Graphene and graphene oxide induce mitochondrial damages after membrane interaction of skin keratinocytes

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Due to their extraordinary physicochemical properties, graphene-based nanomaterials (GBNs) are becoming promising tools for several applications in the field of nanotechnology and biomedicine. However, despite the huge GBNs technologies progress, little is known about their impact on human health, so far. In particular, the cutaneous toxicity of GBNs remains largely unexplored, although skin contact can be considered one of the major exposure routes to GBNs during their production and use. Hence, an *in vitro* study was carried out to evaluate the toxicity of two GBNs (graphene, G, and graphene oxide, GO) toward human HaCaT skin keratinocytes. Initially, GBNs effects were evaluated by means of mitochondrial activity (WST-8 assay) and mitochondrial membrane depolarization (JC-1 assay) up to 72 h exposure. G induced a significant reduction of mitochondrial activity in HaCaT keratinocytes after 48 h exposure, whereas GO was effective already after 24 h exposure. After 72 h exposure, G was significantly less potent than GO (EC_{50} = 62.8 μg/ml and 5.4 μg/ml for G and GO, respectively). G and GO induced also mitochondrial membrane depolarization: 72 h exposure to the highest G or GO concentration (100 μg/ml) increased mitochondrial depolarization by 44% and 56%, respectively, an effect comparable to that of the positive control valinomycin (46% at 0.1 μg/ml). The effects at the mitochondrial level could be due to a perturbation of plasma membrane integrity as shown by epifluorescence microscopy after probing HaCaT cell membranes with a fluorescence dye which revealed significant changes in cell morphology, probably due to G and GO interaction with the plasma-membrane, as demonstrated by confocal images. Further studies are in progress to characterize the mechanisms of HaCaT cells plasma membrane damage by graphene-based nanomaterials. This research work has been funded by the European Union H2020 Programme under grant agreement no. 696656 Graphene Flagship EU Graphene Flagship (no.696656).