Low-intensity Pulsed Ultrasound Stimulation Significantly Enhances the Promotion of Bone Formation Around Dental Implants

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Abstract: The effect of low-intensity pulsed ultrasound stimulation (LIPUS) on bone formation around dental implants was studied histologically and mechanically after a dental implant was inserted into the femur of male Japanese white rabbits. LIPUS daily treatment started on the day after implant placement for 14 days. Histologically, new bone formation was observed around the implant. Implant removal torque values were significantly higher in LIPUS-treated group (44.0 – 58.8 N·cm) than the control group (32.0N·cm). Moreover, bone contact ratio was significantly higher in LIPUS-treated group than the control group. The results suggest that clinical application of LIPUS for dental implants may promote osseointegration.

Key words: Bone formation, Low-intensity pulsed ultrasound stimulation, Dental implant

Introduction

Oral implant is one of the means of restoring aesthetics and function of missing teeth which occupies an important part in prostheses treatment. However, after placement of a dental implant, a long period of time is required before it can endure the load of dental occlusion thereby posing a large burden on patients. Recently, a revolution in dental implant includes more consideration of the patient’s quality of life (QOL). With the development of new techniques, the no-wait bearing period after implant placement has been shortened to allow ‘immediate load’ or ‘early stage load’. Our research group previously reported that application of pulsed electromagnetic field (PEMF) or capacitively coupled electric field (CCEF) promoted bone formation around dental implants in animal models, which would allow early load or immediate load placement. However, it is thought that shortening the stimulation time is necessary for clinical use. Application of PEMF or CCEF would require stimulation for 4-8 hours and would cause physical and mental fatigue in patients.

In 1983, Duarte and Xavier reported that low-intensity pulsed ultrasound stimulation (LIPUS) promoted healing of bone fracture. Since then, there have been several reports on LIPUS. It was reported that LIPUS treatment for 20 minutes a day enhanced bone adhesion and shortened the recovery time of fresh fracture and is being established as a treatment method in the field of orthopedics.

However, the application of LIPUS in oral implant treatment has not been reported yet. In the present study, dental implants were placed in femoral bone of rabbits and the effect of LIPUS on bone formation around the implants was examined.

Materials and Methods

Experimental animals

A total of 36 adult male Japanese white rabbits weighing approximately 2.5 kg (Hokudo Co, Sapporo, Japan) were used in this study (n=6). The rabbits were kept under a 24-hour light/dark cycle and had free access to drinking water at the Animal Experiment Center of Health Sciences University of Hokkaido. This study was approved by the Animal Ethics and Research Committee of Health Sciences University of Hokkaido. All animal experiments complied with the Guidelines for the Care and Use of Laboratory Animals of the University.

Ultrasound treatment

US-700 (Ito Co., Ltd. Tokyo, Japan) and OSTEOSONIC (Ito Co., Ltd. Tokyo, Japan) LIPUS treatment equipment were used in this experiment. Probe L with a surface area of 6 cm² (Ito Co.,
LIPUS treatment was started on the day after implant placement surgery on the skin using a coupling gel (ITO ULTRASOUND GEL, Ito Co., Ltd. Tokyo, Japan). LIPUS was applied under 6 different conditions. The frequency of the ultrasound was 1 MHz or 3 MHz with an intensity of 40 mW/cm² or 100 mW/cm². The pulse duration was fixed at 1.0 msec and pulse cycle was fixed at 10.0 msec (Table 1).

