Introduction: Mini-implants are often immediately loaded for orthodontic treatment; however, changes in interfacial tissues caused by early loading and its effects might compromise the mini-implant’s function. The purpose of this study was to compare the healing of interfacial tissues 1, 4, and 12 weeks after the placement of titanium-alloy mini-implants in New Zealand rabbits; some of the implants were loaded immediately and others were left unloaded. Methods: Eighteen animals were used in the experiment. Each received 4 titanium grade 5 mini-implants (2.0 × 6.0 mm), 2 of which were immediately loaded with 1 N of force. Tissue healing was verified at 1, 4, and 12 weeks after placement. Four different fluorescent molecules were injected into the rabbits to label calcium deposition. After the rabbits were killed, mineralized bone samples with the mini-implants were removed, fixed, cut, stained, and observed with bright-field, polarized, and fluorescence microscopy. Results: After 12 weeks of healing, higher bone contact and bone area were observed than after 1 or 4 weeks, regardless of loading. Differences between the loaded and unloaded groups were not observed (P < .05) at 1 and 4 weeks. The bone deposition rate was higher in the loaded group. Conclusions: The 1-N immediate force application did not compromise bone formation around mini-implants. (Am J Orthod Dentofacial Orthop 2010;137:80-90)
18 weeks was long enough for biologic fixation capable of resisting up to a load of 2 N for 24, 28, or 32 weeks. Aldikacı et al applied a 2-N load after 6 weeks of healing. They used a long loading period of 52 weeks, and the mini-implants maintained stability throughout the study. In 2005, some authors proposed an early loading protocol for mini-implants after just 1 or 2 weeks of healing. Yao et al suggested that 2 weeks should be enough for soft-tissue healing. Kim et al loaded the mini-implants after 1 week and had a 9% failure rate after 10 weeks of continuous loading.

Immediate loading has also been described in clinical studies. Park et al applied 2 N of force for 36 weeks immediately after placing mini-implants in posterior sites. They obtained a success rate of 90%. Motoyoshi et al using an immediate load in the same region and with the same force, had a success rate of 85.5%. The consensus in the early load protocol is that even experienced clinicians can have failures and that the removal of the mini-implant after the treatment is uneventful. Limited information is available about the effects of immediate loading on the biologic sequences of healing during the early phases of tissue integration, when the primary mechanical stability of mini-implants must be substituted for stability obtained through biologic means.

Our aim in this study was, therefore, to evaluate the interface reactions at early and late stages of osseointegration around mini-implants immediately loaded with 1 N. We used bright-field, polarized, and fluorescence microscopy for this evaluation.

MATERIAL AND METHODS

The protocol for the animal study was approved by the standing ethics committee on animal research of Oswaldo Cruz Foundation (Rio de Janeiro, Brazil), and all procedures were conducted in accordance with Canadian Council of Animal Care guidelines.

Eighteen 6-month-old male New Zealand white rabbits, weighing 3.0 to 3.5 kg, were used. The surgical procedures were common to all animals and consisted of placement of 4 mini-implants in the left tibial metaphyses of each animal. All surgeries were performed under sterile conditions in a veterinary operating room.

The titanium-aluminum-vanadium alloy mini-implants had a cylindrical screw design and a hexagonal head (6.0 × Ø 2.0 mm; Conexão Sistemas de Próteses, São Paulo, Brazil). They were machined by turning, cleaned, passivated with nitric acid, and sterilized by 25 Gy of cobalt radiation (Fig 1).

