

# OSTEOCLAST ACTIVITY AROUND LOADED AND UNLOADED IMPLANTS: A HISTOLOGICAL STUDY IN THE BEAGLE DOG

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## KEY WORDS

Bone resorption  
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The mechanisms of bone loss around dental implants are poorly understood. The osteoclast is the most important bone-resorbing cell. Humoral factors seem able to stimulate the differentiation of osteoclasts from mononuclear phagocytes. Bacterial lipopolysaccharides seem to be directly involved in inflammatory bone loss by stimulation of the survival and fusion of preosteoclasts. Excessive load seems to be able to cause bone loss.

The aim of this paper was to evaluate the presence and number of osteoclasts in peri-implant bone in control (unloaded) and test (loaded) implants in order to determine if loading per se could be a contributing factor in peri-implant bone resorption. Forty-eight implants were inserted in the mandibles of 4 beagle dogs. After 3 months, a prosthetic superstructure was inserted on 24 implants, whereas in 24 implants only the healing screws were positioned. Twenty-four implants (12 test and 12 control) were retrieved at 6 months, and 24 implants (12 test and 12 control) were retrieved at 12 months. All implants were osseointegrated. The number of osteoclasts found in the crestal bone in the first 3 mm from the implant surface was evaluated. The mean number of osteoclasts were the following: control implants (6 months),  $5.66 \pm 0.81$ ; control implants (12 months),  $2.55 \pm 1.05$ ; test implants (6 months),  $5.25 \pm 1.55$ ; and test implants (12 months),  $2.5 \pm 1.0$ . No statistically significant differences were observed between the control and test implants. According to our data, loading does not seem to have a relevant importance on the osteoclast activation in peri-implant bone.

## INTRODUCTION

The precise mechanisms of bone loss around dental implants are poorly understood.<sup>1</sup> Osteoblasts and osteoclasts are involved in adaptive modeling and remodeling; these cells are able to sense their mechanical environment and to regulate deposition or resorption of

bone matrix.<sup>2</sup> Modeling changes the amount of bone and determines its geometrical form in relation to the prevailing mechanical loads and their resulting deformations (strains).<sup>3</sup> Remodeling renews existing bone in a sequence of resorption and formation; strain distributions occur during the remodeling process and show a rela-

relationship to the activity of osteoblasts and osteoclasts.<sup>3</sup>

The osteoclast is the most important bone-resorbing cell, and it derives from the monocyte/macrophage lineage.<sup>4-6</sup> Bipotent osteoclast precursors, which are able to form both osteoclasts and monocyte-macrophages, differentiate and become unipotent osteoblast precursors, and these cells fuse together to form the multinucleated osteoclasts, which are cells activated to start bone resorption.<sup>4,7</sup> Osteoclasts highly express the alpha-beta<sub>3</sub> integrin, which binds to a variety of extracellular matrix proteins including vitronectin, osteopontin, and bone sialoprotein.<sup>8</sup> These molecules tend to be located in the sealing zone of actively resorbing osteoclasts, and they seem to have a role in linking the adhesion of osteoclasts to the bone matrix with the cytoskeletal organization and the polarization and activation of these cells for bone resorption.<sup>8</sup> Different factors, local and systemic, may modulate the formation of osteoclasts, and consequently the extent of pathological bone resorption.<sup>7</sup> An increase in the number of mature bone-resorbing osteoclasts from macrophages is one of the cellular mechanisms that produce pathological bone resorption.<sup>7</sup> Humoral factors that stimulate the differentiation of osteoclasts from mononuclear phagocytes also are important in influencing the extent of this bone resorption.<sup>7</sup>

