Orthodontic Mini-Implant Stability under Continuous or Intermittent Loading: A Histomorphometric and Biomechanical Analysis

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ABSTRACT

Background: Both continuous and intermittent loadings are commonly applied in orthodontics. Clinical experiences and some studies believed that longer duration of force produce more effect (tooth movement, suture expansion, bone remodeling) than transient forces applied with the same magnitude. Alternatively, others indicated that interruption or recovery periods of various periods between loadings cause more bone remodeling and less root resorption. Therefore, which force is more favorable for osseointegration and stability of orthodontic mini-implant remains to be elucidated.

Purpose: To evaluate the influence of continuous or intermittent loading on stability of titanium mini-implants.

Materials and Methods: One hundred ninety-two mini-implants were implanted bilaterally in intraradicular zones of mandibular M1 and P2 in 48 beagles. Loadings were delivered consecutively in continuous group, pauses were given for the last 3 or 7 days of each 2-week reactivation period for intermittent group A and B, respectively. The group unloaded was set as control. After 2, 4, 6 and 8 weeks, the animals were sacrificed and microscopic computerized tomography (μCT), histomorphological observation and pull-out test were applied.

Results: The μCT parameters of mini-implants in four groups were gradually increased with loading time prolonged, while the value of peak load at extraction (F_{max}) increased and reached summit at week 6, but dropped slightly at week 8. In continuous group, all measurements were lower than those in intermittent groups at all time points ($p < .05$), and all values in intermittent group B were higher than those in intermittent group A. Histomorphology observation revealed different degrees of bone remodeling with new bone formation in the peri-implants region in different groups.

Conclusions: Intermittent loading regimen is more favorable for obtaining stability than continuous force.

KEY WORDS: continuous force, intermittent force, mini-implants, osseointegration, stability

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INTRODUCTION

Concept of osseointegration (OI) defined as direct contact between bone and implant on the light microscopic level was introduced in 1960s.[1] Thereafter, titanium implants have been widely applied in the clinical treatment of edentulous patients with a success rate of over 90%.[2,3] In addition, they were also used as an anchorage enhancement in orthodontics and dentofacial orthopedics.[4] Compared with traditional anchorage such as a palate bar or extraoral headgear, the major advantages of mini-implants are smaller in size, minimal anatomic limitation for placement, simpler implantation and removal surgery, lower medical cost and patient compliance requirement, as well as the possibility of immediate or early loading.[5] Nevertheless, a problem frequently encountered is loosening or even fall out of the mini-implants.[6,7] Their stability is determined by implant design, surgical technique and loading conditions.[8] And loading conditions (period, magnitude, direction, etc.) of mini-implants attract much interest. However, the data regarding loading period (continuous or intermittent force) of applied forces are rarely reported.

Apart from continuous loading, intermittent force is commonly applied in orthodontics. For example, face masks for maxilla protraction and palatal expanders are intermittently activated, and the forces applied by some orthodontic apparatus, such as rubber rings or elastic chains would dissipate rapidly during loading and thus be interrupted.[9–11] In most circumstances, it remains unclear which force is more favorable and is even not possible to distinguish between continuous and interrupted situations. It might be understandable that longer duration of force produce more effect (tooth movement, suture expansion, bone remodeling) than transient forces applied with the same magnitude, and this is evidenced by some studies.[12,13] Alternatively, interruption or recovery periods between loadings ranging from seconds to weeks have been shown to increase bone remodeling and recruit more osteoclasts.[14–16] Intermittent forces designed with a pause of 3 days before each 1, 2 or 3 weekly reactivation of the springs were reported to cause less root resorptions than continuous forces.[17,18] Although many researchers believe that discontinuous forces cause less resorption without compromising the effect of tooth movement, whether or not the loading protocols are beneficial for integration between bone and mini-implant are still ambiguous and merit investigation.

This study was designed to investigate the impact of continuous or intermittent forces on the stability of loaded mini-implants by micro-computed tomography (micro-CT) and pull-out tests.

MATERIALS AND METHODS

Animals and Mini-Implant Placement

Forty-eight beagles (aged 24 months; 24 male and 24 female; weight 12.5 kg on average) were supplied by Experimental Animal Center of Sichuan University, China. Veterinary records indicated that all beagles were healthy with no malocclusion or periodontal diseases. The study was approved by the Bioethics Committee of Sichuan University.

