Impact of Dynamic and Static Load on Bone Around Implants: An Experimental Study in a Rat Model

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Purpose: *This study aimed to evaluate peri-implant bone reactions to dynamic and static loads in a rat model.* ***Materials and Methods:*** *Two cylindrical titanium implants were placed in the left tibia diaphysis of 39 rats, which were divided into three groups: static load for 4 weeks (S4), static load for 8 weeks (S8), and static load for 4 weeks followed by dynamic load for 4 weeks (S4D4). All implants received a mechanical lateral load. After the experiment, the implants were extracted to determine the attachment strength around the bone and implant. The new bone formation and bone-to-implant contact were measured using plain and polarized light microscopy.* ***Results:*** *Histologic tissue analysis revealed good contact between the bone and implant, and new bone formation around all implants. The S4D4 group had the greatest attachment strength, new bone formation, and complex collagen fiber orientation in the new bone tissue, compared with the other groups. No statistically significant differences in bone-to-implant contact were observed among the three groups.* ***Conclusions:*** *Applying dynamic and static loads to osseointegrated implants increased the amplification of new bone. The attachment strength was significantly improved when dynamic load was used for 4 weeks, compared with when static load was used.* Int J Oral Maxillofac Implants 2016;31:e49–e56. doi: 10.11607/jomi.4372

Keywords: *animal experiment, dynamic load, implant attachment strength test, peri-implant bone, static load*

Infection and overload have been described as negative factors that can cause implant loss. In addition, recent studies have clearly indicated that infection is the major cause of peri-implant bone resorption, and further studies regarding peri-implantitis are expected in the future. However, the correlation between overload and bone resorption remains unclear.

Isidor[1] has reviewed the possibility of bone resorption due to overload in experimental studies, although the causal association between overload and bone resorption remained unclear in the clinical studies that were reviewed. Fu et al[2] reported that peri-implant damage was clearly caused by overload in their systematic review. In addition, they described a protocol to prevent biomechanical complications due to overload. In contrast, many reports have concluded that no correlation exists between overload and the resorption of peri-implant bone[3–5]; therefore, the evidence for any such mechanism remains unclear. Brunski pointed out that dynamic load in a physiologically tolerated range enhanced bone remodeling.[6] Interestingly, two primary studies have indicated that bone resorption due to overload is likely induced by inflammation, such as dental plaque.[7,8] However, the authors also mentioned the possibility of overload causing an increase in the peri-implant bone density when plaque is adequately controlled. Furthermore, Qian et al[4] clearly stated that they found no evidence that early peri-implantitis may be induced by overload alone, although they also stated that peri-implant bone resorption was induced by intricate combinations of multiple factors, such as the implant hardware, clinical handling, and patient characteristics. Similarly, Rungruanganunt et al[5] reviewed 36 publications (including clinical and animal studies), and reported that the impact of static load was related to misfit of the superstructures. These authors also mentioned that static load alone clearly did not negatively impact the implant's survival.

Intraoral loading designs are broadly divided into dynamic load[7,8] (such as occlusal load) and continuously static load,[9] which is associated with incompatibility of the superstructures. Therefore, it is very important to compare these types of overload when considering the occlusal style. Although many studies

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reported the effect of dynamic load alone (such as occlusal load in animals), or mechanically applied static load alone, on the peri-implant bone, few animal studies have compared both dynamic and static loads. Among these studies, Duyck et al[10] compared static and dynamic loads in rabbit tibias, and reported that significant resorption of the peri-implant bone was detected in the dynamic load group compared with that in the static load group, although no significant difference in the bone-to-implant contact ratio (BIC) was observed. Therefore, it appears that the lack of consensus regarding the correlation between implant loss and load is related to *(1)* the lack of a standardized implant design when evaluating overloading and *(2)* few studies having assessed standardized quantitative dynamic and static loads.

In recent years, most commercial implants have been the screw type (with threads), and these provide a superior initial fixation.

