치의과학박사 학위논문

The effect of low intensity pulsed ultrasound on new bone formation during mandibular distraction osteogenesis in rabbits: a comparison with pulsed electromagnetic field

가토 하악골신장술에서 저강도 초음파가 신생골 형성에 미치는 효과: 전자기장 효과와의 비교연구

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치의과학과 구강악안면외과학 전공

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- Abstract -

The effect of low intensity pulsed ultrasound on new bone formation during mandibular distraction osteogenesis in rabbits: a comparison with pulsed electromagnetic field

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Background and Purpose

A disadvantage of distraction osteogenesis (DO) is the long consolidation period. Low-intensity pulsed ultrasound (LIPUS) and pulsed electromagnetic field (PEMF) have been reported to enhance fracture healing, treat nonunion, and accelerate bone maturation during the consolidation stage of DO. However, a comparative study between LIPUS and PEMF has not been reported. Considering that the effect of LIPUS on enhanced consolidation after DO of long bones remains controversial and bone healing patterns in jawbone are different from those in long bone, it is necessary to investigate the LIPUS effects in facial bones. This study was aimed to assess the effect of LIPUS on new bone

formation after rabbit mandibular DO and to compare with PEMF to clarify the optimal timing time and stimulation conditions in the application of LIPUS.

Materials and Methods

Fifty rabbits underwent DO surgery on both sides of the mandible. The distraction started 7 days after mandible osteotomy and proceeded at a rate of 1.5 mm/day for 5 days. The animals were randomly divided into three test groups as follows: Test I compared the enhanced new bone formation between the PEMF (pulse width: 12 μs, pulse frequency: 60 Hz, magnetic intensity: 10 Gauss (= 10^{-3} tesla)) and LIPUS applications (1.5 MHz, 30 mW/cm^2) during consolidation; Test II evaluated the different LIPUS intensities and frequencies (1.5 MHz, 15 mW/cm^2; 1.5 MHz, 30 mW/cm^2; 1.5 MHz, 60 mW/cm^2; 3 MHz, 30 mW/cm^2) whether new bone formation is increased; and Test III investigated the optimal application timing (distraction period versus consolidation period) to enhance consolidation after DO. The rabbits were sacrificed 6 weeks after surgery, and mandible samples were harvested. Bone formation at the distraction site was assessed by microcomputed tomography scan and histological examination.

Results

In Test I, LIPUS stimulation showed significantly higher bone volume (BV) than PEMF stimulation ($p < 0.05$), and higher BV ($p < 0.001$) and bone mineral density (BMD) ($p < 0.05$) compared to the control. The application of LIPUS stimulation with a frequency of 1.5 MHz and an intensity of 30 mW/cm^2 resulted in significantly increased BV ($p < 0.05$) and BMD ($p < 0.05$) than the control in Test II. Test III showed that LIPUS stimulation with a frequency of 1.5 MHz and an intensity of 30 mW/cm^2 resulted in significantly

increased BV ($p < 0.001$) and BMD ($p < 0.001$) during the consolidation period compared with the distraction period, and a higher BV ($p < 0.001$) during the consolidation period compared with the control.

Conclusion

LIPUS accelerated more new bone formation than PEMF. Furthermore, LIPUS stimulation with a frequency of 1.5 MHz and an intensity of 30 mW/cm^2 increased the BMD and BV effectively when it is applied during the consolidation period after mandibular DO.

Keywords: Distraction osteogenesis (DO), Pulsed electromagnetic fields (PEMF), Low intensity pulsed ultrasound (LIPUS), Stimulation intensity, Stimulation pulse frequency, Application time, Enhanced bone formation

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Contents

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I. Introduction

Distraction osteogenesis (DO) technique is used to generate new bone following osteotomy and gradual distraction. The method is based on the tension-stress principle proposed by Ilizarov [1]. Synder et al. first reported the application of DO in the mandibular area in a canine study [2], and mandibular DO in humans was first described by McCarthy et al. in 1992 for the treatment of hemifacial microsomia [3]. Nowadays, DO is widely used for bone lengthening in patients with craniofacial deformities and alveolar bone atrophy. Although DO can regenerate new bone and expand the surrounding soft tissue without bone

graft from donor sites, a long consolidation time is a disadvantage for its clinical application. After finishing the distraction, it is necessary to fix a device on the bone to prevent postoperative relapse for the consolidation period. The long consolidation time may result in enlarged scar formation and the disturbance of mastication and speech; thus, prolonged healing time has an important socioeconomic impact [4]. The acceleration of bone consolidation, therefore, has become a focus in distraction research in recent years. Various surgical and nonsurgical methods have been developed in an attempt to stimulate bone regeneration. Growth hormones, cytokines, bone morphogenetic proteins, ultrasound, electric and electromagnetic stimulation have been reported to accelerate bone healing [5-8]. As an external physical stimulation, pulsed electromagnetic fields (PEMF) and low-intensity pulsed ultrasound stimulation (LIPUS) have been widely studied and clinically used because they are noninvasive and easy to apply in outpatients. These treatments also have US Food and Drug Administration (FDA) approval. The FDA approved the use of PEMF for the healing of nonunion fractures of long bone in 1979. LIPUS was approved to treat fresh fractures of long bone in 1994 and to treat the nonunion of long bone in 2000.

Ultrasound is a form of mechanical energy that is transmitted through biological tissues as acoustic pressure waves and is widely used in medicine as a diagnostic, therapeutic, and operative tool. Webster et al. [9] first reported the effects of ultrasound on protein synthesis in human fibroblasts in vitro. Subsequently, pulsed ultrasound with frequency intervals of 1 to 3 MHz has been found to stimulate healing and increase the mechanical strength of fracture calluses in animal models [10, 11] and promoted the normal repair process involving fractures of the tibia and radius in clinical trials [12, 13]. Its therapeutic effect is known to arise from piezoelectric and angiogenetic effects on cell

membranes [14].

Electrical stimulation to promote fracture healing has been used since the 19th century, and there are relevant reports from as early as 1841 [15]. However, the use of this method did not become widespread until the 1950s when Fukada and Yasuda [16] demonstrated new bone formation in rabbit femora adjacent to a cathode. Friedenberg et al. demonstrated the use of direct current on nonunion fracture in 1971 [17]. Additionally, the augmentation of bone repair by inductively coupled electromagnetic fields was reported by Bassett et al. in 1974 [18]. Subsequently, many reports were published describing the effects of electricity on bone growth and fracture repair up until the late 1970s. Since then, a variety of electromagnetic field devices have been developed and used clinically on patients with long bone fracture, delayed unions or nonunion of long bone and cervical spine fusion. Several animal studies have demonstrated the utility of PEMF on osteoporosis [19], distraction osteogenesis [20], and dental implants [21]. Moreover, PEMF has been clinically applied in various fields, such as postmenopausal osteoporosis [22], pseudoarthroses [23] and cancer therapy [24]. Recent studies have shown a positive effect of PEMF on bone regeneration in DO [25] and mandibular fracture healing [26] and on the management of postoperative pain and edema [27] after orthognathic surgery [28] and implant surgery [29].

Although PEMF and LIPUS have been widely studied for their impact on enhanced bone regeneration [5, 13], a comparative study has not been reported regarding the bone-forming effect of these physical stimulation methods. Most studies have been performed in long bone and vertebrae, and few studies have evaluated the effect of LIPUS or PEMF in mandible. Considering that site-dependent skeletal pathobiology accounts for differences

in matrix composition, bone morphogenetic protein expression, osteoclastic bone degradation, and bone marrow stromal cell compartment, as well as functional differences in turnover and the mechanical properties of bone at various anatomic locations [30, 31], the optimal stimulation conditions such as intensity, pulse frequency and application timing in jaw bone, may be different from conditions in long bone. The purpose of this study was to evaluate the bone-forming effect of LIPUS in a rabbit mandibular DO model compared with PEMF and to find the optimal timing and stimulation conditions of LIPUS in mandible (frequency and intensity).

II. Materials & methods

1. Study design

Rabbits were randomly assigned into three test groups: Test I compared the enhanced new bone formation between PEMF (pulse width: 12 μs, pulse frequency: 60 Hz, magnetic intensity: 10 Gauss (= 10^{-3} tesla)) and LIPUS application (1.5 MHz, 30 mW/cm^2) during consolidation; Test II evaluated the ability of different LIPUS intensities and frequencies (1.5 MHz, 15 mW/cm^2; 1.5 MHz, 30 mW/cm^2; 1.5 MHz, 60 mW/cm^2; 3 MHz, 30 mW/cm^2) to increase new bone formation during consolidation; and Test III investigated the optimal application timing (distraction period versus consolidation period) to enhance consolidation after DO (Fig. 1). In Test I, sham treatment served as a control group (C). So, the test I was subdivided into three groups: the C group received a sham treatment, the P group received PEMF treatment, the L group received 1.5 MHz frequency with 30 mW/cm^2 LIPUS treatment. Test II was subdivided into four groups: the L1 group received 1.5 MHz frequency with 15 mW/cm^2 LIPUS treatment, the L2 group received 1.5 MHz frequency with 30 mW/cm^2 LIPUS treatment, the L3 group received 1.5 MHz frequency with 60 mW/cm^2 LIPUS treatment, the L4 group received 3.0 MHz frequency with 30 mW/cm^2 LIPUS treatment. Test III was subdivided into three groups: the Ld1 group received 1.5 MHz frequency with 15 mW/cm^2, the Ld2 group received 1.5 MHz frequency with 30 mW/cm^2 and the Ld3 group received 1.5 MHz frequency with 60 mW/cm^2 (Fig. 1, Table 1). In Test III, LIPUS was applied one day after the start of DO, whereas LIPUS was applied after the distraction in tests I and II.

