Genotoxic effect of selected hydrogels loaded with superparamagnetic iron oxide nanoparticles potentially applied in regenerative medicine

Lucia Balintova[1], Alessandro Paolini[2], Andrea Masotti[2], Monika Sramkova[1]

[1]*Biomedical Research Center of the Slovak Academy of Sciences, Bratislava, Slovakia*
[2]*Bambino Gesù Children's Hospital-IRCCS, Research Laboratories, Rome, Italy*
lucia.balintova@savba.sk

Advanced methods in regenerative medicine are trying to develop suitable materials that can reproduce and restore the favorable, natural environment needed for skin regeneration. The development of advanced multifunctional materials for injured skin treatment with the ability to provide multiple functions at once is crucial for clinical application. The utilization of hydrogels loaded with nanoparticles in regenerative medicine provides an innovative way to treat skin injuries.

It is important to evaluate the biosafety of nanohydrogels as a degradable biomaterial for use in the biomedical field. The aim of this study was to determine the genotoxic effects of newly prepared nanocomposites. The model system represents different types of skin cell lines, keratinocytes (HaCaT), and fibroblasts (HFF-1). The experiments were focused on determining the genotoxic effect of nanocomposites in *in vitro* conditions. For nanohydrogel build-up, three different hydrogels (Alginate, Pluronic F127, and Gelatin metacryloyl) with different chemical compositions and iron oxide nanoparticles were used. For genotoxicity determination, we used three different methods: comet assay, fpg-modified comet assay, and micronucleus test to determine aneugenic, clastogenic, and DNA damage. We also determine nanoparticle release from hydrogel structure using Prussian blue staining.

Initial results after 24 h nanohydrogel exposure, measured by comet assay showed a significant increase in DNA damage in the case of higher concentration of gelatin metacryloyl nanohydrogel. We didn't observe any DNA damage in the two other nanohydrogels. Subsequently, we used an fpg-modified comet assay to determine if this damage is caused by base oxidation. However, we did not observe any differences between samples with and without fpg-enzyme treatment. We assume that DNA damage is a result of single or double-strand breaks incurred as the attempted repair of UV irradiation-induced base damage in DNA structure. From the results of the micronucleus test, we noticed a higher amount of apoptotic and necrotic cells after gelatin metacryloyl exposure, also the presence of micronuclei was significantly higher. Results of Prussian blue staining showed that nanoparticle release from hydrogel structure depends on hydrogel concentration.

This work was supported by ENM III/2019/861/TENTACLES; DoktoGrant no. APP0316, project VISION (Strategies to strengthen scientific excellence and innovation capacity for early diagnosis of gastrointestinal cancers) No. 857381, CA21108 - European Network for Skin Engineering and Modeling and VEGA No. 2/0121/21