# THE EFFECTS OF ELECTROSTIMULATION ON HUMAN MESENCHYMAL STEM CELL CHONDROGENIC DIFFERENTIATION

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Electrostimulation (ES) is widely used in joint diseases like osteoarthritis or rheumatoid arthritis. Many studies showed safety and efficacy of transcutaneous electrostimulation for joint pain relief and improvement in their physical function [1]. But there is still a lack of knowledge on ES effects for cartilage repair *in vivo*. Cartilage tissue has limited ability to regenerate after trauma or degeneration, so mesenchymal stem cells (MSC) is a promising tool for treatment of studies of chondrogenesis induction, as they have a strong capacity to differentiate into chondrogenic lineage [2]. Increased expression of chondrogenic differentiation markers were observed after application of ES [3]. We hypothesise that efficacy of differentiation is mediated through voltage-gated $Ca^{2+}$ channels.

The aim of this study was to evaluate effects of different intensity ES, as well as pulsed-ES (PES), as potential stimulating factors for improvement of MSC chondrogenic differentiation capacity through alteration of intracellular $Ca^{2+}$ levels.

MSCs were isolated from bone marrows of three different patients. Additionally, human chondrocytes were isolated from articular cartilage of three different patients and compared to MSCs in all of the experiments. ES was performed for 3 days in 12 well plates under 5 V/cm electric field, pulse duration 8 ms and the frequency of pulse was 5.0 Hz. After ES, cell proliferation capacity was analyzed using cell proliferation kit 8 (CCK-8) (Spectrophotometry), intracellular $Ca^{2+}$ concentration was measured using fluorescent dye Cal-520 (Spectrophotometry, fluorescent microscopy). Also, different nanosecond PES was applied to cells in order to analyze the differences in intracellular $Ca^{2+}$ concentration. Chondrogenic differentiation was performed in micromasses of 200 000 cells. Chondrogenesis was evaluated by Safranin-O, Collagen type II antibody staining and by SOX9, Collagen II and Aggrecan gene expression (RT-PCR).

Results. ES has affected MSCs and chondrocyte proliferation, intracellular $Ca^{2+}$ levels and chondrogenic differentiation capacity. Cell proliferation was significantly increased even after 1$^{st}$ day of stimulation (according to CCK-8 staining). Moreover, the amount of intracellular $Ca^{2+}$ was significantly lower in cells after ES stimulation, as compared to non-stimulated cells. On the other hand, PES increased the levels of intracellular $Ca^{2+}$ in both cell types.

In conclusion, ES and PES differently regulate intracellular $Ca^{2+}$ levels of human MSCs and chondrocytes, which might reveal the differences in stimulation of chondrogenic differentiation response in those cells.

This work was funded by the European Social Fund according to the activity "Improvement of researchers" qualification by implementing world-class R&D projects' of Measure No. 09.3.3-LMT-K-712 (grant application code: 09.3.3-LMT-K-712-01-0157, agreement No. DOTSUT-215).

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