# Green exfoliation of graphene; an *in vitro* study of toxicity and biocompatibility

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## INTRODUCTION

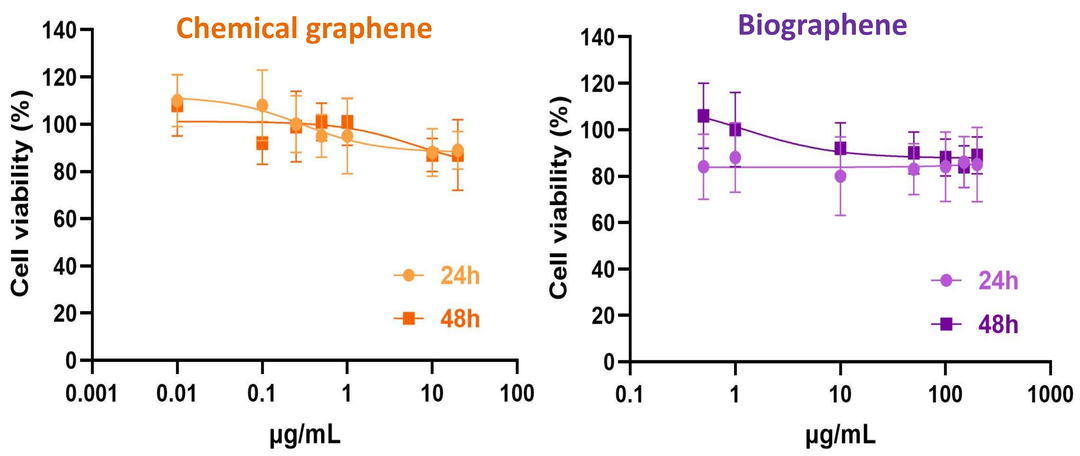
Due to their unique physicochemical properties, the use of graphene-based nanomaterials in biomedical applications has attracted great interest over the last decade. Recently, several green exfoliation methods have emerged as more economical and environmentally friendly approaches for producing graphene from graphite. The aim of this study was to evaluate the toxicity and biocompatibility of graphene that has been synthesized either with chemical (Chemical graphene) or green procedures (Biographene).

## METHODS

Cytotoxicity of the two compounds was assessed *in vitro* in human THP-1-derived macrophages with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT assay) Examination of the redox stage of the cells with ROS- DCFDA assay, as well as evaluation of apoptosis and cell cycle analysis, were performed using Flow cytometry.

