Stability and Bone Response of Immediately Loaded Micro-Implants in Beagle Dogs

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Purpose: *To study the stability and the peri-implant alveolar bone response of immediately loaded micro-implants.* ***Materials and Methods:*** *Micro-implants were implanted into the mandibles of male beagle dogs, immediately loaded in the test group (200-g horizontal force) and unloaded in the control group. The healing bone was then labeled with polyfluorochrome at intervals over 10 weeks. Radiographs were taken at the beginning and end of the study. Seventy-three days after implantation, the dogs were euthanized, and the dissected mandibles were prepared for examination. The tissue specimens were evaluated by light microscopy, fluorescent microscopy, polarized light microscopy, scanning electron microscopy, and energy dispersive x-ray spectroscope.* ***Results:*** *The teeth anchored by the micro-implants moved normally, while the micro-implants remained basically stable. All the microscopy results showed that osseointegrated interfaces were formed between the micro-implants and the alveolar bone. Fluorochrome labels demonstrated that lamellar bone had appeared at 6 weeks after implantation, and was formed more extensively after another 3 weeks.* ***Conclusion:*** *This in vivo study found that the stability and osseointegration of immediately loaded micro-implants were not impaired. Micro-implants inserted in dense cortical alveolar bone in the mandibles of beagle dogs may be loaded immediately in a dog model to achieve satisfactory orthodontic anchorage.* Int J Oral Maxillofac Implants 2008;23:885–890

Key words: *bone-implant interface, immediate loading, micro-implant, osseointegration, stability*

Orthodontic treatment involves moving malpositioned teeth and their supporting structures to more appropriate intraoral locations, which requires anchorage by other teeth, bone, soft tissues, and implants. The micro-implant[1] is a small screw-like implant specifically designed for orthodontic anchorage. Due to its many practical advantages, the micro-implant is drawing increasing attention from orthodontists.

Several researchers have stated that the micro-implant or miniscrew can be loaded immediately after implantation,[1–6] and successful orthodontic treatments have been reported for immediately loaded micro-implants.[1–4] However, further in vivo studies on the stability of immediately loaded micro-implants and histologic evaluations of the implant-bone interface are needed.

The present study was designed to investigate the stability of immediately loaded micro-implants and the bone response to this loading.

MATERIALS AND METHODS

Healthy adult male beagle dogs aged 19 to 22 months and weighing 13.2 to 14.4 kg were selected for the study. The dogs were cared for by veterinarians at the Nanjing Medical University Animal Center. The study was approved by the Animal Ethics Committee of Nanjing Medical University.

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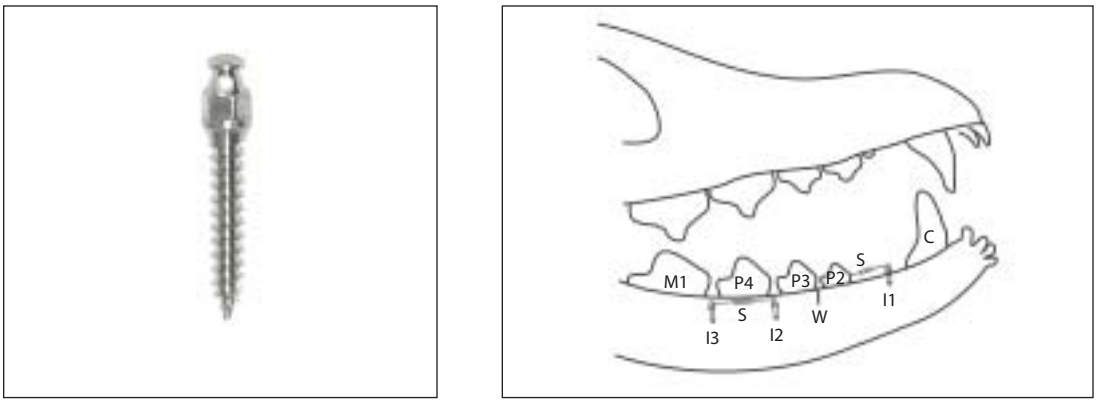

Fig 1 The image on the left shows the micro-implant used in this study. Micro-implants were placed bilaterally in the mandible posterior to the canine (I1), between the third premolar and fourth premolar (I2), and between the fourth premolar and first molar (I3). C = canine, P2 = second premolar, P3 = third premolar, P4 = fourth premolar, M1 = first molar, S = NiTi closed coil spring, W = titanium wire marker.

