**Effect of immediate loading on the biomechanical properties of bone surrounding the miniscrew implants**

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**SUMMARY** The aim of this study was to investigate the effect of immediate loading on the biomechanical properties of bone surrounding a miniscrew implant. Forty titanium alloy miniscrew implants were placed on the buccal side of the maxillae and mandibles in four beagle dogs. Twelve pairs of miniscrew implants were immediately loaded with approximately 150 g of continuous force using nickel–titanium coil springs and the remaining 16 implants were left unloaded for 8 weeks. Nanoindentation testing was performed (peak load 10 mN) and the hardness and elastic modulus were calculated. Two series of indentations (in cortical and trabecular bone) for both the compression and tension sides were made. For each site, five indentations were placed approximately 25 μm from the implant–bone interface and 250 μm from the screw thread. The mean hardness and elastic modulus were generally higher in mandibles than maxillae and were higher in cortical bone than in trabecular bone. The trabecular bone near the implant–bone interface on the compression side was significantly harder than that at other locations in trabecular bone. In conclusion, this is the first study that has investigated the biomechanical properties of bone surrounding a miniscrew implant under immediate loading using nanoindentation testing. The mechanical properties of bone surrounding a miniscrew implant may be influenced by immediate loading.

**Introduction**

Miniscrew implants have been gaining popularity because their small size allows for more placement sites in the oral cavity, less discomfort for patients, easy surgical procedure, and low cost (Miyawaki et al., 2003; Park et al., 2006; Kuroda et al., 2007); clinicians expect reliable anchorage without the need for patient compliance. However, one of the major concerns regarding miniscrew implants is their relatively low survival rate. Previous studies have shown that the cyclic application of mechanical forces modulated the development and regeneration of bone (Turner et al., 1994; Robling et al., 2001). In addition, Majzoub et al. (1999) studied the response of bone to the orthodontic loading of an endosseous implant in the rabbit and observed slight bone apposition on the compression side, while there was either no apposition or resorption on the tension side.

The stability of orthodontic miniscrew implants has been attributed to mechanical factors (mechanical locking) in the initial stage and biological factors after osseointegration (Chen et al., 2010). Recently, the immediate application of orthodontic loading for a miniscrew implant has been studied, and the authors reported that immediate loading does not affect osseointegration of the miniscrew implant, which suggests that the healing period with miniscrew implants can be shortened or even eliminated (Chen et al., 2009, 2010). The immediate loading of miniscrew implants may help to activate bone remodeling and increase the mineral contents at the loaded region.

Recent advances in the nanoindentation test have allowed the measurement of mechanical properties, such as the hardness and elastic modulus, for extremely small volumes of materials where contact radius is less than 100 nm (Oliver and Pharr, 1992; Iijima et al., 2011), and this technique has been used to characterize the mechanical properties of bone in studies near the implant–bone interface (Chang et al., 2003; Clark et al., 2007; Baker et al., 2010). At the early stage, bone healing may depend on the distance from the implant interface and on the anatomic location (Chang et al., 2003). Although it is important to assess the nanostructural changes at the microscopic level of the bone-miniscrew...
implant interface and the bone surrounding the miniscrew implant to understand the process of bone-miniscrew implant integration, no recent study has examined the biomechanical properties of bone surrounding a miniscrew implant. Accordingly, the present study was undertaken to obtain this information. To achieve this aim, the hardness and elastic modulus of bone surrounding a miniscrew implant were measured using nanoindentation testing. We hypothesized that the mechanical properties of the bone surrounding a miniscrew implant are not influenced by immediate loading. This is the first study that has investigated the biomechanical properties of bone surrounding a miniscrew implant using nanoindentation testing.

