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Low intensity pulsed ultrasound  
improves the osseointegration of  
mobile dental implants in dogs

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치 의 학 과

주 동 옥

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잡종견에서 저강도파동형의 초음파가 동요가 있는  
임플란트의 골융합에 끼치는 효과

2010년 2월 25일

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mobile dental implants in dogs

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이 논문을 치의학 박사학위신청 논문으로 제출함.






2009년 10월 일

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# 주동옥의 박사학위논문을 인준함

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2009 년 12월

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# Low intensity pulsed ultrasound improves the osseointegration of mobile dental implants in dogs

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Approved by  
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# ABSTRACT

## Low intensity pulsed ultrasound improves the osseointegration of mobile dental implants in dogs

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Low intensity pulsed ultrasound (LIPUS) has been proven to be an effective treatment for bone fracture healing, and lots of human and animal studies have confirmed its ability to enhance osteogenesis and facilitate bone regeneration.

The aim of this study was to investigate the effects of LIPUS on the osseointegration of dental implants without initial stability in a canine model using histomorphometric (bone to implant contact, BIC) and histologic analysis.

Six, male mongrel dogs (16-20 kg) were included in this study. Right and left mandibular premolars were extracted. Three months after extraction, a total of 36 implants were placed bilaterally into the lower mandible. Holes, 3.5 mm in diameter and 8.5 mm in length, were drilled into the mandibular bone, and implants with a double acid etched surface, 3.25 mm in diameter and 8.5 mm in length, were placed into the holes.

The left side of each dog received LIPUS (BR-SONIC<sup>®</sup>, DENTOVE, Japan) application (15 minutes/day for 7 days)(LIPUS treated group), while the right side as a control (LIPUS untreated group). The output of LIPUS was 3.0 MHz and 240 mW/cm<sup>2</sup>. This experiment was carried on for eight weeks and each two dog was sacrificed at 2-, 4- and 8-weeks after surgery. After sacrificing the dogs, histomorphometric and histologic analyses were performed on the different groups of dogs.

Histologic findings showed limited woven bone formation around the implant border in the 2-week healing period, woven bone formation around the implant border in the 4-week period, and more compact woven bone formation around the implant border in 8-week period in control group. And, 4-week histologic finding in LIPUS group showed similar figures to 8-week in non-LIPUS. 8-week specimen showed similar findings irrespective of LIPUS application. Histomorphometric analysis showed BIC% for all the groups had a tendency to increase as time passed.

The results suggests that LIPUS can improve the osseointegration of mobile dental implants in dogs

# I . Introduction

Dental implants have been widely used in dental clinic for the restoration of lost tooth/teeth from the late of 20th century. But, there are so many challenges to achieve a higher success rate and to shorten the rehabilitation time.

To achieve primary stability of the implant at time of placement is one of the prerequisites for long-term success of dental implants. To enhance bone to implant osteointegration, many methods for improving biomaterial properties have been developed which include optimization of implant material, implant design, surface morphology and osteogenetic coatings. Other methods that have been attempted to enhance endogenous bone healing around biomaterials are different forms of biophysical stimulations such as pulsed electromagnetic fields and low intensity pulsed ultrasounds (LIPUS).<sup>1)</sup>

Ultrasound is an acoustic pressure wave, which is produced by vibrations in a piezoelectric material, that transmits mechanical energy into biological tissues. The ultrasound signal is delivered by a transducer that is coupled to the skin with water-based gel.<sup>2)</sup> The use of LIPUS for the treatment of established nonunions was approved by The Food and Drug Administration in USA, 2000, and has been widely used in medicine as a diagnostic and therapeutic tool.<sup>3)</sup>

There are several theories about the effect of LIPUS on bone, for example, differential absorption of LIPUS, generation of acoustic streaming and cavitation which have been shown to affect diffusion rates and membrane permeability, and temperature increase in tissues and cells.<sup>4)</sup>