However, when measuring the attachment strength between the bone and implant, it is difficult to distinguish between osseointegration and the mechanical fitting force, as significant resistance may be related to the screw fit, even if osseointegration is absent. In addition, clinical reports have stated that implant loss is most common in the first year after surgical placement.[11] Although these reports have indicated that a history of periodontitis was the main causal factor, it is also possible that the implants did not achieve osseointegration due to technical difficulties that were encountered by the surgeon. For example, most implants can survive for several months simply because of the mechanical fit between the screw and bone. In addition, the period between implantation and osseointegration is shorter in rat models than in humans; therefore, it is very difficult to clearly identify real osseointegration. Several methods are available to confirm completed osseointegration, although the evidence is reportedly limited for all of these methods.[12] Therefore, most basic osseointegration should be judged using mechanically polished, straight titanium implants.

Many studies have reported overload due to occlusal load in animals and humans, and these models are favorable because they closely mimic the clinical style. However, it becomes difficult to objectively define the quantitative load and cycle that is administered. Furthermore, animal occlusal models require large animals, such as dogs and monkeys, making it difficult to incorporate large experimental groups, and resulting in highly variable mean values. Therefore, it is important to quantitatively assess the load protocol as a numerical value, rather than as an ambiguously qualitative load, and these specific numerical values would be helpful for future studies.

In this study, the authors constructed a basic rat tibial model to assess the peri-implant tissue morphology and attachment strength when a quantitative load was applied to well-osseointegrated, mechanically polished, straight titanium implants. In this model, the authors carefully ensured secure osseointegration, and completely eliminated inflammation and infection, which allowed them to evaluate only the bone's reaction to overload.

MATERIALS AND METHODS

Animals and Surgical Procedures

Thirty-nine Sprague-Dawley rats (12 weeks old, mean body weight: 450 ± 20 g) were used in this study. These rats were divided into three groups: static load for 4 weeks (S4), static load for 8 weeks (S8), and static load for 4 weeks followed by dynamic load for 4 weeks (S4D4). Eight weeks after the implant placement, all rats were sacrificed via a lethal overdose of intravenous sodium pentobarbital.

The experiments were conducted in compliance with the animal care guidelines laid out by the National Institutes of Health (NIH) and the institutional animal care committee of Tokyo Medical and Dental University. This study protocol was also reviewed and approved by the institutional animal care committee (0130200A).

Implant Placement

Two cylindrical, 99.5% commercially pure, titanium implants (diameter: 1.5 mm, length: 40 mm, Nillaco) were used for each rat. Before the placement, the experimental implants were degreased with acetone, rinsed with 70% ethanol, rinsed again with distilled water, dried at room temperature, and sterilized in an autoclave. The animals were then anesthetized using isoflurane (Isoflu, Abbott Laboratories), and a periosteal separation was made on the left medial side tibia to expose the bone. Two parallel implant holes (diameter: 1.6 mm) were drilled using a low-speed hand drill (Kyocera). All implants were placed bicortically, with both ends exposed approximately 10 mm outside of the skin, and the incision was subsequently closed with 6-0 nylon sutures. A healing phase of 4 weeks was set to complete osseointegration.

Static Load Device

The devices[10,13] for static loading were prepared by mounting four 0.98-N super elastic springs (TOMY), as shown in Fig 1. The two implants were connected to each other at both sides, which allowed a continuous static load of 3.92 N to be exerted.

