The effect of varying healing times on orthodontic mini-implant stability: a microscopic computerized tomographic and biomechanical analysis

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Objective. The aim of this study was to evaluate the effect of different healing times on the stability of titanium mini-implants used for orthodontic anchorage.

Study design. Eight male beagles were used and randomized into 4 groups according to different healing times (1, 3, 5, and 7 weeks); each group had 2 beagles. Sixty-four mini-implants were inserted bilaterally in the maxilla and mandible of the beagles. Microscopic computerized tomography (μCT) and pull-out test were used for morphometric and biomechanical analysis, respectively.

Results. All μCT parameters and Fmax (maximum pull-out force) increased with the prolongation of healing time. One week after insertion, all 4 measurements, namely osseointegration, trabecular bone volume density, intersection surface, and Fmax, were lower in the maxilla group than in the mandible group (P < .05). Between the span of 1 and 3 weeks after insertion, a more obvious rising tendency of the 4 values was observed in the maxilla group than in the mandible group. Five and 7 weeks after insertion, the maxilla group expressed higher values of the 4 measurements than the mandible group (P < .05).

Conclusions. Although insertion in the mandible could provide higher primary stability for mini-implants, with the prolongation of healing time, insertion in the maxilla achieves higher osseointegration. The results indicated that insertion in maxilla has a more positive effect on the stability of mini-implants than insertion in the mandible. (Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2011;112:423-429)

In the early 1960s, Branemark et al.1 noted the biocompatibility of titanium implants in bone tissue. Thereafter, the discovery of bone-to-implant contact by light microscopic examinations led to the development of the concept of “osseointegration.”2 Since then, researchers showed an increased interest in investigating the application of titanium implants in dentistry. Several studies reported an implant success rate of >90% in edentulous patients.3,4 The excellent long-term results of implants stimulated orthodontists to consider that dental implants could be used to reinforce orthodontic anchorage. In 1984, Roberts et al.5 suggested that titanium implants could provide firm osseous anchorage for orthodontics and dentofacial orthopedics. Many studies6-8 verified that hypothesis.

For the successful orthodontic treatment, the anchorage has to be well controlled. In contrast to some anchorage methods, such as a palate bar or extraoral headgear, mini-implants can provide effective anchorage without depending on the patient’s compliance.9,10 Although prosthetic implants can provide excellent anchorage, they also have many disadvantages, such as requiring good bone structure and a more complicated surgical procedure, a delay of several months before loading, limited insertion sites, and higher cost. To overcome these disadvantages, mini-implants, or microscrews, were introduced. Their benefits are obvious: minimal anatomic limitation for placement, allowing immediate or early loading, simpler placement and removal surgery, less discomfort after implantation, lower cost,11 and less requirement of the patient’s compliance.9,10 Therefore, mini-implants are more and more commonly used for anchorage enhancement. Nevertheless, the incidence of mini-implant loss caused by fracture or the loosening of screws is generally reported to be between 0% and 30%.9,12-14 This cannot be regarded to be satisfactory. It is also why a few
clinical and experimental studies have attempted to identify the factors improving mini-implant stability. The overall stability of mini-implants consists of primary and secondary stability. Earlier studies have proved that primary stability is critical for the stability of dental implants.15-17 Primary stability refers to the initial stability immediately after insertion and is mainly determined by cortical bone thickness around implants.18-20 Secondary stability is obtained by osseointegration,21 which represents the bone condition of the interface between bone and implant. Structurally, the maxilla has relatively less cortical thickness than the mandible.22,23 Several studies24-26 reported that the primary stability of implant inserted in the mandible is higher than that in the maxilla. However, some clinical studies8,27 reported that mini-implants eventually achieve even better stability in the maxilla. So which insertion site (maxilla or mandible) is more stable for mini-implants? So far, the literature does not provide a clear answer.

Therefore, the present study was designed to investigate the impact of insertion site (maxilla or mandible) and healing time on stability of titanium mini-implants by microcomputerized tomographic (μCT) analysis and pull-out test.

MATERIALS AND METHODS
Mini-implant placement
Eight male beagles (24 months in age, ~12.5 kg in weight) were supplied by the Experimental Animal Center of the Sichuan University. The veterinary records indicated that all of the beagles were healthy without malocclusion or periodontal diseases. All of the beagles in this study were handled according to an experimental protocol approved by the Bioethics Committee of Sichuan University.

The beagles were randomized according to a computer-generated randomization list in sealed opaque envelopes into 4 groups associated with different healing times (1, 3, 5, and 7 weeks), and every group had 2 beagles. Also, the animal backgrounds were blinded to the researcher during analysis. All surgical procedures were performed under systemic (1 mg/kg ketamine and 2 mg/kg intramuscular xylazine; North China Company, China) and local (2% lidocaine with 1:80,000 epinephrine) anesthesia. Sixty-four microscrews (with an outer thread diameter of 1.6 mm; Medicon Company, Tuttlingen, Germany) were prepared for implantation. Based on radiographs and the procedures stated in our previous study,28 the implant sites were chosen at places where 6 mm existed beneath the top of the alveolar crest: between the mesial and distal roots of the second premolar (P2) and the first molar (M1) bilaterally in the maxilla (Fig. 1, A) and mandible (Fig. 1, B) of the beagles.29 A guide drill (with an outer thread diameter of 1.2 mm) was used to mark the insertion site and ascertain the direction of insertion. For each maxilla and mandible, 4 mini-implants were inserted. Each animal received 8 mini-implants, which were not loaded with any force during the experiment. The mini-implants remained and the beagles were kept for various periods of time according to the protocol, before they were killed.