LIPUS has been proven to be highly efficacious in facilitating the healing of fresh fracture<sup>5,6)</sup> and treatment of nonunion fractures.<sup>7,8)</sup> In an *in vitro* model, a great number of studies have reported a stimulatory effect of LIPUS on bone forming cells.<sup>9,10)</sup> Sena et al.<sup>11)</sup> reported that LIPUS stimulates a transient increase in the expression of the early response genes, as well as the bone differentiation marker genes in rat osteoblastic cells. Unsworth et al.<sup>12)</sup> reported

that LIPUS enhanced mineralization in preosteoblast cells. Sant'Anna et al.<sup>13)</sup> demonstrated that the expression of some genes such as Cbfa-1/Runx2, IGF receptor, Alk-3, alkaline phosphatase, osteopontin, TGF- $\beta$ 1 BMP-7 was increased when compared to non-LIPUS controls in bone marrow stromal cells. On the other hand, Schortinguis et al.<sup>14)</sup> reported LIPUS didn't appear to stimulate osteoconduction into a bone defect in the rat mandible.

Based upon the previous enhancement of osseointegration by LIPUS and the main underlying mechanisms of osseointegration process around implants are very similar to those occurring during bone fracture repair, author hypothesizes that LIPUS can improve osseointegration of dental implants without complete initial stability, too. This study evaluated the effect of LIPUS on osseointegration of mobile dental implants in a canine model.

## II. Material and methods

This study was approved by the Animal Research Committee of Chosun University(CDMDIRB-0903-A33).

Six, male mongrel dogs (16-20 kg) were included in this study. Right and left mandibular 2nd-, 3rd-, and 4th premolars were extracted. Three months after extraction, a total of 36 implants (3.25 mm width and 8.5 mm length, double acid etched surface) were placed bilaterally into the lower mandible. 3 holes, 3.5 mm in diameter and 8.5 mm in length, were drilled into the mandibular bone, and implants with a double acid etched surface, 3.25 mm in diameter and 8.5 mm in length, were placed into the holes.

The left side of each dog received LIPUS (BR-SONIC<sup>®</sup>, DENTOVE, Japan) application (15 minutes/day for 7 days), while the right side as a control received no treatment. The output of LIPUS was 3.0 MHz and 240 mW/cm<sup>2</sup>. This experiment was carried on for 2-week, 4-week and 8-week, and each two dog was sacrificed at 2-, 4- and 8-weeks after surgery. After sacrificing the dogs, histomorphometric and histologic analysis were performed on the different groups of dogs.

In this study, the ground sections were used for the histologic and histomorphometric analysis. Histologic examination was performed in a microscope (Olympus BX-51, Japan) equipped with an image system (Visus Image Analysis System (Image & Microscope Technology, Korea). All measurements were performed at magnification of x40 and x100.

Bone-implant contact (BIC%) is defined that the linear surface of the implant directly technique contacted by mineralized bone and expressed as a % of the implant surface on each side of the implant.

The implant samples were fixed in 70% alcohol for 6 days, dehydrated through an alcohol series and embedded in glycolmethacrylate resin (Spurr low-viscosity embedding media, Polyscience, Harrington, PA, USA). Polymerized samples were sectioned using a high-precision diamond disc (low-speed

diamond wheel saw 650, SBT, San Clemente, CA, USA) along the long axis in 200- $\mu$ m thicknesses. Then, using a lapping and polishing machine (OMNILAP 2000, SBT, San Clemente, CA, USA), abraded to 30- $\mu$ m thicknesses. One slide was prepared per implant, stained with a Villanueva osteochrome bone stain (San Clemente, CA, USA) and observed under a light microscope (Olympus BX50, Tokyo, Japan). For the histomorphometric evaluation, BIC within the implant screws was calculated using the following formula:

## Statistical analysis

Means  $\pm$  standard deviation of the BIC% were calculated. Analysis of variance (ANOVA) with Scheffe was used to test the difference between healing periods and BIC% among the groups, and BIC% according to experimental methods within each healing period.



