Clinical and histologic analysis of the stability of microimplants with immediate orthodontic loading in dogs

Yan Chen, Sung Taek Kang, Seong-Min Bae, and Hee-Moon Kyung
Hohhot, China, and Daegu and Seoul, Korea

Introduction: The aims of this study were to evaluate the stability of machined microimplant anchorage after immediate orthodontic loading in dogs and to ascertain clinical and histologic features. Methods: Sixty microimplants were placed in the buccal sides of the maxillae and mandibles of 4 dogs, including the interradicular area. Superelastic nickel-titanium coil springs were activated between 24 pairs of reciprocally loaded implants, producing a force of 200 g, and the remaining 12 unloaded implants served as controls. The distance between 2 microimplants was measured at the beginning and the end of loading. Results: Success rates were 89.58% in the loaded group and 75% in the unloaded group (P < 0.05). The mean displacements of the loaded microimplants were 0.98 mm (±0.57) in the maxilla and 0.53 mm (±0.48) in the mandible. Extrusion and tipping were seen in areas of thin cortical bone in both jaws. Histologic analysis showed remodeling at the periosteal bone was slightly more active in the loaded specimens than in the controls. There was no statistically significant difference in bone and implant contact values of the 2 groups. Conclusions: Immediate loading does not affect osseointegration of orthodontic microimplants, but the anchorage unit is not always absolutely stationary. (Am J Orthod Dentofacial Orthop 2009;136:260-7)

Endosseous integrated dental implants have gained wide acceptance as direct and indirect orthodontic anchorage and have been confirmed to provide absolute and rigid anchorage clinically and histologically. However, dental implants are intended to be permanent, whereas implants for orthodontic anchorage are usually removed after orthodontic treatment. Recently, mini-implants have been introduced as temporary skeletal anchorage devices and have gained popularity because their small size allows more placement sites in the oral cavity and reduces bone healing time.

Not only has immediate loading been used successfully in prosthetics for more than 20 years, but it has also been practiced in orthopedic surgery and bone fracture fixation. Dental implants are subject to high intermittent forces of mastication, but forces acting on orthodontic anchors are lower but continuous, and the direction of loading is different. A focus on immediate loading after placement has been attempted since Gainsforth and Higley introduced implants to orthodontics in 1945, but their low success rate limited their use. With the trend to shorten treatment time and reduce patient discomfort and inconvenience, immediate loading on implants has reemerged as an alternative approach. It is now suggested that the healing period of miniscrew implants can be shortened or even abolished because their primary stability is sufficient to withstand usual orthodontic loading. However, even though this treatment approach has shown promising and predictable results, the literature on the clinical stability of miniscrew implants and the histologic response of the surrounding tissues is limited.

The stability of miniscrew implants has been attributed to mechanical factors in the initial stage and biological factors after osseointegration. It was suggested that micromovement at the bone-implant interface on early loading might induce fibrous tissue formation rather than bone contact. Also, there is concern that the implant might fail because of unavoidable bacterial intrusion and epithelial down-growth.

This study was intended to resolve the controversies about whether with immediately loaded microimplants...
can be as stable and rigid as osseointegrated implants and whether the contact of bone to implant was direct skeletal encapsulation or connective tissue encapsulation.

**MATERIAL AND METHODS**

Four 1-year-old female mongrel dogs (weight, 13-15 kg) were selected. The maxillary and mandibular buccal sides, including the interradicular spaces, were chosen as the recipient sites for 60 microimplants (Fig 1). The recipient sites included the attached gingiva and the movable mucosa area. Each dog received 14 to 16 custom-made threaded titanium-alloy microimplants (AbsoAnchor system, NH1312-07; diameter, 1.2-1.3 mm; length, 7 mm; Dentos, Daegu, Korea). A 0.9-mm pilot hole was drilled before placement.

Approximately 200 g of continuous and constant force was loaded immediately on 24 pairs of microimplants by horizontally stretching the 8-mm superelastic, nickel-titanium closed-coil springs (Tomy, Tokyo, Japan) with a transparent tube between pairs of microimplants for 9 weeks. The remaining 12 microimplants in both jaws were not loaded and served as controls.

