Cytotoxicity Assessment of a Graphene Monolayer in Direct Contact with RAW 264.7 Cells

Iwona Lasocka[a], Lidia Szulc-Dąbrowska[b], Aleksandra Krajewska[c,d], Bartosz Skibniewski[e], Ewa Skibniewska[a], Michał Skibniewski[f]
[a]Department of Biology of Animal Environment, Faculty of Animal Science, Warsaw University of Life Sciences, Warsaw, Poland
[b]Department of Preclinical Sciences, Faculty of Veterinary Medicine, Warsaw University of Life Sciences, Warsaw, Poland
[c]CENTERA, CEZAMAT, Warsaw University of Technology, Warsaw, Poland
[d]CENTERA labs, Institute of High Pressure Physics PAS, Warsaw, Poland
[e]Faculty of Veterinary Medicine, Warsaw University of Life Sciences, Warsaw, Poland
[f]Department of Morphological Sciences, Faculty of Veterinary Medicine, Warsaw University of Life Sciences, Warsaw, Poland

Introduction: Graphene in the form of a monolayer is a promising scaffold material for wound dressings and regenerative therapies. However, its potential biomedical application requires confirmation of non-toxicity and biocompatibility, which are essential for all materials intended for direct contact with the body. Macrophages play a crucial role in the innate immune response and are among the first cells to interact with foreign materials, including biomaterials.

The aim of the study: RAW264.7 macrophages were cultured on a graphene monolayer to evaluate its biological impact.

Material and methods: Graphene, deposited on copper foil (Graphenea, Spain), was transferred onto glass coverslips using an electrochemical delamination method. Cell viability, morphology, and membrane integrity were assessed in direct contact with the graphene nanotopography. Flow cytometry and fluorescence microscopy were applied, using Zombie Green dye for viability analysis and WGA staining for membrane visualization. Additionally, FSC-A and SSC-A parameters were analyzed to assess cell size and internal complexity.

Results: The representative Raman spectrum of the graphene structure is shown in Figure 1. Macrophages cultured on graphene (GM) showed no significant differences in size or granularity compared to the control group (CM) (Figure 2). Cell death levels were comparable between Groups (CM=16.5 ± 2.5%), although slightly higher variability was observed for GM (18.3 ± 5.7%). Fluorescence imaging confirmed intact plasma membranes and typical macrophage morphology, including round to polygonal shapes with visible surface extensions (Figure 3).

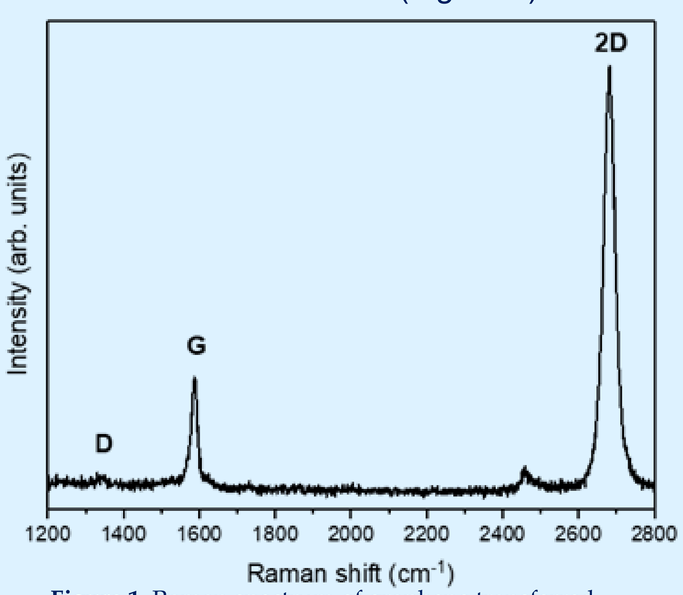

Figure 1. Raman spectrum of graphene transferred onto a glass slide.

