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Low-intensity pulsed ultrasound enhances bone formation around miniscrew implants

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ABSTRACT

Miniscrew implants (MSIs) are currently used to provide absolute anchorage in orthodontics; however, their initial stability is an issue of concern. Application of low-intensity pulsed ultrasound (LIPUS) can promote bone healing. Therefore, LIPUS application may stimulate bone formation around MSIs and enhance their initial stability.

Aim: To investigate the effect of LIPUS exposure on bone formation after implantation of titanium (Ti) and stainless steel (SS) MSIs.

Methods: MSIs made of Ti-6Al-4V and 316L SS were placed on rat tibiae and treated with LIPUS. The bone morphology around MSIs was evaluated by scanning electron microscopy and three-dimensional micro-computed tomography. MC3T3-E1 cells cultured on Ti and SS discs were treated with LIPUS, and the temporary expression of alkaline phosphatase (ALP) was examined.

Results: Bone-implant contact increased gradually from day 3 to day 14 after MSI insertion. LIPUS application increased the cortical bone density, cortical bone thickness, and cortical bone rate after implantation of Ti and SS MSIs ($P < 0.05$). LIPUS exposure induced ALP upregulation in MC3T3-E1 cells at day 3 ($P < 0.05$).

Conclusion: LIPUS enhanced bone formation around Ti and SS MSIs, enhancing the initial stability of MSIs.

1. Introduction

Anchorage control is a key component in clinical orthodontic success. Numerous anchorage devices have been proposed and used for more than a century. However, most of these devices have disadvantages, in that their effectiveness depends on patient compliance and they cannot provide absolute anchorage. The concept of skeletal anchorage was initially introduced to the orthodontic field in the 1980s, reaching worldwide acceptance by the year 2000.[1–5] In the skeletal anchorage approach, screws or miniplates are fixed

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directly onto the bone and provide absolute anchorage for several kinds of tooth movements.

Miniscrew implants (MSIs) made of Ti-6Al-4V alloy offer biocompatibility, improved comfort, relative noninvasiveness, and fewer limitations in placement compared to other skeletal anchorage devices.[6,7] For these reasons, MSI use is generally accepted by orthodontists and patients. However, the clinical use of MSIs has been associated with some risks and complications, particularly screw failure.[8] A recent systematic review found an overall success rate of 86.5% among 4987 MSIs placed in 2281 patients. This rate is significantly lower than the success rate of dental implants for prosthetic restorations.[9] Therefore, increasing the success rate of MSIs in clinical orthodontics is an urgent issue.

Low-intensity pulsed ultrasound (LIPUS) is a form of physical energy that can be delivered to living tissue as acoustic waves. Used extensively as a therapeutic, operative, and diagnostic tool in medicine, LIPUS does not have any known deleterious, carcinogenic, or thermal effects on living tissues. LIPUS is well accepted as a noninvasive and safe tool for the treatment of bone fractures.[10] In previous reports, LIPUS increased the rate of repair of bone fractures at all stages of the healing process[11–14] and increased the mechanical properties of callus.[15,16] Radical changes in density are inherent in a healing tissue, which may lead to gradients in physical strain.[17] Ultrasound can be generated through several possible mechanisms. Microbubble compression and acoustic streaming can have direct effects on cell membrane permeability.[18] Physical force serves as an extracellular signal to various cell types, including bone cells. For example, BMP-2-induced bone formation[19] and cellular mineralization[20] were enhanced after various types of biophysical stimulation of bone cells. Additionally, ultrasound has been shown to enhance protein synthesis.[21,22]

Most MSI failures occur within a week after implant placement. This fact implies that early bone metabolism around the inserted screws might be related to the screw stability. If LIPUS can stimulate bone formation around the MSI, then LIPUS application after MSI implantation may be able to enhance the initial implant stability. Therefore, the aim of this study was to evaluate the effect of LIPUS application on bone formation after placement of MSIs made of Ti-6Al-4V alloy or stainless steel (SS).

2. Materials and methods

2.1. Animals

Forty 6-week-old Sprague-Dawley rats (body weight: 190.0–210.5 g) were used in this study. All animals were treated in accordance with the Guidelines for Animal Experiments at the Laboratory Animal Centre of Tokushima University. Animals were caged individually under automatically controlled conditions, with a temperature of 23 °C, humidity of 50%, and a 12 h:12 h light: dark cycle. Animals were given free access to tap water and rodent chow. All of the protocols of the study were approved by the Ethics Committee of Tokushima University.

2.2. Surgical procedure

Each animal was anesthetized with an intra-abdominal injection of 50 mg/kg sodium pentobarbital (Kyoritsu, Tokyo, Japan). The skin was cleaned and incised with a scalpel blade. The tibia surface was exposed, and the implant site was prepared by a standard surgical technique with sharp drills. All drilling procedures were done under profuse irrigation with sterile saline. For each animal, four MSIs were inserted with a miniature jewellery screwdriver. MSIs measured 1.0 mm in inner diameter, 1.5 mm in outer diameter, and 1.6 mm in length. MSIs were made of Ti-6Al-4V or 316L SS (Nishimura Metal, Sabae, Japan).

2.3. In vivo LIPUS application

The LIPUS exposure system used in this study was modified from a clinical device (Osteotron-D IV; Ito Co, Tokyo, Japan) and was used in both the in vitro and in vivo experiments. Pulsed ultrasound signal was transmitted at a frequency of 1.5 MHz with a spatially averaged intensity of 30 mW/cm^2 and 1:4 pulse rate (2 ms on to 8 ms off). LIPUS exposure was initiated 24 h after MSI implantation. Tibiae on the right side were irradiated with LIPUS for 20 min/d. Tibiae on the other side served as a sham-irradiated control.

2.4. Micro-computed tomography (μ-CT) analysis

Animals were perfusion-fixed with 4% paraformaldehyde (Wako, Osaka, Japan) 0, 3, 7, and 14 days after MSI implantation. The tibiae were resected and dehydrated by incubation in a graded ethanol series (70%, 80%, 90%, 99%, 100%, and 100% ethanol, v/v) for 12 h at each concentration. Tibiae were embedded in methyl methacrylate resin (Technovit 9100; Kulzer, Wehrheim, Germany). The resin blocks were scanned by μ-CT (Latheta LCT-200; Hitachi Aloka Medical, Tokyo, Japan). Images consisted of 936 slices with a voxel size of 24 μm in all three axes. Cortical bone and MSIs in the specimens were imaged and reconstructed in three dimensions. Regions of interest adjacent to the implants were analyzed to determine the cortical bone density (CBD; mg hydroxyapatite/cm^3), cortical bone thickness (CBT; mm) and cortical bone ratio (CBR; %). CBD was defined as the volumetric density of calcium hydroxyapatite. CBR was calculated as the amount of cortical bone area divided by the total area in the tread.

2.5. Scanning electron microscopy (SEM) analysis

After μ-CT analysis, the resin blocks were trimmed and prepared for analysis by SEM (Carry Scope JCM-5700; JOEL, Tokyo, Japan; Fig. 1). Acquired images were analyzed with a digital image analysis software (ImageJ version 1.44; US National Institutes of Health, Bethesda, MD).

2.6. Cell culture

Ti-6Al-4V and 316L SS discs measuring 33 mm in diameter and 1 mm in thickness were used. These discs were perfectly fitted to the bottom of wells in six-well cell culture plates. The discs