The osteodynamic changes during the 9-week period were recorded with bone labeling fluorochromes. Four doses of oxytetracycline (15 mg per kilogram of body weight per day; Oxytet, Kepro, Barneveld, Netherlands) were given for the first 4 days after placement. A 1% calcien subcutaneous injection was given (20 mg per kilogram of body weight per day; Sigma, Steinheim, Germany) at week 4. A dose of 0.16% alizarin red (25 mg per kilogram of body weight per day; Sigma) was injected intramuscularly at week 7.

Mobility of the microimplants was measured with dental tweezers and recorded with a 3-grade scale in which score 0 denoted no mobility and was defined as success. Score 1 indicated palpable mobility, and grade 2 meant visible mobility; both were defined as failure. The mobility examinations were performed immediately after placement and at 9 weeks.

Peri-implant pocket depth was measured on the mesial and distal aspects of each implant with a custom-made periodontal probe before and after loading.

For the assessment of displacement, the distance between the reciprocally forced microimplants in each pair was directly measured with a sharp digital micrometer (Ortho!14, Ortho, Seoul, Korea) in the dog’s mouth before and after the 9 weeks of loading. The measurement point was the mesial middle of the hexagonal head of the implant. Two readings were taken for every pair of microimplants, and the mean values were taken.

The 4 dogs were anesthetized and killed after 9 weeks of loading with 10% formaldehyde and saline solution. The maxillary and mandibular bones including the microimplants were dissected and fixed in 10% formaldehyde for 48 hours at room temperature and then changed to phosphate-buffered saline solution (pH 7.4). Half of the 60 microimplants were unscrewed, and the others were microscopically examined and allocated into the undecalcified group (22) or the decalcified group (8).

The 8 samples in the decalcified group (4 loaded, 4 unloaded) were fixed in 10% formaldehyde neutral buffer solution (pH 7.4) at 4°C for 24 hours and then decalcified in a mixture of formic acid and sodium citrate at 4°C for 6 days. Before being embedded in paraffin with a conventional technique, the specimens were dehydrated in a graded series of ethanol. Serial sections, 5 μm thick, were cut and stained with hematoxylin and eosin, and studied with a light microscope.

Twenty-two undecalcified representative samples (20 loaded, 2 controls) were dipped in Villanueva bone stain solution under negative pressure. Before being embedded in EPON resin (Miller-Stephenson, Danbury, Conn) (Villanueva bone stain, propylene oxide), the specimens were dehydrated and defatted with graded ethanol. Subsequently, the embedded specimens with implants and bone were cut sagittally (mesiodistal) into 500-μm thick slices with a low-speed digital saw (Accutom-50, Struers, Bellerup, Denmark) and then ground down to 50 μm in thickness until the microimplants and surrounding tissues could clearly be examined with light and fluorescent microscopes.

Histomorphometric analysis was performed on combined images including the entire longitudinal sectional implant interface. Six or 7 microscopic images of the Villanueva-stained specimens at 100 times magnification were spliced by using MagicScan32 software (version 4.6, SPSS, Chicago IL) to create an image of a complete microimplant for each specimen. The spliced implant images were quantitatively analyzed by using computer-assisted image analysis software (image and microscope technique; iMT, Goleta, Calif) to calculate the percentage of bone-to-implant contact (BIC). The percentages of BIC were expressed in 2 ways: (1) overall BIC values = (the length of the bone and implant contact area)/(the total length of the implant interface) × 100% and (2) BIC values in the cortical bone = (the length of the bone and implant contact area in cortical bone)/(the length of the implant interface in the cortical bone) × 100%.

**Statistical analysis**

Descriptive statistics including means and standard deviations were calculated for the loaded fixtures before
and after loading by using SAS software (version 8.0, SAS, Cary, NC). One-way analysis of variance (ANOVA) and paired $t$ tests were used to identify differences in BIC values between the mesial and distal sections of the microimplants. Differences were considered significant at a probability less than 0.05.

RESULTS

The dogs remained in good health with a 0 to 0.5 kg weight loss over the experiment duration. All microimplants had initial mechanical stability immediately after placement, and no periosteal pockets were seen. After 9 weeks, the 24 microimplants in the study group and the 6 in the control group were easily unscrewed with a screwdriver, without breakage.