Fig. 1. LIPUS device used in this study

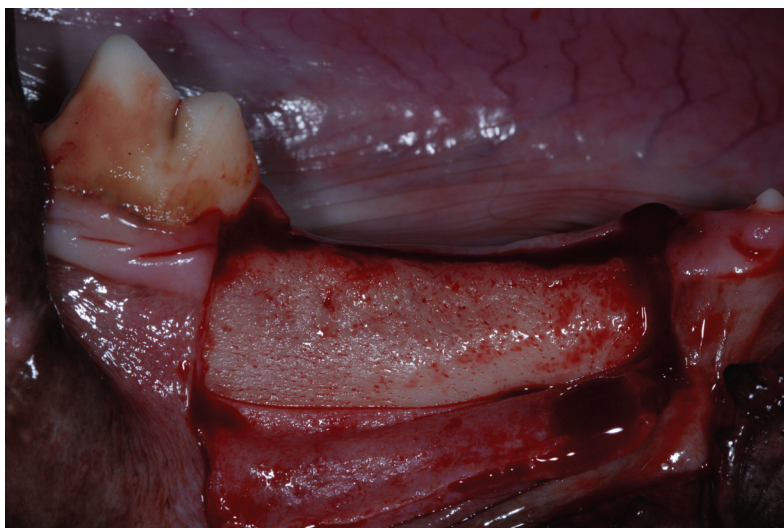


Fig. 2. Clinical photograph of 3 months after teeth extraction. The alveolar ridge was healed normally.



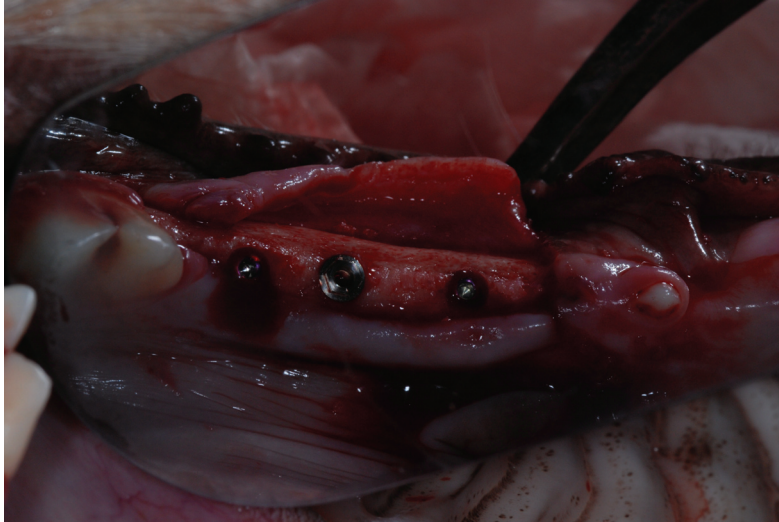


Fig. 3. 3 implants were placed.



Fig. 4. Primary closure was performed.

### III. Results

Good primary stability was achieved at all implants. Healing was uneventful in all the implants. No implant exhibited clinical mobility at 4- and 8-week group, irrespective of LIPUS application.

#### **Histologic analysis**

Histologic findings showed limited woven bone formation around the implant border in the 2-week healing period, woven bone formation around the implant border in the 4-week period, and more compact woven bone formation around the implant border in 8-week period in control group. 8-week specimen showed similar findings irrespective of LIPUS application(Fig. 2 to 10).

#### **Histomorphometric analysis**

As calculated by the 1-way ANOVA test, the BIC% means for the 3 groups were significantly different ( $P<0.05$ ). The mean BIC% value for the control group was  $16.800\pm 7.919$ ,  $34.400\pm 9.854$ , and  $56.075\pm 12.020$ , in 2-week, 4-week and 8-week, respectively. The mean BIC% value for the LIPUS-treated group was  $30.215\pm 2.708$ ,  $45.700\pm 11.869$ , and  $73.175\pm 4.914$ , in 2-week, 4-week and 8-week, respectively. The mean BIC% value for the LIPUS-untreated group was  $37.040\pm 14.764$ ,  $42.270\pm 8.808$ , and  $46.756\pm 5.781$  in 2-week, 4-week and 8-week, respectively. The BIC% values for the control and LIPUS-treated groups showed statistically significant difference between 2-week after healing and 8-week ( $P<0.05$ ), and for the LIPUS-treated group and LIPUS-untreated in 8-week after healing (Table 1). 4-week BIC% value for the LIPUS treated group showed similar value to 8-week BIC% for the LIPUS untreated.