Materials and methods

Sample preparation

Four healthy male beagle dogs (age, 10–15 months; weight, about 10 kg) were selected. They received a soft diet during the experiment. All procedures were approved by the Animal Care Committee of Health Sciences University of Hokkaido. Forty conical titanium alloy (Ti-6Al-4V) miniscrew implants (AbsoAnchor SH-1413-5, Dentos, Daegu, Korea), 1.3 mm in tip diameter, 1.4 mm in neck side diameter, and 5 mm long were used. The recipient sites included the attached gingival and movable mucosa in the posterior region (approximately 5 mm apically to the root furcations of the premolars and approximately 3 mm to the gingival margin between the roots of premolars) of both the maxillary and mandibular buccal sides (Figure 1). The control specimens (unloading) were randomly chosen from a different location.

The dogs were treated with ketamin hydrochloride (Ketalar, Daiichi Sankyo Espha, Tokyo, Japan; 5 mg per kg, i.m.) for analgesia and thiopental sodium (Ravonal, Mitsubishi Tanabe Pharma, Osaka, Japan; 50 mg per kg, i.v.) for anesthesia. Diazepam (Horizon, Astellas Pharma, Tokyo, Japan; 2 mg per kg) and atropine sulfate (Mitsubishi Tanabe Pharma; 0.1 mg per kg) were given intramuscularly for pre-anesthesia.

All the miniscrew implants were placed manually with a hand-driver by the same operator (Y.Y.) after a pilot hole was made with a slow-speed handpiece at a speed of 500 rpm and a pilot drill 1.1 mm in diameter. The mobility of the miniscrew implants was checked with dental tweezers. Approximately 150 g of continuous force (for 8 weeks) was immediately loaded on 12 pairs (maxillae, six pairs; mandibles, six pairs) of miniscrew implants by approximately 10 mm stretched nickel–titanium closed coil springs (Sentalloy Closed Coil Spring, Tomy International, Tokyo, Japan). The remaining 16 implants in both jaws (eight maxillae and eight mandibles) were left unloaded for the same follow-up interval (control specimens). The heads of the miniscrew implants were covered with Dura Seal (Reliance Dental, Worth, IL). Six miniscrew implants were unstable during the experimental period, and only successfully stable miniscrew implant specimens were used for the nanoindentation test.

The four dogs were anesthetized and sacrificed after 8 weeks with thiopental sodium (Ravonal, Mitsubishi Tanabe Pharma; 150 mg per kg, i.v.). The maxillary and mandibular bones including the miniscrew implants were dissected.

Nanoindentation test

Six loaded miniscrew specimens from each pair in both jaws and six unloaded miniscrew specimens were encapsulated in epoxy resin (Epofix, Struers, Copenhagen, Denmark) for the nanoindentation test. All specimens were ground (600-grit sandpaper) and polished using diamond suspensions (particle size of 3, 1, and 0.25 μm) to obtain a suitable surface for nanoindentation. The specimens were then washed by distilled water and lightly cleaned ultrasonically. All nanoindentation testing (ENT-1100a, Elionix, Tokyo, Japan) was carried out at 28°C with a peak load of 10 mN using a Berkovich indenter. Each test consisted of three parts: 10 seconds for loading to the peak value, 1 second of holding at the peak load, and 10 seconds for unloading. Two series of indentations (in cortical bone and trabecular bone)
were made for both the compression side and tension side. For each site, five indentations were placed at approximately 25 µm from the implant–bone interface region and 250 µm from the screw thread (Figure 2). Linear extrapolation methods (ISO Standard 14577) were used for the unloading curve between 95% and 70% of the maximum test force to calculate the elastic modulus. The hardness and elastic modulus were calculated by software available with the nanoindentation apparatus, and their values were compared between the maxillae and mandibles and different anatomic locations (compression side and tension side; 25 µm from the implant–bone interface region and 250 µm from the screw thread).

Statistical analysis
The experimental results were analysed using IBM SPSS Statistics software (version 19.0J for Windows, IBM, Armonk, NY). Because the data for the mean hardness and elastic modulus for the mandible and maxilla of control groups (the data from two regions, 25 µm from the implant–bone interface region and 250 µm from the screw thread, were combined) were normally distributed (Shapiro-Wilk test), Student’s t-test was applied to determine whether significant differences existed among the groups (P < 0.05). The mean hardness and elastic modulus for different test sites were compared by one-way analysis of variance (ANOVA), followed by the Tukey-Kramer honestly significant difference test, with a significance level of 5%.

