ENHANCEMENT OF LOW INTENSITY PULSED ULTRASOUND ON FRACTURE HEALING AT CRITICAL ANGLE

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Introduction: Low-intensity pulsed ultrasound (LIPUS) is one of the promising treatment methods to accelerate fracture healing process by providing mechanical stimulation on bone[4]. Our previous studies also indicated beneficial effect of LIPUS on human periosteal cells[1] in vitro and complex fracture healing[3] clinically. Our current study has provided hints that the stimulatory effect of LIPUS was not just localized to the area of direct stimulation[3]. Most common forms for ultrasound to travel in a solid material are longitudinal and shear waves[7]. This suggested that the combination of the longitudinal wave and the shear wave traveling along and around the bone may be one of the keys to the efficacy of the LIPUS treatment on accelerating the fracture healing process. Based on the physical properties of LIPUS, we hypothesized that LIPUS might enhance the treatment effect on fracture healing at critical angle of application by maximizing the biological effects on periosteum. In this study, we investigated the effect of LIPUS on fracture healing at different incident angles with a rat femoral fracture model.

Materials and Methods: A femoral fracture model in left femur was performed according to our established protocol in 100SD rats[5]. Fractured rats were divided into either control group (n=12) without treatment or LIPUS group with treatment(n=12/group/time point). Fractured rats with treatment were held individually in the standard platform with different incident angle holders(0°, 22°, 35° and 48°) connected with the LIPUS device(Exogen 2000®, Smith & Nephew Inc. West Calwell, NJ), which transmits 200-μsec burst of 1-MHz sine waves repeated at 1kHz with an average intensity of 30mW/cm2 for 20min/day and 6days/week. Radiographies were taken weekly post-treatment at equal magnification in a cabinet X-ray system model 43855C for routine monitoring the fracture healing. The callus width and mineralized tissue of all the X-ray films were measured and analyzed by the Metamorph Image Analysis System. In radiographic qualitative analysis, the fracture bridging rate was examined by two blinded orthopaedic surgeons according to a standardized scoring system. At different time points (week 2,4,6 and 8), the fracture site of femur was harvested. The volumetric bone mineral density and the bone mineral content were measured by using pQCT(Densiscan2000, Scanco Medical, Bassersdorf, Switzerland) while the difference in microarchitecture of the healing femur was assessed by μCT machine(μCT40, Scanco Medical, Zurich, Switzerland). The specimens(n=4/group/time point) were then proceeded for decalcified histology and stained with Hematoxylin & Eosin (H&E) or Safranin O after densitometric measurement. Mechanical torsional testing for the specimens was performed. The callus maturation, bone mineral density and the bone strength were examined qualitatively and quantitatively. Analysis of variance(ANOVA) and student’s t-test with significance level at 0.05 were done for statistical analysis of continuous data.

Results: For qualitative radiographic assessment, the fractures treated with LIPUS at 35° obtained the highest score among the control and treatment groups, especially in the later time points(Figure1). Quantitatively, the fractures treated with LIPUS at 35° healed significantly faster than the control group, as reflected by the increased callus area at week2 and3(p=0.036, 0.015 respectively). The other two treatment groups, 0° and 48°,only showed significant difference(p=0.04, 0.027 respectively) at week3 when compared with the control group. For the 0° and 48° groups, the pattern of the increase of callus area and the bridging rate were similar while the callus of 22° group bridged slower than the other groups. The 3D reconstructed images from μCT of the fractured femora in 35° group also showed that a more extensive bone bridging at the fracture site than the control and other treatment groups. At week6, the mean total bone volume of 35° group was 105.8±16.7mm3 (p=0.028) and the torque at bone failure was 0.35±0.12Nm (p=0.017), showing significantly higher than the control and other treatment groups. Histological analysis also supported these results.

Discussion: Based on the physical properties of the acoustic wave, transmitting the waves in its critical angle might maximize the longitudinal and shear waves traveled to the periosteum at the fracture site so as to increase the efficacy of the LIPUS treatment effect. In this study, the maturation of the callus and the bone strength of fracture group were showed to be enhanced by treating with LIPUS at 35°. Both of the radiographs and the 3D reconstructed images of the fracture site in 35° group demonstrated the faster fracture union rate, as reflected in the significantly larger callus area than the control group without treatment. This implied the earlier maturation of the callus in the 35° group. Transmitting the acoustic wave in its critical angle may indirectly increase in the dose of the longitudinal and shear waves reaching on the periosteum and hence this may stimulates periosteal cell proliferation, differentiation, and osteogenesis activities with the dose dependent effects[1,2]. The torque at bone failure of the 35° group was significantly higher than the control and other treatment groups in the later time points. This was coincident with the results of the μCT measurements on the bone volume density in which the 35° group showed more mineralized tissues than the control and other treatment groups. Our findings substantiated Azuma’s work showing that the longest period of time of the fracture group treated with LIPUS showed a significantly higher maximal torque and suggested that LIPUS had additive effect on the acceleration of fracture healing process[6]. Histology of the 35° group was also supported these findings. In conclusion, this study showed that 35° might be the critical incident angle for treating the fracture with LIPUS. LIPUS treatment at critical angle could maximize the acoustic waves on periosteum and significantly accelerate the fracture healing by increasing the callus maturation and bone strength. These findings demonstrated the potential of LIPUS on fracture healing could be further enhanced by manipulating the basic physical features of acoustic wave.


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