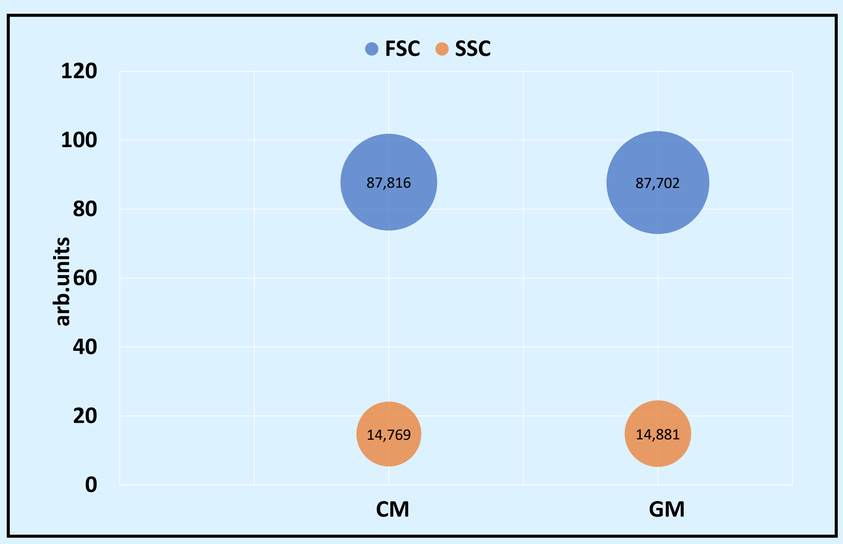

Figure 2. Flow cytometry analysis. Cell size and granularity in macrophages were calculated according to forward (FSC-A) and side (SSC-A) scatter profile. The center of the circle represents the mean value, and the radius of the circle indicates the standard deviation within the group.

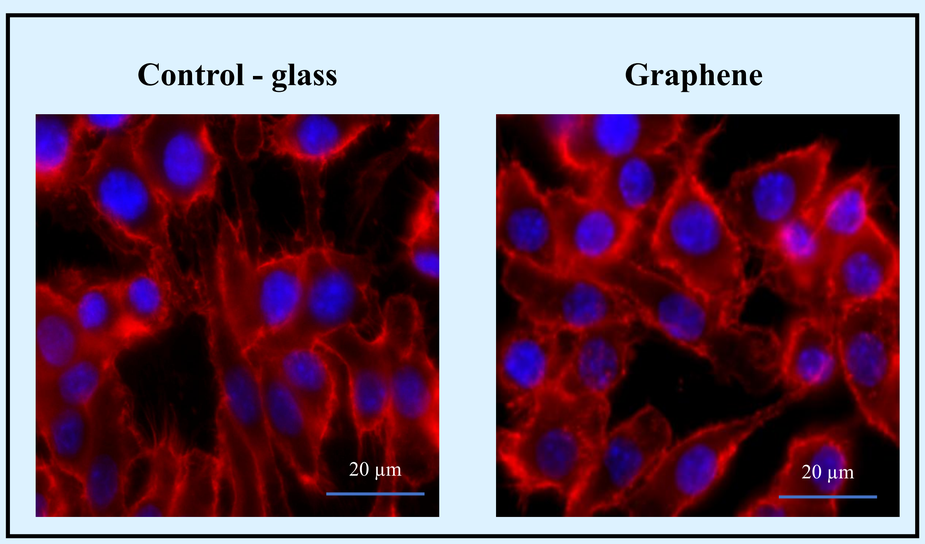

Figure 3. WGA (wheat germ agglutinin) labeling. Cells were stained with WGA (plasma membrane, red fluorescence) and Hoechst 33342 (DNA, blue fluorescence).

Conclusion: Overall, the results indicate that graphene monolayers support macrophage adhesion and growth without adverse effects on viability or morphology, highlighting their potential as biocompatible materials for wound-healing applications.

National Science Centre in Krakow, Poland (grant no. 2022/06/X/NZ3/00289)