The rabbits were acclimatized for a month in a vivarium before the surgical procedure. Immediately before the surgery, the animals were anesthetized with intramuscular injection of tiletamine (5 mg/kg) and zolazepam (5 mg/kg) followed by continuous delivery of 2% halothane and isoflurane throughout the surgery. The hair on the medial surface of the upper portion of the left leg was clipped and the skin was cleansed with iodinate surgical soap. An incision approximately 50 mm in length was made parallel to the longitudinal axis of the tibia, in the medial aspect. The periosteum was elevated by using sterile surgical techniques, and the bone was denuded. Four implantation holes about 5 mm apart were drilled with a 1.6-mm drill under profuse sterile saline-solution irrigation and at low rotary speed. The mini-implants were threaded at the first cortex of the tibia with their longitudinal axes parallel to each other and perpendicular to the external cortical tibia. After placement, the 2 central mini-implants were immediately loaded with reciprocal forces. A nickel-titanium closed-coil spring was attached to the mini-implant’s head, providing 1 N of force. Then the periosteum was closed with resorbable sutures, and the skin was sutured.

After the surgical procedures, each animal had 4 mini-implants, 2 loaded and 2 unloaded, for a total of 72 mini-implants. Thirty mini-implants were used in this study, and the other 42 were analyzed by removal.
torque test and scanning electron microscope analysis in the first part of this study (Table I).

The experimental design was delineated to analyze 3 periods of healing: 1, 4, and 12 weeks. In each assessment period, there was 1 group loaded and another unloaded in a total of 6 groups (6 samples per group). In this manner, after each proposed time, 6 rabbits were killed by exsanguination.

Within 30 minutes after the rabbit’s killing, the placement surgical procedure described above was performed on each animal’s right leg; a new mini-implant per rabbit was placed for a total of 18 mini-implants. The maximum placement torque was measured with a manual torque meter (Table I). Thus, 48 mini-implants were used, from which 30 had the sequential interface reactions analyzed and 18 had the maximum torque insertion measured.

Quadruple polychromatic fluorescence labeling was performed after the second postoperative day (Fig 2). The sequential administration of fluorescent dyes varied between the groups and followed the topographic localization and the rate of new bone formation. The animals received intramuscular injections of oxytetracycline (15 mg/kg of body weight), calcein blue (30 mg/kg of body weight), alizarin-complexone (30 mg/kg of body weight), and xylene orange (90 mg/kg of body weight) diluted in 2% sodium carbonate solution.

After sample preparation, the slides were analyzed by using a fluorescence microscope (BX 51WI, Olympus, Tokyo, Japan) with filters with wavelengths of 450 to 490 nm (blue filter) and 340 to 380 nm (violet filter). The distance between the label marks was measured, and the mineral appositional rate (MAR) was calculated in micrometers per day.27 The MAR was measured in both sides of the unloaded samples and in the tension and compression sides of the loaded samples (2 measurements in each area).

After the healing period of 1, 4, or 12 weeks, the animals were killed, and the left leg of each rabbit was carefully sectioned and removed. The closed-coil springs were cut, and the tibia block specimens containing the mini-implants and at least 2 mm of surrounding tissue were dissected. The specimens were fixed (4% paraformaldehyde solution in 0.1 mol/L sodium phosphate buffer, pH 7.2), rinsed in the same buffer, and dehydrated in a graded ethanol series until absolute. Thereafter, the blocks were embedded in methylmethacrylate (Technovit 7100, Heraeus Kulzer, Dormagen, Germany) and sectioned in the longitudinal plane with a microtome (Exakt Medical Instruments, Oklahoma City, Okla). The thick slides were ground and polished to about 50 μm for fluorescent microscopic examination. Subsequently, the slides were stained with 2% toluidine blue for the bright-field microscopic examination and the histomorphometric measurements. Each tibia block sample was prepared to result in 1 central slide.

Histologic analysis was carried out by bright-field polarized light transmission microscopy. The microphotographic images were acquired by using 10, 20, and 40 objectives with a digital camera (EOS D-30, Canon, Tokyo, Japan) interfaced with a computerized system and the microscope. Before sample observation, a standard slide consisting of a linear gridline in micrometers was photographed for scaling the images. After obtaining the images, the measurements were made on each side of the bone within 1 mm² of the mini-implant surface, which corresponded to the bone interface at the first 2 screw threads. The Image J Launcher software (Java version 1.1.4, for Windows, Microsoft, Redmond, Wash) was used to measure and convert pixels to micrometers.