Using a computer-generated randomization method, beagles were assigned into four groups: continuous group, intermittent group A and B and unloaded control according to different loading regimens, and every group had 12 beagles. 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 1:80000 epinephrine) anesthesia. One hundred ninety-two mini-implants (6 mm height, 1.6 mm diameter, Medicon Company, Tuttlingen, Germany) were prepared for implantation. Referred to the map of safe zones for mini-implant implantation of beagle jaws,[19] the intraradicular zones of the mandibular first molar (M1) and second premolar (P2) were chosen. To avoid drilling in the mucosa, 4–5 mm incisions were made at the keratinized mucosa in the experimental regions. The surface of the mandible was surgically exposed by blunt dissection. All mini-implants were inserted through drilling method (Figure 1A). Firstly, a guide drill was used to ascertain the insertion site and angle. Then the mini-implants were inserted by handheld screwdriver up to no distance between bone and collar. Each animal received four mini-implants (Figure 2A) and they were immediately loaded with a force of 0.98 N by Nickel-titanium (NiTi) closed coil spring between the implanted pairs (Figure 2B). Forces were delivered consecutively in continuous group. In intermittent group A and B, pauses were given for the last 3 or 7 days of each reactivation period by loosening the ligature wires on mini-implants, respectively. For unloaded control, mini-implants were rested without force (Figure 3). For all the three loaded groups, the forces were reactivated and checked at 2, 4, 6, and 8 weeks. To ensure good oral hygiene, the beagles' oral cavities were locally rinsed with 2% chlorhexidine solution twice a day during the study.

Specimen Preparation

After 2, 4, 6, and 8 weeks of loading, three animals were selected randomly from each group and killed with a lethal dose of pentobarbital. Mandibles with mini-implants were removed from the animals and carefully sectioned into small blocks, each containing one mini-implant surrounded by at least 5 mm of bone without soft tissue. All bone/implant blocks were subsequently transferred into 10% buffered formalin at 4°C for fixation.

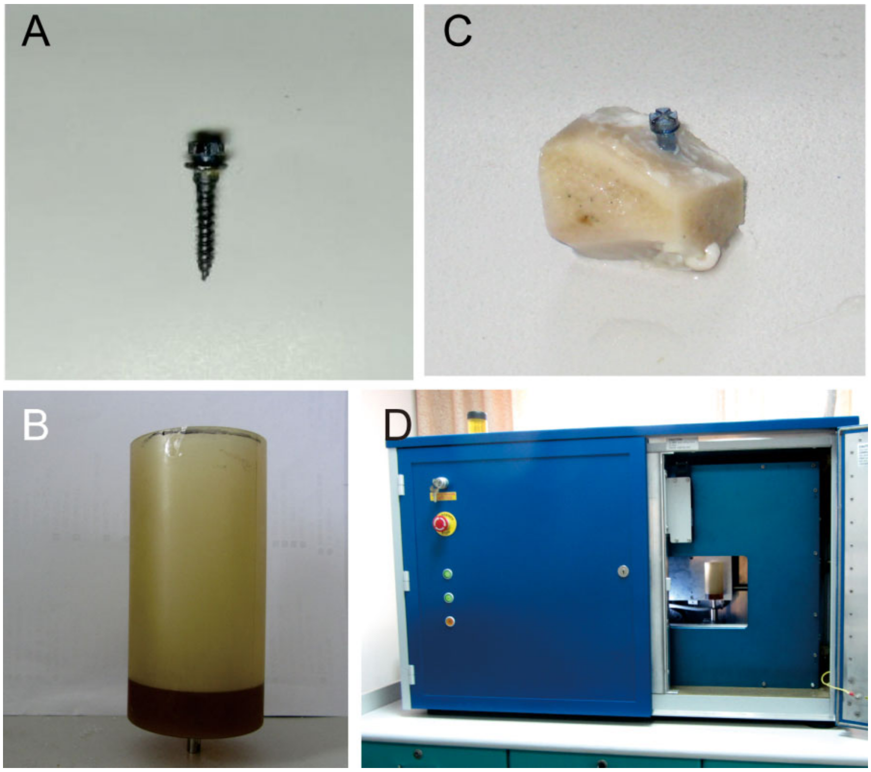

Figure 1 (A) The mini-implant; (B) sample container; (C) the specimen containing one mini-implant surrounded by 5 mm of bone, prepared for μCT investigation; (D) microscopic computerized tomography imaging system with specimen prepared for scanning.