Results
All dogs remained in good health during the experimental period and all miniscrew implants showed initial mechanical stability with no mobility immediately after placement. However, six miniscrew implants (three in the maxillae, three in the mandibles) were unstable during the 8 weeks and showed some mobility. The success rates were 85.0% in the maxillae and 85.0% in the mandibles. Bone–implant contact was visible between the miniscrew implant and the surrounding bone for all of the stable miniscrew implants.

The mean hardness and elastic modulus measured in the maxillary and mandibular control specimens are shown in Table 1. Mandibular trabecular bone showed significantly higher values for the hardness (0.77 GPa, P = 0.0001) and elastic modulus (19.58 GPa, P = 0.008) than the maxillary trabecular bone (hardness, 0.66 GPa; elastic modulus, 18.06 GPa). On the other hand, while mandibular cortical bone showed a significantly higher value of hardness (0.92 GPa, P = 0.03) than that for maxillary cortical bone (0.85 GPa), there was no significant difference in the elastic modulus (P = 0.861). The mean hardness and elastic modulus in maxillary and mandibular specimens at different test sites are shown in Figures 3 and 4; the detailed values are summarized in Table 2. There was no significant difference in hardness or the elastic modulus among the six specimen groups in maxillary and mandibular cortical bone. On the other hand, the mean hardness was significantly higher for trabecular bone on the compression side near the implant–bone interface region (25 µm from the implant–bone interface region) in maxillary (P = 0.0001–0.007) and mandibular specimens (P = 0.001–0.01) when compared with other locations.

Discussion
The mechanical properties, such as hardness and elastic modulus, of bone in different regions vary, and their values should be important for understanding how bone responds and adapts to changes in the mechanical environment. The hardness and elastic modulus of bone may be influenced by the mineral content and crystal structure, and their values may be associated with the clinical primary stability of a miniscrew implant. Higher values of hardness and elastic modulus surrounding the miniscrew implant is associated with higher resistance to deformation of miniscrew implant, which is useful to stand more load under clinical condition. Therefore, the present study used the nanoindentation test to characterize the mechanical properties of bone surrounding miniscrew implants. Recent advances in the nanoindentation test allow the measurement of mechanical properties for extremely small volumes of materials where the contact radius is less than 100 nm, and it is possible to determine the hardness and elastic modulus simultaneously (Oliver and Pharr, 1992; Rho and Pharr, 1999; Seong et al., 2009; Iijima et al., 2011). The nanoindentation test is usually performed based on ISO Standard (ISO 14577), and the reliability has been established (Oliver and Pharr, 1992). The nanoindentation test is a useful method for investigating mechanical properties in small regions, such as interfaces of bone-implant for the miniscrew implant.
Iijima et al. (2011) compared the mechanical properties (hardness and elastic modulus) of commercial orthodontic wires with values obtained by the conventional Vickers hardness test, three-point bending test, and tension test and found that the nanoindentation test did not yield identical values of hardness and elastic modulus compared with those obtained from conventional tests because of their different material volumes sampled, different work-hardening levels, and an oxide layer on the wire surface (Iijima et al., 2011). Butz et al. (2006) used nanoindentation testing to characterize the biomechanical properties of bone and reported that bone integrated with acid-etched titanium was approximately three times harder than that integrated with machined titanium. In this study, the nanoindentation test revealed that the mean values for the hardness and elastic modulus of bone in the mandible were higher than those in the maxilla, except for the elastic modulus in cortical bone and were also higher

<table>
<thead>
<tr>
<th>Bone type</th>
<th>Maxillae Mean</th>
<th>Maxillae SD</th>
<th>Mandible Mean</th>
<th>Mandible SD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hardness</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Cortical bone</td>
<td>0.85</td>
<td>0.16</td>
<td>0.92</td>
<td>0.17</td>
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<td>0.66</td>
<td>0.13</td>
<td>0.77</td>
<td>0.15</td>
<td>0.001</td>
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<tr>
<td>Elastic modulus</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Cortical bone</td>
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<td>3.44</td>
<td>21.44</td>
<td>2.95</td>
<td>0.861</td>
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<tr>
<td>Trabecular bone</td>
<td>18.06</td>
<td>2.37</td>
<td>19.58</td>
<td>3.15</td>
<td>0.008</td>
</tr>
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</table>

Student's t-test ($P < 0.05$).
Figure 4  Mean hardness and elastic modulus obtained in mandible specimens at different test sites.

