Ultrasound in Med. & Biol., Vol. 42, No. 2, pp. 493–502, 2016

0301-5629/$ - see front matter

http://dx.doi.org/10.1016/j.ultrasmedbio.2015.10.022

● *Original Contribution*

HIGH-FREQUENCY, LOW-INTENSITY PULSED ULTRASOUND ENHANCES ALVEOLAR BONE HEALING OF EXTRACTION SOCKETS IN RATS: A PILOT STUDY

KYUNG LHI KANG,*† EUN-CHEOL KIM,†‡ JOON BONG PARK,*† JUNG SUN HEO,†§ and YUMI CHOI*

*Department of Periodontology, School of Dentistry, Kyung Hee University, Seoul, Korea; †Institute of Oral Biology, School of Dentistry, Kyung Hee University, Seoul, Korea; ‡Department of Oral and Maxillofacial Pathology, School of Dentistry and Research Center for Tooth & Periodontal Tissue Regeneration (MRC), Kyung Hee University, Seoul, Korea; and §Department of Maxillofacial Biomedical Engineering, School of Dentistry, Kyung Hee University, Seoul, Korea

(*Received* 5 *February* 2015; *revised* 25 *September* 2015; *in final form* 27 *October* 2015)

Abstract—Most studies of the beneficial effects of low-intensity pulsed ultrasound (LIPUS) on bone healing have used frequencies between 1.0 and 1.5 MHz. However, after consideration of ultrasound wave characteristics and depth of target tissue, higher-frequency LIPUS may have been more effective on superficially positioned alveolar bone. We investigated this hypothesis by applying LIPUS (frequency, 3.0 MHz; intensity, 30 mW/cm^2) on shaved right cheeks over alveolar bones of tooth extraction sockets in rats for 10 min/d for 2 wk after tooth extraction; the control group (left cheek of the same rats) did not receive LIPUS treatment. Compared with the control group, the LIPUS group manifested more new bone growth inside the sockets on histomorphometric analysis (maximal difference = 2.5-fold on the seventh day after extraction) and higher expressions of osteogenesis-related mRNAs and proteins than the control group did. These findings indicate that 3.0-MHz LIPUS could enhance alveolar bone formation and calcification in rats. (E-mail: periokkl@khu.ac.kr)

Key Words: **Low-intensity pulsed ultrasound, High frequency, Tooth extraction socket healing, Alveolar bone formation in rats.**

INTRODUCTION

Low-intensity pulsed ultrasound (LIPUS) has been used for the treatment of fresh fractures since the early 1990s, and for the treatment of non-union and delayed union, from the late 1990s. Food and Drug Administration (FDA) approval was acquired in 2000 for the non-invasive treatment of established non-unions—excluding skull and vertebra—and for acceleration of healing time of radial and tibial fractures. LIPUS is known to be a tolerable, effective and non-invasive therapeutic technique that accelerates healing of fractured long bones (Chung et al. 2011; Tobita et al. 2012; Urita et al. 2013). The beneficial effects of LIPUS, such as reduction of healing time of bone defects, have been reported in systematic reviews (Bashardoust et al. 2012; Ebrahim et al. 2014; Hannemann et al. 2014). The scope of LIPUS application is expanding to include various tissues, such as muscles, oral mucosa and jaw bones (Engelmann et al. 2012; Maeda et al. 2013; Montalti et al. 2013; Takebe et al. 2014; Xie et al. 2011). With respect to the effect of LIPUS on dental health, intra-oral soft tissue wounds and periodontal bone defects were healed fast, osseo-integration of titanium dental implants was accelerated (Gu et al. 2014; Liu et al. 2012; Maeda et al. 2013) and orthodontically induced inflammatory root resorption was reduced (Al-Daghreer et al. 2014). Additionally, the scope of LIPUS application in dentistry has been broadened to include cellular differentiation, cellular proliferation and tissue engineering (Hu et al. 2014; Li et al. 2012; Lim et al. 2013; Yang et al. 2010).

Bone healing is a complex biological process that requires proliferation and differentiation of various cell types; many genes, proteins and signaling pathways; and an extracellular matrix (ECM). The effects of LIPUS on bone formation have been verified in many previous

Address correspondence to: Kyung Lhi Kang, Department of Periodontology, School of Dentistry, Kyung Hee University, Kyung Hee University Dental Hospital at Gangdong, 892 Dongnam-ro, Gangdong-gu, Seoul 05278, Korea. E-mail: periokkl@khu.ac.kr

Conflict of interest: The authors report no conflicts of interest related to this work.