Bacterial infection causes significant morbidity mediated in part by the up-regulation of inflammatory cytokines, and cytokine induction is thought to stimulate osteolysis in periodontal disease.<sup>3</sup> Bacterial lipopolysaccharides seem to be directly involved in inflammatory bone loss, stimulating the survival and fusion of preosteoclasts.<sup>9</sup> Moreover, potential periodontal pathogens have been found to be able to stimulate bone resorption locally when placed beside a bone surface; these data may support their role in the pathogenesis of bone loss in periodontitis.<sup>1</sup>

Little is known about the impact of

loading on the peri-implant bone.<sup>10</sup> Excessive load seems to be able to cause bone loss through the induction of bone microdamage.<sup>10-13</sup> Functional loading provides a site-specific signal for the regulation of bone mass and morphology.<sup>14</sup> One of the forces produced during skeletal loading is hydrostatic pressure.<sup>15</sup> Increased bone resorption was found around loaded implants, and crestal bone resorption was related to overload and damage of the supporting interfacial bone.<sup>16,17</sup>

The aim of the present work was to evaluate the presence and number of osteoclasts in peri-implant bone in control (unloaded) and test (loaded) implants to see if loading per se could be a contributing factor in peri-implant bone resorption.

#### MATERIALS AND METHODS

Sandblasted and acid-etched implants (Bone System, Milan, Italy) were placed in the mandible of 4 male beagle dogs of at least 18 months of age. The 2 premolars and the first molars had been extracted 3 months previously. Each dog received 12 implants in the mandible (6 on the right side and 6 on the left side). The distance between the implants was at least 4 mm. All surgical procedures were performed under general anesthesia (premedication with acepromazine 0.5 mg/kg subcutaneously; anesthesia with Nembutal 15 mg/kg intravenously) and antibiotic prophylaxis. The implant sites were prepared with drills under generously chilled saline irrigation. The implants were then inserted with a tapping instrument. The mucosal tissues were sutured with 3-0 silk sutures. In the first 2 postsurgical weeks the oral cavities were rinsed daily with chlorhexidine-digluconate 0.12%. In addition, the dogs were fed a soft diet. The sutures were removed after 1 week. An oral hygiene regimen was instituted, consisting of plaque removal 3 times a week with a soft tooth brush and 0.2% chlorhexidine gel (SmithKline Beecham, Brentford, UK). No postoperative complications or

deaths occurred. Three months following implantation, second-stage surgery was performed; on 24 implants a prosthetic superstructure was inserted, whereas on 24 implants only the healing screws were positioned. Two dogs were killed after 6 months, and 2 dogs were killed after 12 months. A total of 24 implants were recovered.

#### *Processing of specimens*

The specimens were retrieved and stored immediately in 10% buffered formalin and processed to obtain thin ground sections with the Precise 1 Automated System (Assing, Rome, Italy).<sup>18</sup> The specimens were dehydrated in an ascending series of alcohol rinses and embedded in a glycolmethacrylate resin (Technovit 7200 VLC, Kulzer, Wehrheim, Germany). After polymerization the specimens were sectioned longitudinally along the major axis of the implant with a high-precision diamond disc at about 150 mm and ground down to about 30 mm. Three slides were obtained for each implant. The slides were stained with basic fuchsin and toluidine blue. A double staining with von Kossa and basic fuchsin was done to evaluate the degree of bone mineralization, and one slide per implant, after polishing, was immersed in AgNO<sub>3</sub> for 30 minutes and exposed to sunlight; the slides were then washed under tap water, dried and immersed in basic fuchsin for 5 minutes, and then washed and mounted.

