

Osteogenic Differentiation of Rat Bone Marrow Stromal Cells by Various Intensities of Pulsed Ultrasound

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Introduction Bone growth and repair are under the control of biochemical and mechanical signals. Changes in the tissue strains caused by mechanical stimuli induce production and actions of biochemical signals in the local environment to promote new bone formation [1-3]. One such mechanical stimulus of low-intensity pulsed ultrasound (LIPUS) is a clinically used intervention for accelerating healing of fractures and non-unions. While the FDA approved intensity of 30mW/cm² is used clinically, the actual intensity experienced by the cells at the target site is unknown. It is reasonable to assume that the soft tissues around the repair site attenuate the delivered LIPUS signal and the target cells are exposed to intensities below the applied dose. The aim of this study was to investigate intensities below 30mW/cm² of LIPUS able to provoke phenotypic responses in bone cells. In order to address this question, we studied the effect of 2, 15 and 30mW/cm² LIPUS in rat bone marrow-derived stromal cells (rBMSC) at early (intracellular signaling), middle (alkaline phosphates activity) and late (mineralization) stages of osteogenic differentiation.

Methods rBMSC were cultured at 3x10⁴ cells/well in 12 well plates in an osteogenic media of DMEM with 10% FBS, 50µg/ml ascorbic acid and 10⁻⁸ M dexamethasone (DEX). Cells were exposed to 1.5 MHz LIPUS (repetition rate 1.0 KHz, 20% duty cycle) at intensities 2, 15 or 30 mW/cm²_{SATA} for 20 min. Parallel cultures handled identically, but not exposed to LIPUS, were used as sham controls. For intracellular signaling, cells were harvested 30 min after a single LIPUS treatment and analyzed for activation of ERK 1/2, p38 MAPK pathways by Western blots. Alkaline phosphatase (ALP) activity was assayed after 3, 5 and 7 daily treatments of LIPUS and normalized to DNA content. For mineralization studies, rBMSC cultures were supplemented with 10mM β-glycerophosphate treated daily for 10, 17 and 24 days. To visualize mineral the cultures were stained with Alizarin Red-S. Total mineral was quantitated by photometry of the released dye using 100mM cetylpyridium chloride solution.

Results 2, 15 and 30mW/cm² LIPUS showed increased levels of phosphorylated ERK 1/2 and p38 as compared to sham (Fig.1). The ALP activity on day 5 in 2, 15 and 30mW/cm² treatment groups showed an increase with respect to sham (Fig. 2). This increase in the activity exhibited a trend with the LIPUS intensity. The mineralization was not affected by the LIPUS intensities on day 10 and 17. At day 24, mineralization levels increased significantly for all treatment groups when compared to day 10 values (Fig. 3) with 2mW/cm² showing the highest increase.

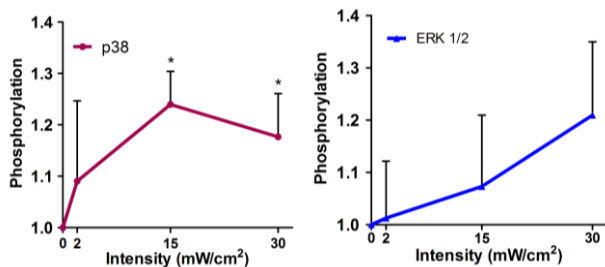


Figure 1: Western blots quantified by intensity. The graphical records represent the phosphorylation observed for 2, 15 and 30mW/cm² normalized with sham and internal control β-tubulin. The data is represented by mean (N=5) ± SEM. *P<0.05 versus sham.

Discussion Our results show that osteogenic cells derived from the bone marrow are responsive to LIPUS intensities far below what is currently being used clinically. This response was seen for 2 and 15mW/cm² by activation of ERK 1/2 and p38 MAPK pathways. Alkaline phosphatase activity being one of the markers of osteoblast maturation and differentiation, showed similar response to 30mW/cm² and lower intensities suggesting that lower intensities may also be able

to initiate osteogenic differentiation. These early effects were seen to influence the mineralization status of the cultures by showing more mineral accumulation in all treatment groups. Interestingly, we observed that 2mW/cm² shows the highest level of mineralization and the levels go down with increasing intensities.

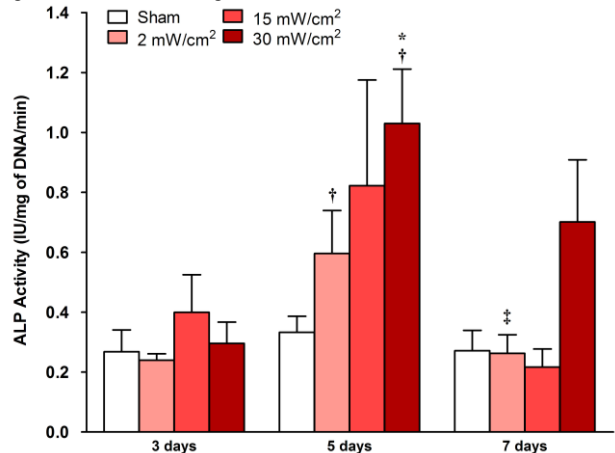


Figure 2: Effect of 20mins stimulations of sham, 2,15 and 30mW/cm² administered daily for 3, 5 and 7 days; on Alkaline Phosphatase(ALP) Activity represented as mean (N=4) ± SEM. †P<0.05 versus day 3. ‡P<0.05 versus day 5. *P<0.05 versus sham.

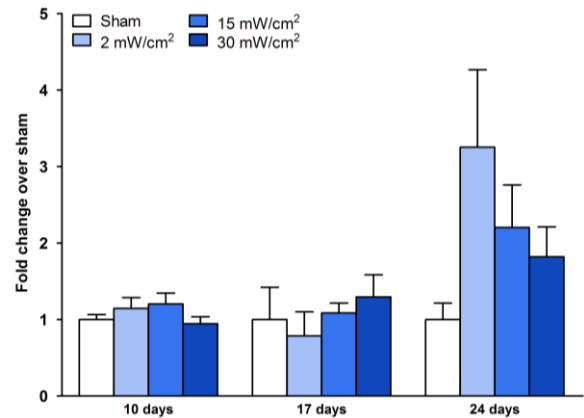


Figure 3: Relative mineralization seen in rBMSC cultures exposed to 0 (sham), 2, 15 and 30mW/cm² LIPUS for daily treatment for 10, 17 and 24 days.

Our mineralization results suggest that lower intensities could possibly cause cells to proliferate for a longer time that collectively undergo mineralization later on. On the other hand higher intensities might influence proliferation only in the early times and provide an early onset, and are possibly less efficient in inducing mineralization. In summary, our data show that LIPUS intensities lower than the currently clinically used (30mW/cm²) show a positive effect on osteogenic differentiation of rat bone marrow stromal cells. This finding warrants further *in vivo* investigations to redefine the most effective LIPUS intensity for clinical use.

References

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Acknowledgements Smith & Nephew, Inc. and the Grainger Foundation.