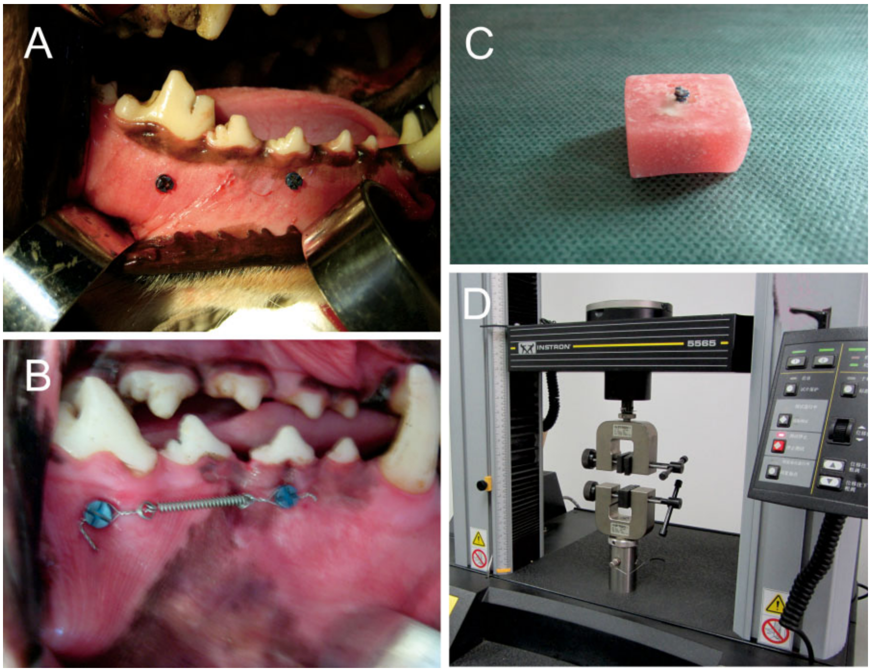

Figure 2 (A) Placement of mini-implants in intraradicular zones of mandibular M1 and P2; (B) loaded with a force of 0.98 N by closed coil spring between the mini-implants pairs; (C) blocks embedded in acrylic resin and prepared for pull-out test; (D) mechanical testing machine (Instron 5565, Instron Corporation, USA).

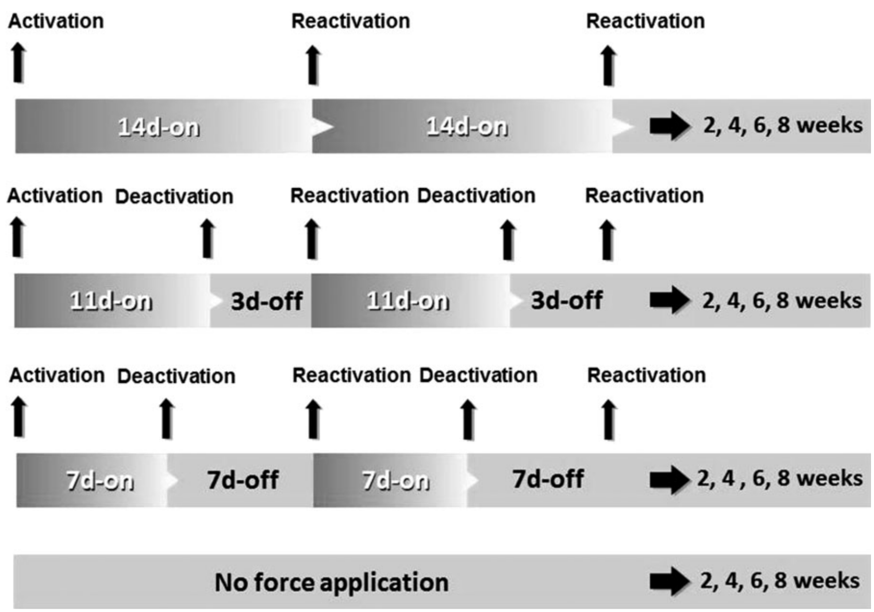

Figure 3 Force application protocol.

Micro-CT Assessment

Specimens were fixed for 2 weeks, and proximal 5 mm of the bone was examined by a micro-CT imaging system (CT80; Scanco Medical, Bassersdorf, Switzerland) (Figure 1). The scan conditions were 70 kV with 300 milliseconds integration time and 114 mA. Microtomographic slices were acquired at 1,000 projections at a spatial nominal resolution of 20 µm. For a detailed qualitative and quantitative three-dimensional evaluation, the images were reconstructed and analyzed by the CT-An (CT-Analyser; Skyscan, Kontich, Belgium) (Figure 4). The titanium and mineralized tissue were segmented from each other and bone marrow, including the immediate implant vicinity, by applying a multilevel thresholding procedure.[20] The peri-implant trabecular bone (PIB) volume of interest included the entire trabecular compartment between the cross-sectional planes 1.0 mm proximally and 1.0 mm distally from the implant's longitudinal axis. The following morphometric parameters were calculated in the PIB: bone volume density (BV/TV), trabecular thickness (Tb.Th), trabecular number density (Tb.N) and intersection surface (IS). The OI was calculated as the ratio of IS to surface areas of intraosseous mini-implant.