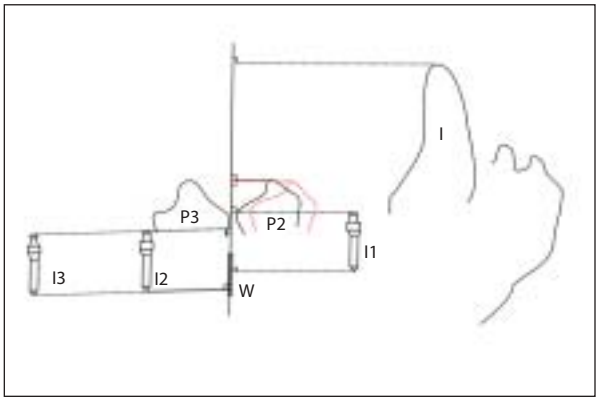

Fig 2 Measurements made on the radiographs. For P2, the black solid line shows the position of the tooth before loading, and the red dashed line shows the position at the end of the study.

All the experimental procedures, including surgical and radiographic procedures and clinical examinations, were performed aseptically under general anesthesia. For each dog, both the mandibular first premolars were extracted, and 1 micro-implant (AbsoAnchor; Dentos, Daugu, Korea) (I1) was implanted bilaterally, posterior to the mandibular canines (C) (Fig 1). One micro-implant provided anchorage for protraction of the mandibular second premolar (P2) on the test side of the mandible, whereas the contralateral micro-implant and P2 acted as a control, with no force applied. Two more micro-implants (I2, I3) were implanted in each side of the mandible between the mandibular third premolar (P3) and fourth premolar (P4) and between the fourth premolar (P4) and first molar (M1), respectively. Micro-implants on one side were loaded, while the contralateral micro-implants acted as controls without traction. Using nickel-titanium (NiTi) closed coil springs, which were activated biweekly, 200 g of calibrated traction force was applied between the I2 and I3 implants, and between the P2 and the I1 implant in each dog. One 5-mm length of thin titanium straight wire (W) was implanted perpendicular to the occlusal plane, posterior to P2, as a marker in each side of the mandible.

Lateral oblique jaw radiographs were taken of the mandibles immediately after the implantation and at the completion of the study. During radiography, the dogs' heads were positioned with a jig comprising 2 ear rods, 1 nose rod, and 2 connection plates. Vertical lines to the wire W were made from the points of the root apices, the heads of implants, and the cusps of the relevant teeth. The distances for W-P2, W-C, W-I1, W-I2, and W-I3 were measured on the radiographs and corrected by the magnification of the P2 on radiographs (Fig 2).

During the study, the dogs were injected intramuscularly 4 times with fluorescent bone labels as follows: 60 mg/kg xylenol orange at 3 weeks after implantation, 5 mg/kg calcein at 6 weeks after implantation, and 45 mg/kg tetracycline at 9 weeks and at 10 weeks after implantation. The anesthetized dogs were euthanized 3 days after the last label was administered. The mandibles were sectioned and 12 bone blocks from each test and control group were obtained. Four randomly chosen bone blocks from each group were frozen, and the bone-implant interfaces evaluated by scanning electron microscopy and energy dispersive x-ray spectroscopy. Other blocks were fixed in neutral buffered formalin, dehydrated in gradient ethanol and acetone, and then

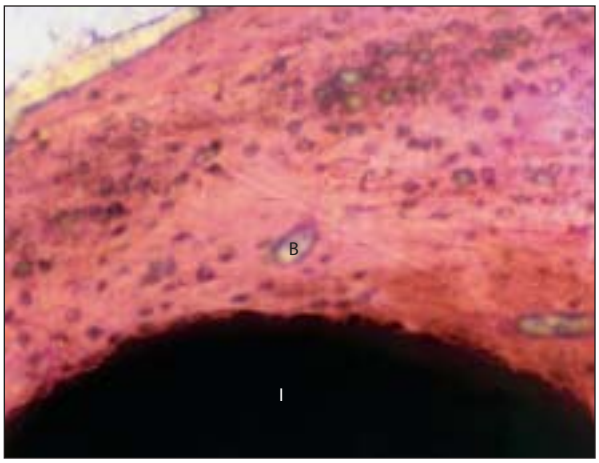

Fig 3 Light microscope image of peri-implant tissues from the test group. The micro-implant is in intimate contact with compact bone, without a fibrous tissue capsule present. I = micro-implant, B = bone (Van Gieson stain; original magnification ×100).