Dynamic Load Device

The dynamic load was set using the following parameters: 1,800 cycles, 3.92 N amplitude, and 3 Hz frequency. An electromagnetic microtester (MMT-250N Shimadzu) was

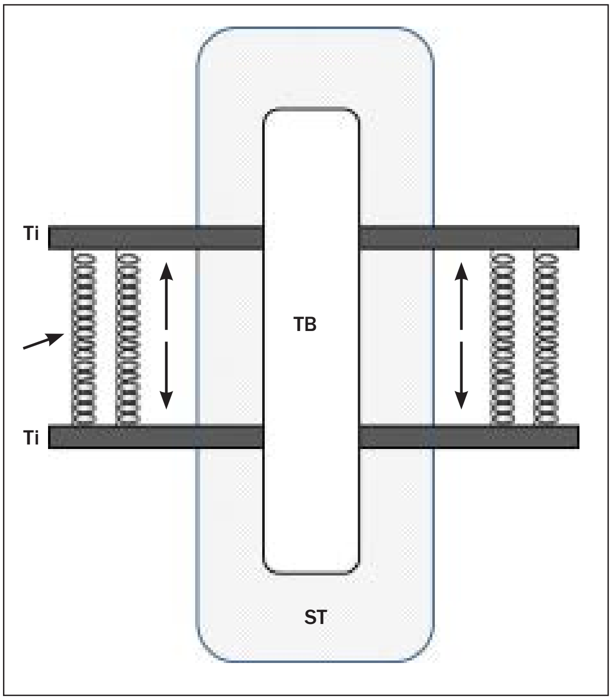


Fig 1 The experimental device for static loading. A total of four springs were mounted between the implants. Ti = titanium implant; TB = tibial bone; ST = soft tissue; Arrow = a 0.98-N super elastic open spring.

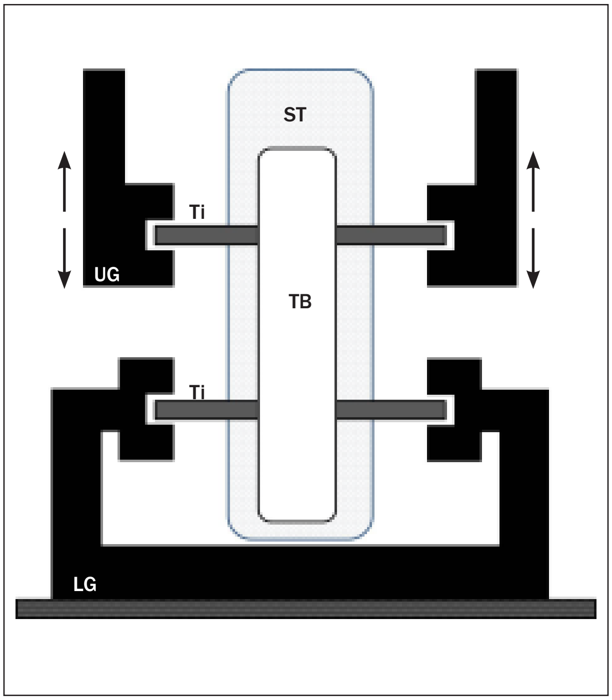


Fig 2 The experimental device for dynamic loading. Both ends of two implants were fixed by the device. Ti = titanium implant; TB = tibial bone; UG = upper grip; LG = lower grip; ST = soft tissue; Arrow = loading direction.

also used. For the dynamic load experiment,[10,13] each implant was fixed to the device for 10 minutes each day, as shown in Fig 2, and the actuator was used to apply the load to the implant. This loading was performed 5 days per week, over a total of 4 weeks.

Histologic Observations

Four weeks (S4) and 8 weeks (S8, S4D4) after the implant placement, all rats were sacrificed via a lethal overdose of sodium pentobarbital. Eighteen tibial cortical block specimens (S4: six specimens, S8: six specimens, S4D4: six specimens) of 20 mm around the implants were then dissected, and the soft tissue was removed from the bone. For histologic observation, the implants and surrounding bone tissues were fixed in 10% formalin immediately after harvesting. The samples were then dehydrated in a graded series of ethanol, defatted using xylene, and embedded in methyl methacrylate resin (Osteoresin, Wako). Thin sections (approximately 70 μm thick) were then prepared using a diamond disc microtome (SP1600, Leica Microsystems).