2. Surgical procedure and distraction protocols

A total of 50 adult male New Zealand White rabbits weighing 3.5-4.0 kg (Dooyeol biotech, Seoul, Korea) were used in this study. The surgery procedure was done in the same manner as our previous study [32], All experimental procedures were undertaken in compliance with the guidelines of the local ethics committee of Seoul National University (SNU-141120-2). An intramuscular and intravenous injection of Zoletil® (0.4 mL/kg; Virbac Laboratories, Seoul, Korea) mixed with Rompun® (10 mg/kg; Bayer Korea, Seoul, Korea) were used for general anesthesia for all operations. After surface disinfection of mandible with 10% betadine (Potadines®; Sam-Il Pharm., Seoul, Korea), a subcutaneous injection was performed with 2% lidocaine containing 1:100,000 epinephrine (Lidocaine HCL Injs.; Yuhan, Seoul, Korea). The skin of the inferior border of the mandible was surgically opened for a length of approximately 7 cm, and the periosteum was elevated under additional local anesthesia to reduce pain. Distraction osteogenesis surgery was performed on both sides of the rabbit mandible. A custom-made titanium external distractor (Hyrex external fixator®; Jaeil Medical Co., Seoul, Korea) was fixed by two titanium mini screws (3 mm diameter x 8 mm length), which were placed across both cortices. A vertical osteotomy was performed on the mandibular body between the first premolar and the mental foramen of the bilateral mandibles by a fissured bur with copious sterile saline irrigation (Fig. 2). After the half cut of the mandible, a distractor was surgically fitted on the lateral aspect of the mandible, while the remaining half of the mandible was osteotomized by a fissured bur with copious sterile saline irrigation. After performing the same process to the opposite side, irrigation was carried out with sufficient normal saline. The surgical site was closed with 4-0 absorbable nylon (Ailee Co., Ltd., Busan, Korea).

The intramuscular injection of Cefazolin® (55 mg/kg; Chong Kun Dang Pharm., Seoul, Korea) was administered for 3 days to all rabbits. After a 7-day latency period, both sides were gradually distracted at a rate of 1.5 mm/day for 5 days. The rabbits were sacrificed after 4 weeks of the consolidation phase, and the new formation of bone was evaluated on both sides.

3. LIPUS stimulation

Two devices were used for LIPUS stimulation. One was a commercial ultrasound device (Exogen 4000+®, Smith & Nephew Inc., Memphis, USA) for a 1.5 MHz frequency with an intensity of 30 mW/cm^2. This commercial device is not adjustable and has a fixed frequency and intensity setting. A custom-made device (Hyemin Co., Seoul, Korea) was used for the other energy settings (Fig. 3). A treatment of 20 minutes per day was initiated one day after the distraction phase or after the distraction phase and continued for 4 weeks, with a cycle of 5 days stimulation and 2 days off.

4. PEMF stimulation

The PEMF generator was made in the electrical engineering laboratory of Dankook University College of Engineering. The PEMF device was identical to that used in our previous study [33]. The PEMF device was constructed with two major assemblies, namely, the internal animal cage assembly and the external PEMF assembly (Fig. 3). During PEMF stimulation, the animals were placed in a 40 x 18 x 25 cm internal animal cage, which was

fabricated from high-density polyvinyl chloride plastic (5 mm thickness). The animals were allowed free access to water and a pelleted commercial natural product diet. The internal animal cage was placed in the external PEMF assembly, which was constructed of polyvinyl chloride plastic (Helmholtz coil holder assembly). The two Helmholtz coils (10 turns) were placed 18 cm apart. The PEMF generator was set to produce PEMF with a pulse width of 12 ms, a pulse frequency of 60 Hz, and a magnetic intensity of 10 G (= 10^{-3} T). The PEMF treatment (2 h/day) was started after the distraction phase and continued for 4 weeks with a cycle of 5 days stimulation and 2 days off.

5. Microcomputed tomographic evaluation of the new bone formation

New bone structures of the distracted calluses were evaluated using micro-CT analysis, which calculates morphometric parameters from a selected ROI. The BV/TV indicates the fraction of mineralized tissue, while Tb.Th and Tb.Sp provide detailed information concerning the thickness and organization of the trabeculae, respectively.

The animals were sacrificed at 4 weeks post-distraction. The experimental sites (a central distraction gap) were stored in 10% formalin for 1 week. Micro-CT scans (Sky-Scan 1172® Microfocus X-ray system; Bruker micro-CT, Kontich, Belgium) with CT software, including CTAn 1.15, CTvol and NRecon Reconstruction (Bruker microCT) were used for quantitative measurement of new bone formation. The SkyScan1172® Microfocus X-ray system was equipped with a microfocus X-ray tube with a focal spot of

2 mm, producing a cone beam detected by a 12-bit cooled X-ray CCD camera fiber-optically coupled to a 0.5 mm scintillator. An aluminum filter was used for optimized images. The final 1000 x 524 pixel images were reconstructed using NRecon reconstruction and analyzed with CTAn 1.15 software. The beam-hardening effect was reduced by a second-order polynomial correction algorithm for all samples. The region of interest (ROI) in two-dimensional images to measure the newly formed bone, was a rectangular area of the central position in the distracted callus. The total new BV and the BV of initial defect in the ROI were determined according to the maximum volume of new bone formation and thickness of the existing bone, respectively. The pixel zone representing ossification in the defined ROI was then reconstructed in 3D by creating a volume of interest (VOI) in the lower and upper ranges of the threshold in grayscale units. After using CTAn 1.15 on each reconstructed bitmap file, we obtained the bone volume (BV), tissue volume (TV), BV ratio (BV/TV), trabecular number (Tb.N), trabecular thickness (Tb.Th), and trabecular separation (Tb.Sp) using a CT-analyzer in a direct 3D-based analysis of a the surface-rendered volume model according to the manufacturer's instructions. To measure bone mineral density (BMD), we converted the attenuation data for the ROI or VOI to Hounsfield units and expressed them as a BMD value using a phantom (Bruker microCT). This phantom contained rods of calcium hydroxyapatite (CaHA) with a standard density corresponding to mouse or rat bone, which ranges from 1.26 to 1.65 g/cm^3. BMD values were expressed in grams per cubic centimeter of CaHA in distilled water. A zero value for BMD corresponded to the density of distilled water alone (no additional CaHA), and a value greater than zero corresponded to non-aerated biologic tissue.

6. Bone structure and VEGF evaluation with histomorphological and immunohistochemical study

After micro-CT reconstruction, the distracted area of mandible was sampled in the direction parallel to the buccal surface of the mandible. The sectioned samples were decalcified using an EDTA solution (7%, pH = 7.0) for 10 days, with a solution change every 2 days. The samples were dehydrated in 70% ethanol and sectioned longitudinally along the axial plane. They were embedded in paraffin by positioning the center portion of the bone chip up. After cleaning for 10 min with xylene, 3-4-μm thickness specimens were prepared. For detecting bone structures, specimens were stained with Masson's trichrome. For immunohistochemical (IHC) analysis, an antivascular endothelial growth factor (VEGF) antibody was used. The specimens were incubated with anti-VEGF antibody (anti-rabbit; Sigma-Aldrich, Darmstadt, Germany) (1:100 dilution) for 1 h at room temperature, washed twice with PBS, and then incubated with the appropriate secondary antibody, namely, anti-mouse/rabbit IgG conjugated with horseradish peroxidase, and visualized with a Vectastain kit (Vecta Laboratories, NSW, Australia) according to the manufacturer's instructions. Images were evaluated using a transmission and polarized light Axioskop microscope, Olympus BX51 (Olympus Corporation, Tokyo, Japan).

7. Statistical analysis

All data are presented as the mean ± standard error of the mean or standard deviation. Statistical analyses were performed with SPSS 21® (IBM Co., Armonk, USA). Between-

group comparisons of data from the animal studies were conducted through one-way analysis of variance (ANOVA) according to the Bonferroni method for the post hoc test. Values of $p < 0.05$ were considered to be statistically significant.

III. Results

1. Microcomputed tomographic analysis of the new bone formation

Test I

PEMF stimulation increased the BV, BV/TV, BMD, Th.Th and Th.N and decreased the Th.Sp, but the differences were not statistically significant. LIPUS stimulation led to a greater than 33% increase in the BV and BV/TV values compared to the control, and greater than 13% compared to the PEMF stimulation. The LIPUS group showed a significant increase in BV and BV/TB compared to the control group ($p < 0.001$) and PEMF group ($p < 0.05$) and a significant increase in BMD compared to the control group ($p < 0.05$). However, the values of other parameters, namely, Tb.Th, Tb.N and Tb.Sp, did not show significant differences between groups. The values for the microarchitecture parameters determined by micro-CT analysis for Test I are shown in Table 2 and Figure 4, 5.

Test II

The BV and BV/TV values of groups L1, L2, and L3 were greater compared to the control. Group L2 showed statistically significant increase compared to the control group ($p < 0.05$) and L1 group ($p < 0.05$). Group L2 also showed a significant increase in BMD compared to the control group ($p < 0.05$). By contrast, decreased BV was seen in group L4, but it was not statistically significant. The parameters of Tb.Th, Tb.N, and Tb.Sp did not show significant differences between groups. The values for the microarchitecture parameters determined by micro-CT analysis for Test II are shown in Table 3 and Figure 6,

7.

