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● *Original Contribution*

FRACTURE HEALING ENHANCEMENT WITH LOW INTENSITY PULSED ULTRASOUND AT A CRITICAL APPLICATION ANGLE

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Abstract—Low-intensity pulsed ultrasound (LIPUS) was shown to have dose-dependent enhancement effect on the osteogenic activity of human periosteal cells that played an important role in fracture healing. It was hypothesized that the stimulatory effects of LIPUS on the periosteal cells could be optimized by adjusting the ultrasound delivered at its critical angle to the surface of bone. This increased the transmission of ultrasound waves on periosteum. By using a rat femoral fracture model, the stimulatory effects of LIPUS transmitted at 0°, 22°, 35° and 48°, and the sham-treatment control were investigated. Treatment efficacy was assessed using radiography, micro-computed tomography (micro-CT), histomorphometry and torsional test. The results showed that callus mineralization and bridging, biomechanical properties were significantly enhanced in the 35° group over the control and 0° groups after week 8. LIPUS transmitted at 35°, which could be the critical application angle, showed the best enhancement effects among all the other groups. LIPUS transmitted at a critical application angle may have greater enhancement effects in fracture healing. (E-mail: louis@ort.cuhk.edu.hk) © 2011 World Federation for Ultrasound in Medicine & Biology.

Key Words: **Low-intensity pulsed ultrasound, LIPUS, Critical angle, Bone, Fracture healing, Callus, Femur.**

INTRODUCTION

Fracture healing is a complex process of overlapping events resulting in an extended period from fracture to remodeling (Einhorn 1998; Leung and Ko 2001). The long recovery time can cause long-term bed occupancy, heavy resource consumption and medico-economic costs. Therefore, accelerating the fracture healing process is always an important task of orthopaedic surgeons and researchers. Numerous clinical studies have demonstrated the enhancement effects of low-intensity pulsed ultrasound (LIPUS) on fracture healing in acute fractures (Cook et al.1997; Duarte 1983; Heckman et al. 1994; Kristiansen et al. 1997; Xavier and Duarte 1983), delayed union, nonunion cases (Gebauer et al. 2005; Jingushi et al. 2007; Nolte et al. 2001) and complex fractures (Leung et al. 2004). Moreover, LIPUS also accelerated bone strength *in vivo* (Azuma et al. 2001; Erdogan et al. 2006; Wang et al. 1994; Yang et al. 1996) and stimulated the osteogenic activities of major cell types involved in bone healing *in vitro* (Ebisawa et al. 2004; Korstjens et al. 2008; Leung et al. 2004; Lu et al. 2009; Parvizi et al. 1999; Wang et al. 2004; Yang et al. 2005; Zhang et al. 2003). All these studies have demonstrated that LIPUS is one of the most promising noninvasive therapies for accelerating the healing process in fracture repair. Our research group also demonstrated LIPUS enhanced gene expression level related to the callus formation, angiogenesis and callus remodeling in osteoporotic fracture (Cheung et al. 2011). The osteogenic effect of LIPUS on bone-lengthening (Chan et al. 2006a, 2006b) and augmentation of bone-tendon junction healing has also been reported in the recent years (Lu et al. 2006, 2008, 2009; Qin et al. 2006a, 2006b).

Ultrasound is a form of longitudinal pressure wave. As ultrasound waves travel through the body, they get attenuated, scattered (Wells 1969) and absorbed (Hadjiargyrou et al. 1998; Parvizi et al. 2005) depending