Two static variables were measured. The fractional bone-to-implant contact (% BIC) consisting of the linear bone-to-implant contact (μm) of the total of the mini-implant surface, which corresponded to the bone interface at the first 2 screw threads. The Image J Launcher software (Java version 1.1.4, for Windows, Microsoft, Redmond, Wash) was used to measure and convert pixels to micrometers.

Statistical analysis
Statistical analyses for reporting means and standard deviations of data from MAR, % BIC, and % BA were performed for all groups. To quantify the significance of the differences, the data were evaluated by 2-way analysis of variance (ANOVA) followed by the post-hoc Tukey test and the t test ($P < 0.05$).

RESULTS
The 48 mini-implants were placed without macrodeformation or fracture. The rabbits had no complications such as infection or leg fracture during the healing process.

All mini-implants monocortically placed into the rabbits’ tibia bones were clinically immobile after the
surgical procedure. At sample preparation, 3 mini-implants exhibited excessive clinical mobility and were considered lost samples, yielding an overall success rate of 90%. These 3 failed mini-implants were in the 1-week loaded, 4-week unloaded, and 12-week unloaded groups.

The maximum placement torque was between 108.02 and 84.40 N mm (mean, 98.33 N per millimeter; SD, ± 9.52 N mm).

By polarized light, no micrographic signs of chronic inflammation at the interface at any healing interval were observed. The analysis showed monocortical fixation for all mini-implants. Bone deformations and microfractures were observed primarily in the 1-week groups; even so, the native bone around the mini-implants was preserved throughout the experiment (Fig 3).

Corticalization was observed in some samples in the 4-week loaded group. The increase of cortical thickness was found only in the endosteal bone region, indicating primary bone formation in this area. Corticalization was also observed in the 12-week groups with periosteal and endosteal bone formation (Fig 4).

After 1 week of healing, the mini-implants were embedded into the bone with the top and the peripheral portions of the pitches of the screw in close contact with the native bone. The portions between the pitches were filled by wound tissue, and no histologic difference was found between the loaded and unloaded samples (Fig 5, A and B). Thus, the native lamellar bone was predominant, and it was not jeopardized by the immediate loading or the placement technique in this initial healing phase, providing mechanical stability for the mini-implants during the first healing period. In the 4-week groups, the relationship between the native bone, the mini-implant, and the interfacial tissue was maintained. The wound-tissue aspect was distinct between the loaded and the unloaded samples. The latter seemed to be less dense than the former, indicating a modified healing process (Fig 5, C and D). After 12 weeks of healing, there was significant bone formation between the threads of the mini-implant in both the loaded and unloaded samples. Then most of the region initially filled by wound tissue was replaced by newly formed bone. There was no difference in the histologic findings between the compression and the tension sides of the loaded mini-implants; however, the tendency of differences between the loaded and unloaded samples was maintained. The loaded samples exhibited more organized tissues (Fig 5, E and F).
The amount of osseointegration quantified by direct bone-to-implant contact (% BIC) and the area of bone observed between the threads of the screw (% BA) are listed in Tables II and III. The % BIC results ranged from 37.03% to 39.73% in the 1-week and 4-week groups, increasing to 66.01% and 70.96% after 12 weeks of healing (Table II). Similarly, the % BA results indicated significant increases in the 12-week groups.

**Fig 4.** Sequential corticalization: **A,** absence of bone deposition in endosteal or periosteal sides at 1 week; **B,** first sign in the endosteal region in the 4-week groups (*white arrows*); **C,** at 12 weeks, the increase of bone thickness was in both the endosteal and periosteal sides.

