

The evaluation of cellular response of mammalian cells co-incubated with Fe₃O₄/graphene oxide to magnetic field

Magdalena Jedrzejczak¹, Karolina Urbas², Rafał Rakoczy³, Ewa Mijowska²

¹Laboratory of Cytogenetics, West Pomeranian University of Technology, Dra Judyma 6, 71-466 Szczecin, Poland

²Department of Environmental and Chemical Engineering, West Pomeranian University of Technology, Szczecin, Piastow Avenue 45, 70-311 Szczecin, Poland

³Institute of Chemical Engineering and Environmental Protection Process, West Pomeranian University of Technology, Piastow Avenue 42, 71-065 Szczecin, Poland
mjdrzejczak@zut.edu.pl

Abstract

The biological effectiveness of magnetic fields (MFs) on tumor cells has been studied since the 1970s. Some of the MF molecular mechanisms that affect tumor cells still remain unclear, but it has been shown that the magnetic field can inhibit cancer cell growth and induce apoptosis (Feng et al. 2013). Localized hyperthermia induced by a magnetic field (ablatherm) is an alternative cancer treatment. It is a form of non-invasive therapy that gives low side-effects and can be an alternative to the traditional treatment – surgery – that can cause harm and often dreadful side-effects. An important element of that type of treatment is magnetic properties of hybrid composed of Fe₃O₄ deposited on graphene oxide platform (GO-Fe₃O₄) that determine the desirable effect on tumor cells.

The aim of the study was to evaluate the cellular response of L929 fibroblast cells to a rotating magnetic field incubated with the graphene oxide nanosheet/Fe₃O₄ nanoparticles (GO-Fe₃O₄).

The three types of nanomaterials: graphene oxide, iron oxide and hybrid of Fe₃O₄ deposited on graphene oxide (GO, GO-Fe₃O₄, and Fe₃O₄, respectively) tested in the present experiment were synthesized using methods described previously by Urbas et al. (2014). L929 mouse fibroblasts were seeded into 96-well plates at the density of 7.4×10^3 per well, and cultured in DMEM supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 0.4% streptomycin/penicillin and 10 mM Hepes. Cells were maintained in standard cell culture conditions at 37°C, 5% CO₂ and 95% humidity. After 24-h incubation period, the three types of nanomaterials (GO, GO-Fe₃O₄, and Fe₃O₄) were added to the cell culture to obtain final concentrations of 0.0, 3.125, 6.25, 12.5, 25.0, 50.0 and 100.0 µg/ml. The cells were incubated with nanomaterials for 48 h. Experiments on the influence of rotating magnetic field (RMF) on L929 cell cultures, incubated with three types of NPs, were performed using an exposure system previously described by Masiuk et al. (2008). Microplates with cells were placed in a glass container in the centre of the coil. All experiments were temperature-controlled at 37°C±0.5°C. Magnetic induction was determined inside each hole of the 96-well plate using the Hall probe. The recorded values of magnetic induction were 10.06, 6.58, 3.95, 2.36, 1.57 and 1.23 mT (average values of different wells on a culture plate) for the magnetic field frequency of 50 Hz.

Firstly, the effects of nanomaterials and rotating magnetic field on the cultured L929 cells were tested using the WST-1 Cell Proliferation Reagent. The WST-1 solution was added after 48-h incubation with nanoparticles and 8-h exposition to a rotating magnetic field of each microplate well, and incubated. The absorbance was measured at 450 nm using a Sunrise Absorbance Reader. Cytotoxicity effects of RMF and nanoparticles were also determined using the LDH CytoTox 96[®] Non-Radioactive Cytotoxicity Assay. Absorbance of the tested probes was measured at 490 nm using a spectrophotometer.

The influence of the rotary magnetic field on the activity of L929 cells (without nanoparticles) showed that the mitochondrial metabolism was highest in cells incubated at 10.06 mT and lowest in cells incubated at 1.23 mT of RMF. When cell cultures were exposed to NPs and rotating magnetic field, the most significantly reduced cellular activity was recorded for graphene oxide particles at a concentration of 6.25-100.0 µg/ml, 10.06 mT of RMF, while in the case of GO-Fe₃O₄ and Fe₃O₄ nanoparticles it was 100 µg/ml at 10.06 mT of RMF.

In summary, it can be concluded that the combination of NPs and the magnetic field may have a synergistic effect on cellular metabolism. Cancer cell lines will be utilized in further studies to determine the adequate co-effect of NPs and RMF for therapeutic aspects of the presented method.

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References

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