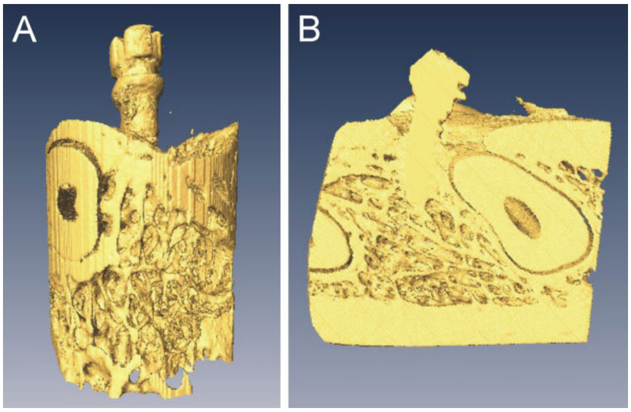

Figure 4 Three-dimensional constructed image by CT-Analyzer. (A) The mini-implant and peri-implant bone in volume of interest; (B) intraosseous surface of mini-implant.

Biomechanical Test

The pull-out test was carried out with Materials Test Systems (Instron 5565, Instron Corporation, Norwood, MA, USA) (Figure 2D) and a cross-head speed of 0.05 mm/second was applied. The applied load was monitored and the peak load at extraction (F_{max}) was obtained from the data file. Six bone/implant blocks in each group were selected out after 48 hours of fixation, embedded in polymethylmethacrylate (PMMA; Dental Products, Heraeus, Germany) (Figure 2C), with the mini-implants head exposed so that the testing machine could tightly clamp the block and pull the mini-implants head by a jig. Each specimen was modified to ensure that it was vertical to the longitudinal axis of the mini-implants, and mini-implants were aligned with the axis of the testing machine to ensure that no bending moment was created during the test and only axial pull-out strengths were recorded.

Histomorphology Observation

After 2 weeks of fixation, the rest osseous specimens of the 8-week loading group were dehydrated with gradient alcohol and chloroform by turn, and then embedded with methyl methacrylate. The embedded osseous specimens were sectioned in longitudinal direction parallel to the longitudinal axis of the mini-implant by a microtome (SP 1600; Leica Instruments, Nussloch, Germany). Three to four sections of 60 μm thick were obtained from each specimen. These sections were stained with 1% toluidine and observed by light microscope (DXM1200, Nikon, Tokyo, Japan) for a qualitative observation.

Statistical Analysis

Statistical analysis was carried out with the Statistical Package for Social Sciences (Windows v11.0, SPSS Inc., Chicago, IL, USA). Analysis of variance was used to evaluate the differences in morphometric and biomechanical parameters of the four groups. The Student-Newman-Keuls test (S-N-K) was used to investigate the differences between groups. *P*-values less than 0.05 were considered to be the level of statistical significance. Spearman or Pearson correlation coefficients were calculated to assess the relationship between insertion patterns, healing times, OI and F_{max} of the pull-out test. This coefficient test was used at the level of $p < .05$.

RESULTS

The survival rates of mini-implants were 100% for the four groups, two mini-implants in continuous group and one in intermittent group A were lightly loose, and the others remained stable throughout the study. The μCT images demonstrated that both OI and PIB density were markedly enhanced in continuous, intermittent groups and unloaded control with the prolongation of time (Figure 5).

As shown in Figure 6, all four measurements OI, BV/TV, IS and F_{max} were significantly lower in continuous group than in other three groups 2 weeks after placement ($p < .05$). The four values of intermittent groups are in the middle, and no significant differences were found between group A and B ($p < .05$). The mean value of OI was 24.31% in continuous group and 33.78% in intermittent group B (Figure 6A). For pull-out test, F_{max} was 302.94 N in continuous group and 361.26 N in intermittent group B (Figure 6D). From week 2 to 4, a similar rising tendency of four measurements was observed in all groups. Four weeks after placement, intermittent group B expressed significantly higher values of four measurements than continuous group ($p < .05$), while parameters in intermittent group A were lower than those of group B without significant differences ($p > .05$) (Figure 6). Six weeks after placement, OI, BV/TV, IS and F_{max} were still significantly higher in intermittent group B than in continuous group ($p < .05$), and only BV/TV was higher in group A than continuous group with significant difference ($p < .05$) (Figure 6). From a longitudinal view, continuous group exhibited a linear pattern of increase for tomographic and biomechanical measurements, while intermittent groups displayed a curvilinear-decelerating pattern of augmentation (Figure 6). Thus, the disparities between continuous and intermittent groups were smaller at week 6 than those at week 4. Eight weeks after placement, all values were still significantly higher in intermittent group B than in continuous group ($p < .05$), with the exception of F_{max}; while the differences between intermittent group A and continuous group were not significant ($p > .05$). Unloaded control still expressed higher values than the continuous and intermittent groups (Figure 6). Furthermore, the increasing tendency of OI, BV/TV and IS tampered, and

