Effects of graphene and graphene oxide on skin HaCaT keratinocytes

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Graphene-based nanomaterials represent a new generation of two-dimensional carbon-based nanomaterials with extraordinary physical and chemical properties, making them promising candidates for several applications, such as nanoelectronics, energy technology and biomedicine as new components for biosensors, tissue engineering and drug delivery. Although there are high expectations for their potential use, graphene-based nanomaterials may pose potentially adverse environmental and human health risks, and little is known about their emissions and the toxic effects in humans, so far. Moreover, even though skin is the organ with the highest exposure risk to nanomaterials during their manufacturing, use and disposal, no data are available on their toxicity after cutaneous exposure.

This study is aimed to preliminarily evaluate the *in vitro* toxicity of some graphene-based nanomaterials, including graphene (G) and two different graphene oxides (GO), at the skin level. The effects of these nanomaterials towards skin HaCaT keratinocytes were evaluated by means of mitochondrial damage (WST-8 assay), cytotoxicity (resazurine assay), cell proliferation (sulforhodamine B assay) and plasma membrane damage (propidium iodide uptake) after different exposure times (24 up to 72 h). The obtained results showed that both G and GO induced significant mitochondrial damages, plasma membrane rupture and cell viability reduction only after 72 h exposure, without significant effects after shorter exposure times (i.e. 24 h). On the contrary, no effects were observed on cell proliferation. Moreover a different potency between G and GO was observed after 72 h exposure by the WTS-8 assay (EC_{50} values of 62.8 μg/ml and 18.6 μg/ml for G and GO, respectively) and the propidium iodide assay (EC_{50} values of 44.3 μg/ml and 13.0 μg/ml for G and GO, respectively), suggesting that G is slightly less toxic than GO.

These results show that G and GO seem to exert a moderate cytotoxic effect, observable only at relatively long exposure times, which involves a reduction of mitochondrial activity, cell viability and a damage at the plasma membrane level. Further studies are in progress to evaluate the mechanisms of plasma membrane rupture by graphene-based nanomaterials..