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on the nature of the tissue they encounter. Bone has one of the highest attenuation coefficients among biologic tissue types. When an ultrasound longitudinal wave hits an interface between two media with different acoustic impedances (*i.e.*, soft tissue and bone) at an angle with respect to the normal, significant amount of waves lose in the transmission due to the impedance mismatch. The longitudinal wave is partially reflected and partially transmitted. The transmitted wave has both shear and longitudinal components due to an effect known as modal conversion. The percentage of the incident ultrasound waves that gets transmitted depends on the three main separated issues: impedance mismatch, attenuation and also the angle of incidence of the waves (Antich and Mehta 1997; Mehta and Antich 1997). The first two issues are the fact that is impossible to change but we could modify the incident angle of ultrasound to enhance the waves reaching the target site. The first critical angle corresponds to the angle of incidence after which incident longitudinal waves travel along the second medium surface and only shear waves (*i.e.*, no longitudinal waves) are refracted at a media boundary from incident longitudinal waves into the second medium. The second critical angle is the angle at which incident longitudinal waves are totally reflected and shear waves travel along the second medium surface. At incident angles greater than the second critical angle, neither shear nor longitudinal waves can be refracted. In the case of a LIPUS wave traveling from soft tissue into bone, the amount of transmitted shear waves can be maximized when the angle of incidence falls between these two critical angles.

Previous clinical study on complex fractures showed that the fracture callus appeared on both of the anteromedial and posterolateral surfaces of the fracture site, with the transducer placing at the anteromedial region. This interesting finding implied that the stimulatory effect of LIPUS was not just localized to the area of direct stimulation (Leung et al. 2004). In addition, it was found that LIPUS was effective in stimulating human periosteal cells, which might be one of the mechanisms in enhancing fracture healing (Leung et al. 2004). Based on these findings, it was hypothesized that the transmitted shear waves inside the bone played a key role in healing the entire fracture. In the present study, the biological effects of four different incident angles at 0°, 22°, 35°, 48° and the sham-treatment control were compared. The first, second critical angles (*i.e.*, 22° and 48° respectively) and the angle in between these two critical angles (*i.e.*, 35°) for bone were determined *in vitro* (unpublished data). By using our established closed femoral fracture rat model, radiology, bone microarchitecture, histomorphometry and biomechanics were employed to confirm our study hypothesis.

MATERIALS AND METHODS

Fracture model and groupings

One hundred eighty 8-month-old female Sprague-Dawley rats, weighting from 250 to 300 g, were used and housed at the Research Animal Laboratory in the authors' institution with 12-h light-night cycle. Free cage movement was allowed with access to standard rat chow and tap water. After 1 week of acclimatization, a closed fracture was created following intramedullary pinning in left femoral shaft in each rat according to a previous protocol (Einhorn and Bonnarens 1984; Leung et al. 2009). Under anesthesia with sodium pentobarbital (50 mg/kg per mL; Alfasan, Woerden, Holland), a medial parapatellar arthrotomy was performed. A 1.2 mm × 28 mm Kirschner wire (Sanatmetal Ltd., Eger, Hungary) was inserted into the medullary canal of the femur after reaming with 18G needle. After wound closure, the closed fracture was created by a three-point-bending fracture apparatus that consists of a frame, animal support system, blade and a 500 g steel weight. An arc support in the apparatus was used to position the left thigh of the rat and the rat was positioned supinely on the support system to create a transverse fracture in the midshaft with a force from a 500 g steel weight dropped from a height of 35 cm. Anteroposterior and lateral radiographs were taken to confirm the transverse fracture in the midshaft of the femur post-surgery. All the animal surgeries and fracture procedures were performed by one single experienced orthopaedic surgeon to ensure the consistency. The Animal Experimentation Ethics Committee of the Chinese University of Hong Kong approved the care and experimental protocol of this study (Ref. no: 08/026/MIS).

The rats were randomly assigned to five groups: four with different LIPUS incident angles (0°, 22°, 35° and 48°), and the control group with sham treatment (perpendicular to the bone long-axis) (Fig. 1). Animals were sacrificed at week 2, 4, 6 and 8 post-surgery, with a sample size of 11 in each group. Mechanical test ($n = 8$) and histomorphologic observation ($n = 3$) for each time point were performed. There was no mechanical testing on samples from week 2 time point, as the callus was too soft and not appropriate for torsional test.

Angle of incidence

The rationale of choosing the four incident angles (0°, 22°, 35° and 48°) was based on the calculation and a previous *in vitro* study that used a bovine bone as model (unpublished data). Figure 2 depicted the modal converter (angle holder) interfacing soft tissue that was on top of bone to illustrate the calculations for modal converter (angle holder) angles needed to achieve the