

## Cross-striated collagen fibers formation with low amplitude, high frequency vibrations

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human Adipose derived Stem Cells (hASCs) are observed to express genes and proteins associated with the osteoblast phenotype under osteogenic conditions and with mechanical stimulation. New investigations have been carried out to understand the effects of Low Amplitude, High frequency vibrations (HFVs) *in vitro*. In this work hASCs were cultured in proliferative or osteogenic media and daily unstimulated/ stimulated at 30 Hz for 45 minutes for 28 days to evaluate the effects of HFVs on the differentiation of hASCs toward bone tissue.

We treated four groups of cells (control in proliferative medium-CP, control in osteogenic medium-CO, treated in proliferative medium-TP and treated in osteogenic medium-TO) for 21 and 28 days. The tissue was incubated in digestion buffer and after suspended in DMEM supplemented with 10% FBS. The hASCs were cultured in proliferative medium (PM) at 37°C with 5% CO<sub>2</sub>. In order to stimulate the cells, we used a custom made "bioreactor". At the end of the culture period the DNA content was evaluated, Alizarin red test was used to determine the presence of calcium deposition. Quantitative real-time RT-PCR was performed to check the expression of the investigated osteogenic genes. The extracellular matrix (ECM) extraction to evaluate the amount of the ECM constituents. We quantified the level of the most important osteogenic protein: Col-I, Col-III, osteocalcin (OC), osteopontin (OP), alkaline phosphatase (ALP), osteonectin (ON) and fibronectin (FN). Ultrastructural investigation with TEM were performed after 21 and 28 days. Statistical analysis: a two-way analysis of variance (ANOVA) was performed.

Alizarin red test after 21 days culture showed strong differences between treated and control samples. The results of osteogenic gene expression reveal the strong increase of their content (Col-I, OP) for the mechanically treated samples, in both media at 21 days. The results obtained from the protein content analysis at 21 days seem to confirm previous observations: both the osteogenic medium and the mechanical treatment induce the early translation of osteogenic genes. TEM observations showed that in the sample treated with HFVs for 21 days, long collagen fibers of indeterminate length are present; at 28 days fibers appear shorter with the classical arrangement cross-striated in the extracellular space. From our results, the HFV treatment seems to have effect only after 21 days treatment and it seems to be ineffective in the long term trial, only collagen fibers appear more structured after 28 days.

Keywords: hASCs, bioreactor, HFV, osteogenesis, TEM