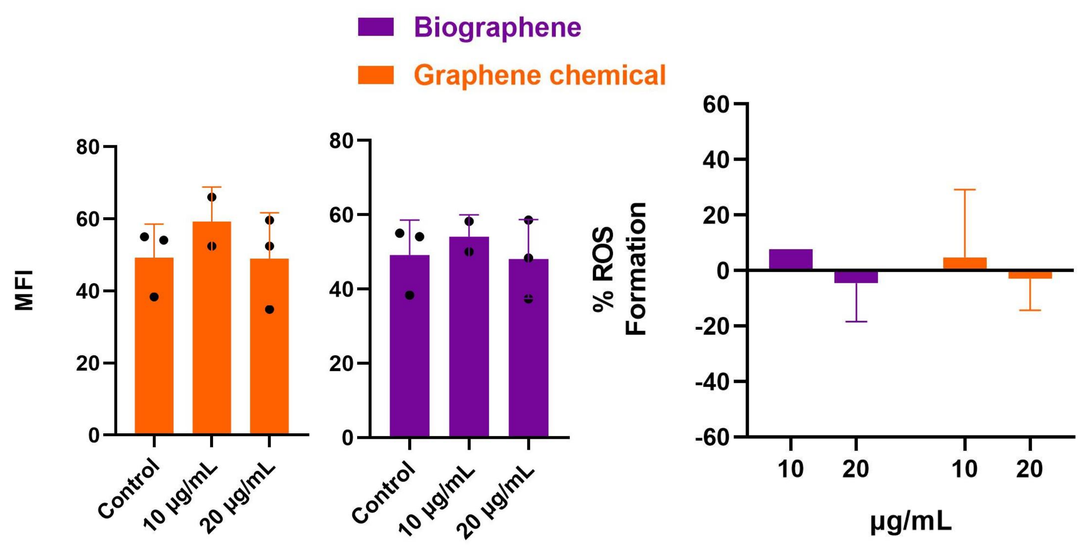
## RESULTS

### CELL VIABILITY



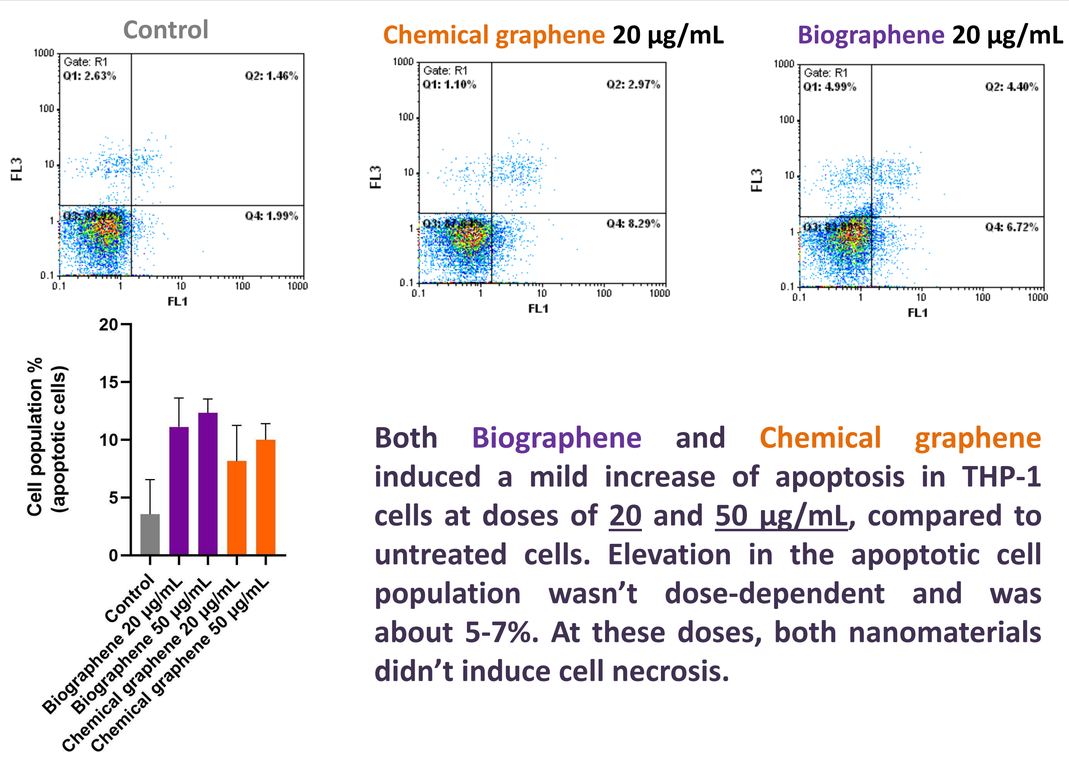

In both nanomaterials, toxicity was dose- dependent rather than time- dependent. Chemical graphene's exfoliation requires the use of toxic solvent DMF, and thus its toxicity assessment was limited to low doses (≤20 μg/mL). At these doses both nanomaterials weren't cytotoxic, however Biographene's lack of chemicals make it biocompatible even at 10x higher doses (200 μg/mL).

### REDOX STAGE



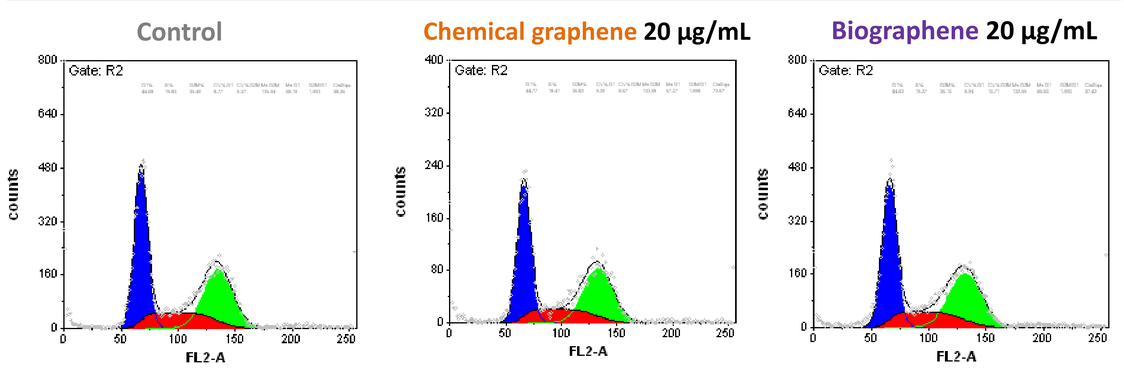

None of the two nanomaterials seemed to induce oxidative stress in THP-1 cells, as neither of them generated intracellular reactive oxygen species (ROS) at the doses of 10 and of 20 μg/mL after 24h of treatment.

### INDUCTION OF APOPTOSIS




Both Biographene and Chemical graphene induced a mild increase of apoptosis in THP-1 cells at doses of 20 and 50 μg/mL, compared to untreated cells. Elevation in the apoptotic cell population wasn't dose-dependent and was about 5-7%. At these doses, both nanomaterials didn't induce cell necrosis.

### CELL CYCLE ANALYSIS




| | G0/G1 | S | G2/M |
|---|---|---|---|
| Control | 44.68 ± 2.09 | 19.83 ± 1.20 | 35.49± 1.03 |
| Biographene 20 μg/mL | 44.63 ± 1.35 | 19.27 ± 1.50 | 36.10 ± 3.10 |
| Chemical graphene 20 μg/mL | 44.77 ± 2.50 | 18.41 ± 2.45 | 36.82 ± 1.57 |

At the dose of 20 μg/mL none of the nanomaterials induced cell cycle arrest at G0/G1 or G2/M phase. This result suggested that no damage in DNA or microtubules occurred after treatment of THP-1 with the two compounds.

## CONCLUSION

Although both materials seem to be safe at low doses, green exfoliated- graphene- Biographene could be used at higher doses. Moreover, it's sustainable and economical way of production make it an ideal candidate for biomedical applications (i.e., biosensing, drug delivery etc.). Further research on the activation of molecular pathways of inflammation by Biographene could prove its value for use in such applications

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