Table 1. Percentage of bone to implant contact (BIC%) (mean  $\pm$  S.D.)

<b>Weeks</b>	<b>Groups</b>	<b>LIPUS-treated</b>	<b>LIPUS-untreated</b>
<b>2 weeks</b>		30.215 $\pm$ 2.708*	37.040 $\pm$ 14.764
<b>4 weeks</b>		45.700 $\pm$ 11.869	42.270 $\pm$ 8.808
<b>8 weeks</b>		73.175 $\pm$ 4.914* $\star$	46.756 $\pm$ 5.781 $\star$

LIPUS = low intensity pulsed ultrasound;  $\S$ \*: statistically significant between 2-week and 8-week ( $P < 0.05$ );  $\star$ : statistically significant between LIPUS treated and LIPUS untreated ( $P < 0.05$ ).

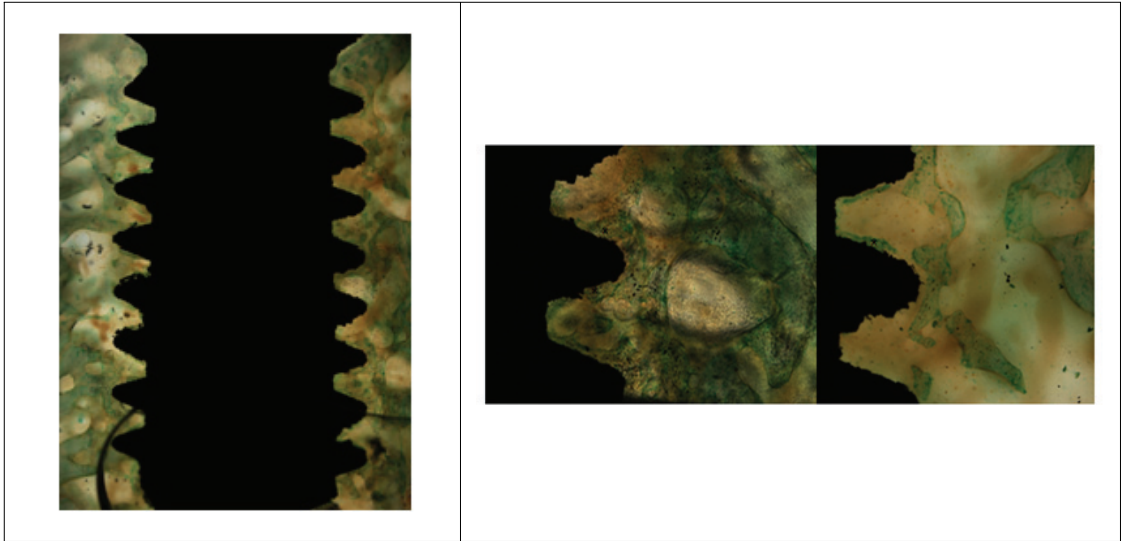


Fig. 5. Histologic findings in LIPUS treated group at 2-week after surgery : limited woven bone formation around the implant border (left x 40, right x 100).