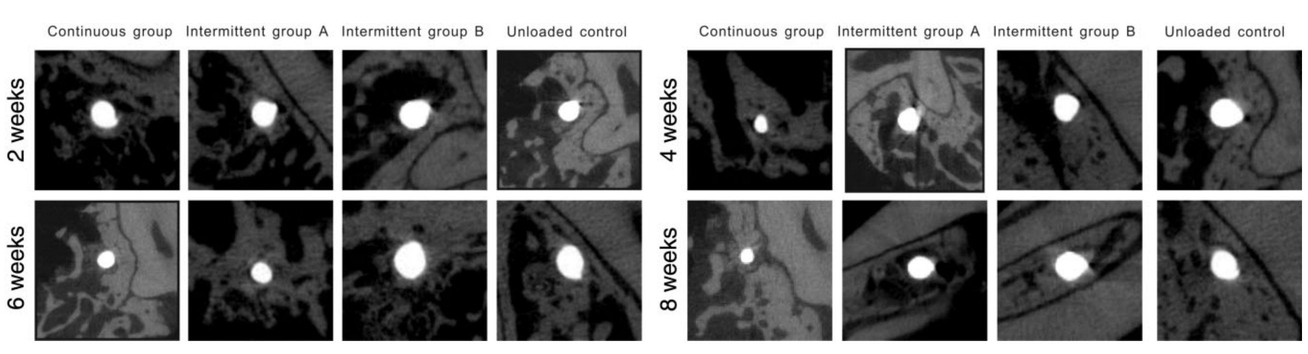

Figure 5 Peri-implant trabecular bone: representative specimens with median trabecular bone volume density values of different groups and healing times.