studies. Katano et al. (2011) reported that LIPUS accelerates delayed fracture healing through promotion of endochondral ossification and bone remodeling using radiologic and histologic analysis. Rutten et al. (2008) suggested that LIPUS accelerates clinical fracture healing of delayed unions of the fibula by increasing osteoid thickness, mineral apposition rate and bone volume through histomorphometric and histologic analysis, indicating increased osteoblast activity. In dentistry, LIPUS group had an increased number of osteoblasts and increased blood vessel dimensions compared with the control group when LIPUS was applied to the interpremaxillary suture after expansion in rats (Toy et al. 2014). After miniscrew placement in rat tibias, bone–miniscrew adhesion was significantly better in the LIPUS-treated group than in the control group on histomorphometric evaluation (Miura et al. 2014). In addition, the effects of LIPUS on bone were proven from a molecular biology perspective. Runt-related transcription factor 2 (Runx2), collagen (col) type 1 and osteocalcin (OCN) are known osteogenic markers expressed during bone healing process. Runx2 is known as the first marker of premature osteoblasts in osteoblast differentiation and is expressed at a very early stage of bone healing (Götz et al. 2008). It is a key transcription factor associated with osteoblast differentiation and skeletal morphogenesis and activates other genes involved in the formation of bone matrix (Franceschi et al. 2009). In Runx2 gene knockout mice, both intramembranous and endochondral ossification were severely inhibited (Komori et al. 1997). Collagen is the main organic component of the bone ECM and col type 1 constitutes about 95% of the collagen content in bones (Niyibizi and Eyre 1994). Collagen type 1 is secreted by differentiating osteoblasts and expressed in the initial phase of biosynthesis and proliferation of the ECM. Additionally, collagen is considered the "scaffolding" of bones because the mineral is deposited after the matrix is formed, which means bone calcification occurs after collagen is formed (Owen et al. 1990). As bone calcification begins, OCN, a marker of mature osteoblasts, is secreted by osteoblasts and increases only in the latter stages of osteoblast differentiation (Tanaka et al. 2007). It is frequently used as a biochemical marker for bone formation and regarded as the most bone-specific, non-collagenous protein. Therefore, OCN expression levels can be used as a measure of osteoblast activity and bone calcification. Previous *in vitro* studies reported that LIPUS promotes Runx2 and OCN mRNA expression in human osteoblasts (Chen et al. 2003) and stimulates mRNA expression of the bone matrix proteins alkaline phosphatase (ALP) and OCN in UMR-106 cells (Warden et al. 2001). Also, mRNA and protein levels of ALP, Col-1 and Runx2 were significantly increased by LIPUS exposure compared with controls in the human PDL cell line (Inubushi et al. 2008). Fávaro-Pípi et al. (2010) reported that LIPUS improves bone repair in rats and upregulates osteogenic genes, bone morphogenetic protein 4, OCN and Runx2, mainly at the late stages of recovery. These findings indicate that LIPUS increases the expression of osteogenic genes or proteins, and as a result, differentiation into osteoblasts is activated, more osteoblasts function and more new bone is formed and calcified.

Several important parameters of LIPUS, such as intensity, frequency, duty cycle and application time, must be considered before its application. In the early period of ultrasound research, the proper therapeutic intensity range was not established. Tsai et al. (1992) concluded that an intensity of 500 mW/cm^2 accelerates fibula healing in rabbits, whereas 1.0 W/cm^2 is deleterious to fracture treatment. Warden (2003) reported the benefits of use of low-intensity ($<$100 mW/cm^2) pulsed ultrasound for fracture repairs. Yang et al. (2010) reported that the intensity was generally $<$100 mW/cm^2, and the range 20–50 mW/cm^2 was frequently used for therapeutic purposes. As a result of the many studies of intensity and other parameters of therapeutic ultrasound, LIPUS treatment is widely used on a daily basis (frequency, 1.0–3.0 MHz; repetition rate, 1.0 kHz; pulse duration, 200 μs; spatial average, temporal average (SATA) intensity, 30–50 mW/cm^2 for 20 min). For frequency, the depth of the target tissue should be considered first, because lower-frequency ultrasound can penetrate thicker tissue, and higher-frequency ultrasound has a greater superficial effect on tissue. To date, most *in vivo* and *in vitro* LIPUS studies have used ultrasound devices at frequencies between 1.0 and 1.5 MHz. To the best of our knowledge, only three published English-language *in vivo* studies have used 3.0-MHz LIPUS for oromaxillofacial tissue healing in animals (Ishihara et al. 2014; Maeda et al. 2013; Takebe et al. 2014). Maeda et al. (2013) reported that 3.0-MHz ultrasound was effective in promoting wound healing of palatal gingiva. Ishihara et al. (2014) suggested that using only 3.0-MHz LIPUS could promote the regeneration of nasal bones in rabbits. Takebe et al. (2014) reported that 1.0-MHz LIPUS had a greater effect on new bone formation in sinuses than 3.0-MHz LIPUS until 8 wk after rabbit sinus augmentation with β-tricalcium phosphate (β-TCP).

The alveolar bone, which houses teeth in the mouth, is covered by only oral mucosa. In a previous study, the physically measured maxillary mucosal thickness values of the natural tooth and missing tooth areas of cadavers were 1.43 $\pm$ 1.24 and 1.06 $\pm$ 0.68 mm, respectively, for the buccal region (Ueno et al. 2011). Compared with long bones, which are surrounded by thick muscles and skin, the alveolar bone is superficial. Therefore, we hypothesized that low-intensity, high-frequency