**Fig 5.** Histologic results. **A** and **B,** Interfacial tissue (*IT*) and preserved native bone (*NB*) in close contact with the mini-implant (*M-i*) surface; original magnification, 20 times. **C** and **D,** Slight difference in the interfacial tissue of the loaded and unloaded sample; original magnification, 20 times. **E** and **F,** Bone penetrating in the internal pitch area. Newly formed bone is more organized with lamellar aspect in the loaded group; original magnification, 40 times.
Table II. Histomorphometric values: % BIC rate

<table>
<thead>
<tr>
<th>Healing time</th>
<th>Unloaded</th>
<th>Loaded</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 week</td>
<td>39.73 ± 5.7ª</td>
<td>37.68 ± 6.1ª</td>
</tr>
<tr>
<td>4 weeks</td>
<td>37.03 ± 5.0ª</td>
<td>38.76 ± 3.4ª</td>
</tr>
<tr>
<td>12 weeks</td>
<td>66.01 ± 10.9b</td>
<td>70.96 ± 7.1b</td>
</tr>
</tbody>
</table>

Term: Two-way ANOVA interaction: 1 × 2
Load: Loaded × unloaded → P = 0.495
Time: 1w × 4w → 1w × 12w → 4w × 12w → P = 0.927

Mean values with the same superscript letters are not significantly different (P > 0.05). Mean values with different superscript letters indicate statistically significant differences (P < 0.05).

1w, 1-week groups; 4w, 4-week groups; 12w, 12-week groups.

Table III. Histomorphometric values: % BA rate

<table>
<thead>
<tr>
<th>Healing time</th>
<th>Unloaded</th>
<th>Loaded</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 week</td>
<td>66.40 ± 3.3ª</td>
<td>69.57 ± 2.6ª</td>
</tr>
<tr>
<td>4 weeks</td>
<td>64.45 ± 7.3ª</td>
<td>70.35 ± 3.7ª</td>
</tr>
<tr>
<td>12 weeks</td>
<td>86.13 ± 5.4ª</td>
<td>87.14 ± 4.2ª</td>
</tr>
</tbody>
</table>

Term: Two-way ANOVA interaction: 1 × 2
Load: Loaded × unloaded → P = 0.128
Time: 1w × 4w → 1w × 12w → 4w × 12w → P = 0.998

Mean values with the same superscript letters are not significantly different (P > 0.05). Mean values with different superscript letters indicate statistically significant differences (P < 0.05).

1w, 1-week groups; 4w, 4-week groups; 12w, 12-week groups.

Table IV. Comparisons of means of the tension and compression sides

<table>
<thead>
<tr>
<th>Time</th>
<th>Area</th>
<th>% BIC mean ± SD</th>
<th>% BA mean ± SD</th>
<th>% BIC</th>
<th>% BA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 week</td>
<td>Compression</td>
<td>37.10 ± 6.78</td>
<td>70.32 ± 3.25</td>
<td>P = 0.8105</td>
<td>P = 0.4576</td>
</tr>
<tr>
<td></td>
<td>Tension</td>
<td>38.27 ± 5.11</td>
<td>68.89 ± 2.29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 weeks</td>
<td>Compression</td>
<td>39.16 ± 4.85</td>
<td>72.56 ± 3.21</td>
<td>P = 0.7330</td>
<td>P = 0.0577</td>
</tr>
<tr>
<td></td>
<td>Tension</td>
<td>38.36 ± 1.43</td>
<td>68.14 ± 3.10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 weeks</td>
<td>Compression</td>
<td>70.52 ± 6.78</td>
<td>87.18 ± 2.23</td>
<td>P = 0.8578</td>
<td>P = 0.9780</td>
</tr>
<tr>
<td></td>
<td>Tension</td>
<td>71.40 ± 8.18</td>
<td>87.10 ± 5.98</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Test for independent samples

ranging from 64.45% to 70.35% after 1 and 4 weeks of healing, reaching 86.13% and 87.14% in the 12-week groups (Table III). The statistical analysis confirmed that, although there was no significant bone growth in the 1-week and 4-week groups, significant formation of new bone was indicated by both % BIC and % BA after 12 weeks, regardless of loading (Tables II and III). The compression and tension areas did not show significant differences at any healing time (Table IV).

A striking result came from the 2-way ANOVA analysis, which proved that the load did not enhance or impair the healing process in terms of the histomorphometric parameters (% BIC, P = 0.495; % BA, P = 0.128). Also, the healing time had a significant influence, in which the 12-week groups showed a relevant increase in bone around the mini-implants (% BIC, P = 0.0001; % BA, P = 0.0001) (Tables III and IV).