However, 4 microimplants from the study group and 3 from the control group were unstable in the 9 weeks. Of the 7 unstable microimplants, 1 from the loaded group and 1 from the unloaded group had grade 2 mobility, and the rest had grade 1 mobility. The latter were mobile but could still serve as orthodontic anchorage. Three loosened implants were found in the maxillae and 4 in the mandibles. One microimplant in the maxillary second premolar area fractured after 1 week of loading. The success rates were 89.58% in the loaded group and 75% in the unloaded group ($P > 0.05$).

Figure 2 shows the distribution of the microimplants.

The gingivae around the nonloaded microimplants were clinically healthier than those around the loaded microimplants, and no peri-implant pocket was seen in either group except for the unstable microimplants.

Some loaded microimplants had significant displacement and tipping; the average relative displacements were 0.98 mm ($\pm 0.57, n = 16$) in the maxilla and 0.53 mm ($\pm 0.48, n = 16$) in the mandible, after continuous orthodontic loading for 9 weeks. The displacements were greater in areas of thinner cortical bone.

The implants in the movable mucosa area showed more threads out of the bone because of the thick soft tissues, but incomplete placement did not seem to affect the stability of the microimplants.

The findings from the light microscope examination of the slides stained with hematoxylin and eosin showed that the bone direction was irregular in the thread area, and more new bone cells could be seen at the interface area interspersed with unmineralized tissue.

On viewing the Villanueva-stained slides under a general light microscope, there was an average of only 5.5 threads of the microimplant screw in the cortical bone (range, 2-8 threads), with the apex of the implants in the bone marrow cavity.
Osseointegration was visible between the microimplants and the surrounding bone in all stable specimens. Resorption lacunae, observed as thin compact bone and a highly porous, spongy inner structure (Fig 3), were less evident around the loaded specimens. Osseointegration looked good at the tip third of the implants if there was cortical bone and poor at the collar third. Resorption at the bone surface and proliferation at the collar area and the bone marrow were seen on the same slide. Slightly more bone apposition was seen at the surface of the surrounding bone in the loaded specimens than in the unloaded ones. There was no evidence of fibrous connective tissue encapsulation at the bone-implant interface of any stable specimen.

At high magnification under the light microscope, longitudinal sections of the Villanueva-stained slides showed incomplete osseointegration in most loaded and unloaded segments (Fig 4). Corticalization of trabecular bone and new bone formation interspersed with some unmineralized areas were generally observed at the bottom threads more often in the loaded specimens.

The Villanueva-stained slides were also evaluated under a fluorescent microscope. The longitudinal sections in Figure 5 correspond to and resemble the findings in Figure 4. Incomplete bone resorption and new compact bone tissue growing into the threads of the microimplants along the endosteal surfaces, where lack of oxytetracycline labeling suggested no previously existing bone in the first week after surgery, were evident by week 9. A slightly wider area void of oxytetracycline was seen in the control specimens, suggesting that resorption was less around the loaded specimens. At the cortical bone area, internal remodeling with cutting-filling cones in mature secondary osteons was observed with slightly more calcein lines around the loaded implants than around the controls (Fig 5).

With infection, loss of marginal bone height around the neck of some implants was evident by the exposed threads that were completely embedded in the bone when the microimplants were placed (Fig 6). In the loosened specimens, inflammatory cells, bacteria, and necrosis were aggregated in the fibrous connective tissues around the microimplants. Three implants placed into tooth roots were covered by inflammatory infiltrate composed of plasma cells without bone tissue (Fig 7).

