Abstract: The purpose of this experiment was to investigate whether low-intensity pulsed ultrasound (LIPUS) irradiation can inhibit dentoalveolar ankylosis in transplanted rat teeth. LIPUS irradiation (the pulsed ultrasound signal had a frequency of 3.0 MHz, a spatial average intensity of 30 mW/cm², and a pulse ratio of 1:4) was performed on the face over the re-planted teeth of rats for 4 weeks. After the rats were euthanized, we measured mobility (Periotest value [PTV]) of the transplanted and control teeth using a Periotest. Finally, we performed histological evaluation to detect ankylosis. PTVs tended to be significantly lower for re-planted teeth than for control teeth. Histological evaluation revealed that the roots of all re-planted teeth were coalescent with alveolar bone. Furthermore, no ankylosis was observed in three-fifths of the re-planted teeth following LIPUS irradiation. These results indicate the potential efficacy of LIPUS to inhibit dentoalveolar ankylosis.

Keywords: low-intensity pulsed ultrasound; dentoalveolar ankyloses; re-planted teeth.

Introduction

In clinical practice, traditional dental prostheses are used in cases of missing or damaged teeth to restore masticatory function. However, autotransplantation is more advantageous than prostheses such as dentures or implant placement in terms of success and periodontal sensation in the alveolar bone; this type of transplantation induces formation of new supporting tissue, continued root formation, eruption, and maintenance of occlusal contact with opposing teeth (1-3). Sugai et al. reported a success rate of approximately 90% for autotransplantation; however, in the other 10% of cases, the prognosis was poor, and one-third of these cases experienced postoperative dentoalveolar ankylosis (7). Dentoalveolar ankylosis can obstruct the primary orthodontic treatment plan, necessitating tooth extraction or other surgical treatments. However, reduction of patients’ burden, operative invasiveness, and the need for multiple corticotomies are critical as well.

In recent years, novel bone fracture treatments have been used in the field of orthopedics. Rutten et al. reported that low-intensity pulsed ultrasound (LIPUS) promotes healing of impaired fractures (9). In addition, Khanna et al. reported soft-tissue healing with LIPUS (10). More-
over, Rego et al. reported a significant decrease in areas of root resorption lacunae in LIPUS-irradiated samples and suggested that LIPUS can decrease traumatic inflammatory reactions (11). Furthermore, El-Bialy et al. reported that LIPUS may enhance the pluripotent characteristics of periodontal ligament (PDL) cells (12). Thus, these reports suggest that LIPUS can stimulate bone externally and may decrease traumatic inflammation as well as enhance PDL repair between the alveolar bone and dental root surfaces. We anticipated the inhibition of coalescence between the alveolar bone and tooth root surfaces associated with an increase in periodontal liga-

In this study, we used Periotest values (PTVs) and histological examination to investigate whether local LIPUS irradiation enhances periodontal tissue regeneration after autotransplantation without increasing the risk of ankylosis.

**Materials and Methods**

Ten 6-week-old male Sprague-Dawley rats (weight, 168.5 ± 15.2 g) were used in this study. All rats were fed a solid and powder diet and given water *ad libitum* during the experimental period. The rats were administered general anesthesia through intraperitoneal injections of sodium pentobarbital (Somnopentyl: Kyoritsu Seiyaku, Tokyo, Japan) at a dose of 60 mg/kg body weight. For re-plantation, xylocaine was used for local anesthesia (Dentsply Sankin, Tokyo, Japan; 0.02-0.03 mL).

The rats’ left maxillary first molars (M$_1$s) were autotransplanted and replaced as described by Kvinnsland et al. (13). The left M$_1$s were extracted using mosquito hemostatic forceps, rotated once anteriorly so that all roots were removed from the socket while leaving part of the attached mesial gingiva intact and then immediately repositioned. Next, self-curing resin was used to attach an anterior temporary cap (UNIFAST III, GC, Tokyo, Japan) to the maxillary and mandibular incisors of all rats (Super-Bond, Sun Medical, Tokyo, Japan) to disocclude molar regions by using a technique modified from that of Eui-Seok et al. (14).

**LIPUS irradiation**

We divided the 10 rats into two groups of five each. In one group, right and left M$_1$s were irradiated with LIPUS [LIPUS(+)], whereas irradiation was not used in the other group [LIPUS(−)]. The left M$_1$s were re-planted and defined as traumatic teeth [Tr(+)], whereas the right M$_1$s were not extracted and were defined as non-traumatic teeth [Tr(−)]. The teeth were thus categorized into four groups: Tr(−)LIPUS(−), Tr(−)LIPUS(+), Tr(+)

LIPUS(−), and Tr(+)LIPUS(+).