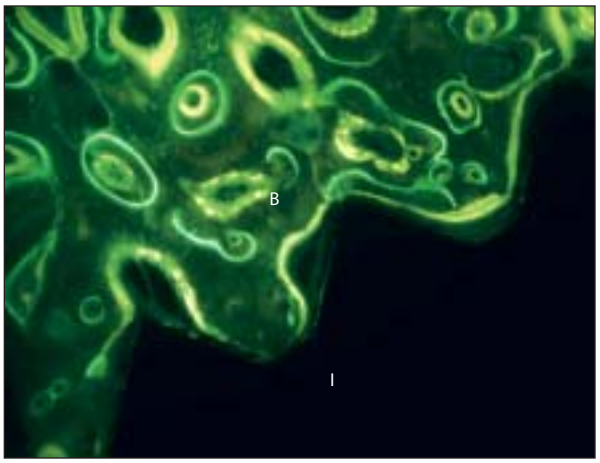

Fig 4 Polyfluorescent image of peri-implant tissues from the test group. The peri-implant bone partially contacts the micro-implant surface and shows active remodeling. Lamellar bone formation is indicated by the green calcein label at 6 weeks, and by the yellow tetracycline labels at 9 weeks and 10 weeks. I = micro-implant, B = bone (original magnification ×40).

embedded in polyester resin. Four micro-implants embedded in polymerized bone blocks from each group were sectioned longitudinally at 100-μm thicknesses with a Leica 1600 Sawmicrotome (Leica Microsystems, Wetzlar, Germany) and stained with Van Gieson. Four other blocks from each group were sectioned perpendicular to the long axis of each micro-implant at 100-μm thicknesses and observed using Van Gieson staining. Two slides from each sectioned block were chosen at random for evaluation by light microscope, fluorescent light microscope, polarizing light microscope, and scanning electron microscope.

RESULTS

All micro-implants were clinically stable and surrounded by radiopaque bone 73 days after implantation. From the radiographic measurements in both groups, there were negligible changes in the distances between C and W, indicating that W was basically stable. When protracted by I1, the P2 cusps in the test group moved a mean distance of 3.04 ± 0.31 mm, and the P2 cusps in the control group moved a mean distance of 0.36 ± 0.13 mm. The mean displacements of the P2 root apices for the test and control groups were 0.17 ± 0.03 mm and 0.13 ± 0.04 mm, respectively. As no archwire was used, P2 showed tipping, rendering measurements of movements for the root apices irrelevant. The mean displacements of the heads of the micro-implants for the test and control groups were 0.62 ± 0.10 mm and 0.21 ± 0.06 mm, respectively.

When histologic sections of the specimens were observed under the light microscope, in all instances the micro-implants were in intimate contact with compact bone, without the presence of fibrous tissue capsules (Fig 3). When evaluated with the NYD-1000 Software (Pathology Department, Nanjing Medical University), the mean bone-implant contact area percentages for the test and control specimens were 59.47% ± 12.10% and 62.34% ± 11.26%, respectively.

The polyfluorescent images showed that the peri-implant bone partially contacted the micro-implant surfaces, with active remodeling in both the test and control groups. At 3 weeks, the fluorescent xylenol orange label tinting showed a diffuse pattern, hinting that only woven bone was being formed at this time. After 6 weeks, lamellar bone had formed, so one green fluorescent calcein label band was present at 6 weeks and 2 yellow fluorescent tetracycline label bands were present at 9 and 10 weeks (Fig 4). Twenty sites were chosen at random, and the distances between the 2 yellow bands were measured. The mean bone formation rates (BFR/d) for the test and control specimens were 1.42 ± 0.21 μm/d and 1.33 ± 0.34 μm/d, respectively.

The polarizing light images showed that secondary osteon existed in the old bone, while primary osteon was present near the interface of the micro-implant and bone. The lamellar bone near the interface, though a little disordered, was basically perpendicular to the interface (Fig 5). Marked lamellar bone formation displayed by polyfluorescence, and primary osteon presence as shown by polarizing light microscopy, indicated active tissue remodeling adjacent to the immediately loaded micro-implants.

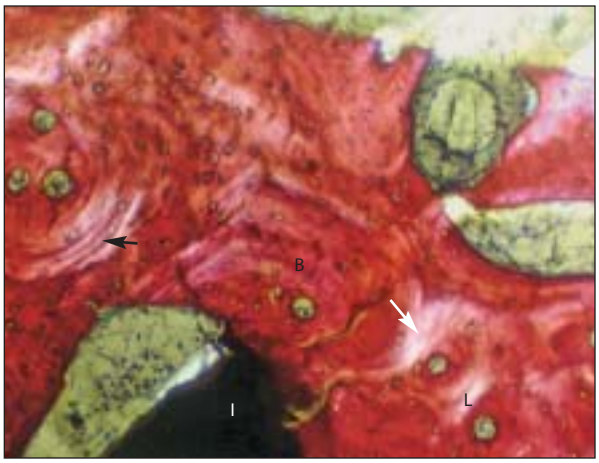

Fig 5 Polarizing light microscope image of peri-implant tissues from the test group. The lamellar bone near the implant interface, though a little disordered, is basically perpendicular to the interface. Primary osteon is indicated by the white arrow and secondary osteon is indicated by the black arrow. I = micro-implant, B = bone, L = lamellar bone (original magnification ×40).