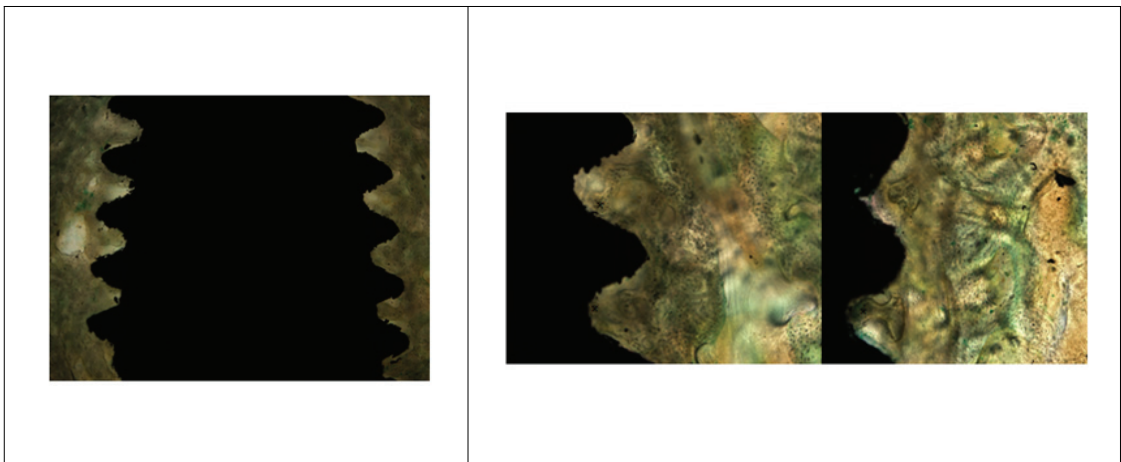


Fig. 6. Histologic findings in LIPUS treated group at 4-week after surgery: more compact woven bone formation around the implant border compared to at 2-week after surgery (left x 40, right x 100).

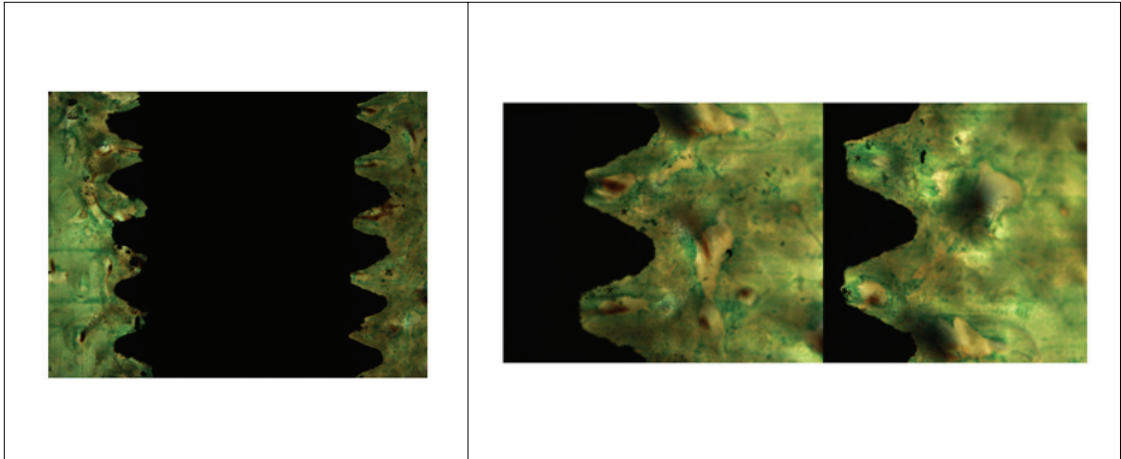


Fig. 7. Histologic findings in LIPUS treated group at 8-week after surgery: more compact woven bone formation around the implant border compared to at 4-week after surgery (left x 40, right x 100).