These sections were then stained with toluidine blue and observed under a light microscope (Eclipse 50i, Nikon) that was equipped with a digital camera (Digital Sight DS-SM5, Nikon). Quantitative morphologic evaluation of the histologic sections was also performed, and the length of the direct contact between the bone and implant (BIC) was measured. In addition, the sections were observed under a polarized light microscope, and the average thickness of the hypodermal tissues was determined in the tissues adjacent to the implant (radius: –500 μm). Furthermore, the new bone formation ratio (NBF) was estimated using the bone tissue on the implant surface.

BIC = Bone contact length (pixel)/Total length (pixel)

NBF = New bone area (pixel)/(New bone area + existing bone area) (pixel)

Evaluating Attachment Strength

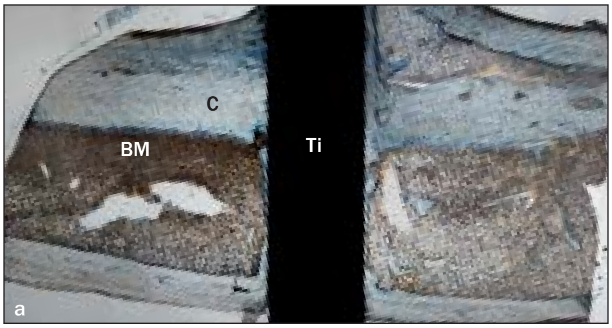
To evaluate the mechanical attachment strength between the implant and bone, 19 tibias (S4: six samples, S8: six samples, S4D4: seven samples) were evaluated using a material testing machine (Autograph AG-X, Shimadzu). The testing was performed at a crosshead speed of 5 mm/minute, and the load and displacement data were recorded in a computer. Using these data, the maximum force value was considered to be the attachment strength.

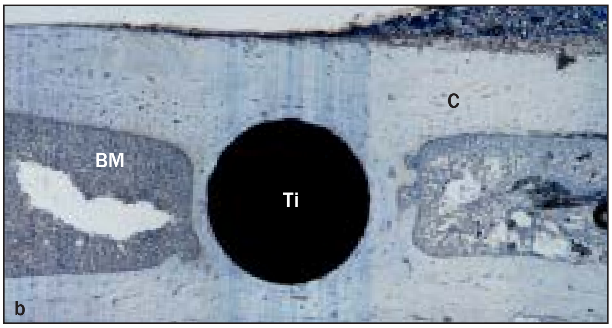
Statistical Analysis

All data were expressed as mean ± standard deviation, unless otherwise stated. The mechanical strength test results, BIC, and NBF were statistically analyzed using Welch's *t* test with Holm's correction for multiple comparisons. All statistical analyses were performed using "R" software (http://www.r-project.org/), and differences were considered statistically significant if the *P* value was $< .05$.

Fig 3 *(left)* A tibia dissected for histologic evaluation after cutting the extra-implant ends. Implants were fixed firmly, with healthy bone.

Fig 4 *(below)* Light microscopy observations using toluidine blue stain. (a) An axial cross section of the implant. *(b)* A horizontal cross section of the implant. The implant and surrounding bone have good contact, and amplification of the bone is found outside of both cortical bones. Ti = titanium implant; C = cortical bone; BM = bone marrow.





RESULTS

Macroscopic Observation

During the experimental period, the general condition of the rats and the tissues surrounding the implants were examined every day. At 4 weeks after the implant placement, two rats' implants had not developed osseointegration; therefore, they were excluded from the analysis. Based on this exclusion, the S4 and S8 groups included 12 rats, while the S4D4 group included 13 rats. However, after osseointegration, amplification was observed in the tibial bone surrounding the implants for all remaining samples (Fig 3).

Histologic Observation

Toluidine blue–stained tissue samples were observed via plain light microscopy (Fig 4), and the BIC between the implant and bone was measured (Fig 5). Polarized light microscopy was used to evaluate the NBF (Fig 6), and bone formation was observed inside and outside the bone in all samples from each group. The collagen fibers ran regularly inside the bone for all groups, although irregular collagen fibers were clearly visible in the new bone area; these changes were used to define the new bone and calculate its area.