#### *Histomorphometry*

Histomorphometry of the number of osteoclasts found in crestal bone in the first 3 mm from the implant surface was carried out using a light microscope (Laborlux S, Leitz, Wetzlar, Germany) connected to a high-resolution video camera (3CCD, JVC KY-F55B, JVC Professional Products, Milan, Italy) and interfaced to a monitor and PC (Intel Pentium III 1200 MMX, Intel Ireland Ltd, Kildare, Ireland). This optical system was associated with a digitizing pad (Matrix Vision GmbH, Oppen-

TABLE 1

| Statistical evaluation of osteoclasts around implant at 3 mm from crestal bone |       |        |        |         |
|--------------------------------------------------------------------------------|-------|--------|--------|---------|
|                                                                                | Mean† | SD     | SE     | P-value |
| Control implants (unloaded)                                                    |       |        |        |         |
| 6 months (Group 1)                                                             | 5.667 | 0.8165 | 0.3333 |         |
| 12 months (Group 2)                                                            | 2.5   | 1.049  | 0.4282 |         |
| Loaded implants                                                                |       |        |        |         |
| 6 months (Group 3)                                                             | 5.25  | 1.545  | 0.4459 |         |
| 12 months (Group 4)                                                            | 2.5   | 1      | 0.2887 | .0001*  |

†Number of osteoclasts.

\*Significant at 95% (according to the analysis of variance test).

weiler, Germany) and a histometry software package with image capturing capabilities (Image-Pro Plus 4.5, Media Cybernetics, Immagini & Computer Snc, Milan, Italy).

#### Statistical evaluation

The differences in the percentages of crestal bone remodeling in the groups were evaluated with the analysis of variance (ANOVA). The significance of the differences observed were evaluated with the Bonferroni test for multiple comparisons (Table 1). The number of osteoclasts were expressed as a mean  $\pm$  SD and SE. Statistically significant differences were set at  $P < .05$ .

#### RESULTS

All implants appeared to be osseointegrated from a clinical and radiographical point of view. No implants were mobile. Histologically, it was possible to observe around all implants the presence of compact, mature bone with well-formed Haversian systems. This bone was in close contact with the metal surface, and no gaps or fibrous tissue were observed at the interface. No epithelial downgrowth was observed. In some areas, marrow spaces were abutting on the implant surface. Osteoclasts, in the process of actively resorbing bone, were present around all implants.

In Group I (unloaded implants after 6 months), the mean number of osteoclasts observed at the level of the crestal bone in the first 3 mm from the implant surface was  $5.66 \pm 0.81$  (Figures 1 and 2).

In Group II (unloaded implants after 12 months), the mean number of osteoclasts was  $2.55 \pm 1.05$  (Figures 3 and 4). In Group III (loaded implants after 6 months), the mean number of osteoclasts was  $5.25 \pm 1.55$  (Figures 5 and 6). In Group IV (loaded implants after 12 months), the mean number of osteoclasts was  $2.5 \pm 1.0$  (Figures 7 and 8).

#### Statistical evaluation

The statistical evaluation showed that there were statistically significant differences in the numbers of osteoclasts in crestal bone between Group I and Groups II and IV, but no statistically significant differences were observed between Group I and Group III (Tables 1 and 2).

#### DISCUSSION

Osteoclast-mediated bone resorption occurs around dental implants and has an important role during initial and late-healing periods, as well as in the long-term success of an implant.<sup>19</sup> Bony craters and Howship's lacunae were signs of bone resorption in the neck area around the dynamically loaded implants in comparison with the statically loaded and control implants.<sup>11</sup> Our results show that no differences were found in the number of osteoclasts at the level of the crestal bone, in the first 3 mm from the implant, in both control (unloaded) and test (loaded) implants. The osteoclasts decreased only in function of time, and statistically significant differences were found only between the 6- and 12-

