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Comparison of direct and indirect methods of cytotoxicity evaluation using different chitosan bioglass composites for bone regeneration

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Evaluation of the material's biocompatibility and its supportive role in tissue regeneration is one of the most important procedures before *in vivo* research and clinical trials. Also, the lack of toxicity caused by the biomaterial during preliminary *in vitro* research is crucial for the future development of tissue regeneration procedures and it can be proven by direct and indirect toxicity tests. The aim of this study was to compare these two methodologies which are described in ISO guidelines (ISO 10993-5:2009 Part 5). Cell proliferation was measured using WST-1 assay, and the cytotoxicity was measured by LDH test kit. Obtained results didn't show decreased proliferation and cytotoxicity was detected by the indirect method. The direct method used

in the research caused lower proliferation compared to the controls and cytotoxicity higher than 30% of positive control. The results obtained indicate that the molecular surface of biomaterials has an impact on the results. Based on the presented findings we confirm that the indirect method does not give a clear picture of the cell condition after exposure to a surface. The comparison of both methods shows that it is pivotal to investigate the biomaterials at the very early stages using both indirect and direct methods to access the influence of the released toxins and surface of the material on the cell condition.

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