Test III

For all parameters, no significant differences were shown between groups L1, Ld1 and the control. The BV and BV/TV values of group L2 were significantly increased compared to the control group ($p < 0.001$) and group Ld2 ($p < 0.001$). The BMD of group L2 was significantly increased compared to group Ld2 ($p < 0.001$). The values of BV and BV/TV were 21% greater for group Ld3, and 14% greater for group L3, compared to the control; however, these results were not statistically significant. Thus, all parameters of the microstructures in groups Ld1, Ld2 and Ld3 were similar to those of the control group. The values for the microarchitecture parameters determined by micro-CT analysis for Test III are shown in Tables 4, 5, 6 and Figures 8, 9, 10, 11.

2. Bone structure and VEGF evaluation with histomorphological and immunohistochemical analysis

Test I

After 4 weeks of consolidation, distracted space was filled and bridged with a new bone (Fig. 12). Histological observations of MT-stained sections of all groups showed no inflammatory reactions in and around the regions where the new bone had formed. Additionally, the well-formed calcified inorganic bone matrix could be seen in groups P and L. The new bone region was further investigated by IHC staining for VEGF, which is a protein-related to angiogenesis. VEGF expression was more apparent in groups P and L

compared to the control group.

Test II

After 4 weeks of consolidation, distracted space was filled and bridged with new bone (Fig. 13). Histological observations of MT-stained sections of groups L2 and L3 showed denser bone with reduced dead spaces compared to the control. By contrast, skin showed severe inflammation, and there was a delayed bone healing reaction although new bone had formed in the MT-stained section of group L4. This could be seen in more detail in the histological section under magnification (x100). Because of the inflammatory reaction, IHC staining for VEGF could not be carried out for group L4. VEGF expression was seen in the control and groups L1, L2 and L3.

Test III

After 4 weeks of consolidation, distracted space was filled and bridged with new bone (Fig. 14). In MT-stained sections of groups L2 and Ld2, L2 showed more densely mineralized new bone compared to Ld2, whereas group L1 displayed a well-formed bone matrix similar to group Ld1. VEGF expression was seen in all groups.

IV. Discussion

DO has been used as an alternative bone formation method to augment bone grafts in the reconstruction of congenital and acquired craniofacial deformities [34, 35]. One of the major disadvantages of DO is a long consolidation period for the new bone formation at distracted sites. LIPUS and PEMF have been applied to shorten the consolidation period and accelerate new bone formation [36, 37], while a comparative study on the osteogenesis stimulation effect of both physical stimulations has not yet been reported. In the present study, the effect of LIPUS on the acceleration of new bone formation at the distracted was compared with that of PEMF, and the optimal stimulation conditions (intensity and pulse frequency) and the optimal application timing of LIPUS were investigated. Our major findings were that LIPUS enhanced bone regeneration more efficiently than PEMF in the rabbit mandibular DO model, and LIPUS with a 1.5 MHz frequency and an intensity of 30 mW/cm^2 was the most effective condition for bone regeneration. LIPUS application after distraction resulted in more bone formation than application during distraction.

It has been suggested that there are functional differences in the formation, resorption, and mechanical properties of flat and long bones [31]. The jaw bone originates from the neural crest and deposits bone intramembranously, while other skeletal bones are derived from the mesoderm and form by endochondral ossification [38]. Craniofacial autologous bone grafts offer superior outcomes to long bone grafts in the reconstruction of maxillofacial bone defects [39]. In contrast to tibial fractures, which mainly heal through endochondral ossification, mandibular fractures heal via endochondral and intramembranous ossification, with a lesser degree of endochondral ossification compared

to tibial fractures [40]. These developmental and functional differences imply the existence of site-specific bone metabolism processes. Osteoblasts from femurs exhibited higher alkaline phosphatase activity, increased matrix mineralization, and expressed more osteogenic-related marker genes, while osteoblasts from mandible proliferated at the highest rate and showed elevated expression of angiogenesis-related factors in an in vitro study. Moreover, mandibular osteoblasts had a stronger pro-angiogenic effect on human umbilical vein endothelial cells than femur osteoblasts [39]. Related to the anti-osteoporotic and adverse effects of zoledronate, the delayed callus, cartilage and bone formation and remodeling by zoledronate were more profound in the mandible compared to the tibia [40]. Zoledronate-treated bone marrow stromal cells derived from the jaw and long bones exhibited differences in cell proliferation, alkaline phosphatase activity, expression of osteogenic and chondrogenic-related marker genes, and in vivo bone formation capacity. LIPUS and PEMF have been utilized to shorten the consolidation period and accelerate new bone formation for long bones in several studies [36, 37]. Considering the different bone metabolisms in the jaw and long bones, it is logical to speculate that the optimal stimulation conditions such as intensity, pulse frequency and application timing in jaw bone could be different from the optimal conditions in long bones. However, comparative studies with different conditions for improved new bone formation in jawbone have not been reported. Different from our initial hypothesis, our results showed that the optimal stimulation energy input for LIPUS was a frequency of 1.5 MHz and an intensity of 30 mW/cm^2 in mandible, which was identical with that for long bone. Based on our results, it appears that the reaction of bone-forming cells and support structures to the external physical stimulation energy input may be similar in the jaw bone and long bone,

even though they have different site-specific bone metabolisms. In terms of stimulation timing, LIPUS stimulation during consolidation resulted in significantly increased BV and BMD compared to stimulation during the distraction period.

PEMF is a type of electrophysical stimulation used for bone regeneration. PEMF has been shown to be effective for enhanced osteogenesis in animal experiments with distraction osteogenesis [20, 41], and it was shown to decrease the healing time in limb lengthening procedures in clinical trials [42]. Although the exact action mechanism for the accelerated bone formation is not fully understood, several studies have shown that the PEMF signal stimulates calcium uptake [43] and the calcification of bone-forming tissues in cell models [44, 45]. PEMF reduced osteoblast-like cell proliferation and increased alkaline phosphatase activity, osteocalcin synthesis, and collagen production [46].

LIPUS is a form of mechanical energy that is transmitted through biological tissues as an acoustic wave. Even though the exact action mechanism of LIPUS in bone regeneration is not clear, it is known that LIPUS exerts micromechanical stress on osteogenic cells directly to stimulate their proliferation and osteoblastic activity, and the cavitation and streaming effect of ultrasound can increase the local blood supply [47-49]. It has been hypothesized that LIPUS can increase the formation of new blood vessels [50], the secretion of prostaglandin E2 [49] and growth factors [51]. The effect of ultrasound may also be related to the piezoelectric properties of biological tissue [23]. The electrical potential produced by LIPUS through the piezoelectric effect may increase bone formation and accelerate bone healing [52, 53]. The activation of nitric oxide pathways and increasing the expression of aggrecan messenger RNA have also been proposed as potential mechanisms by which LIPUS stimulates bone healing [11, 54].

LIPUS has been found to have a positive effect on the acceleration of bone healing in many studies. However, few studies have investigated the application of LIPUS for bone healing in the maxillofacial region. Fedotov et al. first reported the use of ultrasound in maxillofacial surgery in their study of mandible fractures in rabbits [55] and showed that LIPUS stimulated mandibular bone healing in this animal model. Harris et al. reported a beneficial effect of LIPUS for the treatment of mandibular osteoradionecrosis [56]. In a study of rabbit mandible fracture by Erdogan et al. [57], LIPUS improved bone healing of the mandibular fracture. In studies with LIPUS application on the distracted mandible of rabbits, LIPUS was shown to have a positive effect on bone regeneration [58-60]. Although many studies have shown the accelerative effects of LIPUS on mandible bone healing, controversial results have been reported concerning its effect on mandible healing of distracted bone and bony defects. Schortinghuis et al. [61] reported that rat mandible with a critical size defect model showed no response to LIPUS treatment. In another study, Schortinghuis et al. [62] concluded that LIPUS has no stimulatory effect on bone healing in rat mandibular defects covered by an e-PTFE membrane. They stated that this finding was due to two reasons. One is that less ultrasound energy was transmitted through the membrane, and the other was that the mandibular bone of rats is not responsive to the ultrasound signal. Schortinghuis et al. [63] also reported that ultrasound treatment did not appear to stimulate bone formation in a severely resorbed vertical distracted human mandible. The authors explained that no effect was found because ultrasound may stimulate the endochondral ossification process in the extremities, but not the intramembranous ossification process present in the mandible.

