Laser-induced Graphene *in vitro* cytotoxicity

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Laser-induced graphene (LIG) is a versatile and promising material with a wide range of potential applications, and research in this area is ongoing to explore new ways to use this innovative material [1]. The method of LIG synthesis is considered fast, low-cost, and scalable since it can be synthesized by using a laser to burn a pattern onto a substrate (i.e., polymers or paper) [2]. Laser-induced graphene has many potential applications, including energy storage devices, sensors, and biomedical applications [3]. However, little is known about LIG toxicity, which is extremally necessary for medical devices, tissue engineering, wearable sensors, or regenerative medicine applications. For biocompatibility assessment, *in vitro* testing is an essential tool providing valuable information about the potential risks associated with using chemicals and materials in biological systems. In particular, cytotoxicity and proliferation assays are commonly used to assess the potential toxicity of graphene-based materials on living cells. Herein, we reported the *in vitro* study about LIG toxicity in mice fibroblasts (L929) and osteoblast precursor cells (MC3T3).

The cell viability was tested using polyimide discs (PI) and LIG discs through a direct contact test. The LIG was obtained by the direct irradiation of a laser (1.5 W laser power at 405 nm) on PI substrates. The presence of LIG was confirmed by scanning electron microscope images and Raman spectroscopy. For the experiment, the cells were seeded in 96-well plates (10^4 cells per well), and 24h later, the discs were carefully placed on the cell layer. After 48 hours, the cell viability was tested with the XTT protocol. For the cell proliferation assay, cells were cultured at the bottom of each well (6 discs per sample). This way, the cells would grow on top of the discs, and the growth would be compared with cells grown on the plate substrate (Control cells). The cells were cultured for 5 days; then, the cell viability was performed with an XTT assay. The direct contact cytotoxicity test results of both PI and LIG means are statistically different from control cells in terms of cell viability. Still, they are not different from each other. Although the cell viability is lower than the controls, it remained over 70% in both scaffolds, which is considered non-cytotoxicity. While in the proliferation assay, both platforms led to a lower cell proliferation than controls (cells grown on a substrate specifically modified for cell growth). The cell growth on top of LIG scaffolds was even lower ($p<0.05$), probably due to LIG's surface effect not giving rise to the most favorable conditions for efficient cell adhesion, growth, and proliferation.

References

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