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ACCELERATION OF CRITICAL BONE DEFECT HEALING BY ULTRASOUND TREATMENT IN A
OSTEOPENIA MODEL**Abstract**

Bone defects, usually caused by trauma, inflammation and tumors, may lead to loss of limb functions and substantially affect the quality of human life. When bone is impaired, it is essential to find effective methods to accelerate bone healing. Low-intensity ultrasound (LIUS), which is a unique form of mechanical energy, can transmit through and into living tissues as dynamic acoustic radiation force in the local region. The aim of this study was to evaluate the effect of LIUS induced healing in a rat tibial critical defect. A total of 40 skeletally mature female Lewis rats were used in this study. Bilaterally, the surgical bone defects of 2-3 mm in diameter were made at proximal tibia. Subsequently, the defects of the left tibia (LIUS group) received daily treatment of low-intensity pulsed ultrasound. A modified repetitive frequency at 100 Hz and an ultrasound characteristic frequency of 1 MHz and a pulse width of 200 s at an intensity of 30 mW/cm² were used for the stimulation for 20 minutes/day. After 2 weeks, the rats were euthanized and the tibias were assessed using micro-CT scanning, histology and mechanical testing. Trabecular bone volume fraction (BV/TV), connectivity density (Conn.D), trabecular thickness (Tb.Th), trabecular separation (Tb.Sp), trabecular number (Tb.N), and tissue mineral density (mgHA/cm³) were evaluated. Osteomeasure software was used to evaluate the newly formed trabecular bone in the defections of tibia. Four-point bending was used for examining the stiffness and strength using a MTS mechanical testing system. In comparison to control group, LIUS treated tibia showed increased in BV/TV, Conn.D, Tb.N, and BMD, and significant decrease in Tb.Sp. Osteomeasure analysis showed a similar trend, which is in line with the micro-CT results. BV/TV and Tb.Th were higher in LIPUS group than those in control group, and Tb.Sp was lower in LIUS group than that in control group. Ultimate load (N) at mid-shaft of tibia showed an increasing trend, but no significant increase was observed following the exposure to LIPUS, when compared to control group ($p=0.056$). The histological analysis and μ CT data revealed that new bone formed in both LIUS therapy and control with tibia defect; and LIUS therapy can significantly increase new trabecular bone volume. Moreover, bone stiffness and ultimate load of LIUS treated bone were higher than no-therapy group. Two weeks seem to be the key period for bone regeneration after bone defect surgery. Performing LIUS on bone defect could efficiently accelerate bone healing.