Dental implant placement surgery

Seventy-two PLATON TYPE I dental implant (PLATON JAPAN Co., Ltd. Tokyo, Japan) (3.3 mm diameter, 10.0 mm length) were used in the experiment. The surface of the PLATON TYPE I implant was sand-blasted, acid-etched and treated with glow discharge on titanium alloy. Dental implant placement was performed under general anesthesia by intravenous injection of pentobarbital sodium 16.2 mg/kg (Somnopentyl, Kyoritsu seiyaku Co., Tokyo, Japan) into the ear vein. After preparing the surgical field, local anesthesia was done using lidocaine hydrochloric acid containing 2% epinephrine (Xylestesin A injection solution, ESPE, Germany). A full thickness flap to include the skin, fascia and periosteum was done to expose the femur. A hole was drilled to penetrate the osteo-epiphysis marrow of the femur using low-speed drill (800 rpm, IMPLANTOR II®, Kyocera, Kyoto, Japan) with physiological saline solution used as a coolant. The implant was then placed in the hole followed by suturing of the periosteum and skin. Implant placement was performed according to the method of PLATON implant system. Ampicillin sodium 30 mg/kg (Viccillin® Meiji Seika Kaisha, Ltd. Tokyo, Japan) was administered to the femur by intramuscular injection for two days starting on the day of the operation. The experimental period lasted for two weeks. ^5^ LIPUS was applied in 6 different conditions on the right and left sides.

Resonance frequency analysis (RFA)

Implant stability quotient (ISQ) was measured with an RFA device (Ostell®, transducer type F4 L5, Integration Diagnostics AB, Sweden). ISQ was measured immediately after implant placement and 2 weeks later.

Measurement of implant removal torque value (RTV)

RTV was measured to evaluate the affinity of the implant for bone or fixation force. RTV was measured with a torque wrench (Torque gauge 2400, ATG-N®, 15 BTG-N® Tohnichi Mfg., Tokyo, Japan).

Sample removal and specimen preparation

After RTV measurement of the right femur, the rabbit was sacrificed by overdose of pentobarbital sodium. The surrounding bone including the implant in the left femur was also removed. The removed tissues were embedded in polyester resin (Rigolac® Okenshoji Co., Ltd, Tokyo Japan). Sections were thinly sliced by a cutting machine (BS3000®, Exakt, Germany) in an axial direction in relation to the implant. Sections were again treated with machine grinding (MG4000®, Exakt, Germany), polished to a thickness of 50µm and stained with basic fuchsin or methylene blue. Sections

Table 1. Conditions of LIPUS irradiation. Six conditions were used in this study. Vibration frequency, ultrasound wave output and exposure time were changed while pulse width and pulse cycle were the constant conditions.

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<th>Vibeational frequency (HZ)</th>
<th>Ultrasound wave output (mW/cm²)</th>
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were observed under a photon microscope (BX-50© Olympus Tokyo) at a magnification of 8x and 25x.

**Observation by contact microradiography (CMR) and measurement of bone implant contact rate (BIC)**

Pictures of samples with a thickness of 120µm were taken using soft x-ray generation device (Sofron Model BSTI 1505CX© Souken Laboratory, Tokyo, Japan) and ultrafine particle film for soft x-rays (MIN-R 2000© Kodak, Tokyo, Japan). The bone implant contact ratio (BIC) was measured. Computer analysis with NIH Image®1.61 (National Institutes of Health, Bethesda, MD, U.S.A) was done with image analysis software. BIC was calculated as the ratio of the contact length of the implant and the newly-formed bone adjacent to the implant as peripheral length (contact length/implant peripheral length of implant and newly-formed bone) x 100%.

**Statistical analysis**

Significant differences in RTV and BIC were assessed with Games-Howel multiple comparison procedure using Stat View software (StatView® ver5.0, SAS®©, USA). Differences between group means were considered to be statistically significant at a level of \( p < 0.05 \).

**Results**

**Laboratory animals**

No anomalous variation in the laboratory animals was observed for the entire 2 weeks of the experiment period. No abnormal findings in the rabbits that were subjected to LIPUS irradiation were observed.

**Change in ISQ value**

In the control group, ISQ value was lower 2 weeks later than at the beginning of the experiment. The LIPUS irradiation group had higher ISQ value 2 weeks after (Fig. 2).