The results of the BIC comparison are shown in Fig 7a, and approximately 50% bone contact was observed in all three groups. The BIC was 50.4% ± 34.8% in the S4 group, 44.3% ± 20.7% in the S8 group, and 50.9% ± 23.7% in the S4D4 group; these differences were not statistically significant.

The results of NBF comparison are shown in Fig 7b, and approximately 10% to 20% new bone was observed in the three groups (vs the preexisting bone). Specifically, the NBF value was 10.6% ± 4.10% in the S2 group, 11.9% ± 3.55% in the S8 group, and 17.1% ± 3.35% in the S4D4 group. Significant differences were observed between the S4D4 and S8 groups, and between the S4D4 and S4 groups.

Measuring Mechanical Strength

The result of the mechanical strength tests were 10.4 ± 4.81 N in the S4 group, 16.1 ± 7.35 N in the S8 group, and 28.9 ± 12.7 N in the S4D4 group (Fig 7c). Based on these values, a significant difference was observed between S4 and S4D4, and between S8 and S4D4 (both $P < .05$), although no significant difference was observed between S4 and S8. These results revealed a trend whereby the strength of the osseointegration increased when dynamic load was applied to the osseointegrated implant.

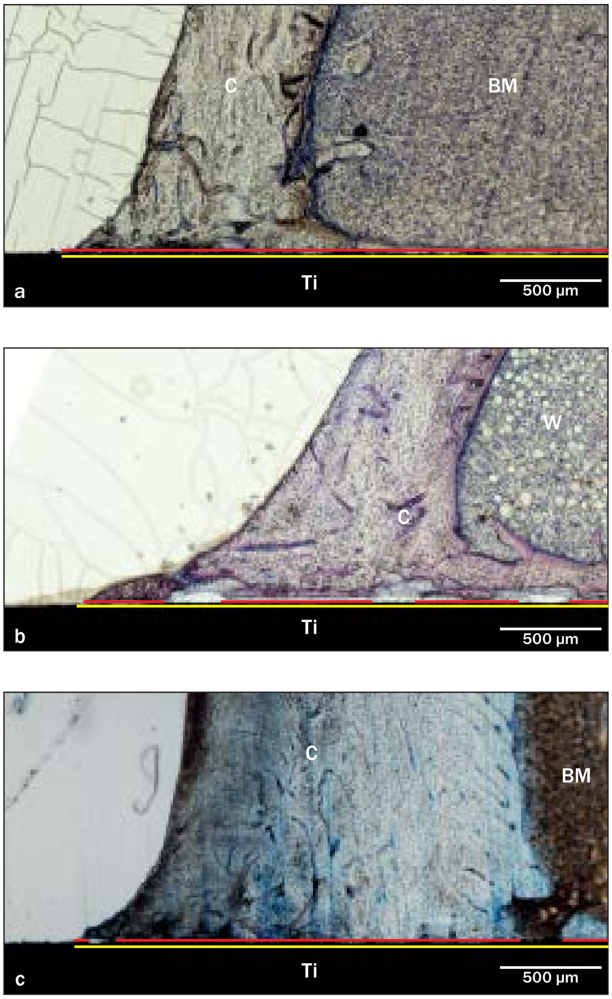


Fig 5 Light microscopy observations using toluidine blue stain. *(a)* S4, *(b)* S8, and *(c)* S4D4. The bone-to-implant contact ratio was measured using these samples, and the bone surrounding the implant exhibited good osseointegration for all the groups. Ti = titanium implant; C = cortical bone; BM = bone marrow; red line = bone contact length; yellow line = total length.