Motivated by the absence of a comparative study between LIPUS and PEMF

stimulation on bone regeneration in the distracted jaw bone, the present study evaluated new bone formation upon LIPUS and PEMF stimulation in comparison with a control. LIPUS stimulation resulted in significantly increased BV than PEMF stimulation, as well as increased BV and BMD compared to the control. Previous results have been inconsistent in studies of PEMF stimulation for fracture healing and enhanced bone regeneration in DO animal experiments [64-66]. The previous experiments were carried out in different animal models, such as rat radius, rabbit fibula, and sheep, with different stimulation intensities and application durations. In the study of a rabbit tibial lengthening model by Fredericks et al. [20], 1-hour daily exposure to low-frequency, low-amplitude PEMF during the distraction and consolidation period accelerated the biomechanical strengthening of distracted rabbit tibia; PEMF with a pulse width of 30 ms and a pulse frequency of 1.5 Hz was used throughout the experimental period. On the other hand, Taylor et al. reported no positive effect of PEMF on a rabbit tibial distraction model with 1 hour of exposure for 20 days during the consolidation period, even though the same stimulation conditions were applied. They found that PEMF was effective only when applied in the early stage of the consolidation period because there was a significant positive difference in the mineral apposition rate during the interval 1–2 weeks post distraction and this difference was no longer evident by the interval 2–3 weeks post distraction. The authors assumed that this difference resulted from the stimulation timing, namely, application only during the consolidation period in their study, and they suggested that PEMF application in the early phase may be more effective [41]. The present study applied much higher intensity—2 hours per day during the 4-week consolidation period. The inconsistent results in previous studies and the less effective bone formation by PEMF compared to LIPUS stimulation

found in the present study led to choose LIPUS for further evaluating the optimal stimulation conditions.

To produce favorable outcomes with LIPUS therapy, the energy conditions should be optimized. It was reported that ultrasound intensities of less than 100 mW/cm^2 spatial average and temporal average are nonthermal [67]. Duart and Pilla et al. demonstrated that low-intensity ultrasound treatment (30-57 mW/cm^2) showed minimal temperature changes at the bone fracture site of rabbits [10, 68]. Another different rat study reported that the mechanical properties of newly formed bone were better when ultrasound was applied at an intensity of 50 mW/cm^2 compared to 100 mW/cm^2 and a no-treatment control [11]. Lai et al. investigated the healing effect of ultrasound at four different frequencies (0.5, 1, 1.5 and 2 MHz) in rabbit fibular fracture [69]. In the present study, the application of LIPUS stimulation at a frequency of 1.5 MHz and an intensity of 30 mW/cm^2 resulted in significantly increased BV and BMD. This observation was consistent with previous reports. El-Bialy et al. [58] used the same study design as the present study and reported that LIPUS had a positive effect on mandibular DO in rabbits. Shimazaki et al. [70] also reported that ultrasound accelerated bone maturation in a rabbit tibial DO model.

The effective timing for the application of LIPUS during DO has differed in previous studies. LIPUS accelerated bone maturation in DO when applied during the consolidation phase [36, 70], while LIPUS was effective when it was applied during the distraction phase in other studies [59, 71]. In the present study, the same LIPUS energy setting was applied during different time periods. One period started on the 2nd day of distraction, and the other started during consolidation. LIPUS was found to have a positive effect when it was applied during the consolidation period at a frequency of 1.5 MHz and

an intensity of 30 mW/cm^2, while no significant effect was found when applied using other stimulation conditions during distraction and consolidation; these findings were similar to those of Chan et al. [36] Different from our results, Xie et al. [60] reported that higher radiopacity and microhardness and increased bone formation upon histological examination was observed for LIPUS applied at 1.5 MHz and 30 mW/cm^2 immediately after and 2 weeks after finishing distraction of rabbit mandible; moreover, LIPUS treatment during the first 4 weeks after finishing distraction had no more effect on new bone formation than the control. Sakurakichi et al. [72] showed that LIPUS (1.5 MHz and 30 mW/cm^2) increased BMD and mechanical strength most effectively when applied during the distraction phase of rabbit tibia, while new bone formation was more enhanced when LIPUS was applied during the consolidation period.

Clinically, LIPUS is noninvasive, easy to use, and free of side effects. Our results suggest that LIPUS can be used to shorten the consolidation period after DO and to shorten the healing time of fracture and other bone surgeries. Randomized clinical studies are needed to evaluate the effects of LIPUS on human bone.

V. Conclusion

LIPUS accelerated more new bone formation than PEMF. Furthermore, LIPUS stimulation with a frequency of 1.5 MHz and an intensity of 30 mW/cm^2 increased the BMD and BV effectively when it is applied during the consolidation period after mandibular DO.

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Figures and figure legends

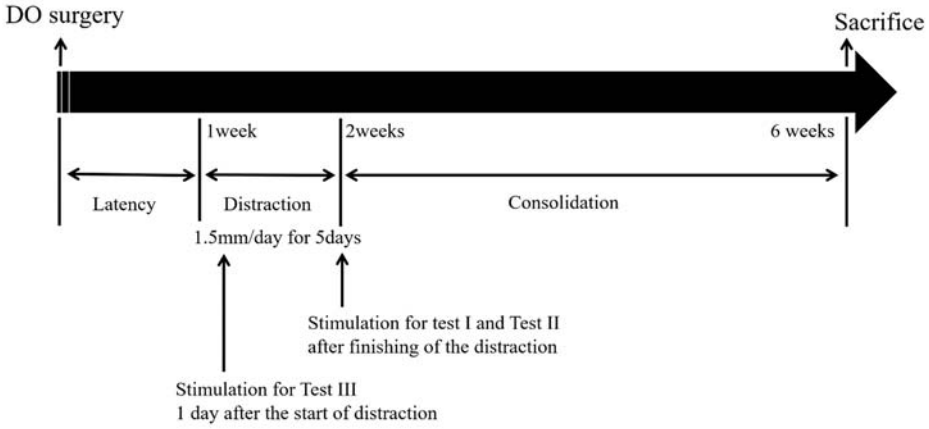

Figure 1. Schematic experimental protocol.

Rabbits underwent placement of a mandibular distraction device in their bilateral mandibles. This was followed by a 7-day latency period, gradual distraction, and consolidation. Rabbits were divided into three test groups. Arrowheads indicate the timing of stimulation start point: Test I and II after finishing of distraction, test III at one day after the start of distraction. All rabbits were sacrificed at week 4 of the consolidation phase and new bone formation was evaluated on both sides.

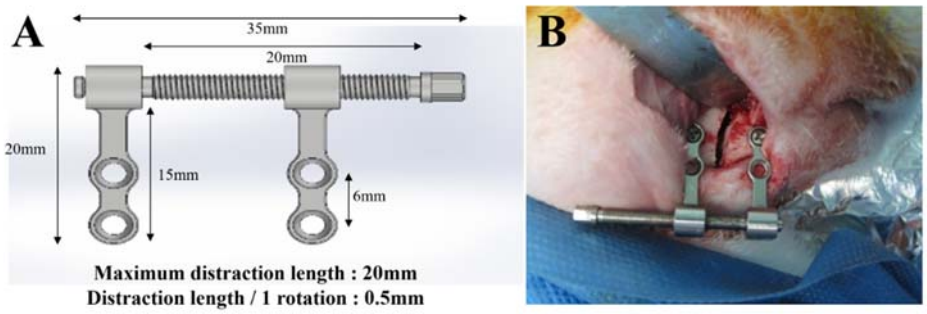

Figure 2. Distraction device and application on the rabbit mandible.

(A) Design of custom-made distraction device. (B) Fixation of distraction device on the osteotomized mandible.

Figure 3. Stimulation devices for low intensity pulsed ultrasound (LIPUS) and pulsed electromagnetic field (PEMF).

(A) Commercial LIPUS device (Exogen 4000+®, Smith & Nephew Inc., Memphis, USA). (B) Handy type of custom-made LIPUS device (Hyemin Co., Changwon, Korea). (C) Custom-made LIPUS device (Hyemin Co., Changwon, Korea). (D) Custom-made PEMF device.

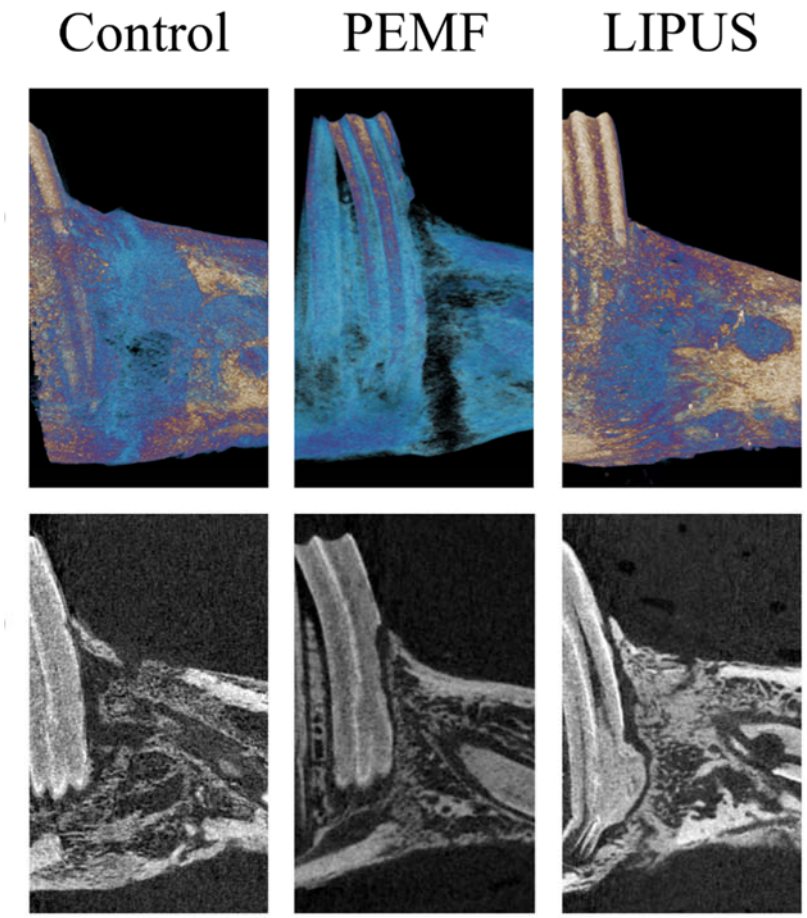

Figure 4. Demonstrative picture of a 3-D reconstruction and artificial coloring and sagittal cut of Micro-CT images of new bone formation 6 weeks after surgery at the distraction site in Test I (PEMF vs. LIPUS).