Fluorescence microscopy images showed a gradual increase of deposition of the dyes. In the 1-week groups, no specific marks could be seen in the bone around the mini-implants (Fig 6). Bone deformation and microfractures were clearly identified in the slides of this period. A weakly stained bone was observed after 4 weeks; however, some loaded samples had an unspecific alizarin-complexone dye, which was the last fluorescence label administered. This label was restricted to the endosteal region, indicating the beginning of the mineral deposition just in that region of the loaded sample. The 12-week groups showed well-defined labels in all peri–mini-implant bone, in both the endosteal and peri–mini-implant bone, in the endosteal and peri-osteal regions, allowing for rate measurements (Fig 6).

The regions labeled by fluorochromes were easily identified in all 12-week specimens (Fig 7); however, the calcein blue dye was not visible when the blue filter was used. It was visible with the violet filter. Nevertheless, because of the need to have all labels in the same view to proceed with the MAR calculations, these data were discarded. The statistical analyses showed significant differences between the unloaded and loaded groups (P = 0.024 and P = 0.008, respectively), whereas the tension and compression sides of the loaded group had no significant difference (P = 0.934) (Table V). This indicates accelerated healing in the loaded sample without a difference between the areas of tension and compression.

DISCUSSION

These findings indicate that immediate loading did not compromise the healing process in the tissues.
around the mini-implants, although the quality and the rate of bone formation had been altered. The analysis of the undecalcified sections provided an overview of the sequential healing evolution, since the primary mechanical stability of the mini-implants obtained by the friction and close contact with native bone until the late biological attachment provided by the new bone formation at the bone–mini-implant interface. The fluorescence analysis allowed for comparison of the effects of the immediate load application to the bone formation rate as well as to the topographic localization of the early healing events.

The first prerequisite for the success of implants is minimal damage to the host tissues during the surgical procedure.\(^1,2,5\) Copious irrigation to prevent superheating during drilling, preservation of the periosteal tissue, and maintenance of cleanliness are well-described precautions. Furthermore, Motoyoshi et al.\(^\text{17}\) described the recommended implant placement torque in the range of 50 to 100 N mm. They concluded that low values are insufficient for establishing mechanical stability, and high values could generate excessively high stress levels, resulting in degeneration of the interfacial bone. In our study, the mean placement torque was 98.3 N mm, indicating a good relationship between stability and interfacial stress. Additionally, all samples were clinically stable after placement, and the healing process resulted in osseointegration of most samples after the maximum experimental interval. Thus, we assumed that the surgical technique and the postsurgical conditions were suitable for the immediate loading sequential analysis.

Within the framework of the time of loading, the proposed healing interval before orthodontic force application has been gradually decreased since the first protocol described.\(^10,11,23,24\) Now some experienced research groups have described a success rate of about 90% with an immediate orthodontic loading