Histomorphometric evaluation of the longitudinal sections indicated no significant difference of osseointegration between the loaded and unloaded specimens, or between the tension and compression sides of the loaded samples. The overall BIC value for the stable microimplants was 23.91% (±7.73%; range, 13.13%-40.31%), whereas the BIC value of the unstable implants including the implants in the roots was less than 10%. The comparisons of the BIC values between the mesial and distal sections of the loaded and unloaded samples are shown in Table I. BIC values were associated with the quantity of surrounding cortical bone. The thin cortical bone area showed lower BIC values in the microimplants. Table II shows the BIC values in the cortical bone area.
DISCUSSION

This study demonstrates the anchorage capability of microimplants; they sustained 200 g of immediate horizontal loading for 9 weeks with a high success rate (89.58%). The unloaded group had a 75% success rate. The chi-square test showed no statistically significant difference because of the small sample size of the unloaded group. Even though Roberts et al.1 stated that a 4-month closed healing period was necessary for orthodontic implants, for 1-stage microimplants...
healing in an open situation; immediate loading does not affect their success rate both in clinical trials\textsuperscript{12} and in-vivo tests.\textsuperscript{4}

The microimplants with a 1.2-mm diameter can be placed in any areas of the maxilla and the mandible, including interradicular areas, even 2 in 1 interradicular area. The implants were easily unscrewed after 9 weeks of loading with a screwdriver without breakage. Park et al\textsuperscript{8} reported that 3 pure titanium screws of 1.2-mm diameter were broken in a clinical trial during unscrewing. This suggests that titanium-alloy microimplants can withstand high mechanical stress to resist breakage, and that stability is sufficient for continuous loading.

However, immediate loading also has some disadvantages. The displacement and tipping of some microimplants in the loaded group showed that the anchorage unit was not absolutely stationary. Because of dependence on bone quality and quantity, displacement was greater in the maxilla and more obvious in thin cortical bone areas.

In a clinical trial, some screws showed displacement, tipping, and extrusion after 9 months of 400-g loading.\textsuperscript{11} Compared with the 0.4-mm displacement of implants loaded after a 2-week healing period, the average displacement values of 0.98 mm in the maxilla and 0.53 mm in the mandible in our study are greater. Apparently, even though a 2-week healing period was not sufficient to allow osseointegration, it could compensate for bone damage. This discrepancy in implant displacement suggests that the healing period might be critical in reducing displacement and tipping. This was further confirmed by several dental implant studies showing that orthodontic loads do not change the positions of osseointegrated implants.\textsuperscript{13-16}

When an immediate load is applied, the surrounding tissue does not integrate with the implant, and resorption is possible. Histologically, displacement and tipping could be ascribed to temporary bone resorption after primary damage by motor drilling and secondary damage caused by immediate loading that might cause microfractures of the peri-implant calli on the compressive side and bone proliferation on the tensile side.

These results prove that immediate loading does not prevent bone formation, but it activates the physiologic
bone adaptation and stimulates remodeling of the original surrounding bone. The osteodynamic changes show surrounding bone remodeling in process in both the study and control specimens. Relatively more new bone formation and remodeling activities around the periosteal surface and beneath it in the study specimens were extrapolated by the absence of the initial oxytetracycline and calcein label near the endosseous surface established. As a physiologic time marker, the oxytetracycline label provides demarcation between original bone and new bone formed during the experimental period. The originally nonvital interface shows increased remodeling in bone trabeculae and the most composite bone around implants at 9 weeks. Similar findings were reported in previous studies. Majzoub et al\textsuperscript{17} and Wehrbein et al\textsuperscript{15} addressed more bone remodeling activity, with significant evidence of this activity on the compression side compared with the tension side. Akin-Nergiz et al\textsuperscript{18} admitted that continuous loading stimulated turnover from immature to mature bone, so that an increase in the velocity of lamella bone production was observed.

Compared with unloaded samples, there was slightly greater bone apposition in the surface of surrounding bone in the loaded ones. Similar to Aldikaqti et al\textsuperscript{19} and Majzoub et al\textsuperscript{17} a slight increase of bone apposition was found in the marginal crestal area of the loaded implants when compared with the nonloaded implants. Roberts et al\textsuperscript{20} found that marginal bone apposition might depend on factors such as force magnitude, bone structure, and deformation of the bone from orthodontic forces.

All stable microimplants had a certain quantity of osseointegration, and connective tissue would not interpose between the bone and the implant when loaded prematurely. BIC values, the index of osseointegration, ranged from 13.13\% to 40.46\% with an average of 23.91\%. This means that, if the overall BIC values are over 10\%, the microimplants can sustain orthodontic loading.