The ultrasound exposure system (OSTEOTRON D2: ITO Co., Tokyo, Japan) used in this study consisted of an ultrasound generator device and a circular transducer with a diameter of 30 mm. The pulsed ultrasound signal had a frequency of 3.0 MHz, a spatial average intensity of 30 mW/cm², and a pulse ratio of 1:4 (2 ms on, 8 ms off) (15). Before undergoing LIPUS, general anesthesia was induced using 4% isoflurane and maintained under 1-2% isoflurane. An ultrasound gel was applied to the cheek for LIPUS irradiation (Fig. 1). The left (re-planted) and right M$_1$s regions were alternately irradiated with LIPUS for 20 min. The irradiation period was 4 weeks (15). LIPUS irradiation was administered to the maximum under sedation in order to reduce pain to the animal, which was then euthanized. In addition, a minimum number of rats were used for a given experiment. The experiments were approved by the Animal Experimentation Committee of Nihon University School of Dentistry (AP11D007), Japan.

**Histological examination and Periotest measurements**

Any coalescence of the tooth root and the alveolar bone was defined as dentoalveolar ankylosis. The rats were euthanized using an overdose of sodium pentobarbital 4 weeks after re-plantation. The maxillary specimens were removed, fixed in 10% neutral buffered formalin, and embedded in gypsum to create highly reproducible PTVs. In addition, all measurements were conducted by a single examiner (TK) to eliminate interexaminer error and at least 3 times to reduce intraexaminer error, and the mean values were calculated.

The specimens were then decalcified in 10% ethylene-
diamine tetraacetic acid solution (pH 7.0-7.4) at room temperature for 6 weeks and embedded in paraffin wax (TEC5CM and TEC5EM; Sakura Seiki, Tokyo, Japan). The specimens were sagitally sliced into serial sections of 3-μm thickness including the mesiobuccal root apex, distobuccal root apex, and periodontal tissue. If one of 20 serial sections exhibited ankylosis, the tooth was judged as ankylosed. In this study, we examined the mesiobuccal root apex to detect ankylosis because in all specimens, the root and bone were closest in the mesiobuccal root.

Statistical analysis
Results are expressed as means ± standard deviations, and P values < 0.05 were considered to indicate statistically significant differences. Daily body weight changes were analyzed using unpaired Student’s t-test. To analyze the differences among the Tr(−)LIPUS(+), Tr(−)LIPUS(−), Tr(+LIPUS(+), and Tr(+LIPUS(+) groups, the χ² test was used. Statistical analyses were performed using the SPSS software (ver. 16 for Windows; SPSS, Inc., Chicago, IL, USA).

Results

Changes in rat body weight
The body weights of the rats decreased on days 1-3 and recovered thereafter (Fig. 2). No significant differences were noted in weight change among the groups (P > 0.01).

Periotest values
PTVs for M’s are enlisted in Table 1. PTVs were significantly lower in the Tr(+LIPUS(−) group (−0.5 ± 1.08) than in the other groups (P = 0.008). No significant difference was observed between the Tr(−)LIPUS(−) and the Tr(−)LIPUS(+) groups (P = 0.168), but a significant difference was observed between the Tr(−)LIPUS(+) and the Tr(+)LIPUS(+) groups (P = 0.008).

Histological findings
Figure 3 presents the histological images. In the Tr(−) LIPUS(−) group, the PDL space was preserved, and the cementum layer surrounding the root was relatively thin. The root was clearly separated from the alveolar bone. Histological findings in the Tr(−)LIPUS(+) group were similar to those in the Tr(−)LIPUS(+) group. In contrast, dentoalveolar ankylosis was clearly observed on the distal surfaces of the mesiobuccal root apices in the Tr(+) LIPUS(+) group. A magnified image of the islet in Fig. 3c is shown in Fig. 3c’. The cementum of the root was connected to the alveolar bone, with no clear border in between. Moreover, ankylosis was observed in two of five Tr(+LIPUS(+) specimens, with histological findings similar to those observed in the Tr(+LIPUS(−) group. In the un-ankylosed roots, the cementum and alveolar bone formed close interfaces, and slight root resorption was observed in the dentin and cementum.

The overall incidence of dentoalveolar ankylosis is
summarized in Table 2. Dentoalveolar ankylosis was noted in all Tr(+)LIPUS(−) specimens and in two of five Tr(+)LIPUS(+) specimens; this difference was significant \( (P = 0.0384) \).