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**Fig 6.** Fluorescence microscopy. **A** and **B**, Longitudinal section of mini-implant and surrounding bone after 1 week of healing without dye deposits. Bone deformation (BD) and microfractures (MF) are shown in both the load and unloaded groups. **C** and **D**, the 4-week groups. Unspecific alizarin-complexone dye marked in the endosteal region of the loaded sample (A). **E** and **F**, the 12-week groups. Great level of fluorochrome labeling shown in all surrounding bone, regardless of the loading. CCS, Closed-coil spring; M-i, mini-implant. Original magnification, 4 times.
even so, they suggested that immediate loading could be used with no compromise in the stability of the mini-implants. In agreement, we found a 10% fail rate, and the lost samples were not related to immediate loading. On the contrary, Costa et al20 and Melsen and Costa 21 postulated that premature loading resulted in fibrous tissues in the interface. Szmukler-Moncler et al22 and Cope29 addressed these controversial conclusions by suggesting that micromotion is more detrimental than premature loading, and, when these forces are acting simultaneously, the results could be misinterpreted. Orthodontic loading could be applied up to threshold, above of which it would damage to the osseointegration process.16,22,29,30 The overloading limit depends on the quality and quantity of native and newly formed bone and on the implant design. Isidor,30 using finite-element analysis, concluded that loads resulting in interfacial strains higher than 6700 μm/day are harmful to the healing process. The transfer of stress at the bone–mini-implant interface after load application is influenced by implant design and surface geometry.31 Load concentration tends to occur at the first threads of the screw-design implants after lateral force application, possibly resulting in bone resorption around these areas.19,32 Büchter et al16 demonstrated that immediate tip forces higher than 9 N resulted in cervical resorption around the mini-implant or failure of the mini-implant. Oyonarte et al31,33 compared the bone response in the proximity of machined-threaded implants and porous-surface implants after orthodontic loading. They found significant cervical bone resorption around the machined-threaded implants and concluded that implant geometry resulted in high stress concentration and, consequently, bone loss. In our study, bone resorption around the first threads of the mini-implant after 1, 4, or 12 weeks of loading was not found. Because we did not apply a finite-element model to complement our in-vivo study, we could not correlate the loading areas with the histologic response, but it could be suggested that the 1 N force associated with the mini-implant design used in this study did not reach the overloading limit.

Hoshaw et al34 stated that the placement process of screw-shaped implants causes microdamage in the adjacent bone, and the damage concentration decreases after 4 weeks of healing. Despite the different dimensions and loading protocol, in our study, many microfractures and bone deformation in the 1-week groups were also found. After 4 weeks of healing, some were still present, but they were not found in the 12-week groups. The bone modeling and remodeling processes not only filled the interface with newly formed bone but also regenerated bone in the regions of damage. Regarding the histomorphometric findings, no significant statistical difference was found between the 1-week and 4-week groups in terms of % BIC and % BA values, regardless of the immediate loading. Similar to our previous study, in which the removal torque values increased in the 12-week groups, the % BIC and % BA means increased significantly after 12 weeks of healing; however, no statistical difference was found between the loaded and unloaded groups.35 Moreover, the areas under tension and compression also had no statistical difference, signifying that the immediate load did not influence the amount of osseointegration.

The increase of the static histomorphometric values (% BIC and % BA) after several weeks of healing is well described, not only for the delayed loading protocol but also for the immediate loading protocol.1,33,36,37 Nevertheless, bone formation at the areas of tension and compression remains controversial. Consistent with our findings, Wehrbein and Diedrich5 observed no micromorphologic differences between the compression and

**Table V.** MAR rate values compared with 1-way ANOVA and post-hoc Tukey test

<table>
<thead>
<tr>
<th>Group</th>
<th>Mineral appositional rate (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12wUn (n = 16)</td>
<td>2.96 ± 0.8 μm/day</td>
</tr>
<tr>
<td>12wLo compression (n = 10)</td>
<td>3.68 ± 0.5 μm/day</td>
</tr>
<tr>
<td>12wLo tension (n = 10)</td>
<td>3.78 ± 0.5 μm/day</td>
</tr>
<tr>
<td>ANOVA</td>
<td>P = 0.00035</td>
</tr>
<tr>
<td>Post-hoc Tukey test</td>
<td></td>
</tr>
<tr>
<td>12wUn vs 12wLo compression:</td>
<td>P = 0.024</td>
</tr>
<tr>
<td>12wUn vs 12wLo tension:</td>
<td>P = 0.008</td>
</tr>
<tr>
<td>12wLo compression vs 12wLo tension:</td>
<td>P = 0.934</td>
</tr>
</tbody>
</table>