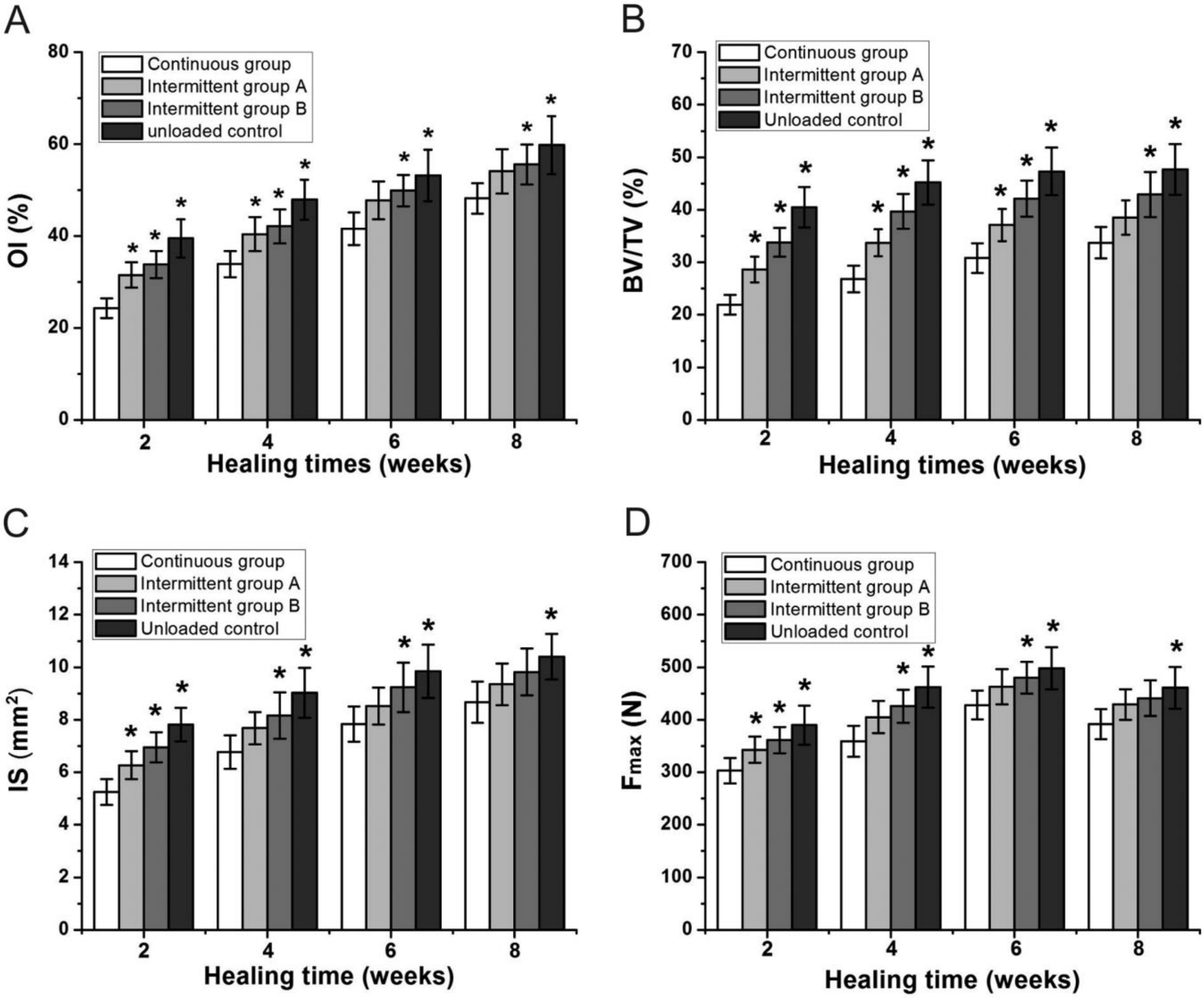

Figure 6 Four measurements of various groups at different healing times. (A) osseointegration (OI); (B) trabecular bone volume density (BV/TV); (C) intersection surface (IS); (D) maximum force (F_{MAX}) of pull-out test. Data are mean ± SD obtained at different healing times. The white, light gray, gray and dark gray columns represent continuous group, intermittent groups A and B and unloaded control. Asterisks indicate statistical differences between the groups they stand and the continuous group ($p < .05$).

a slight drop of F_{max} following the summit at week 6 was also observed. The average value of OI was 55.63% in intermittent group B, significantly higher than the value of 48.19% in the continuous group ($p < .05$) (Figure 6A). The results of pull-out test in continuous and unloaded control were 392.35 N and 461.76 N, respectively (Figure 6D).

For histomorphology observation, different degrees of bone remodeling in the peri-implant region were observed at all groups after 8 week of orthodontic loading. In continuous group, the obvious absorption lacunae filled with fewer collagen fibers, were observed in both cancellous and cortical bone around mini-implants while phenomenon of bone remolding, such as collagen fibers deposition and immature osteoid formation, were also observed. In intermittent group A, there were obvious absorption lacunae in both cancellous and cortical bone around mini-implants, but the collagen fibers deposition and immature osteoid formation were better than continuous group. In cortical bone regions, some new bone was found rounding the mini-implants. In intermittent group B, there were obvious collagen fibers deposited in bone trabecula, cortical bone and bone-implant interface, and newly formed Haversian system was found in cortical bone, which was inconsistent with direction of original lamellar bone. In unloaded control group, changes of cancellous and cortical bone were similar to group B with more maturely

TABLE 1 Correlation among Loading Patterns, Healing Times, Osseointegration (OI) of the Micro-Computed Tomography Analysis and F_{max} of the Pull-Out Test

	Loading Patterns	Healing Times	OI (%)	F_{max} (N)
Loading patterns			$r = 0.276$ $p = .029^*$	$r = 0.319$ $p = .036^*$
Healing times			$r = 0.594$ $p < .001^{**}$	$r = 0.263$ $p = .039^*$
OI (%)	$r = 0.271$ $p = .029^*$	$r = 0.537$ $p < .001^{**}$		$R = 0.371$ $p = .032^*$

r, Spearman correlation coefficient; *R*, Pearson correlation coefficient. $^*p < .05$; $^{**}p < .01$.

formed Haversian system. Active osteoblasts and immature osteoid were observed more than the other groups, non-mineralized collagen and newly formed woven bone were arranged around mini-implants (Figure 7).

All pairs of parameters in the correlation test demonstrated statistically significant differences ($p < .05$). Both OI and F_{max} were correlated with different loading patterns and healing times, the correlation coefficients were $r = 0.276$ or 0.319 for loading patterns, and $r = 0.594$ or 0.263 for healing times, denoting a moderate correlation between healing times and OI. The correlation coefficient between OI and F_{max} was 0.371, denoting a moderate correlation (Table 1).

