine the platelets width). TEM and SEM allowed us also to observe in detail the interaction of GO and CXYG with cell membranes and organelles. Cytotoxicity was assessed by means of AlamarBlue, CFDA-AM (5-carboxyfluorescein diacetate acetoxymethyl ester) and neutral red uptake. Oxidative stress status was determined through reactive oxygen species (ROS) levels, and mitochondrial membrane potential (MMP). Other enzyme activities or expression levels of particular genes informed about effects of interest. Negligible cytotoxicity was observed at concentrations of GO or CXYG lower than 16 µg/ml in both cell lines. Both graphene derivatives interacted with the plasma membrane in both cell lines damaging it. However, at low concentrations (showing barely any decrease in cell viability) GO and CXYG appeared inside cells. Strikingly, in the case of PLHC-1, GO and CXYG accumulations not surrounded by any membrane could be observed, suggesting that internalization occurs through a non-endocytosis mechanism. These results open the door for the use of graphene as carrier of other substances. In parallel, an increase of ROS levels and a reduction of MMP were detected, together with the observation of physical interaction of platelets with mitochondrial membranes. Therefore, oxidative stress associated with damage of cellular ultrastructure constitutes probably an important cause of toxicity. When cells were first pre-exposed to GO or CXYG or co-exposed to GO or CXYG and some environmental pollutants, all of them inducers of cytochrome P4501A (CYP1A), an enhancement of the expression of this cytochrome was observed at the transcriptional level together with an increase of the CYP1A dependent EROD (ethoxyresorrufin-*O*-deethylase) enzyme activity. This effect did not occur when cells were post-exposed to graphene derivatives, suggesting that graphene platelets favor the accumulation of pollutants inside the cells possibly by passive diffusion after damaging cell membranes.

References

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