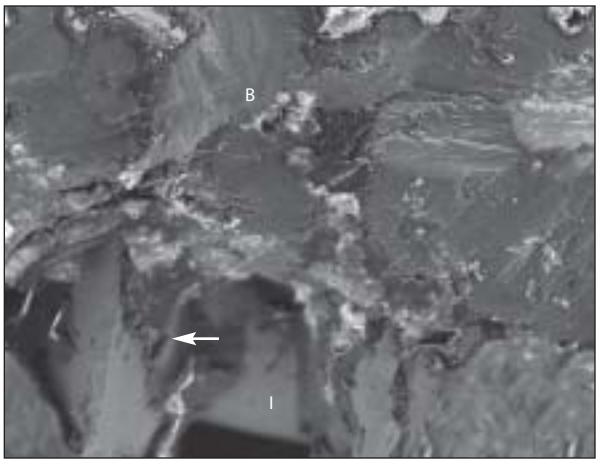

Fig 6 SEM image of peri-implant tissues from the test group. The peri-implant bone directly contacted the implant surface, as indicated by the white arrow. I = micro-implant, B = bone (original magnification ×80).

A small amount of bone was removed to expose part of the micro-implant surface, and the specimen was evaluated by scanning electron microscope (SEM). The finding that the peri-implant bone directly contacted the implant surface corresponded with that of the light microscope. Some fragments of bone were still attached to the surface of the exposed micro-implant (Fig 6).

One randomly chosen spot near the interface from each specimen was examined by energy dispersive x-ray spectroscope (EDX). Calcium and phosphorus constituted the majority of the chemical elements detected. For calcium, the mean percentages for the test and control specimens were 56.52% ± 10.12% and 54.31% ± 12.96%, respectively. For phosphorus, the mean percentages for the test and control specimens were 24.30% ± 3.06% and 21.83% ± 2.43%, respectively.

DISCUSSION

Anchorage control always plays an important role in orthodontic therapy, especially for fixed appliances. Many of the conventional means for the enhancement of anchorage are less than ideal, because they either risk mobility of the anchorage unit or rely too much on the compliance of patients. Some methods are uncomfortable, inconvenient, or unhygienic.

Implant anchorage circumvents many of these shortcomings, but conventional dental implants have certain drawbacks. Cost, invasiveness, waiting time for osseointegration, and limitation of available implantation sites severely restrict their use for orthodontic anchorage.[7] The onplant, which had the appearance of a button, was marketed in 1989.[8] The mini-implant, a 2-stage screw-like implant, was reported in 1997.[9] Subsequently, many types of small-sized implant anchorage were introduced,[2–5,10] most of which were one-stage modifications of the mini-implant.

Many factors, including the loading schedule and implantation site, can affect the stability and osseointegration of micro-implants.[5,11,12] The stability of the implants was determined clinically by 2 aspects (ie, no loosening and little movement during loading period). Chen et al[6] indicated that the removal torque values of miniscrews when loaded immediately were greater than 0.89 kg • cm, which was sufficient for them to fulfill their purpose as anchors. The orthodontic force is usually less than 200 g. In the present study, the P2 moved significantly, while the micro-implants were clinically firm and moved relatively little. Therefore, the stability of the immediately loaded micro-implants was adequate for clinical anchorage when using a 200-g horizontal force.

The occurrence of osseointegration or lack thereof will determine the stability of the micro-implant. An unstable micro-implant may lead to unfavorable tooth movement, tissue damage adjacent to the implant, and complaints from the patient.