C, control group without any stimulation; P, stimulation with PEMF device (pulse width: 12 µs, pulse frequency: 60 Hz, magnetic intensity: 10 Gauss (= 10^{-3} tesla)) during consolidation; L, stimulation with LIPUS device with a frequency of 1.5 MHz and an intensity of 30 mW/cm^2 during consolidation.

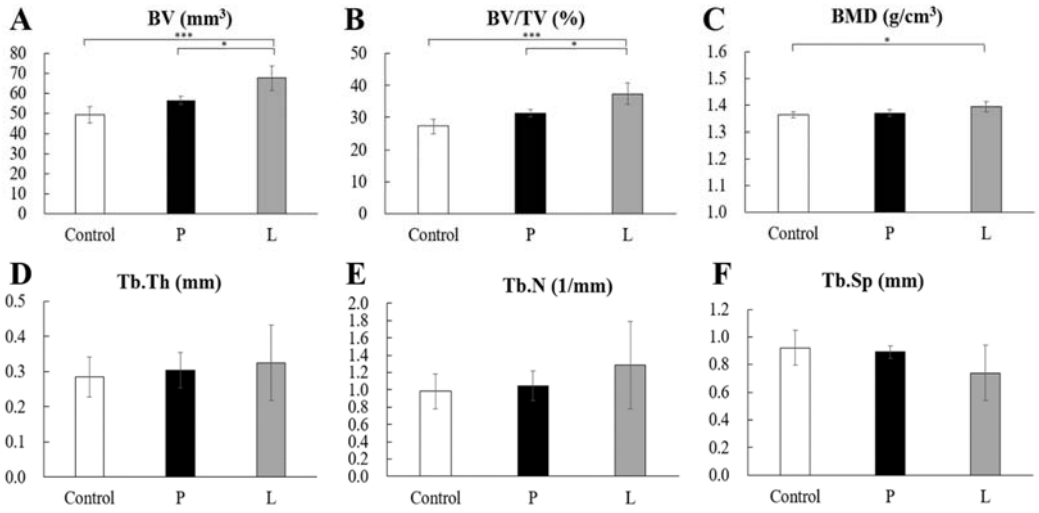

Figure 5. Micro-CT analysis of new bone formation 6 weeks after surgery at the distraction site in Test I (PEMF vs. LIPUS).

(A) bone volume (BV) (mm^3), (B) bone volume/total volume (BV/TV) (%), (C) bone mineral density (BMD) (g/cm^3), (D) trabecular thickness (Tb.Th) (mm), (E) trabecular number (Tb.N) (1/mm) and (F) trabecular separation (Tb.Sp) (mm).

C, control group without any stimulation; P, stimulation with PEMF device (pulse width: 12 μs, pulse frequency: 60 Hz, magnetic intensity: 10 Gauss (= 10^{-3} tesla)) during consolidation; L, stimulation with LIPUS device with a frequency of 1.5 MHz and an intensity of 30 mW/cm^2 during consolidation.

*$p < 0.05$, ***$p < 0.001$ (ANOVA according to the Bonferroni method for the *post hoc* test)

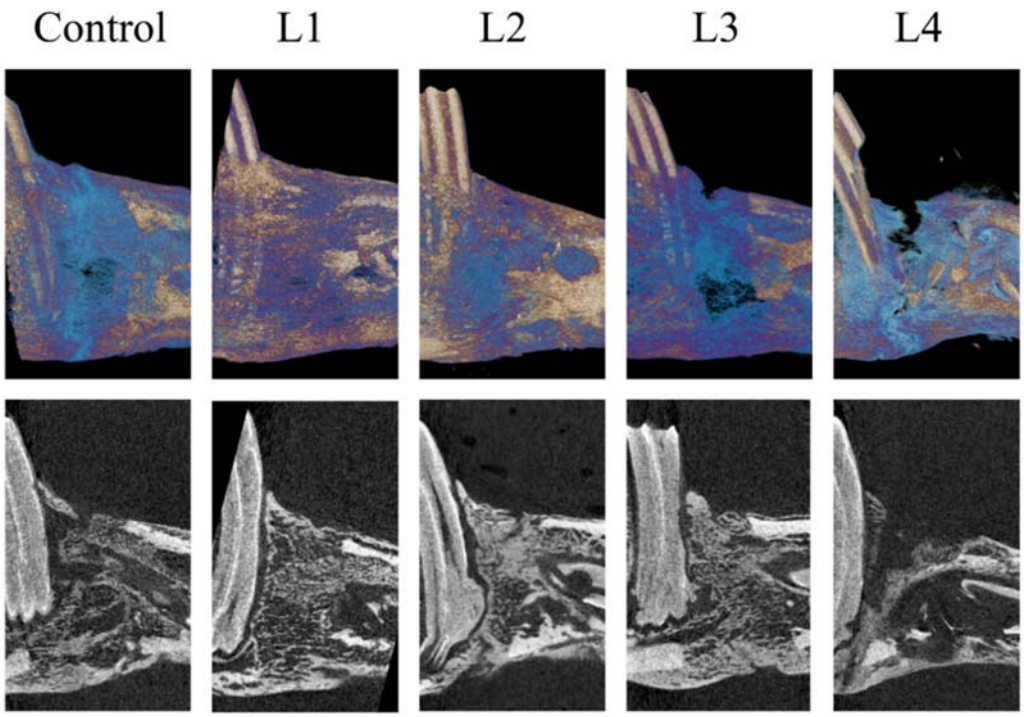

Figure 6. Demonstrative picture of a 3-D reconstruction and artificial coloring and sagittal cut of Micro-CT images of new bone formation 6 weeks after surgery at the distraction site in Test II depending on the different intensity and frequencies of LIPUS.

C, control group without any stimulation; L1, 1.5 MHz, 15 mW/cm^2; L2, 1.5 MHz, 30 mW/cm^2; L3, 1.5 MHz, 60 mW/cm^2; L4, 3 MHz, 30 mW/cm^2.

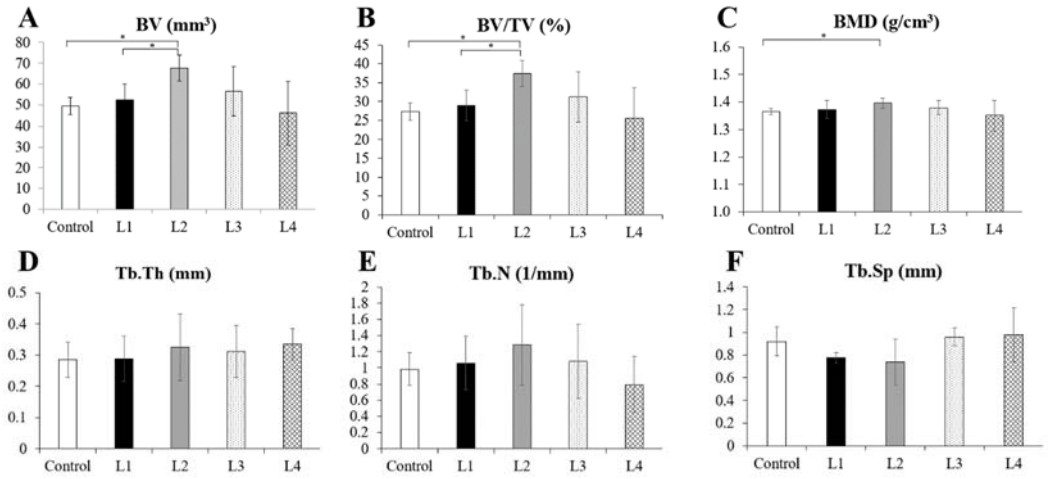

Figure 7. Micro-CT analysis of new bone formation 6 weeks after surgery at the distraction site in Test II depending on the different intensity and frequencies of LIPUS.

(A) bone volume (BV) (mm^3), (B) bone volume/total volume (BV/TV) (%), (C) bone mineral density (BMD) (g/cm^3), (D) trabecular thickness (Tb.Th) (mm), (E) trabecular number (Tb.N) (1/mm) and (F) trabecular separation (Tb.Sp) (mm).

