CHARACTERIZATION OF THE MECHANISMS OF OXIDATIVE STRESS INDUCTION BY GRAPHENE-BASED MATERIALS IN HUMAN KERATINOCYTES

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Graphene based materials (GBMs) are innovative 2D nanomaterials consisting of a single atom thick sheet of sp2–hybridized carbon atoms, experimentally discovered in 2004 and isolated from its three-dimensional parent material, graphite. GBMs have unique physicochemical properties, including high surface-to-volume ratio, strong mechanical strength, capability of bio-functionalization as well as extraordinary electrical and thermal conductivity. Despite the huge GBMs technology progress, little is known about their impact on human health, so far. In particular, skin contact represents one of the major exposure routes to GBMs during their production and disposal as well as during their use as bendable or foldable mobile devices, protective coatings and multi-touch screens. Recently, with the aim to characterize the in vitro biocompatibility of GBMs towards skin keratinocytes, we have demonstrated their ability to reduce mitochondrial activity and damage plasma membrane. Therefore, the present study was aimed to characterize the mechanisms involved in GBMs effect on mitochondria, focusing on reactive oxygen species (ROS) production.

Initially, two GBMs (few layer graphene, FLG, and graphene oxide, GO) were evaluated for their effect toward human HaCaT skin keratinocytes by means of ROS production after 24 h exposure, using three assays: NBT (nitroblue tetrazolium), DCF-DA (2',7'-dichlorofluorescin diacetate) and luminol assays. Exposure of HaCaT cells to FLG or GO induced a significant concentration-dependent ROS production. At the highest concentration tested (100 μg/ml), FLG increased ROS production by 9%, 25% and 39% at the NBT, DCF-DA and luminol assay, respectively. The increase of ROS production induced by GO was 36%, 39% and 21% at the NBT, DCF-DA and luminol assay, respectively.

Moreover, analyzing the kinetic of ROS production by a time-dependency DCF-DA assay up to 72 h, FLG and GO induced also a time-dependent ROS production. After the longest exposure time, the highest concentration (100 μg/ml) of FLG and GO increased ROS production by 85% and 124%, respectively.

To evaluate the mechanism of the increased ROS production induced by FLG and GO, their effects were investigated in presence of specific inhibitors of the major ROS-producing enzymes: HaCaT cells were pre-exposed for 1 h to diphenyliodonium (inhibitor of flavin systems), rotenone (inhibitor of mitochondrial complex I), apocynin (inhibitor of NADPH oxidase), L-NG-monomethyl arginine citrate (inhibitor of nitric oxide synthase), allopurinol (inhibitor of xanthine oxidase), indomethacin (inhibitor of cyclooxygenases) and subsequently to FLG or GO for 72 h. Among the selected inhibitors, rotenone (5 μM) and allopurinol (100 μM) significantly reverted or even abolished GBMs-induced ROS production. Intriguingly, the same inhibitors significantly reduced also GBMs-induced mitochondrial depolarization, suggesting that mitochondrial electron transport

chain complex I and xanthine oxidase may be involved not only in the mechanism of GBMs-induced ROS production but also in the mechanism of GBMs-induced mitochondrial dysfunction.