P23

Effect of the oxidation status of reduced graphene oxide on the genotoxicity of human bronchial cells

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Graphene-based materials (GBM) are a broad family of novel carbon-based nanomaterials suitable for many nanotechnology applications. However, the increasing market of these materials raises concerns on their possible impact on human health, especially via the inhalation route in occupational settings. Several studies have focused on the assessment of the influence of different physico-chemical properties on the toxicity of GBM. However, the broad variability of materials and cellular systems used preclude the identification of the key physico-chemical parameters. Here, we evaluated the in vitro cytotoxicity and genotoxicity towards human bronchial cells of four reduced graphene oxide (rGO) materials that only differ on the carbon-to-oxygen (C/O) ratio.

The physicochemical characterization of the selected rGOs was conducted by means of oxygen content, microscopy and spectroscopy analyses. The effect on viability of human bronchial epithelial (16HBE14o-) cells was evaluated by the WST-8 assay, whereas the effects on reactive oxygen species (ROS) production by the fluorescent DCFDA probe. Then, the in vitro genotoxicity of the four materials, was evaluated towards human bronchial epithelial (16HBE14o-) cells by the micronucleus (MN) test (assessing chromosome damage) and the comet assay (assessing DNA damage).

Each material presented a different oxygen content, ranging from 1 to 12 %. According with the WST-8 assay, all the materials significantly reduced cell viability after 3 and 24 h exposure (EC50 values ranging from 4.6 to 30.8 µg/mL), with slightly different potencies not dependent on the C/O ratio content. A similar observation was evidenced by the analysis of ROS production but highlighted the capability of all the materials to significantly increase ROS level after 3 and 24 h exposure. The results obtained by the in vitro comet assays indicate that none of the materials induced DNA damage after exposing 16HBE14o- cells to 1.5-50 µg/mL, for 3 and 24 h. Regarding the MN test, two rGOs showed an increase in MN frequency after exposing cells to 1.5-50 µg/mL, for 24 h. However, the genotoxic response did not correlate with the content of oxygen of the materials.

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Reduced graphene oxide; oxidation status; comet; micronuclei.