C, control group without any stimulation; L1, 1.5 MHz, 15 mW/cm^2; L2, 1.5 MHz, 30 mW/cm^2; L3, 1.5 MHz, 60 mW/cm^2; L4, 3 MHz, 30 mW/cm^2;

*$p < 0.05$ (ANOVA according to the Bonferroni method for the *post hoc* test)

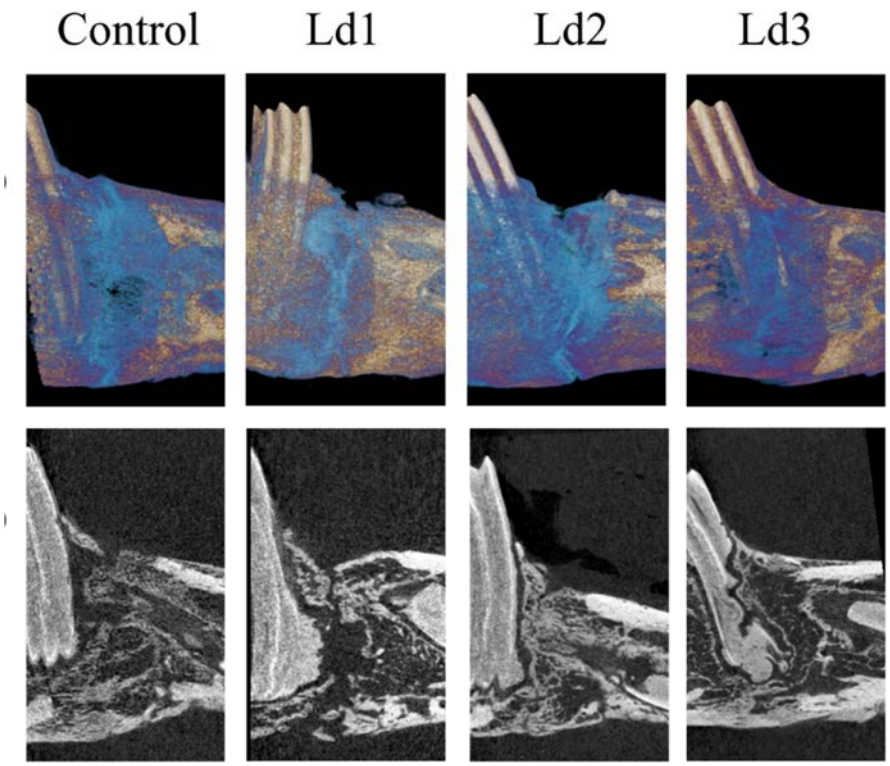

Figure 8. Demonstrative picture of a 3-D reconstruction and artificial coloring and sagittal cut of Micro-CT images of new bone formation 6 weeks after surgery at the distraction site in Test III with two different stimulation timing of LIPUS.

C, control group without any stimulation; Ld1, LIPUS stimulation with 1.5 MHz, 15 mW/cm^2 started 1 day after distraction beginning; Ld2, LIPUS stimulation with 1.5 MHz, 30 mW/cm^2 started 1 day after distraction beginning; Ld3, LIPUS stimulation with 1.5 MHz, 60 mW/cm^2 started 1 day after distraction beginning.

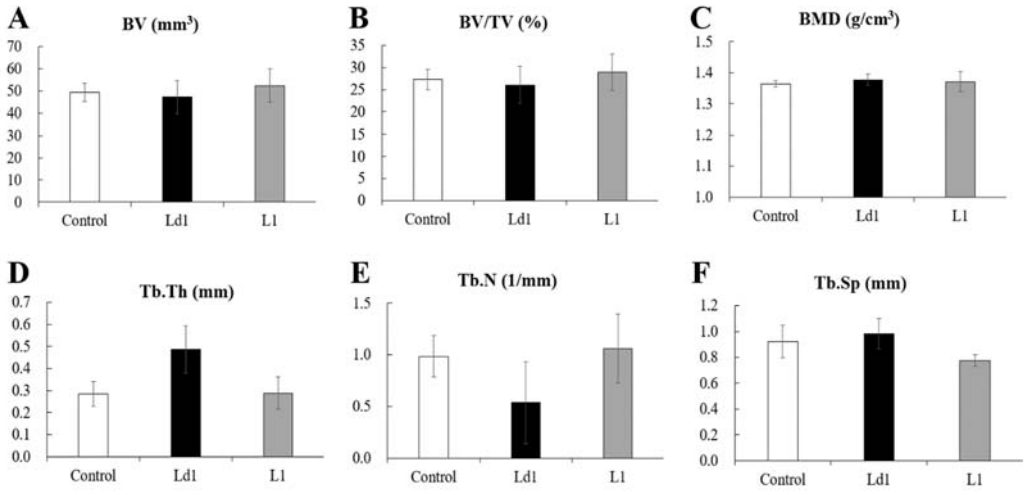

Figure 9. Micro-CT analysis of new bone formation 6 weeks after surgery at the distraction site in Test III with two different stimulation timing of LIPUS (1.5 MHz, 15 mW/cm^2).

(A) bone volume (BV) (mm^3), (B) bone volume/total volume (BV/TV) (%), (C) bone mineral density (BMD) (g/cm^3), (D) trabecular thickness (Tb.Th) (mm), (E) trabecular number (Tb.N) (1/mm) and (F) trabecular separation (Tb.Sp) (mm).

C, control group without any stimulation; Ld1, LIPUS stimulation (1.5 MHz, 15 mW/cm^2) started 1 day after distraction beginning; L1, LIPUS stimulation (1.5 MHz, 15 mW/cm^2) during consolidation.

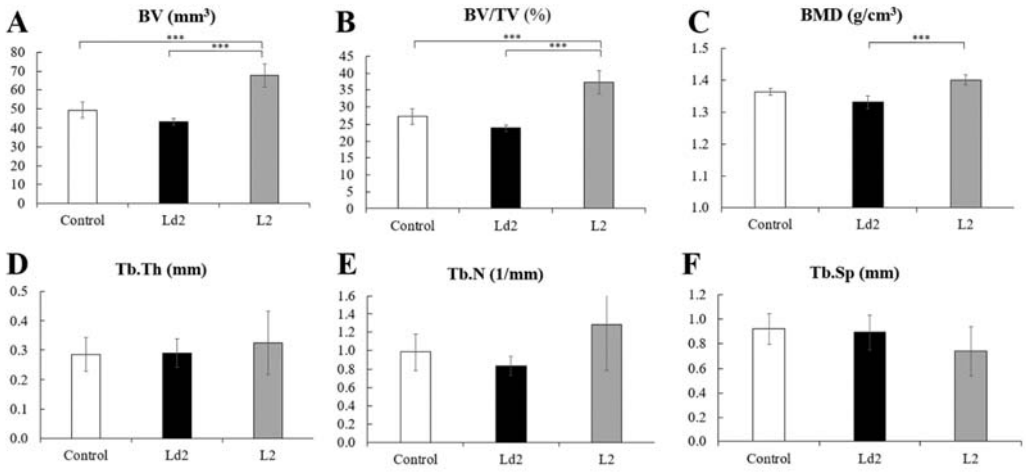

Figure 10. Micro-CT analysis of new bone formation 6 weeks after surgery at the distraction site in Test III with two different stimulation timing of LIPUS (1.5 MHz, 30 mW/cm^2).

(A) bone volume (BV) (mm^3), (B) bone volume/total volume (BV/TV) (%), (C) bone mineral density (BMD) (g/cm^3), (D) trabecular thickness (Tb.Th) (mm), (E) trabecular number (Tb.N) (1/mm) and (F) trabecular separation (Tb.Sp) (mm).

C, control group without any stimulation; Ld2, LIPUS stimulation (1.5 MHz, 30 mW/cm^2) started 1 day after distraction beginning; L2, LIPUS stimulation (1.5 MHz, 30 mW/cm^2) during consolidation.

***$p < 0.001$ (ANOVA according to the Bonferroni method for the *post hoc* test)

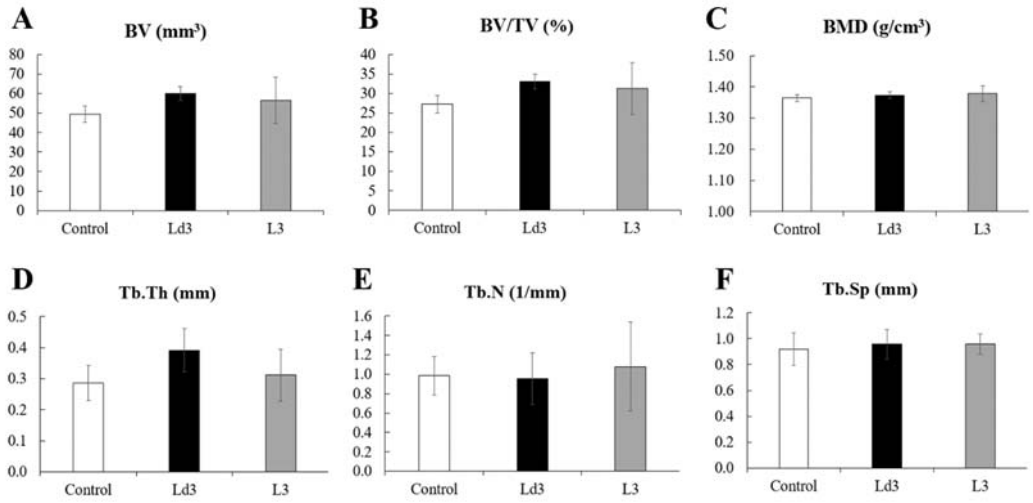

Figure 11. Micro-CT analysis of new bone formation 6 weeks after surgery at the distraction site in Test III with two different stimulation timing of LIPUS (1.5 MHz, 60 mW/cm^2).

(A) bone volume (BV) (mm^3), (B) bone volume/total volume (BV/TV) (%), (C) bone mineral density (BMD) (g/cm^3), (D) trabecular thickness (Tb.Th) (mm), (E) trabecular number (Tb.N) (1/mm) and (F) trabecular separation (Tb.Sp) (mm).

C, control group without any stimulation; Ld3, LIPUS stimulation (1.5 MHz, 60 mW/cm^2) started 1 day after distraction beginning; L3, LIPUS stimulation (1.5 MHz and an intensity of 60 mW/cm^2) during consolidation.