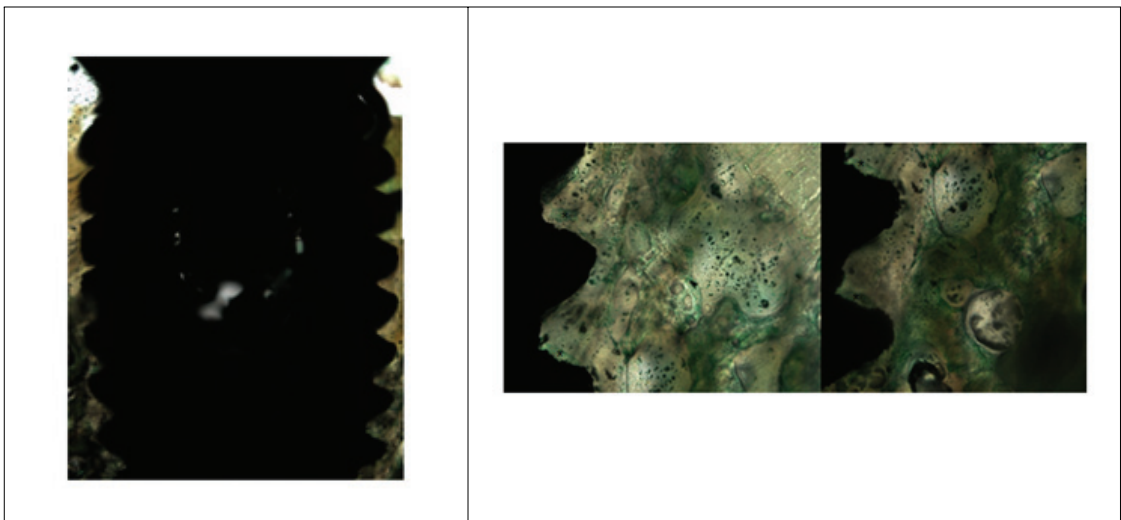


Fig. 8. Histologic findings in LIPUS untreated group at 2-week after surgery : limited woven bone formation around the implant border (left x 40, right x 100).

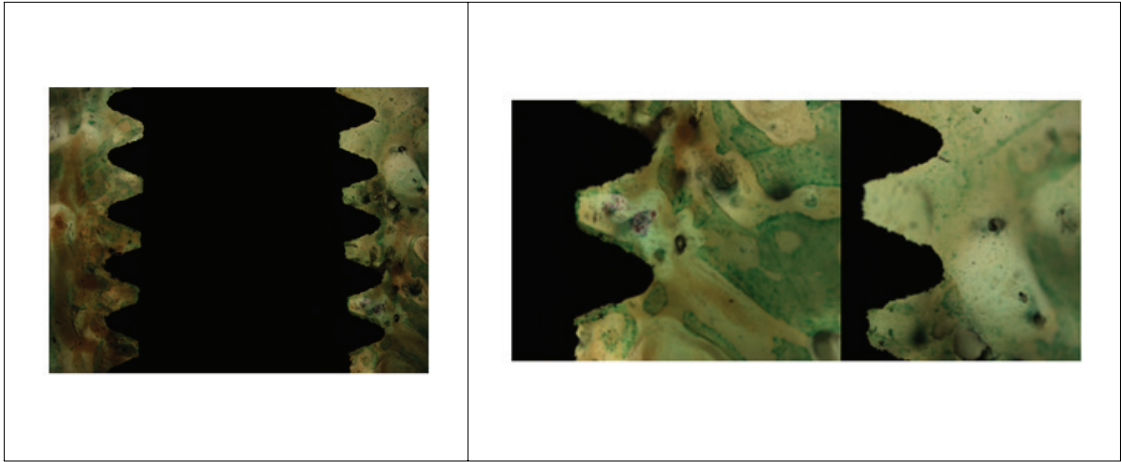


Fig. 9. Histologic findings in LIPUS untreated group at 4-week after surgery: more compact woven bone formation around the implant border compared to at 2-week after surgery (left x 40, right x 100).

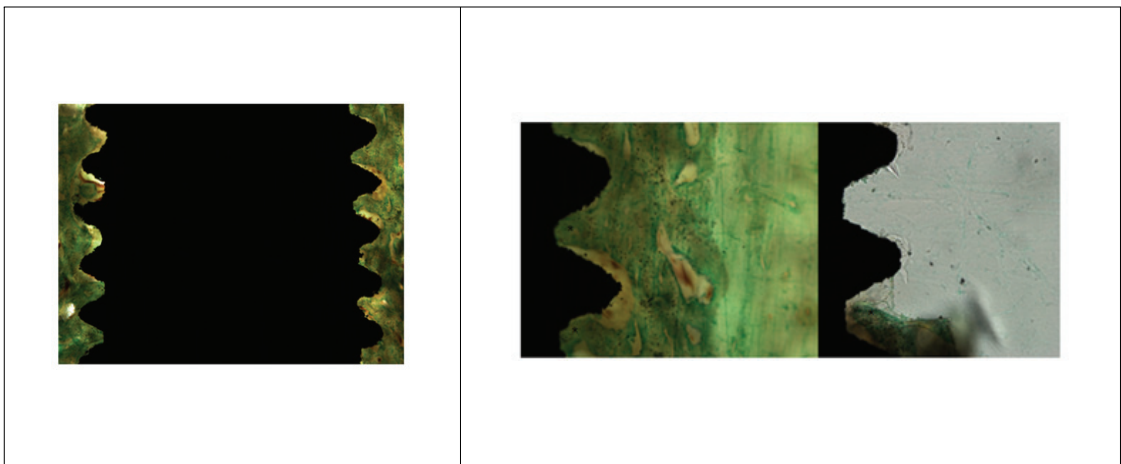


Fig. 10. Histologic findings in LIPUS untreated group at 8-week after surgery: more compact woven bone formation around the implant border compared to at 4-week after surgery (left x 40, right x 100).