12wUn, 12 weeks unloaded; 12wLo, 12 weeks loaded.
tension side of the implants. The implants were loaded after a healing period of 25 weeks. In addition, Melsen and Lang\textsuperscript{38} stated that the quantity of osseointegration was not influenced by the applied load (1-3 N) after 12 weeks of healing. On the contrary, Büchter et al.\textsuperscript{37} in an immediate loading study, analyzed bone response around 2 geometrically different mini-implants loaded with tip forces varying between 1 and 9 N. They concluded that forces of 5 or 6 N resulted in increased bone–mini-implant contact. The force variation depended on the geometry of the mini-implant. Wehrbein et al.\textsuperscript{7} suggested that bone generation activity close to implants could be influenced by the magnitude of force and the quality of the bone. They concluded that the 1-N force did not cause an increase in bone deposition in alveolar bone, but 2-N forces resulted in moderate bone apposition in the midsagittal palatal bone, especially in the areas under compression. Such bone formation depends on several clinical factors including the magnitude of the force, the quality of the supported bone, and the geometry of the implant. The different experimental protocols used in these studies could explain the varied findings.

The most interesting finding of our study was related to the bone deposition rate. The rate was significantly higher in the loaded implants than in the unloaded implants after 12 weeks ($P = 0.024$ and $P = 0.008$, respectively). The areas under compressive and tensile stresses were not statistically different ($P = 0.934$). The bone regeneration process could be accelerated by early biomechanical stimulation. This was suggested by Sarmiento et al.\textsuperscript{39} who provided experimental radiographic, histologic, and mechanical evidence during the fracture healing process. In the implant field, Rubin et al.\textsuperscript{40} tested the early biomechanical stimulation in porous titanium-alloy implants and found that exposure to low-amplitude mechanical strain during the healing period enhanced precocious biologic fixation. In addition, increased bone deposition has been found in the bone around the loaded implants when compared with bone surrounding unloaded implants.\textsuperscript{5,7,8,27,33} In this study, the bone deposition rate was higher in the loaded groups. Hence, it can be suggested that healing was accelerated by early loading. Supporting this suggestion, the woven bone produced along the endosteal surface is the primary response to the cortical healing defects, and, in our study, some endosteal corticalization was seen in the loaded samples after 4 weeks.\textsuperscript{2,41,42} Moreover, fluorescence alizarin-complexone dye was observed in the endosteal areas of these samples.

However, the accelerated healing process did not result in greater osseointegration (% BIC or % BA) in the loaded group after 12 weeks. Huja and Roberts\textsuperscript{27} described the continuous and accelerated remodeling process within 1 mm of the loaded implant surface as a possible mechanism whereby the loaded implant maintains the integration with less mineralized bone. A high rate of bone remodeling produces incompletely mineralized lamellar tissue in contact with the implant surface. This mechanism has been suggested to be important to prevent microdamage and crack accumulation at the interface.\textsuperscript{22,27,33,43,44} Thus, it could be suggested that immediately load influenced the quality of the newly formed bone, quickly producing less mineralized bone. This could be correlated with the findings of the first part of this study, in which a lower removal torque value was found in the 12-week loaded group compared with the unloaded 12-week group.\textsuperscript{35} The well-described easy removal of mini-implants in clinical studies could be also related to this suggestion.\textsuperscript{9,25,45} Quantification of the bone mineral content in bone-tissue growth under immediate loading conditions is necessary to confirm this hypothesis.

Self-drilling mini-implants have recently been described.\textsuperscript{10} Self-tapping mini-implants were used in this study. The choice between these 2 mini-implant types should be made carefully. The main differences between them are the immediate postsurgical conditions: the self-drilling mini-implant has more bone–mini-implant contact and better primary stability. On the other hand, the implant placement torque could be higher depending on the characteristics of the host bone. It could generate a high stress level, resulting in local ischemia and necrosis of the bone at the interface.\textsuperscript{17} Thus, the results of this immediate-loading study should not be extrapolated to self-drilling mini-implants.

Some important features—eg, soft-tissue inflammation around the mini-implants, exposure to the oral environment, and variations in cortical thickness—can influence the healing process but were not considered in this animal study. So, the extrapolation of these results to clinical applications should be done with caution. Our data showed that osseointegration was reached regardless of immediate loading. It can be suggested that the immediate loading protocol resulted in accelerated healing and modified new bone formation without jeopardizing the stability of the mini-implants during the experimental period.