Table 2  Mean hardness and elastic modulus obtained in maxillary and mandibular specimens at different test sites.

<table>
<thead>
<tr>
<th>Bone type</th>
<th>Control</th>
<th>Compression side</th>
<th>Tension side</th>
<th>P value</th>
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<td></td>
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<td>0.85±0.16</td>
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<td>18.11±2.39</td>
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<tr>
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<tr>
<td>Hardness</td>
<td>Cortical bone</td>
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<td>0.93±0.16</td>
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<td>Elastic modulus</td>
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<td>19.75±3.45</td>
<td>19.40±2.87</td>
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</table>

One-way ANOVA followed by Tukey-Kramer multiple range test (p < .05). Identical letters indicate that mean values were not significantly different (p < .05).

in cortical bone than in trabecular bone. These trends are in agreement with previous findings regarding the mechanical properties of bone obtained by nanoindentation testing (Rho and Pharr, 1999; Seong et al., 2009).

The immediate loading of miniscrew implants is beneficial for the patient because the treatment period can be shortened. However, the surrounding tissue does not integrate with the implant when a load is applied immediately, and resorption is possible. Histologically, displacement and tipping could be ascribed to temporary bone resorption after primary damage caused by immediate loading, which might cause microfractures of the peri-implant calli on the compression side and bone proliferation on the tension side (Chen et al., 2009).
Serra et al. (2010) investigated the healing of interfacial tissues at early (1 and 4 weeks) and late stages (12 weeks) of osseointegration around miniscrew implants immediately loaded with 1N; they found that there was no difference in the histologic findings between the compression and the tension side of the loaded specimens, although the bone deposition was higher in the loaded specimens and some endosteal corticalization was seen in the loaded specimens after 4 weeks. The results of the present study showed that there was no significant difference in hardness or elastic modulus among different locations of cortical bone and trabecular bone on the tension side. On the other hand, the implant–bone interface region (25 µm from the implant–bone interface region) on the compression side of trabecular bone showed significantly greater hardness compared with other locations, suggesting that the internal remodeling of mechanically loaded bone resulted in an increase in mineral density in this region. These results support previous findings that immediate loading activates physiologic bone adaptation and stimulates remodeling of the original surrounding bone (Majzoub et al., 1999; Chen et al., 2009). The present study also supports the previous finding by Majzoub et al. (1999), who observed bone apposition in mechanically loaded bone. Chang et al. (2003) stated that the elastic modulus of bone surrounding an implant is a more important factor than hardness, and previous studies have shown that the elastic modulus gradually increased from the implant surface radially outward into the peripheral bone (Chang et al., 2003; Clark et al., 2007), although these studies did not investigate the effect of loading. On the other hand, the present study showed that the elastic modulus tended to increase in implant–bone interfacial regions of trabecular bone of the maxillae and cortical bone of the mandible on the compression side, although there was no significant difference among them. In the present study, the trabecular bone might change to the cortical near-cortical-like bone due to the immediate loading. Further research using a scanning electron microscopy with elemental analysis and microcomputed tomography are necessary to elucidate this hypothesis.

Conclusions

Under the conditions in this study, the following conclusions can be drawn:

1. The mean values for the hardness and elastic modulus of bone in the posterior region were generally higher in mandibles than in maxillae and were higher in cortical bone than in trabecular bone.
2. The trabecular bone near the implant–bone interface on the compression side showed significantly greater hardness than that in other locations.

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References

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