Specimen preparation
The animals were killed with a lethal dose of pentobarbital. Maxillas and mandibles with mini-implants were separated from the animals and carefully sectioned into small blocks, each containing 1 mini-implant, which was surrounded by ≥5 mm of bone without soft tissue (Fig. 2, A). All bone_mini-implant blocks were subsequently transferred into 10% buffered formalin at 4°C for fixation.

μCT assessment
After 2 weeks of fixation, the specimens were prepared for μCT investigation; the proximal 5 mm of the bone was examined by a μCT imaging system (μCT 80; Scanco Medical, Bassersdorf, Switzerland). The scan conditions were 70 kV with 300-ms integration time and 114 mA. Microtomographic slices were acquired with 1,000 projections at a spatial nominal resolution of 20 μm (Fig. 2) for a detailed qualitative and quantitative 3-dimensional evaluation, the images were reconstructed and analyzed by the SkyScan CT Analyzer (Kontich, Belgium; Fig. 3). The titanium and...
mineralized tissue were segmented from each other and from the bone marrow, including the immediate mini-
implant vicinity, by applying a multilevel thresholding procedure. The peri-implant trabecular bone (PIB) volume of interest included the entire trabecular compartment between the cross-sectional planes 1.0 mm around the microscrews. The following morphometric parameters were calculated in the PIB: trabecular bone volume density (BV/TV) and area of intersection surface (IS). Osseointegration (OI) was calculated as the ratio of IS to the surface area of the intraosseous mini-
implant.

Biomechanical test

After the μCT analysis, pull-out testing was performed by using a mechanical testing machine (AG-IS; Tensile Test Equipment, Kyoto, Japan; Fig. 1, C). The bone specimen was embedded in polymethylmethacrylate (Dental Products, Heraeus, Germany), vertical to the longitudinal axis of the mini-implant, with the mini-implant head was exposed so that the testing machine could tightly clamp the block and pull the mini-implant head by a jig (Fig. 1, D). For pull-out testing, the mini-implant was aligned with the axis of the testing machine to ensure that no bending moment was created during the test and that only axial pullout strengths were recorded. The mini-implants were pulled out at a crosshead speed of 0.05 mm/s. The applied load was monitored, and the peak load at extraction (maximum pull-out force, $F_{\text{max}}$) was obtained from the data file.

Statistical analysis

SigmaStat software (SPSS, Chicago, IL, USA) was used throughout. Differences in morphometric and biomechanical parameters were analyzed by Student $t$ test.
P values of <.05 were considered to be the level of statistical significance.

RESULTS

No mini-implant was found to be loose in either maxilla or mandible group. We generated high-resolution tomographic images with the aid of the μCT system, which could enhance x-ray transmission through the titanium implant. These images clearly depicted the bone-implant interface, identified bone in contact with the implant, quantified the OI. Thus, we demonstrated that OI was markedly enhanced in both maxilla and mandible with the prolongation of healing time before loading (Fig. 4).

One week after insertion, all 4 measurements, namely OI, BV/TV, IS, and F max, were lower in the maxilla group than in the mandible group (P < .05; Fig. 5). The average value of OI was 22.138% in the maxilla group and 26.667% in the mandible group (Fig. 5, A). In the pull-out test results, F max was 223.95 N in the maxilla group and <257.62 N in the mandible group (P < .05; Fig. 5, D).

Between the span of week 1 and week 3, a more obvious rising tendency of the 4 values was observed in the maxilla group than in the mandible group. Three weeks after insertion, the maxilla group expressed higher values of the 3 measurements than the mandible group except BV/TV, but there were no significant differences (P > .05; Fig. 5).

All 4 values went up with the prolongation of healing time in both maxilla and mandible groups. Five weeks after insertion, all 4 values were higher in maxilla group than in the mandible group (P < .05). The increase of OI and IS values in both groups slowed down at 5 weeks after insertion, whereas F max increased more quickly and BV/TV did not change greatly. Seven weeks after insertion, all 4 values were higher in the maxilla group than in the mandible group (P < .05). The average value of OI was 44.237% in the maxilla group, significantly higher than the value of 36.293% in the mandible group (P < .05). The pull-out test results in the maxilla group were 394.69 N, significantly higher than the value of 325.85 N in the mandible group (P < .05; Fig. 5).