## IV. Discussion

LIPUS therapy is a recently developed method for application of mechanical stress, and used clinically to promote bone healing. Author evaluated the effect of LIPUS on osseointegration of dental implants without initial stability in a canine model.

The main underlying mechanism of osseointegration processes around implants are very similar to those occurring during bone fracture repair. In peri-implant healing, blood vessels are damaged and this results in hemorrhage and the formation of a blood.<sup>15)</sup> During the initial phase after implant placement, osteoid tissue and new trabecular bone substitute the blood clot filling the gap between the implant and the alveolar bone gradually.<sup>16)</sup> This woven bone is then replaced into lamellar bone which directly contacts with most of the implant surface, completing the biological process of osseointegration.<sup>17)</sup>

Botticelli et al.<sup>18)</sup> stated that a circumferential gap about 1-1.25 mm wide and 5 mm deep lateral to the implant may heal with new bone. In this study, the defects had high capability of new bone formation because 1 mm less of space lateral to the implant was present. The observation that the gap of 1 mm or less present lateral to the implants formed with new bone is in agreement with findings presented by Berglunch et al<sup>18)</sup>.

Histomorphometric analysis showed the BIC% values from 2 week and onward were consistently higher for this study. BIC% for all the groups had a tendency to increase compared with the non-LIPUS group as time passed, especially, had significantly increased between 2-week after healing and 8-week for the LIPUS treated group.

Albrektsson and Johansson<sup>19)</sup> stated that approximately 50% bone implant contact is necessary to provide success for implant restorations. Based on the data obtained from this study, author might postulate that the mobil, double acid etched surface implant(s) with LIPUS treatment would be sufficiently anchored in the surrounding bone to provide resistance to functional loading

after 4 weeks of healing.

As stated above, lots of studies generally demonstrated the positive effect of LIPUS on bone regeneration, while Schortinghuis et al.<sup>20,21)</sup> have suggested that LIPUS didn't stimulate bone formation. And the LIPUS application affects during the soft callus formation phase and not during the remodeling phase.<sup>22,23)</sup> In this study, the histomorphometric analysis showed BIC% for both LIPUS-treated and -untreated group have a tendency to increase as time passed. This result may be related to an already optimal healing tendency in the maxillofacial region because of good blood supply and perfusion as Schortinghuis et al.<sup>24)</sup> have suggested or to an abnormal application direction of the LIPUS.

As there are few researches on osseointegration of mobile dental implants like this study, author couldn't compare to any other studies. But, base on the histologic findings and histomorphometric analysis, LIPUS can be considered to promotes osseointegration of mobile dental implant.

Although numerous studies have confirmed its ability to enhance osteogenesis and facilitated bone regeneration, the underlying mechanism of the signal transduction (mechanotransduction) pathway involved in cellular responses to ultrasound which is still unclear will be needed as a further research.



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## 잡종견에서 저장도파동형의 초음파가 동요가 있는 임플란트의 골융합에 끼치는 효과

주동욱

지도교수: 김병욱

조선대학교 대학원 치의학과

저장도파동형의 초음파(low intensity pulsed ultrasound, LIPUS)는 골절부위의 치유에 효과적이라고 보고되고 있으며, 사람과 동물을 이용한 많은 연구에서도 골생성이 향상되었고 골재생이 촉진되었음이 입증되고 있다. 따라서 이 연구의 목적은 저장도파동형의 초음파가 잡종견에서 동요가 있는 임플란트의 골융합을 촉진시킬 수 있는지를 조직학적 및 조직형태측정학적으로 평가하는 것이다.