**Measurement of RTV**

In the irradiation group, the mean RTV was 58.8 N·cm in the group subjected to 3 MHz and 40 mW for 20 min, 53.0 N·cm for those subjected to 3 MHz and 40 mW for 10 min, 52.0N·cm for those subjected to 1 MHz and 40 mW for 20 min and 47.0 N·cm for those subjected to 3 MHz and 100 mW for 10 min (Fig.3). In the control group, RTV was 32.0 N·cm. Fig. 3 shows a no significant difference between horizontal lines. There were significant differences in RTV among the 5 LIPUS groups and control group although a significant difference was not observed between the irradiation group and the control group subjected to 1 MHz and 40 mW for 10 min.

**Measurement of BIC**

Fig. 4 shows a graph of BIC 2 weeks after implant placement. The mean values of the irradiation groups were as follows: 59.1% for those subjected to 3 MHz and 40 mW for 20 min, 46.4% for those subjected to 3 MHz and 40 mW for 10 min, 46.42% for those subjected to 1 MHz and 40 mW for 10 min, 40.1% for those subjected to 1 MHz and 40 mW for 20 min.
Figure 5. CMR image and histological findings. A representative section of a femur with implant of the indicated group (1)–(6) is shown. The sections were stained with basic fuchsin-methylene blue.

(1) Control group
(2) 1 MHz-40 mW/cm²-10 min irradiation group
(3) 1 MHz-40 mW/cm²-20 min irradiation group
(4) 3 MHz-100 mW/cm²-10 min irradiation group
(5) 3 MHz-40 mW/cm²-10 min irradiation group
(6) 3 MHz-40 mW/cm²-20 min irradiation group
(a) CMR images (original magnification x8, bar=1.0 mm)
(b) Uncalcified grinding section (original magnification x8, bar=1.0 mm)
(c) Uncalcified grinding section (original magnification x25, bar=0.3 mm)

(2) – (6) In LIPUS irradiation groups, the more purplish red color newly formed bone-like tissue around the implants can be observed than (1) control group.
LIPUS applied to bone fractures used vibration frequency of 1.5 MHz, ultrasound wave output of 30 mW/cm², pulse width of 0.2 msec and pulse cycle of 1.0 msec. This is because the output of the machine used is fixed. In the present study, the vibrational frequency, the output and the stimulation time were modified. Although a significant difference was not observed, the newly formed bone-like tissue had a more complex histological features after the 20-min stimulation than after 10-min stimulation. Moreover, the newly formed bone-like tissue had more complex histological features when subjected to vibrational frequency of 3 MHz than in those subjected to 1 MHz. The effect of LIPUS in promoting bone formation was most evident under the conditions with vibration frequency of 3 MHz for 20 min. However, additional studies are still needed.

Ultrasound wave decreased during connective tissue formation. One of the factors in which ultrasound wave takes part in decreasing ultrasound intensity is the content of collagen in tissues. Ultrasound intensity decreases in blood and tissues and the absorption of ultrasounds induces the growth of tendon and the cartilage. Furthermore, it is directly proportional to vibrational frequency. The attainment depth at 3 MHz is about 1/3 of 1 MHz as it reaches 3 times depth compared with a vibrational frequency of 1 MHz. The vibrational frequency is indirectly proportional to vibration frequency at 3 MHz. It reaches 3 times depth compared to 1 MHz. The rise in temperature of the limited part is misleading because energy is converted into thermal energy on the surface. It is thought that there is no tissue damage caused by the rise in temperature because the output is minimal. It is thought that there is no harmful effect because of the vibration and heat.

The point where RFA is uniting excellently with the implant can be measured in a continuous and non-destructive manner. RFA was performed before measuring the implant rotation torque value which is a destruction measurement method. ISQ decreased on day 30 after implant placement but it increased after 60 days and 90 days later. ISQ index acquired osseointegration more than ISQ 55 in the placement of implants in human mandibular bone. Because all LIPUS irradiation groups showed ISQ 55 or more, it is thought that osseointegration was promoted at the early stage.

