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Evaluation of the Influence of Graphene Oxide on Spheroid and Monolayer Culture under Flow Conditions

Authors : A. Zuchowska, A. Buta, M. Mazurkiewicz-Pawlicka, A. Malolepszy, L. Stobinski, Z. Brzozka

Abstract : In recent years, graphene-based materials are finding more and more applications in biological science. As a thin, tough, transparent and chemically resistant materials, they appear to be a very good material for the production of implants and biosensors. Interest in graphene derivatives also resulted at the beginning of research about the possibility of their application in cancer therapy. Currently, the analysis of their potential use in photothermal therapy and as a drug carrier is mostly performed. Moreover, the direct anticancer properties of graphene-based materials are also tested. Nowadays, cytotoxic studies are conducted on in vitro cell culture in standard culture vessels (macroscale). However, in this type of cell culture, the cells grow on the synthetic surface in static conditions. For this reason, cell culture in macroscale does not reflect in vivo environment. The microfluidic systems, called Lab-on-a-chip, are proposed as a solution for improvement of cytotoxicity analysis of new compounds. Here, we present the evaluation of cytotoxic properties of graphene oxide (GO) on breast, liver and colon cancer cell line in a microfluidic system in two spatial models (2D and 3D). Before cell introduction, the microchambers surface was modified by the fibronectin (2D, monolayer) and poly(vinyl alcohol) (3D, spheroids) covering. After spheroid creation (3D) and cell attachment (2D, monolayer) the selected concentration of GO was introduced into microsystems. Then monolayer and spheroids viability/proliferation using alamarBlue® assay and standard microplate reader was checked for three days. Moreover, in every day of the culture, the morphological changes of cells were determined using microscopic analysis. Additionally, on the last day of the culture differential staining using Calcein AM and Propidium iodide were performed. We were able to note that the GO has an influence on all tested cell line viability in both monolayer and spheroid arrangement. We showed that GO caused higher viability/proliferation decrease for spheroids than a monolayer (this was observed for all tested cell lines). Higher cytotoxicity of GO on spheroid culture can be caused by different geometry of the microchambers for 2D and 3D cell cultures. Probably, GO was removed from the flat microchambers for 2D culture. Those results were also confirmed by differential staining. Comparing our results with the studies conducted in the macroscale, we also proved that the cytotoxic properties of GO are changed depending on the cell culture conditions (static/ flow).

Keywords : cytotoxicity, graphene oxide, monolayer, spheroid
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