DISCUSSION

Nowadays, the systematic comparison between continuous and intermittent forces is far from enough, and the lack of proper animal models mainly accounts. Both absolute anchorage and constant force are required to apply the force continuously or intermittently with same magnitude. Forces applied by some orthodontic apparatus, such as helical springs or rubber rings, would not be satisfactory because they dissipate rapidly during loading. In our model, NiTi closed coil spring could ensure the constancy of force.[21,22] Moreover, mini-implants could provide absolute skeletal anchorage and greater control of the forces generated by reciprocal anchorage.[23,24]

Our previous study demonstrated that the prolonged integration between mini-implants and surrounding bone took for an approximate duration of 5–7 weeks.[25] The experimental duration of present study was 8 weeks, covering the time the mini-implants require to obtain adequate OI and long-term stability for clinical application. Longer durations of 12 weeks were chosen in previous studies in which a similar loading design was used.[17] Moreover, the two weekly reactivation periods, and alternative frequencies of 11 days on and 3 days off or 7 days on and 7 days off are feasible for clinical revisits. Thus, patient-related cooperation problems were eliminated by operator-controlled intermittent force application in this study.

The nondestructive μCT analysis allows for comprehensive observation of the bone-implant interface within the same specimen three dimensionally.[19] Results of μCT study showed that augmented μCT parameters were measured with the prolongation of healing time. OI, BV/TV and IS were all observed to be highest in unloaded control, middle in intermittent groups, and lowest in continuous group throughout the experiment. Moreover, traditional histomorphology also verified that less absorption lacunae and more collagen fibers, immature osteoids were observed in intermittent groups than continuous group. Our data indicated that mini-implants could gain OI with the prolongation of time, no matter which loading pattern was applied and continuous force is not so beneficial for the establishment of bone-implant integration as intermittent forces. When compared between the intermittent groups, the 7/7-day loading regimen assumes more favorable for mini-implants to achieve OI than the 11/3-day loading cycle, although not apparently.

Information on biomechanical performance of mini-implants is also necessary for clinicians for better implant designs, as μCT may underestimate the morphometric parameters.[26] Using pull-out testing, we measured the holding power of mini-implants at different time points, and found that mini-implants in intermittent groups obtained better stability at any time points in terms of F_{max}. With time prolonged, there was a greater rising tendency of mini-implant stability in continuous group than in intermittent groups. In accordance with μCT results, the 7/7-day protocol is more benefit for obtaining biomechanical performance than 11/3-day cycle.

With increasing demand for higher clinical efficiency and shorter rehabilitation time, immediate and continuous activation of mini-implants is proposed.[27] However, strains at bone-implant interface caused by micromotions of immediately/continuously loaded