By measuring the cortical bone area, the mean BIC values of the loaded samples for the maxilla and the mandible were 37.51\% and 38.56\%, respectively. In the unloaded samples, the BIC values were 37.47\% for the maxilla and 23.96\% for the mandible. With the same measurement criteria, Kim et al\textsuperscript{21} reported that, after 1 week of healing, the mean BIC value was 23.4\% for loaded mini-implants with a 1.6-mm diameter. In another dog experiment, Ohmae et al\textsuperscript{16} reported BIC values of 25\% for loaded microimplants and 18.9\% for unloaded ones, after a 6-week healing period. These indicate that immediate loading does not affect osseointegration of the implant, so that a healing period does not seem to be necessary for orthodontic microimplants.

Roberts et al\textsuperscript{22} found no significant difference in the percentage of BIC associated with a supplemental load. Melsen and Lang\textsuperscript{23} also believed that the degree of osseointegration is independent of loading. The BIC values were similar for the loaded and unloaded specimens in the maxilla and higher for the loaded than the unloaded ones in the mandible because of neck infection and resorption.

The BIC value of the microimplants might be affected by the thickness of the cortical bone. To satisfy the biomechanical requirement for immediate orthodontic load, microimplants should be embedded in the compact bone area at least to 3 or 4 threads; this is the key to initial stability and secondary osseointegration. Because the device is mechanically placed into bone like a peg, the tip area of submerged microimplants might have less chance to become infected. Thus, it is advisable to place the implant at an angle to increase the BIC area, but damage to tooth roots should be avoided. Nevertheless, because bone structure is complicated on the buccal sides of the maxilla and the mandible, their physiologic characteristics should be considered.

| Table I. Comparison of mean BIC values in the mesial and distal sections |
|-----------------------------|-----------------------------|-----------------------------|
|                             | Mesial section              | Distal section              |
| Overall microimplants       | 36.97 (± 16.08)             | 35.93 (± 12.21)             |
| Maxillary loaded samples    | 28.26 (± 15.57)             | 46.74 (± 14.9)              |
| Maxillary unloaded samples  | 36.87                       | 39.62                       |
| Mandibular loaded samples   | 41.13 (± 14.96)             | 33.89 (± 12.86)             |
| Mandibular unloaded samples | 29.08                       | 17.93                       |

*NS,* Not significant.

| Table II. Comparison of BIC values in cortical bone in loaded and unloaded samples |
|-----------------------------|-------------------------------|
|                             | Loaded                        | Unloaded                     |
| Maxillary implants          | 37.51 (± 14.39) (n = 4)       | 37.47 (n = 1)                |
| Mandibular implants         | 38.56 (± 9.85) (n = 11)       | 23.96 (n = 1)                |
Generally, apart from infection and insufficient bone quality, a traumatic operation is thought to be the main cause of implant failure, if the biocompatibility of the titanium implants has been ascertained. Three microimplants failed because of misplacement into tooth roots, and the surrounding bone tissue was absent histologically. Zarb\textsuperscript{24} stressed that failure of implants with tight BIC might have been due to traumatic surgery and early loading, since 1-stage implants could not eliminate the bacterial and epithelial intrusions. Despite minimal trauma with a strict surgical protocol and abundant saline-solution irrigation during pilot drilling, it was impossible to prevent some bone cell destruction on the surface of the implants. Therefore, good oral hygiene is important to reduce infection.

**CONCLUSIONS**

These results suggest that microimplants can serve as anchorage with immediate and moderate loading, but their stability is not absolute. Because the cortical bone thickness through which the implant penetrates is an important prognostic indicator, the implant should be placed at an angle. This will not only improve the stability of the implant, but also reduce the risk of damage to roots and other anatomic structures.

Immediate loading does not inhibit osseointegration of microimplants but stimulates bone adaptation. The BIC value was not affected by the immediate load and was also not different between the compression and tension sides of the loaded implants.

If there is enough bone, immediate force on orthodontic anchorage is recommended, but the position of microimplants should allow adequate distance from vital organs in expectation of some implant displacement.

**REFERENCES**