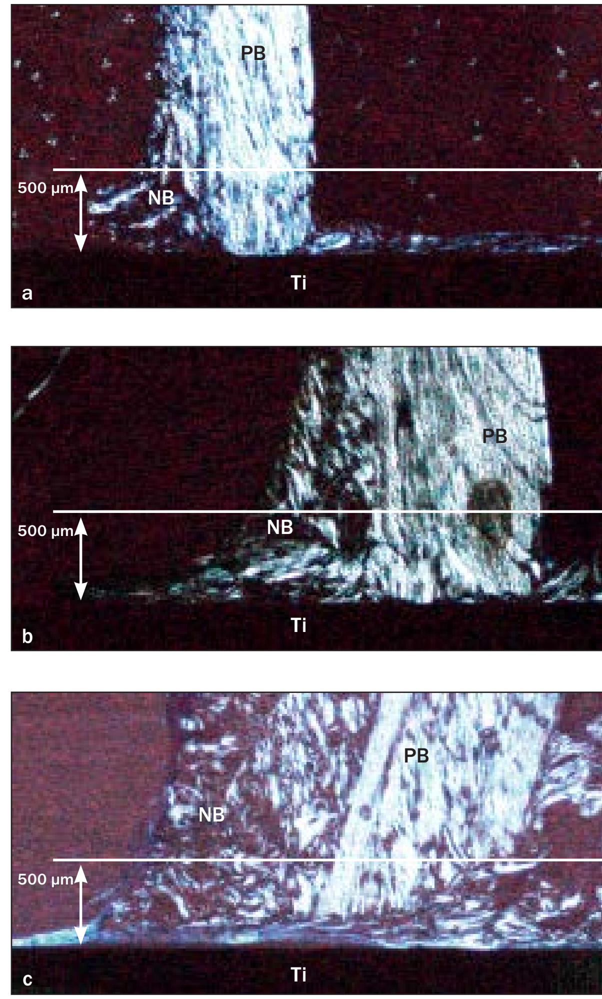


Fig 6 Polarized light microscopy observations using toluidine blue stain. *(a)* S4, *(b)* S8, and *(c)* S4D4. The new bone formation ratio was measured using these samples. Collagen fibers ran regularly in the preexisting bone for all groups, compared with irregularly in new bone. Fibers that were closer to the periphery of the implant ran more parallel in the S4 and S8 groups, whereas the pattern was mostly irregular in the S4D4 group, ranging from oblique to almost perpendicular. Ti = titanium implant; NB = new bone; PB = preexisting bone.

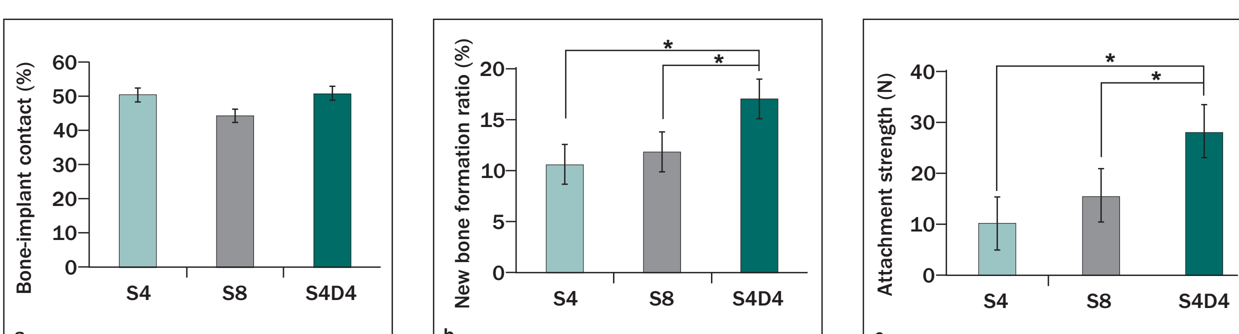


Fig 7 Bone-to-implant contact ratio (BIC), new bone formation (NBF) ratio, and attachment strength. *(a)* BIC (S4, S8, and S4D4: n = 6). No significant differences in BIC were observed among the three groups. *(b)* NBF (S4, S8, and S4D4: n = 6). *Significant differences were observed between S4 and S4D4, and between S8 and S4D4 ($P < .05$). *(c)* Attachment strength (S4 and S8: n = 6; S4D4: n = 7). *Significant differences were observed between S4 and S4D4, and between S8 and S4D4 ($P < .05$).

