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Cytotoxicity of Graphene Nanoribbons

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Graphene, a two-dimensional (2-D) carbon nanostructure possesses unique nanoscopic properties, suitable for various material and biomedical science applications [1,2]. Investigating the cyto- and bio-compatibility is an important step towards the development of graphene for various in vitro and in vivo biomedical applications. Future use of graphene for a variety of commercial materials science applications would lead to their release in the environment. Recently, macroscopic amounts of graphene can be prepared by the longitudinal unzipping of carbon nanotubes [3]. These nanoparticles (oxidized graphene nanoribbons, hereafter referred as O-GNR) may be suitable for various biomedical applications provided they are non-toxic in vitro and in vivo.

In this study, we report the cytotoxicity screening of O-GNRs, dispersed in PEG-DSPE (1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[amino(polyethylene glycol)]) in National Institute of Health 3T3 mouse fibroblast cells (NIH-3T3), Henrietta Lacks cells (HeLa) derived from cervical cancer tissue, Michigan cancer foundation-7 breast cancer cells (MCF7) and Sloan Kettering breast cancer cells (SKBR3) [4]. Various endpoint assays (lactate dehydrogenase (LDH) release, lysosomal integrity, cellular metabolism and cell proliferation) were performed.

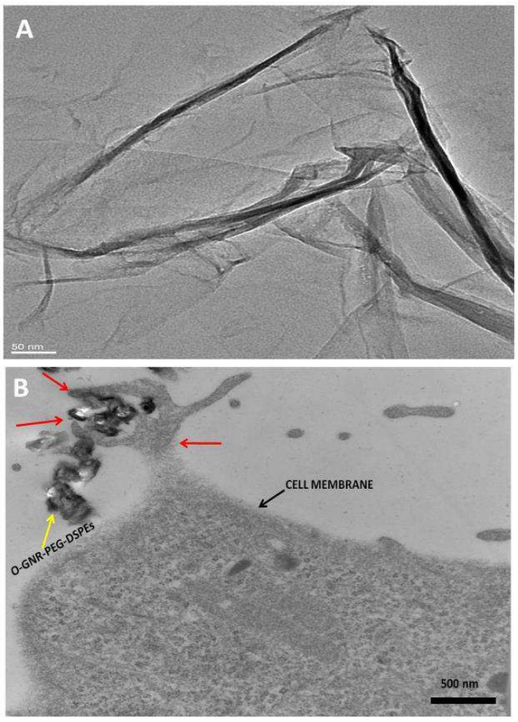

Figure 1: (A) Representative transmission electron microscopy (TEM) image showing the unzipping of carbon nanotubes forming graphene nanoribbons. (B) Representative TEM image of a HeLa cell engulfing O-GNR-DSPE aggregate (red arrow).

In general, all the cells showed a dose-dependent (10-400 µg/ml) and time-dependent (12-48 h) decrease in cell viability. Compared to MCF7 or SKBR3 cells, the degree of cytotoxicity was significantly higher in HeLa cells. After 48 hours, MCF-7 and SKBR cells were ≈100% viable when incubated with O-GNR-PEG-DSPE at 10 µg/ml concentration. However, the cell viability decreases above this concentration with ≈22% cell death at the highest treatment concentration (400 µg/ml). In contrast, HeLa cells showed ≈5-25% cell death (depending on the assay, and the time point) at the lowest treatment concentration of 10 µg/ml. Cell viability reduced significantly with an increase in the treatment concentration with the CD50 values > 100 µg/ml (depending on the time point, and the assay). In comparison to other cell lines, HeLa cells showed a significantly higher uptake of O-GNR- PEG-DSPEs (assessed by transmission electron microscopy of cells). Additional analysis confirms that the significantly higher toxicity exhibited by HeLa cells is due to an increase in the uptake of O-GNR-PEG-DSPEs. These results suggest a heterogenous toxicity behavior of O-GNR-PEG-DSPEs, which is significantly different compared to graphene nanoparticles prepared by modified Hummer's method (oxidation of graphite, followed by mechanical exfoliation) or its variations.

References:

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[2] Schedin F, Nat Mater 2007;6:652-5
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[4] Mullick Chowdhury S, Biomaterials 34 (2013) 283-293