Mesoporous bioactive glasses – Part II: Biological response

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ABSTRACT

Preclinical studies of bioactive glasses (BG) are first carried out in cell culture models (for this purpose primary culture or cell lines can be used). The cell culture model is a convenient way of screening the effectiveness of a BGs formulation. The cell culture models represent a major tool for the investigation of the normal physiology of the cells and possible toxic interaction between materials and biological system (1). The main advantage of using cell culture is consistency, reproducibility and shorter duration time of experiments. The cytotoxicity of a proposed material to a specific cell type can be studied either by directly seeding the cells on the surface of the material or by indirect evaluation by exposing the cells to the extraction fluid (2, 3).

In this work three different cell lines were used. Namely the MG-63 (human osteosarcoma cell line), MEFs (mouse embryonic fibroblasts cells) and ST-2 (mouse bone marrow stromal cell line) in the indirect elution test and indirect test in tissue cell culture inserts. Cytotoxicity of SiO_2-CaO and SiO_2-CaO-ZnO bioactive mesoporous glasses (MBGs) has been evaluated. Selection of cell types was based on the specific applications of MBGs. Human osteosarcoma cell lines could be a suitable model for orthopaedic implant materials. Mouse fibroblasts can be used for determining the cytotoxic potential of wound dressing material. Because of pluripotency of the stromal cell lines, they could be used as a model in both mentioned applications.

In the elution test method, extracts were obtained by placing the test materials in separate cell culture media incubated in standard conditions (24 h, 37°C). The obtained fluid extract was applied to a cultured-cell monolayer and jointly incubated for 2 days. In another indirect approach, the cells are in indirect contact with the material, but they are in the constant contact with released ions from the investigated material. Cells were located in the cell culture inserts, which represents a barrier between the cells and cell culture medium with the immersed material. The viability of the cells was obtained in 1 and 3 days (Fig.2).

To determine the viability of the cells in the contact with material was used WST-8 method. WST-8-(2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium is bioreduced by cellular dehydrogenases to an orange formazan product. The amount of formazan produced is directly proportional to the number of living cells.

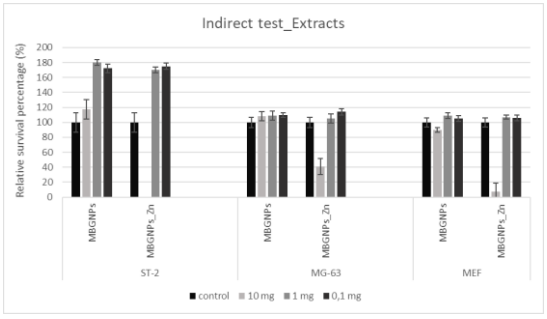

Fig. 1: Cytotoxicity testing – indirect elution test method. Influence of MBGNPs and MBGNPS_Zn powder extracts (10 mg/mL, 1 mg/mL, 0.1 mg/mL) on the viability of ST-2, MG-63 and MEF cells.

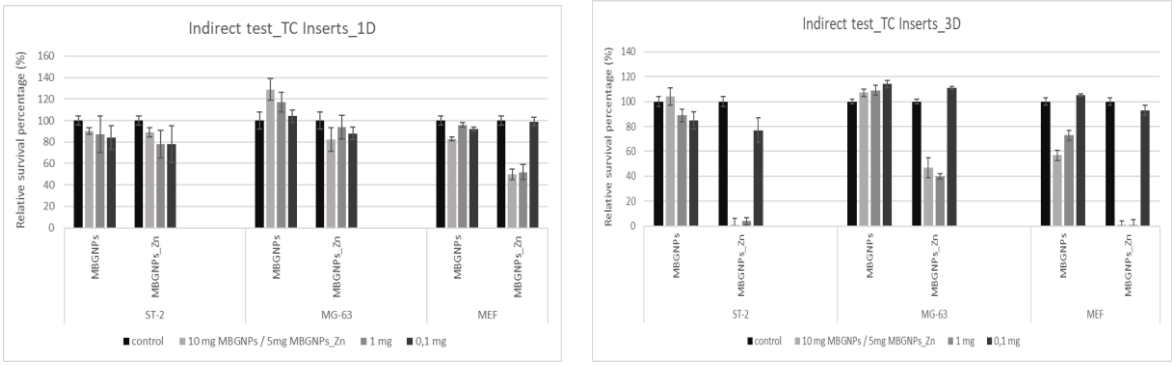

Fig. 2: Cytotoxicity testing – indirect method with use cell tissue culture inserts. Investigation of indirect influence of constantly releasing ions from MBGNPs and MBGNPS_Zn powder on the viability of ST-2, MG-63 and MEF cells after 1 day and 3 days of culture.

In the cytotoxicity testing, a negative result indicates that an MBGNPs material is free of harmful extracts for all tested concentrations with standard elution testing method and with the other approach which implies the use of tissue cell culture inserts (Fig. 1, Fig. 2). The cell viability of about 90 % characterizes a great biocompatibility potential of the MBGNPs. On the other hand, a positive elution cytotoxicity test result of MBGNPs_Zn (10 mg/mL), was an early warning sign, that a material contains extractable substances that could be of clinical importance (Fig. 1). Based on this fact MBGNPs_Zn undergone further investigation, titration in the elution test method, by means of which 5mg/mL concentration of the powder was determined as nontoxic for all tested cell lines (data not shown). Further cytotoxicity tests with seeded cells in cell tissue culture inserts on MBGNPs_Zn (5mg/mL) powder in constant contact with the cells, demonstrated that MBGNPs_Zn stays nontoxic for all cell lines after 1 day. However, the relative survival percentage of all tested cell lines was lower than 50 %, after 3 days, which indicate the cytotoxic effect of the material (Fig. 2).

In conclusion, cytotoxicity assay is a good first step toward ensuring the biocompatibility of proposed biomaterial. The material showed a variable cytotoxic potential which was related to the cell type. Obtained results confirmed the cell-line dependent sensitivity and support the necessity of the combination of at least two evaluation methods for *in vitro* cytotoxicity test.

Keywords: biomaterials, cytotoxicity, cell lines, indirect methods

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