DISCUSSION

Many different research designs (eg, animal experiments,[7,8] clinical studies,[14] and finite element analyses[15] have been used to investigate the effects of load on implant status, and these studies have evaluated static load,[16] dynamic load,[17] occlusal load of arch[18] or tibia load,[19] and various load timings.[20] However, their results are conflicting, as several groups have reported a clear correlation between overload and peri-implant bone resorption, while others have reported no correlation between these factors. Therefore, the aim of this study was to assess the effect of different load types on osseointegrated implants, and the present study is the first report to directly and quantitatively compare static and dynamic loading using histologic and attachment strength assessment.

In the literature, several groups have reported that occlusal overload may increase bone density when plaque is well controlled.[3–5] The results of the present study support their conclusion that the loss of an osseointegrated implant is not directly caused by overload alone. However, their conclusions were based on various load types and animal sizes, as well as the use of occlusal loads (which are preferable when considering clinical situations). Furthermore, their data were qualitative, rather than quantitative; therefore, it is difficult to directly replicate their experiments and provide a direct comparison of the results. Thus, the authors of the present study selected quantitative measurements to assess the load and frequency, rather than the magnitude of the occlusal load, which is typically evaluated using qualitative methods.

Very few study designs have attempted to quantify dynamic load.[10,21] However, Hattori et al[22] have reported that the mean number of occlusal contacts is 26.1 sites in humans, that the mean magnitude of the occlusal force resultants is 776.7 N, and that the mean occlusal force magnitude on each occlusal contact is 26.2 N. In addition, Duyck et al[10] applied a static load of 29.4 N and a dynamic load of 14.7 N to rabbit tibias in their preliminary experiments. In contrast, the authors of the present study used rats, which are smaller than rabbits, to ensure an ample load could be applied, even compared with those used in the previous studies.

In this study, the authors hypothesized that overload on healthy osseointegrated implants could not be the only cause of implant loss. Therefore, they used threadless, mechanically polished, straight implants to eliminate any mechanical fit, which would allow them to focus exclusively on osseointegration. They actually did not observe any bone resorption for the implants that exhibited complete osseointegration and healthy peri-implant tissue. It should also be noted that, although a screw implant and straight one differ in their shape, they shared similar osseointegration processes, since both of the bone-implant interfaces were mainly subjected to the normal loads.

Using these experimental methods, dynamic loads were applied immediately after implant placement as a pilot study to investigate the effects on the peri-implant bone. In that study, inflammatory cells and soft tissues appeared all around implants at approximately 4 weeks, and none of the implants developed osseointegration, because of the poor initial fixation. In contrast, the S4, S8, and S4D4 groups (which were allowed to develop adequate initial fixation) developed adequate osseointegration. These results indicate that immediate loading of the implant (eg, when using threadless implants) may prevent initial fixation, thereby markedly inhibiting osseointegration. Similarly, a review by Esposito et al[23] concluded that a high insertion torque value and initial fixation are important factors for immediate loading of implants. Therefore, mechanical fit and osseointegration must be clearly differentiated in loading experiments, as initial fixation is one of the most important factors for achieving osseointegration.