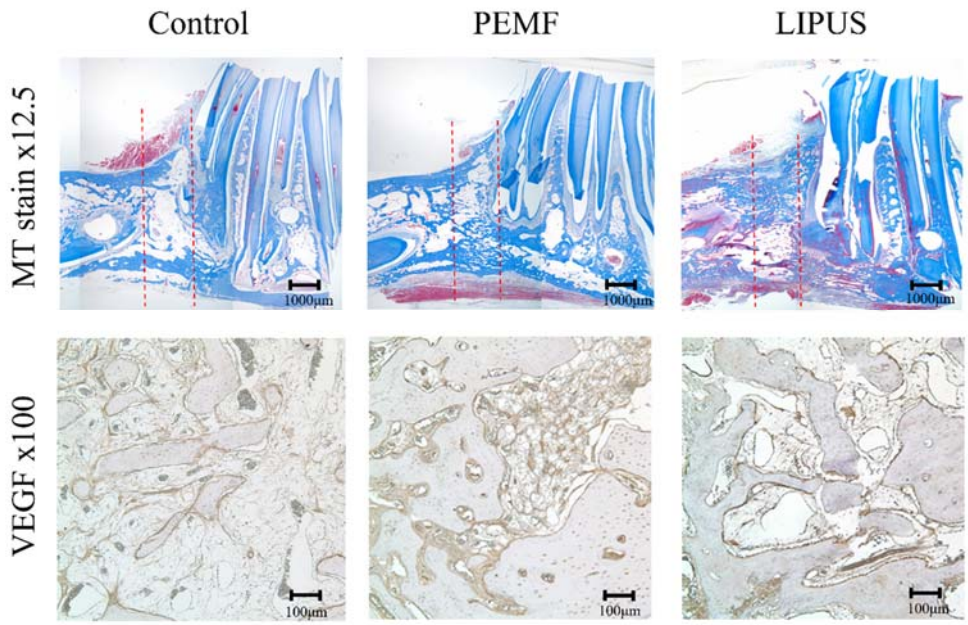

Figure 12. Histological images in the Test I.

(Upper row) Masson's trichrome (original magnification, x12.5) staining. Broken red lines indicate the distracted sites. (Lower row) Immunohistochemical (IHC) staining for vascular endothelial growth factor (VEGF) at the distracted site (original magnification, x100).

C, control group without any stimulation; P, stimulation with PEMF device (pulse width: 12 μs, pulse frequency: 60 Hz, magnetic intensity: 10 Gauss (= 10^{-3} tesla)) during consolidation; L, stimulation with LIPUS device with a frequency of 1.5 MHz and an intensity of 30 mW/cm^2 during consolidation.

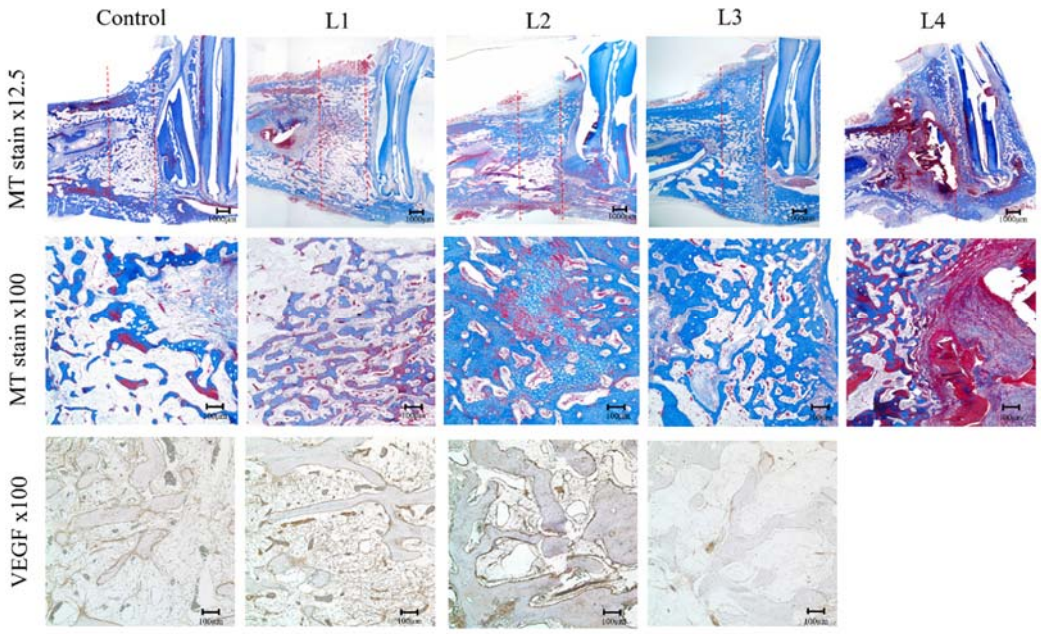

Figure 13. Histological images in the Test II.

(Upper row) Masson's trichrome staining (original magnification, x12.5). Broken red lines indicate the distracted sites. (Middle row) Masson's trichrome staining (original magnification, x100). (Lower row) Immunohistochemical (IHC) staining for vascular endothelial growth factor (VEGF) (original magnification, x100).

C, control group without any stimulation; L1, 1.5 MHz, 15 mW/cm^2; L2, 1.5 MHz, 30 mW/cm^2; L3, 1.5 MHz, 60 mW/cm^2; L4, 3 MHz, 30 mW/cm^2.

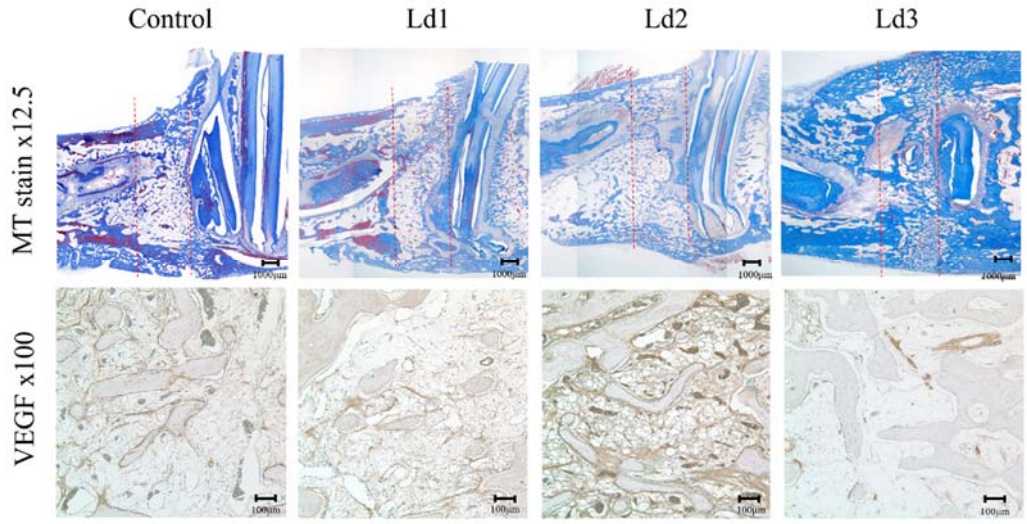

Figure 14. Histological images in the Test III.

(Upper row) Masson's trichrome staining (original magnification, x12.5). Broken red lines indicate the distracted callus region. (Lower row) Immunohistochemical (IHC) staining for vascular endothelial growth factor (VEGF) (original magnification, x100).

C, control group without any stimulation; Ld1, LIPUS stimulation with 1.5 MHz, 15 mW/cm^2 started 1 day after distraction beginning; Ld2, LIPUS stimulation with 1.5 MHz, 30 mW/cm^2 started 1 day after distraction beginning; Ld3, LIPUS stimulation with 1.5 MHz, 60 mW/cm^2 started 1 day after distraction beginning.

Tables

Table 1. Design of experimental groups.

Test I	**Test II**	**Test III**
PEMF vs. LIPUS during consolidation	Different LIPUS intensity during consolidation	1-day after beginning of distraction osteogenesis
Control	LIPUS 1.5 MHz/15 mW	LIPUS 1.5 MHz/15 mW
C	**L1**	**Ld1**
PEMF	LIPUS 1.5 MHz/30 mW	LIPUS 1.5 MHz/30 mW
P	**L2**	**Ld2**
LIPUS	LIPUS 1.5 MHz/60 mW	LIPUS 1.5 MHz/60 mW
L	**L3**	**Ld3**
	LIPUS 3.0 MHz/30 mW	
	L4	

N = 10 in each group (5 rabbits, both sides of mandible).

Table 2. Micro-CT analysis in Test I.

	Control	PEMF	LIPUS
BV (mm^3)	49.360 ± 4.125	56.618 ±2.091	67.714 ± 6.181
BV/TV (%)	27.233 ± 2.276	31.237 ± 1.154	37.359 ± 3.410
Tb.Th (mm)	0.285 ± 0.057	0.305 ± 0.050	0.325 ± 0.108
Tb.N (1/mm)	0.984 ± 0.200	1.048 ± 0.170	1.285 ± 0.501
Tb.Sp (mm)	0.921 ± 0.126	0.891 ± 0.045	0.738 ± 0.200
BMD (g/cm^3)	1.364 ± 0.011	1.370 ± 0.012	1.395 ± 0.019

Data are presented as mean ± standard deviation.