DISCUSSION

Anchorage is the resistance to unwanted tooth movement and anchorage control is critical in orthodontics. Recently, mini-implant has been widely used as an absolute anchorage during orthodontic therapy. To ascertain the effect of different insertion sites in maxilla or mandible and healing time on the stability of orthodontic mini-implants, we used 8 male beagles and randomized them into 4 groups according to different healing times of 1, 3, 5, 7 weeks. Then, we inserted 8 mini-implants between the mesial and distal roots of the second premolar and first molar bilaterally in the maxilla and mandible of each beagle without applying any force throughout the experiment. After that we examined the stability of the mini-implant in maxilla or mandible at different time points, by μCT analysis and pull-out test.

The μCT analysis is nondestructive and allows investigating mechanical parameters with the same spec-
imen, thereby reducing the number of animals used for preclinical trials. However, μCT may underestimate the morphometric parameters when there is new bone and cartilage formation at the interface. It is able to comprehensively observe the interface between bone and implant in 3 dimensions, which is much more precise and convenient than traditional histomorphometry. The results of our study indicated that higher μCT parameters were measured with the prolongation of healing time. OI, BV/TV, and IS were all observed to be significantly higher in the mandible than in the maxilla at week 1. Because the rate of increase of these 3 parameters differed between the 2 groups, they were significantly higher in the maxilla group than in the mandible group at weeks 5 and 7 after insertion.

Both pull-out and torque removal tests are generally used in the fields of orthopedics, neurosurgery, and maxillofacial surgery to evaluate the biomechanical performance of implants. In the present study, the mini-implant we used is a kind of spiral implant which can effectively transform the pull-out force to shearing force and transfer the latter to the contacting bone. Therefore, the pull-out test was considered to be a better method than the torsion test to reflect the bone condition of the interface. Information on the biomechanical performance of these mini-implants is im-

Fig. 5. Four measurements at different healing times. A, osseointegration (OI); B, trabecular bone volume density (BV/TV); C, intersection surface (IS); D, maximum force ($F_{\text{max}}$) of pull-out test. Data are mean ± SD obtained at different healing times. The white column represents the maxilla group and the gray column the mandible group. Asterisks indicate statistical differences ($P < .05$).
portant to the clinicians because it can provide for better implant designs, fewer clinical failures, and improved guidelines for clinical use. Furthermore, because the µCT cannot show osteoid clearly, the biomechanical test is needed to verify the calculated parameters of µCT. In the present study, we attempted to measure the holding power of the mini-implant in maxilla and mandible at different time points by pull-out testing. We found that mini-implant inserted in mandible achieved better stability from the time immediately after insertion to week 1 in terms of F_{max}. With the prolongation of healing time, there was a greater rising tendency of mini-implant stability in the maxilla group than in the mandible group. In weeks 5 and 7, the stability of mini-implant in maxilla was significantly higher than in mandible.

Using high-resolution µCT-based morphometry and biomechanical testing, we demonstrated that mini-implant inserted in mandible could achieve higher primary stability than maxilla. This coincides with various other studies. The anatomic characteristics differ between the 2 jaws: maxilla has relatively thin cortices that are interconnected by a network of trabeculae. In contrast, the mandible is composed of thick cortices with more radially oriented trabeculae. The quantity of the bones, principally the cortical bone thickness, predominate the primary stability at the time of placement. While trabecular bone shows a poor degree of bone mineralization and helps little in the initial stability, the mandible, with a thicker bone cortex, was considered to be able to achieve higher initial stability.

The results of this study also indicated that the maxilla can provide higher overall stability for mini-implants than the mandible after 3 weeks of healing time. This might be due to the achievement of secondary stability. The reasons that mini-implant inserted in maxilla can gain higher secondary stability may be as follows. First, some studies suggest that the secondary stability of implant is correlated closely with osteogenesis of trabecular bone. The maxilla has a network of trabeculae, although the mandible is composed of radially oriented trabeculae. Compared with radially oriented trabeculae in mandible, the structure of a network arrangement in maxilla may be of more benefit to form larger contact areas between trabecular bone and the mini-implant. Second, because the intraosseous length of the mini-implant is definitive, the thicker the cortical bone is, the thinner the cancellous bone around the mini-implant will be, so the osteogenesis of trabecular bone around the mini-implant in maxilla should be more than that in the mandible. Third, the blood supply of maxilla is more abundant than that of mandible. The good blood supply can provide better material foundation for osteogenesis and osseointegration of mini-implant over a long period of time. In contrast, the posterior mandible is deficient of vascularization. Tolstunov referred to this zone as “ischemic zone.”

The results of this study show that during the first week, both the OI and the F_{max} of the titanium mini-implants was weak, but those in mandible had significantly higher values than those in the maxilla. After 3 weeks of healing time, integration of the titanium mini-implant was significantly stronger, in both the maxilla and the mandible. However, the rate of maxilla stability increase was greater than that of the mandible. These data suggested that immediate or early loading ought to have a more negative influence on stability of mini-implants in the maxilla than in the mandible. These data may serve as the basis for further clinical studies in humans to help orthodontists make optimal plans and achieve successful treatment outcomes.

In conclusion, although mini-implants in the mandible group obtained higher initial stability, with the prolongation of time, the maxilla can provide better eventual stability for mini-implants than the mandible.

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