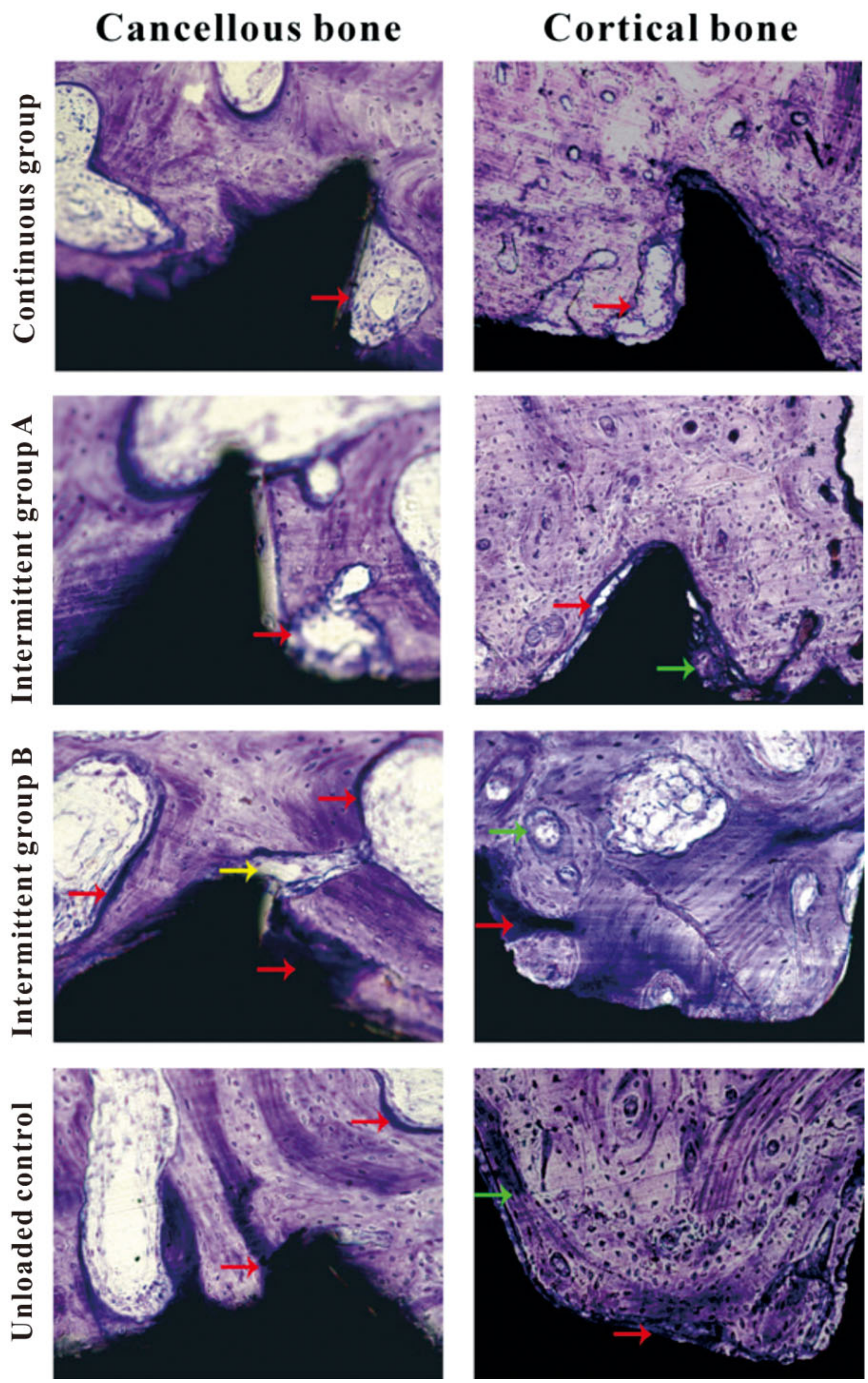

Figure 7 In continuous group, obvious absorption lacunae (red arrow) filled with fewer collagen fibers were observed in both cancellous and cortical bone around mini-implants. In intermittent group A, there were obvious absorption lacunae (red arrow) around mini-implants. In cortical bone regions, some new bone (green arrow) was found rounding the mini-implants. In intermittent group B, there were obvious collagen fibers (red arrow) deposited in bone trabecula, cortical bone and bone-implant interface, and the newly formed Haversian system (green arrow) was found in cortical bone, which was inconsistent with direction of original lamellar bone. In cancellous bone, there was some trabecular bone fractured due to the mini-implants insertion, and the phenomenon of bone remodeling was seen in the micro-fissures (yellow arrow). In unloaded control group, non-mineralized collagen (red arrow) and newly formed woven bone (green arrow) were arranged around mini-implants.

mini-implants at early stages may not only cause considerable root resorption, but compromise the OI. Thus, immediate or early loading (especially of high magnitude) should be avoided if primary stability is desired.[8,28] Conversely, low-intensity delayed loads may induce tolerated micromotion without affecting peri-implant mineralized bone formation.[29] Similarly, intermittent force is expected to be more favorable for mini-implant stability than continuous force, as they are maintained over a shorter duration, thus producing less micromotions and fractures around bone-implant interface to achieve higher OI at later stages.[8,29] The findings of present study that all the histomorphometric and biomechanical measurements were superior in intermittent groups over continuous group throughout the experiment verified our hypothesis, even though the disparities between them decreased with time prolonged.

In vivo, bone cells sense and respond to mechanical loading, but their sensitivity to the stimulus wanes quickly after initiation, which is called desensitization or mechanosensory saturation.[30] Thus, anabolic effects of mechanical loading diminished, and the osteogenic response also tends to saturate toward the end of a loading bout. Implicit in this phenomenon is the existence of a recovery period, which is confirmed to be important for restoring mechanosensitivity and maximizing osteogenic response of the desensitized bone cells to loading.[31,32] Therefore, introduction of recovery periods into orthodontic loading are postulated to be necessary for OI and stability of microscrews. In our study, we testified that 3–7 days of interbout recovery periods resulted in a higher OI and stability of mini-implants than continuous manner. Nevertheless, the optimal length of recovery periods between loading bouts, and the proper frequency of loading/resting alteration for orthodontic therapy are still poorly defined. Additional investigations in humans are needed to help orthodontists choose optimal loading protocol and achieve successful treatment outcomes.

CONCLUSIONS

In conclusion, mini-implants under intermittent loadings obtained higher OI and biomechanical stability than the continuous force, and the 7/7-day loading cycle is more favorable for bone-implant integration than the 11/3-day regimen.

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