UTILIZATION OF GRAPHENE-BASED MATERIALS AND MESENCHYMAL STEM CELLS IN REGENERATIVE MEDICINE

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Introduction
One of the challenge of regenerative medicine is to obtain a native and functional cartilage tissue *in vitro*, that can be used for articular cartilage repair. Recent evidence indicate innovative approaches that utilized stem cells (SCs) and biomaterials investigations as one of the promising tool that may be used in biomedical applications. Mesenchymal stem cells (MSCs) possess huge proliferative capacity, high paracrine activity and low immunogenicity. Moreover, MSCs can be differentiated into cells of mesodermal origin. There is also evidence that physical stimulation of the differentiation process is very important, but it is still not well known. Thus, in this study, we tested MSCs together with graphene-based materials, that possess unique physical, chemical, and biological properties to improve the effectiveness of chondrogenic differentiation of MSCs.

Materials and Methods
GO-based materials were prepared according to the Marcano method and then were modified with different types of metal nanoparticles (ITME Institute). Next, GO materials were sterilized and utilized as culture surface dedicated for human umbilical cord Wharton's jelly mesenchymal stem cells (hUC-MSCs). The influence of GO-based materials on biological properties of hUC-MSCs was evaluated. Proliferation test was performed by Scepter 2.0 Automated Cell Counter (Merck Millipore). Cell viability was measured by LSR Fortessa flow cytometer by FITC Annexin V Apoptosis Detection Kit (BD Biosciences). Moreover, the influence of GO on induction of chondrogenic differentiation process of hUC-MSCs was studied by gene expression (real-time PCR; Life Technologies) and protein level analysis (alcian blue staining; Sigma Aldrich).

Results and Discussion
The results revealed that graphene-based substrates constitute non-toxic surfaces for culture of hUC-MSCs. We observed, that GO may slightly modulate proliferation and survival of hUC-MSCs. Moreover, the obtained results indicate a positive effect of analyzed graphene-based surfaces on the induction of chondrogenic differentiation of hUC-MSCs. Interestingly, we revealed, that modification of GO with metal nanoparticles induce the positive effect of this process. We observed a significant increase in the number of colonies containing proteoglycans (e.g. aggrecan) that are formed on native and modified GO-based substrates. In addition, we observed an increased size and rate of formation of microbodies during the experiment. Moreover, the results of the gene expression analysis indicate the stimulation of the chondrogenic differentiation process in hUC-MSCs cultured on both GO scaffolds: native and modified. Quantitative analysis demonstrated an increase level of the transcripts of genes associated with chondrogenesis (e.g. SOX9 and COL2A1).

Conclusions
Performed studies provide evidence that tested graphene-based substrates (native and modified) constitute a suitable surface for hUC-MSCs propagation and may induce differentiation of MSCs towards cartilage cells. However, further studies are required to optimize new protocols for cell preparation for orthopedic applications in the future.

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