

Effect of pulsed electromagnetic fields (PEMFs) on chondrogenic phenotype maintenance of MSCs in presence of pro-inflammatory cytokines: preliminary results

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The aim of the study was to evaluate *in vitro* the effects of PEMFs on maintenance over time of chondrocyte phenotype of conditioned Mesenchymal Stem Cells (MSCs), in presence of pro-inflammatory cytokines (IL- β 1). MSCs, taken from bone marrow, were pellet-cultured in medium conditioning towards the chondrogenic lineage. Two targets were pursued: the first was to standardize the method to obtain chondrocyte pellets in terms of type/amount of withdrawal, time/degree of differentiation and amount of extracellular matrix production; the second was to extend over time chondrocyte differentiation, checking the phenotype maintenance, after adding pro-inflammatory IL- β 1 cytokine in culture medium with/without the application of PEMFs (device provided by IGEA-Carpi). The pellets obtained were cultured for different times (21, 28, 34 days), verifying the presence of type-II collagen (as index of chondrocyte differentiation) both by means of TEM analysis and immunoreaction. The best differentiation was obtained after 28 days of culture; in such pellets the studies were performed in triplicate for 15 days, identifying four experimental conditions: 1) without IL- β 1 and PEMFs; 2) with IL- β 1, without PEMFs; 3) without IL- β 1 and with PEMFs; 4) with IL- β 1 and PEMFs. The parameters of applied PEMFs were 1.7mT and 75Hz, and the time of application was 4 hours/day. Medium was changed every 3-4 days and stored for the evaluations of PGE2 (indicative of inflammation) and proteoglycans (indicative of chondrogenic differentiation). At the end of the experiment, each pellet was fixed with paraformaldehyde 4% and embedded in paraffin; sections (5 μ m thick) were obtained and stained with Toluidin Blue in order to evaluate metachromasia.

The results show that: 1) only the pellets treated with IL- β 1 without PEMFs did not show metachromasia, indicating a chondrocyte de-differentiation towards fibroblastic phenotype; 2) only in pellets treated with IL- β 1 and with PEMF application, after about 12 days of treatment the amount of PGE2 in medium decreases (31%), indicating the anti-inflammatory effect of PEMFs, while the proteoglycan production slightly increases (2%).

In conclusion, pulsed electromagnetic fields could be proposed in preventing chondrocyte de-differentiation due to inflammation induced by IL- β 1; this with the final aim to integrate regenerative medicine techniques to apply in the healing of joint cartilage lesions with bio-physic energy devices, useful to obtain a stable-in-time recovery of physiologic function of articular surfaces that suffered a severe injury.

Keywords: Pulsed Electromagnetic Fields (PEMFs), Mesenchymal Stem Cells (MSCs), chondrocyte phenotype, pro-inflammatory cytokines, metachromasia, type-II collagen