When wonder materials meet DNA; graphene's toxic and genotoxic footprint

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Background: Graphene and related two-dimensional materials are widely promoted as "wonder materials", yet their long-term safety profile at realistic exposure levels, especially regarding DNA integrity in human barrier tissues, remains poorly unknown.

Aims: We aimed to characterize the toxic and genotoxic footprint of graphene-related materials (GRMs) in human skin cells, focusing on HaCaT keratinocytes, and to translate these findings into robust in vitro DNA-damage testing strategies and regulatory-relevant guidance.

Methods: We investigated acute and subchronic exposures to well-characterized graphene oxide (GO), few-layer graphene (FLG) and small FLG at cytotoxic and non-cytotoxic doses using primary human skin cells and HaCaT keratinocytes. Endpoints included viability, ROS production, Ca^{2+} signaling, metabolomics, mitochondrial function, proliferation and genotoxicity assays (comet, γ-H2AX, micronucleus), followed by optimization of GRM-adapted in vitro DNA-damage assays and guidance for regulatory risk assessment.

Results: GRMs induced dose-dependent oxidative stress, Ca^{2+} dysregulation and cell death, but also profound metabolic rewiring at sublethal doses, including a shift towards glycolysis, increased TCA cycle intermediates, glutamine dependence and mitochondrial damage in HaCaT cells. Subchronic, non-cytotoxic exposures to GO and FLG in HaCaT keratinocytes triggered DNA damage, activation of the DNA damage response, and, after prolonged exposure, persistent genotoxic lesions comparable to those induced by arsenite. These findings led to the refinement of micronucleus and comet-based assays, the proposal of GRM-specific testing batteries, and their positioning within emerging European regulatory frameworks for graphene-enabled products.

Conclusions: Our work reveals that wonder graphene materials leave a measurable toxic and genotoxic footprint in human skin cells, even at sublethal doses, driven by oxidative stress, metabolic reprogramming and sustained DNA damage. It also underscores that tailored in vitro genotoxicity batteries are essential to capture these risks and to support experimentally based regulation of graphene and other 2D materials.