C, control group without any stimulation; PEMF, pulsed electromagnetic fields; LIPUS, low intensity pulsed magnetic fields; BV, bone volume; TV, total volume; Tb.Th, trabecular thickness; Tb.N, trabecular number; Tb.Sp, trabecular separation; BMD, bone mineral density.

Table 3. Micro-CT analysis in Test II.

	Control	L1	L2	L3	L4
BV	49.360 ±	52.376 ±	67.714 ±	56.467 ±	46.122 ±
(mm^3)	4.125	7.528	6.181	11.833	14.977
BV/TV	27.233 ±	28.897 ±	37.359 ±	31.207 ±	25.446 ±
(%)	2.276	4.153	3.410	6.633	8.263
Tb.Th	0.285 ±	0.288 ±	0.325 ±	0.311 ±	0.335 ±
(mm)	0.057	0.072	0.108	0.083	0.051
Tb.N	0.984 ±	1.060 ±	1.285 ±	1.079 ±	0.792 ±
(1/mm)	0.200	0.332	0.501	0.457	0.345
Tb.Sp	0.921 ±	0.776 ±	0.738 ±	0.958 ±	0.981 ±
(mm)	0.126	0.045	0.200	0.078	0.239
BMD	1.364 ±	1.371 ±	1.395 ±	1.378 ±	1.350 ±
(g/cm^3)	0.011	0.033	0.019	0.026	0.055

Data are presented as mean ± standard deviation.

C, control group without any stimulation; L1, 1.5 MHz 15 mW/cm^2; L2, 1.5 MHz 30 mW/cm^2; L3, 1.5 MHz 60 mW/cm^2; L4, 3 MHz 30 mW/cm^2; BV, bone volume; TV, total volume; Tb.Th, trabecular thickness; Tb.N, trabecular number; Tb.Sp, trabecular separation; BMD, bone mineral density.

Table 5. Micro-CT analysis in Test III with 1.5 MHz and 30 mW/cm^2 LIPUS intensity.

	Control	Ld2	L2
BV (mm^3)	49.360 ± 4.125	43.254 ± 1.623	67.714 ± 6.181
BV/TV (%)	27.233 ± 2.276	23.864 ± 0.895	37.359 ± 3.410
Tb.Th (mm)	0.285 ± 0.057	0.290 ± 0.047	0.325 ± 0.108
Tb.N (1/mm)	0.984 ± 0.200	0.835 ± 0.101	1.285 ± 0.501
Tb.Sp (mm)	0.921 ± 0.126	0.893 ± 0.141	0.738 ± 0.200
BMD (g/cm^3)	1.364 ± 0.011	1.330 ± 0.020	1.395 ± 0.019

Data are presented as mean ± standard deviation.

C, control group without any stimulation; Ld2, LIPUS stimulation (1.5 MHz and 30 mW/cm^2) started 1 day after beginning of distraction; L2, LIPUS stimulation (1.5 MHz and 30 mW/cm^2) during consolidation; BV, bone volume; TV, total volume; Tb.Th, trabecular thickness; Tb.N, trabecular number; Tb.Sp, trabecular separation; BMD, bone mineral density.

Table 6. Micro-CT analysis in Test III with 1.5 MHz and 60 mW/cm^2 LIPUS intensity.

	Control	Ld3	L3
BV (mm^3)	49.360 ± 4.125	59.929 ± 3.528	56.467 ± 11.833
BV/TV (%)	27.233 ± 2.276	33.064 ± 1.946	31.207 ± 6.633
Tb.Th (mm)	0.285 ± 0.057	0.392 ± 0.070	0.311 ± 0.083
Tb.N (1/mm)	0.984 ± 0.200	0.955 ± 0.266	1.079 ± 0.457
Tb.Sp (mm)	0.921 ± 0.126	0.957 ± 0.114	0.958 ± 0.078
BMD (g/cm^3)	1.364 ± 0.011	1.373 ± 0.011	1.378 ± 0.026

Data are presented as the mean ± standard deviation of the mean.

C, control group without any stimulation; Ld3, LIPUS stimulation (1.5 MHz and 60 mW/cm^2) started 1 day after beginning of distraction; L3, LIPUS stimulation (1.5 MHz and 60 mW/cm^2) during consolidation; BV, bone volume; TV, total volume; Tb.Th, trabecular thickness; Tb.N, trabecular number; Tb.Sp, trabecular separation; BMD, bone mineral density.

- 국문 초록 -

가토 하악골신장술에서 저강도 초음파가 신생골 형성에 미치는 효과: 전자기장 효과와의 비교연구

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1. 목적

골신장술은 골절단 후 점진적으로 신장시켜 연조직과 경조직의 길이 신장을 동시에 도모하여 골결손을 치료하는 방법이지만, 골신장술 후 기능적 부하를 받을 수 있을 정도의 단단한 뼈가 되기까지 시간이 많이 걸리는 문제점이 있다. 골절이나 골절 후 유합이 지연되는 경우 치유를 촉진하거나 골신장술 후 골이 완전히 성숙되는 시간을 단축하기 위하여 성장호르몬, 사이토카인, BMP등의 주입, 전기적 자극, 전자기장 및 레이저 같은 외부자극 등을 활용하는 방법들이 제시되었다. 최근 하악골 신장술 후 저강도 맥동 초음파(Low intensity pulsed ultrasound: LIPUS)를 이용한 골형성 촉진에 대한 연구가

보고되었지만, 현재 많이 사용되고 있는 맥동 전자기장(Pulsed electromagnetic field: PEMF)에 대한 비교연구가 없으며, 골신장술에서 효과적인 초음파 자극 세기와 자극 시점에 대해 알려진 바가 미미하다. 본 논문은 토끼의 하악골 신장술에서 저강도 맥동 초음파가 신생골 형성에 미치는 효과를 맥동 전자기장 효과와 비교하고, 효과적인 저강도 맥동 초음파 자극 세기와 자극 시점을 평가해 보고자 하였다.

2. 연구방법

토끼의 양쪽 하악골을 절단하여 골 신장장치를 하악골에 고정하고, 일주일 동안의 잠복기를 거친 후 하루에 1.5 mm씩 신장하여 5일 동안 총 7.5 mm을 신장하는 모델을 확립한 후, 총 50마리의 토끼를 대조군과 실험군으로 나누고, 임의로 각 세부 군당 5마리씩 배정하였다. 대조군은 골신장술은 하였지만 자극을 받지 않았다. 실험군은 테스트 I, II, III으로 나누었다. 테스트 I에서는 맥동전자기장(주파폭: 12 μs, 주파수: 60 Hz, 자극세기: 10 Gauss (= 10^{-3} tesla)과 맥동초음파(1.5 MHz, 30 mW/cm^2)의 골형성 비교를 하였고, 테스트 II에서는 서로 다른 자극세기를 가진 4가지 저강도 초음파 자극군(1.5 MHz, 15 mW/cm^2; 1.5 MHz, 30 mW/cm^2; 1.5 MHz, 60 mW/cm^2; 3 MHz, 30 mW/cm^2)의 골형성을 비교 평가하였으며 테스트 III에서는 2가지 자극 시점(골신장 기간, 골경화기간)에서의 골형성 정도를 평가하였다. 전자기장 자극군의 경우, 골신장이 끝난 다음날부터 하루에 2시간씩 5일간 총 4주 동안 자

극을 주었다. 저강도 맥동 초음파 자극군의 경우 자극 세기를 달리하여 골신장이 끝난 다음날부터 하루에 20분씩 5일간 총 4주 동안 자극을 주었다. 4주의 골경화기간이 끝난 이후 모든 토끼를 안락사 시켜 조직을 채취하였다. 조직은 Micro-CT를 이용하여 골신장 부위의 신생골 형성정도를 분석하고, Masson's trichrome 염색, vascular endothelial growth factor 면역 염색을 하여 조직의 반응과 골형성 활성도를 조사하였다.

3. 결과

테스트 I에서는 저강도 맥동 초음파 자극군이 맥동 전자기장군에 비하여 골체적이 유의하게 높았으며 ($p < 0.05$), 대조군과의 비교에서 저강도 맥동 초음파 자극군만 골체적 ($p < 0.001$)과 골밀도 ($p < 0.05$)에서 유의하게 높았다. 테스트 II에서는 1.5 MHz, 30 mW/cm^{2}의 저강도 맥동 초음파 자극군이 대조군에 비해 골체적과 골밀도에서 유의하게 높았으며 ($p < 0.05$), 1.5 MHz, 30 mW/cm^{2}의 저강도 맥동 초음파 자극을 골경화시기에 적용하는 것이 골신장기간에 적용한 경우 ($p < 0.001$)와 대조군에 비해 ($p < 0.001$) 골체적이 유의하게 높았고, 골밀도에서도 골경화기간에 적용하는 것이 골신장기간에 적용하는 것 보다 유의하게 치밀한 골형성을 보였다 ($p < 0.001$).

4. 결론

가토의 하악골 신장모델에서, 저강도 맥동 초음파 자극군이 맥동 전자기장군에 비하여 더 좋은 골형성 촉진을 보였으며, 1.5 MHz, 30 mW/cm^{2}의 저강도 맥동 초음파 자극을 골경화기간에 적용하여 골체적과 골밀도를 효과적으로 향상시킬 수 있었다.

주요어: 골신장술, 저강도 맥동 초음파, 맥동 전자기장, 자극강도, 맥동주파수, 자극 시기, 골형성 촉진

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