### Table 2 Dentoalveolar ankyloses rate of M1: Tr(±) LIPUS(±)

<table>
<thead>
<tr>
<th>Ankyloses</th>
<th>No-ankylosis</th>
<th>Total</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tr(−)LIPUS(−)</td>
<td>0</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Tr(−)LIPUS(+)</td>
<td>0</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Tr(+)LIPUS(−)</td>
<td>5</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Tr(+)LIPUS(+)</td>
<td>2</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>7</td>
<td>13</td>
<td>20</td>
</tr>
</tbody>
</table>

**Discussion**

In clinical dentistry, autotransplantation is widely used to replace missing teeth and to correct anodontia. The advantages of this technique include functional adaptation and preservation of the alveolar ridge. Certain studies have reported good prognoses after tooth transplantation, with recent success rates exceeding 90% \((7,16)\). However, other studies have demonstrated failure of approximately 23% of transplanted teeth, most commonly because of dentoalveolar ankylosis \((4-8)\). Therefore, we aimed to investigate whether LIPUS could regenerate PDL and prevent dentoalveolar ankylosis after autotransplantation.

During LIPUS irradiation, we maintained non-interfering occlusion to prevent occlusal stimuli, which could decrease the incidence of ankylosis \((17)\). We fixed M1 specimens from young rats in gypsum to facilitate high reproducibility of PTV measurement. Uemura et al. and Miura et al. reported a strong correlation between PTV and the anchor-screw-to-bone contact rate on field-emission scanning electron microscopic (FE-SEM) images \((18,19)\). This helped in confirming that the PTVs were accurate. Our LIPUS irradiation method conformed to that used by Rego et al. \((11)\) and Ikai et al. \((15)\). However, we were able to include only 10 rats because it was difficult to reproduce definite ankylosis models, and thus, we used the minimum number of rats required to demonstrate effectiveness. A future study with a larger subject group is warranted to support our findings. The left M1 was selected as the traumatic tooth and the right M1 as the non-traumatic tooth. The teeth were irradiated from each direction, with a time lag, to provide LIPUS irradiation of the same strength on the right and left sides. We assumed that ankylosis had occurred when the border between the cementum and the adjacent alveolar bone was indistinct.

Among non-traumatic teeth, no differences were noted in results between the LIPUS(−) and LIPUS(+) groups \((P = 0.168)\); thus, LIPUS had no effect on PTVs in these specimens. However, a significant difference was observed between the Tr(−) and Tr(+) teeth. PTV of the traumatic tooth group was markedly lower than that of other groups, indicating possible ankylosis. Cambell et al. used the Periotest to diagnose traumatic permanent incisors in children and reported that PTVs were markedly lower for teeth with ankylosis than for those without ankylosis \((20)\). This study suggests that significantly lower PTVs indicate ankylosis.

Histological examination showed narrowing of the PDL space in traumatic teeth with and without LIPUS treatment. Levy and Mailand reported a slight increase in cellular cementum in the apical zones of supraerupted teeth under occlusal hypofunction at 15 days \((21)\). All distal surfaces of the mesial roots of traumatic teeth that did not receive LIPUS showed ankylosis. The border between the cementum and the adjacent alveolar bone was indistinct; such coalescence might indicate the onset of ankylosis. However, no ankylosis was observed in three of the five traumatic specimens from the LIPUS group, suggesting that LIPUS irradiation inhibits ankylosis. PDL contains several types of regenerative stem cells, including fibroblasts. Webstar et al. reported that ultrasound stimulation improves collagen synthesis in human fibroblasts \((22)\). Enhanced collagen synthesis and angiogenesis may be related to PDL growth, which could help explain why LIPUS irradiation improves PDL repair between the tooth root and alveolar bone, thereby inhibiting dentoalveolar ankylosis.

However, two-fifths of traumatic teeth in our study had ankylosis despite having undergone LIPUS, perhaps because of failure to irradiate the entire tooth. Therefore, additional studies are required to verify optimum irradiation conditions for establishing valid transplantation; combined examination of periodontal tissue repair and inflammatory inhibitory effects, along with development of an artificial tooth that can regenerate as a natural tooth, is necessary.

In conclusion, PTV was significantly lower in the traumatic tooth group compared with the other groups. Our study demonstrated that lower PTVs are associated with ankylosis and that LIPUS stimulation helped prevent ankylosis. These results highlight the therapeutic implications of ultrasound stimulation in periodontal regeneration and suggest the efficacy of LIPUS in inhibiting dentoalveolar ankylosis. Nevertheless, additional studies are warranted in the future to evaluate its full potential.
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Conflict of interest
The authors have no competing financial interest to declare.

References