CONCLUSIONS

1. The titanium-aluminum-vanadium mini-implants were appropriate as anchorage for 1 N of immediate loading.
2. The immediate 1-N load caused no significant changes in the amount of osseointegration

The most interesting finding of our study was related to the bone deposition rate. The rate was significantly higher in the loaded implants than in the unloaded implants after 12 weeks ($P = 0.024$ and $P = 0.008$, respectively). The areas under compressive and tensile stresses were not statistically different ($P = 0.934$). The bone regeneration process could be accelerated by early biomechanical stimulation. This was suggested by Sarmiento et al.\textsuperscript{39} who provided experimental radiographic, histologic, and mechanical evidence during the fracture healing process. In the implant field, Rubin et al.\textsuperscript{40} tested the early biomechanical stimulation in porous titanium-alloy implants and found that exposure to low-amplitude mechanical strain during the healing period enhanced precocious biologic fixation. In addition, increased bone deposition has been found in the bone around the loaded implants when compared with bone surrounding unloaded implants.\textsuperscript{5,7,8,27,33} In this study, the bone deposition rate was higher in the loaded groups. Hence, it can be suggested that healing was accelerated by early loading. Supporting this suggestion, the woven bone produced along the endosteal surface is the primary response to the cortical healing defects, and, in our study, some endosteal corticalization was seen in the loaded samples after 4 weeks.\textsuperscript{2,41,42} Moreover, fluorescence alizarin-complexone dye was observed in the endosteal areas of these samples.

However, the accelerated healing process did not result in greater osseointegration (% BIC or % BA) in the loaded group after 12 weeks. Huja and Roberts\textsuperscript{27} described the continuous and accelerated remodeling process within 1 mm of the loaded implant surface as a possible mechanism whereby the loaded implant maintains the integration with less mineralized bone. A high rate of bone remodeling produces incompletely mineralized lamellar tissue in contact with the implant surface. This mechanism has been suggested to be important to prevent microdamage and crack accumulation at the interface.\textsuperscript{22,27,33,43,44} Thus, it could be suggested that immediate loading influenced the quality of the newly formed bone, quickly producing less mineralized bone. This could be correlated with the findings of the first part of this study, in which a lower removal torque value was found in the 12-week loaded group compared with the unloaded 12-week group.\textsuperscript{35} The well-described easy removal of mini-implants in clinical studies could be also related to this suggestion.\textsuperscript{9,25,45} Quantification of the bone mineral content in bone-tissue growth under immediate loading conditions is necessary to confirm this hypothesis.

Self-drilling mini-implants have recently been described.\textsuperscript{10} Self-tapping mini-implants were used in this study. The choice between these 2 mini-implant types should be made carefully. The main differences between them are the immediate postsurgical conditions: the self-drilling mini-implant has more bone–mini-implant contact and better primary stability. On the other hand, the implant placement torque could be higher depending on the characteristics of the host bone. It could generate a high stress level, resulting in local ischemia and necrosis of the bone at the interface.\textsuperscript{17} Thus, the results of this immediate-loading study should not be extrapolated to self-drilling mini-implants.

Some important features—eg, soft-tissue inflammation around the mini-implants, exposure to the oral environment, and variations in cortical thickness—can influence the healing process but were not considered in this animal study. So, the extrapolation of these results to clinical applications should be done with caution. Our data showed that osseointegration was reached regardless of immediate loading. It can be suggested that the immediate loading protocol resulted in accelerated healing and modified new bone formation without jeopardizing the stability of the mini-implants during the experimental period.

CONCLUSIONS

1. The titanium-aluminum-vanadium mini-implants were appropriate as anchorage for 1 N of immediate loading.
2. The immediate 1-N load caused no significant changes in the amount of osseointegration
(histomorphometric parameters) after 1, 4, or 12 weeks of healing ($P < 0.05$).

3. The bone deposition rate was higher in the loaded groups than in the unloaded groups, indicating accelerated healing.

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