Three of the main factors needed for the establishment of osseointegration are a precise fit of the implant, a careful surgical technique to avoid the

generation of frictional heat, and the prevention of implant movement by avoiding early loading.[13] Some researchers hold that immediate loading results in the formation of a capsule of fibrous connective tissue (pseudoperiodontium) around the implant.[13] Over time, this tissue layer will become increasingly thick, and the implant may fall out. In one early study,[14] the bones in which the implants were immediately loaded totally fractured after 1 week. It was thought that the continuous load progressively weakened the bones to less than the structural strength required to resist routine functional loading and that immediate loading could compromise initial implant stability and threaten vitality of adjacent bone. A 6-week healing period is apparently required for implant stability, because by then sufficient lamellar bone has formed adjacent to the implant. Immediate loading may cause micromovement between the bone and implant, destroy the 3-dimensional lattice of woven bone, and impede the normal healing process.[15]

However, a 3-week healing period in dogs was sufficient for orthodontic loading, and the lack of failure of the loaded implants indicated that the critical healing time was actually shorter than 6 weeks.[16] Other studies of immediately loaded implants for prostheses have demonstrated that the osseointegrated interface can form between the implants and bone despite the absence of a healing period before loading.[17,18] Therefore, it is possible that the loading period can determine entirely the type of interface present for implant anchorage, in particular for the micro-implant.

In the present study, despite immediate loading, the achievement of osseointegration indicated that a 200-g orthodontic force did not prevent the periimplant tissues from forming lamellar bone. The success of this study and one other study[5] might have been due to preventing infection, providing soft foods, and using careful operative techniques when placing self-tapping implants into dense cortical bone, which provided initial stability and reduced micromovement.

Following micro-implant placement in bone, tissue remodeling commences with the resorption of blood clot, angiogenesis, formation of skeletal matrix, and calcification. Woven bone is formed during the early period of calcification, followed by the formation of more highly calcified lamellar bone. Newly formed lamellar bone around the implant tends to be orientated parallel to the direction of force acting on the bone-implant interface (Fig 5). Gradually, primary osteon is formed by the newly formed lamellar bone, which is somewhat disordered. Later, the primary osteon is replaced by secondary osteon, which is composed of more ordered concentric layers of lamellar bone. Immediate loading, which may be unfavorable bone strain, could potentially induce unfavorable bone formation conditions during the early healing period, in particular during the "demineralization period" at 2 to 3 weeks after implantation. However, in the present study, when a horizontal force of 200 g was applied to the micro-implant, woven bone, newly formed lamellar bone, and primary osteons appeared, which indicated active remodeling to form skeletal tissues.

Roberts et al[19] reported that osseous contact on less than 10% of the implant surface area is all that is necessary to resist orthodontic loads. In the present study, the mean surface area osseous contact of the immediately loaded specimens was 59.47% ± 12.10%, equivalent to approximately 3% to 4% of the surface area of conventional-sized implants. Owing to its small surface, it can be supposed that it can be easily removed when it is no longer needed.

Fluorescent light microscopy demonstrated bone modeling and remodeling at the bone-implant interface. Over the 10-week healing period, active remodeling with osteons and lamellae was observed in the bone adjacent to the micro-implants. Haider et al[20] reported that woven bone formation showed a diffuse pattern from fluorescent labeling. Xylenol orange labels were used in the present study, and hinted during the first 3 weeks that woven bone was formed and that no lamellar bone was present. The green labels of calcein indicated that during the second 3 weeks, lamellar bone began to appear. But green labels were less extensive than the first yellow tetracycline labels, suggesting that lamellar bone formed more extensively during the third 3 weeks than during the second 3 weeks. The mean new bone formation rate (BFR/d) for the test group was 1.42 ± 0.21 μm/d, which was not significantly different from the control group. Roberts et al[19] considered that the BFR/d of lamellar bone in dogs was 0.6 μm/d, while that of woven bone was approximately 30 to 50 μm/d. Fluorescent microscope analysis found no evidence that the immediate loading of micro-implants impaired normal bone remodeling.

Because healing at implant sites is influenced by many factors, in particular by primary stability of the implants, osseointegration may not occur in all clinical situations. The patient's cortical alveolar bone may be less dense at some sites compared to that in dogs. Therefore, further studies in patients are needed on the stability and histology of immediately loaded micro-implants at different alveolar bone sites. However, other clinical and experimental studies[1–6] support the positive results of the present in vivo animal study.

CONCLUSION

When micro-implants were placed in the alveolar bone of mandibles in male beagle dogs and subjected to either immediate loading (200-g horizontal force) or allowed to heal unloaded, there were no clinically significant differences in their stability and histology. Immediate loading did not cause implant instability and did not impair normal osseointegration.

ACKNOWLEDGMENTS

This study was supported by grants from Medical Science and Technology Development Foundation of Jiangsu Province of China (no: Z200313), the National Natural Science Foundation of China (no: 30572068), and the Education Department of Jiangsu Province of China (no: 07KJD320149). The editorial assistance of Professsor Roger J. Smales, School of Dentistry, University of Adelaide, Adelaide, Australia, is gratefully acknowledged.

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