GRAPHENE OXIDE-BASED BIOMATERIALS AS A POTENTIAL TOOL FOR CARTILAGE TISSUE REGENERATION

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Introduction

Currently, combining biomaterial scaffolds with living stem cells for tissue regeneration is the main approach for tissue engineering. Human adipose mesenchymal/stromal stem cells (hAT-MSCs) are promising candidates for tissue regeneration through pro-angiogenic, immunomodulatory and anti-apoptotic activity [1] as well as differentiating into specific tissues, in particular bone and cartilage [2].

Graphene Oxide (GO) is a biophysical phenomenon that accelerates biological processes by several orders of magnitude [1,2]. Herein, we ventured to assess the influence of several compositions of GO-based hybrid biomaterials on chondrogenic and osteogenic differentiation [3] by comparing *in vitro* cell cultures conditions and selected markers expression of hAT-MSCs grown under 2-dimentional or 3-dimentional cell culture conditions.

Materials and Methods

hAT-MSCs, obtained from adult young male and female donors, were seeded at density of 4,200 cells/cm^2 for 2D culture and 30,000 cells/micromass for 3D culture. Both cultures, 2D and 3D, were expanded in a standard growth medium (αMEM, Macopharma; 10% human plated lysate, Macopharma) on cell culture plates, 6-well (Eppendorf) coated with 10 µg/cm2 dedicated GO-buffer. Subsequently, cells were collected in 3, 7 and 14 days. Medias were changed every 3-4 days. Changes in cell morphology between defined time point frames were carried out using phase-contrast microscopy (Nikon Eclipse TS100) with MicroPublisher Camera (Qimaging). To assess the induction of chondrocyte differentiation, the cells were fixed using 4% paraformaldehyde (POCH) and stained with 1% Alcian Blue 8G (Merck Millipore) up to 30 min. The cells were observed under phase-contrast microscope (Nikon Eclipse TS100).

RNA isolation was performed using the Universal RNA / miRNA Purification Kit (EURx). Reverse transcription was carried out using the NG dART RT kit (EURx). Analysis of the expression of selected genes (normalizing and characteristic for cartilage and bone cells) was performed by means of real-time PCR analysis.

Results and Discussion

Our preliminary studies show differences between hAT-MSC 2D and 3D culture in the tested media including differences in morphology and deposition of cartilage matrix proteoglycans depending on tested GO-based biomaterials. Quantitative analysis of gene expression revealed these observations. Part of tested GO-based scaffolds may enhance hAT-MSCs differentiation toward chondrogenic and osteogenic cells *in vitro*. Thus, these data may suggest, combining hAT-MSCs with appropriately biofunctionalized biomaterial such as GO-based scaffolds, on the one hand, provides them with a niche for growth, and on the other, it can increase their biological potential, due to which we will obtain more effective implants for the treatment of bone-cartilage defects.

Conclusions

Obtained preliminary results may indicate positive effect of GO-based biomaterials on chondrogenic and osteogenic differentiation. Our data allow us to choose most promising GO-based biomaterials for the purpose of preclinical application.

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