There are many reports that LIPUS does not influence cell proliferation. In in vitro experiments, it was reported that irradiating LIPUS increases the levels of aggrecan mRNA and PGE₂ by induction of Cox-2 in cartilage cells. Moreover, LIPUS irradiation induces the expression of IGF-1 and BMP-2 in cartilage cells at an early stage. An increased in Cbfa-1 mRNA was not observed although an increased in Cox-2 and OPN m-RNA in the rat marrow stem cell after LIPUS irradiation. Changes in irradiated C3T3-E1 cells were noted after LIPUS irradiation from 40mW/cm² to 120 mW/cm². Although the groups subjected to 40 mW/cm²'s and 120 mW/cm² indicated a high value than the control.

**Discussion**

Ultrasound stimulation is used in physiotherapy. Raising the temperature of deep tissue is expected to promote metabolic rate, pain relief and hyperthermia. The intensity of ultrasound used for the treatment of fracture is 30~40 mW/cm², which is considerably low compared to the intensity of 1,000~3,000 mW/cm² used in physiotherapy. Moreover, the increased in temperature of the deep tissue is assumed not to be caused by pulse wave. On the other hand, it is thought that the minute vibration by an ultrasound wave gives some signals to cells during bone formation. Although the mechanism is not clear, a study showed that recovery from mechanical stiffness in osteotomy occurred soon after LIPUS irradiation. LIPUS irradiation in rat after femoral fracture increased dynamic strength. Azuma et al reported that LIPUS irradiation of rat femoral fracture increased the bone mineral content at the fracture site and increased maximum twist strength at the early stage.

Heckman performed a multi-random-facilities double-blind, placebo-controlled clinical trial on the fresh fibula fracture and reported that LIPUS irradiation promoted recovery. LIPUS irradiation shortened the bone healing period in a multi-random facilities, double-blind test placebo-controlled clinical trial. Moreover, when experimenting on LIPUS intended for plastic surgery implant, LIPUS irradiation promoted bone formation in the thigh bone after placement of porous-coated titanium implants.

**CMR images and histologic observation**

Figs. 5 (1) – (6) show the results of CMR image and staining with basic fuchsin and methylene blue. Existing bone is pink in color with basic fuchsin and heavy methylene blue dyes and the bone-like tissue is purplish red in color (fig. 5). In the LIPUS irradiation group, the existing bone was stained in pink and the bone-like tissue was stained in purplish red, which were observed in the surroundings of the implant. The newly-formed immature bone which stained purplish red likewise was seen in x-ray image as an opaque structure in CMR image. The x-ray image of the opaque structure can be observed more in the implant surroundings in LIPUS irradiation group. The opacity of the irradiated group with 3 MHz for 20 min was higher than the control group and a space in between the implant and the existing bone was observed. A few newly-formed bone which is purplish red in color was also observed.

**The condition of LIPUS**

subjected to 1 MHz and 40 mW for 10 min. 45.1% for those subjected to 3 MHz and 100 mW for 10 min. Fig. 4 shows no significant difference between horizontal lines. The BIC in LIPUS groups were significantly higher than the control group (mean, 35.0%).
group in the amount of calcium, a positive correlation was not obtained after statistical analysis. The group subjected to 40 mW/cm² showed a higher amount of calcium than those subjected to 120 mW/cm².  

Mechanical stimulation with LIPOS promoted calcification at an early stage. Due to increasing permeability of cell membrane, an increased in the factor which it takes part in the bone formation was detected. Further research might still be necessary because there are lots of uncertain points. One point is the change in the appearance depending on the stimulus parameter of LIPOS and the cell strain. Therefore, we consider that LIPOS promotes calcification because it has some influences on the effect of cell differentiation but not on cell proliferation.

References

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