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EFFECTS OF ELECTRICAL STIMULATION CONDITIONS ON HUMAN MESENCHYMAL STEM/STROMAL CELLS OSTEOGENIC DIFFERENTIATION: REFINING PROTOCOLS TOWARDS ENHANCED IN VITRO BONE FORMATION

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Summary: Endogenous electrical fields are known to drive key cellular processes such as cell proliferation, migration, differentiation and tissue development. Electrical stimulation (ES) has been described as a promising strategy for bone regeneration treatments in several clinical studies [1, 2]. However, the underlying mechanism by which ES augments bone formation is still poorly understood. Thus, this work aims to study the effects of five different ES protocols on the viability, proliferation and osteogenic differentiation of human bone marrow-derived mesenchymal stem/stromal cells (hBMSCs). For that, we started by developing an electro-bioreactor device (similar to the one described in [3]) based on a custom-made lid containing medical-grade stainless steel wire 316LVM electrodes and which was able to fit a standard 6-well tissue-culture treated polystyrene plate. The lid was fabricated by fused deposition modelling (FDM) using C8 material, which was proven non-cytotoxic in our previous work [4]. ES protocols based on electric potential (constant-DC or pulsed-AC) were performed using a power source equipment, while the protocol based on electric current application was done using a custom-made current pump electric circuit. The electrical fields delivered by the electro-bioreactor system to the cell cultures were predicted by computer modelling. More specifically, the impact of electrode size and positioning as well as of the culture medium volume on the distribution/magnitude of electrical fields was evaluated by conducting a Finite Element (FE) analysis using the AC/DC module of COMSOL Multiphysics. The different ES protocols (STIM 1 OM - 1.2V DC, 1 hour/day; STIM 2 OM - 1.2V DC, 1 sec/day; STIM 3 OM - 0.03 mA DC, 1 hour/day; STIM 4 OM - 1.2V AC, T=10 sec, 1 hour/day; and STIM 5 OM - 1.2V AC, T=2 sec, 1 hour/day) were applied every 2 days during 2 weeks while culturing the cells in osteogenic induction medium in an incubator at 37ºC and 5% CO_2. Cells cultured in standard growth medium (BM CTRL) and osteogenic medium (OM CTRL) for 14 days without ES were used as controls. The *in vitro* experimental results showed that none of the different ES protocols impaired normal cell viability, morphology and metabolic activity. Moreover, the osteogenic differentiation of hBMSCs was supported by all ES protocols as confirmed by the positive alkaline phosphatase (ALP)/Von Kossa stainings performed after 14 days of culture. The osteogenic differentiation of hBMSCs under the different ES protocols was also assessed quantitatively by the determination of ALP activity, calcium content and osteogenic marker genes expression (RT-qPCR analysis). In overall, this study highlights the advantages of using computer modelling in the definition and optimization of ES protocols for improved *in vitro* stem cell-based osteogenesis towards the development of novel tissue engineering strategies for bone regeneration.

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