이 연구에는 6마리의 숫컷 잡종견(16-20 Kg)의 하악이 이용되었다. 무치악 부위를 만들기 위해서 잡종견의 하악 제2소구치(p2), 제3소구치(p3) 제4소구치(p4)까지 발거한 다음, 3개월간의 치유기간을 부여하였다. 3개월 후, 하악의 좌, 우측에 각각 3개씩 총 36개의 임플란트를 식립하였는데, 임플란트 직경보다 더 큰 3.5 mm 드릴로 8.5 mm 까지 골삭제를 한 후 직경 3.25 mm, 길이 8.5mm 임플란트를 식립하였다. 좌측은 LIPUS (BR-SONIC<sup>®</sup>, DENTOVE, Japan) 조사군으로, 그리고 우측은 LIPUS 비조사군으로 설정하였다. 초기고정이 완전히 상실된 상태로 골내에서는 덮개나사를 장착할 수 없었으므로 식립전에 커버스크류를 장착한 채로 임플란트의 회전없이 수동적으로 골내로 삽입하였다. 수술 직후부터 항생제(Gentamicin 0.1 ml/kg, Daesung, Korea)를 7일간 근육주사하였고, 1주일에 3회씩 0.2% chlorhexidin digluconate(부광약품, 한국)를 사용하여 구강위생관리를 4주간 시행하였다. 술 후 1주 후에 발사를 시행하였고, 2주, 4주 그리고 8주에 각각 2마리씩 희생시켰다.

LIPUS(BR-SONIC<sup>®</sup>, DENTOVE, Japan)는 제조회사의 지시대로 3.0 MHz, 240 mW/cm<sup>2</sup> 출력으로 수술 1주일 후에 1주일 동안 하루에 15분씩 조사하였다.

조직학적인 소견을 보면, 대조군은 수술 2주후에 임플란트 주위에 교직골이 부분적으로 생성되어 있었으며, 8주후에는 보다 더 치밀한 교직골이 임플란트 주위에 생성되

어 있었다. 반면, LIPUS 조사군의 4주군은 LIPUS 비조사군의 8주 소견과 유사한 양상을 나타냈으며, 8주에는 LIPUS 조사와 무관하게 비슷한 양상을 나타냈다.

조직형태계측학적분석을 보면 시간이 경과됨에 따라 LIPUS 조사와 무관하게 골-임플란트 접촉률이 증가됨을 알 수 있었다.

이 연구를 통해서, LIPUS가 잠종전에서 동요가 있는 임플란트의 골융합을 촉진시킬 수 있음을 알 수 있었다.

# 저작물 이용 허락서

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논 문 제 목	한글: 잡종견에서 저장도파동형의 초음파가 동요가 있는 임플란트의 골융합에 끼치는 효과				
	영문: Low intensity pulsed ultrasound improves the osseointegration of mobile dental implants in dogs				

본인이 저작한 위의 저작물에 대하여 다음과 같은 조건 아래 조선대학교가 저작물을 이용할 수 있도록 허락하고 동의합니다.

- 다 음 -

1. 저작물의 DB구축 및 인터넷을 포함한 정보통신망에의 공개를 위한 저작물의 복제, 기억장치에의 저장, 전송 등을 허락함.
2. 위의 목적을 위하여 필요한 범위 내에서의 편집과 형식상의 변경을 허락함. 다만, 저작물의 내용변경은 금지함.
3. 배포·전송된 저작물의 영리적 목적을 위한 복제, 저장, 전송 등은 금지함.
4. 저작물에 대한 이용기간은 5년으로 하고, 기간종료 3개월 이내에 별도의 의사 표시가 없을 경우에는 저작물의 이용기간을 계속 연장함.
5. 해당 저작물의 저작권을 타인에게 양도하거나 출판을 허락을 하였을 경우에는 1개월 이내에 대학에 이를 통보함.
6. 조선대학교는 저작물 이용의 허락 이후 해당 저작물로 인하여 발생하는 타인에 의한 권리 침해에 대하여 일체의 법적 책임을 지지 않음.
7. 소속 대학의 협정기관에 저작물의 제공 및 인터넷 등 정보통신망을 이용한 저작물의 전송·출력을 허락함.

동의여부 : 동의( ○ ) 반대(     )

2010년 2월

저작자: 주 동 옥 (인)

**조선대학교 총장 귀하**