No previous study has reported a mechanical strength resistance model using mechanically polished straight titanium implants as a method for assessing dynamic and static loads. In this study, the authors measured the attachment strength between these implants and bone in the S4, S8, and S4D4 groups, and observed significant differences between the S4D4 and S4 groups, and between the S4D4 and S8 groups (both $P < .05$). However, no significant difference was observed between the S4 and S8 groups. These results indicate that the bone around an implant reacts more strongly to dynamic load, compared with the reaction to static load. Baker et al[24] conducted a similar experiment using mechanically polished straight implants that were placed in rabbit tibias, and they reported a mean resistance force of 33.3 N, which is similar to the results of the present study. However, Zheng et al[25] reported a resistance of $>$ 300 N for a microscrew implant that was placed in beagles' jawbones for 8 weeks, which is much higher than the results of the present study. This discrepancy is likely attributable to the use of a screw implant, which provided a superior mechanical fit. Moreover, they used the jawbone of a beagle, which has a greater quantity of bone compared to a rat tibia, and this may also have contributed to the higher resistance. The authors of the present study suggest that the degree of the attachment strength between the implant and bone should be assessed quantitatively in an experimental model without any mechanical fit. From a clinical perspective, and based on these findings, it is highly possible that the occlusal force in the healthy oral environment contributes to amplification of peri-implant bone.

When considering the direction of the load, most stress is born on the neck of the implant, and bone microfractures typically occur in this region.[15] In addition, the lateral and oblique mechanical stress is greater than that exerted on the axis.[26] In this context, chewing stress frequently adds axial, lateral, and oblique loads to oral implants. Thus, lateral loads were intentionally applied in the present study, which resulted in new bone amplification around the implant, rather than implant loss. In the future, the authors plan to investigate the effects of oblique loads in an additional study.

Interestingly, the authors did not observe any significant difference in BIC between the three groups. However, the average BIC of 48.3% in the present study is similar to that reported in several previous studies.[27] This result indicates that there is no histologic change in the peri-implant bone, regardless of whether several loads are applied over 4 to 8 weeks. Similar to the results of the present study, many experimental studies have found no significant difference in BIC, regardless of the load type or loading time.[7,10] In contrast, several other reports have indicated a significant difference in BIC, due to loading time, between the load and no-load groups.[18,28] Therefore, further standardization in terms of experimental design is needed to provide reproducible experimental results.

Regarding NBF, the authors of the present study observed a significant difference between the S4 and S4D4 groups, and between the S8 and S4D4 groups (both $P < .05$). Although various designs have been used to evaluate bone amplification via polarized light microscopy, no significant change in the BIC and NBF of peri-implant tissues, regardless of the load type, was observed in one study.[29] Although that finding contradicts the results of the present study, further detailed studies are needed, given the limited number of quantitative reports.

Based on the collagen fibers' orientation, the polarized light microscopy analysis in the present study revealed notable difference in the new bone's properties. Interestingly, previous studies have reported that collagen fibers were oriented perpendicular or oblique to the direction of the load.[30–32] In addition, bone regeneration and collagen fiber orientation are likely related, regardless of the implant material or its surface properties.[33,34] In this study, the collagen fibers' orientation depended on the type of load that was applied. For example, the collagen fibers ran more oblique or perpendicular in the S8 group, compared with the fibers in the S4 group. In addition, the pattern was more complicated in the S4D4 group than in the S8 group. Furthermore, despite the absence of a significant difference in the BIC, the dynamic load group exhibited significantly greater mechanical strength resistance and NBF. Therefore, based on these results, it appears that the orientation of collagen fiber in new bone likely contributes to its mechanical strength.

CONCLUSIONS

Within the limitations of this study, the application of dynamic and static loads to a smooth osseointegrated titanium implant in a rat tibia did not affect the peri-implant bone resorption. New bone amplification was histologically observed around all the implants after osseointegration was achieved, and the attachment strength and NBF were significantly increased after applying dynamic load for 4 weeks. However, no differences in bone histologic structure were observed in the BIC when dynamic or static loads were applied. These results suggest that dynamic loading of implants may provide superior mechanical and tissue benefits, compared with static loading or no loading.

ACKNOWLEDGMENTS

The authors report no conflicts of interest related to this study.

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