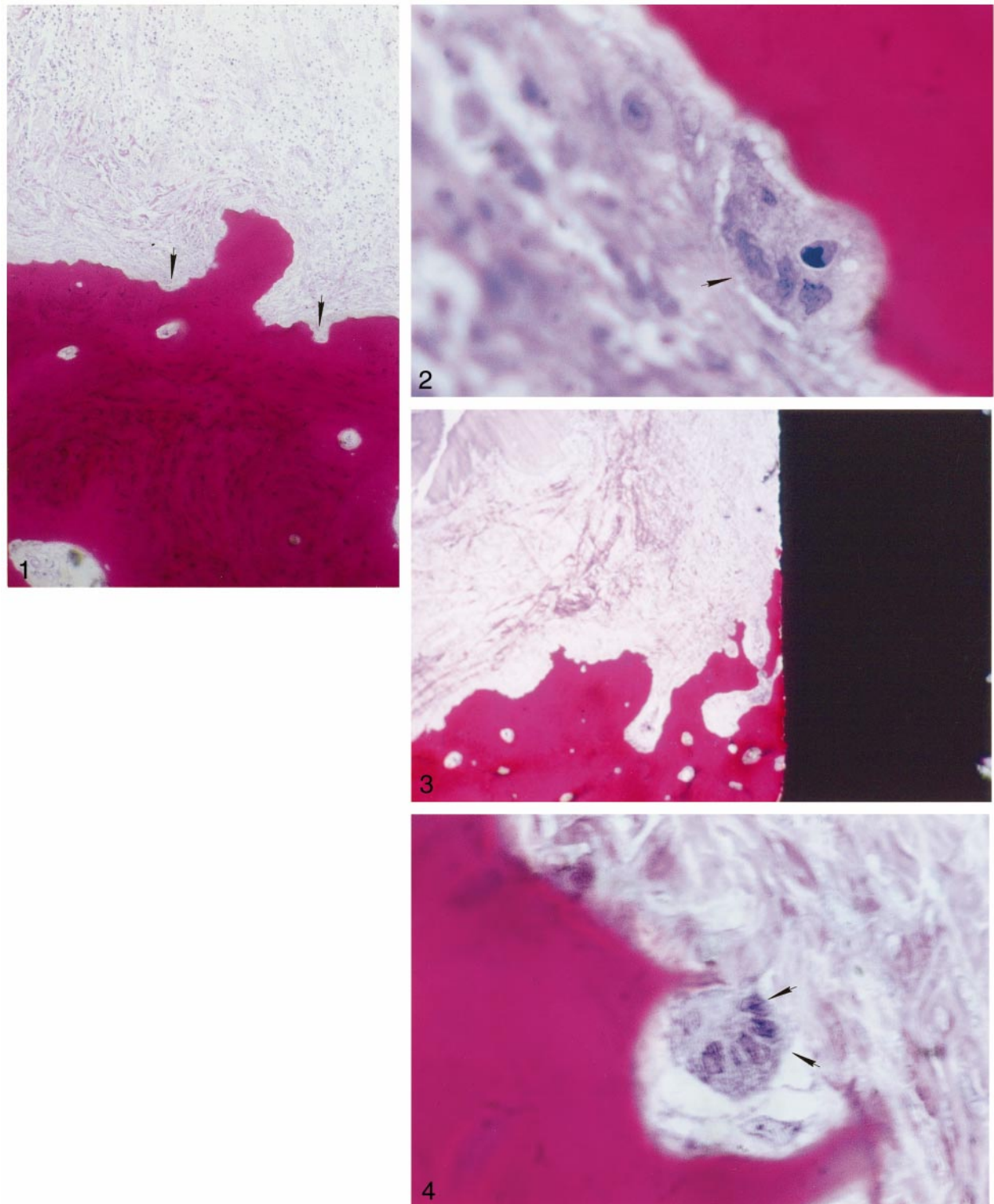
TABLE 2

| Significance of the differences with the Bonferroni test for multiple comparisons ( $P < .5$ ) |              |
|------------------------------------------------------------------------------------------------|--------------|
| Comparison                                                                                     | Significance |
| 1 vs 2                                                                                         | Yes          |
| 1 vs 4                                                                                         | Yes          |
| 1 vs 3                                                                                         | No           |
| 3 vs 2                                                                                         | Yes          |
| 3 vs 4                                                                                         | Yes          |
| 4 vs 2                                                                                         | No           |

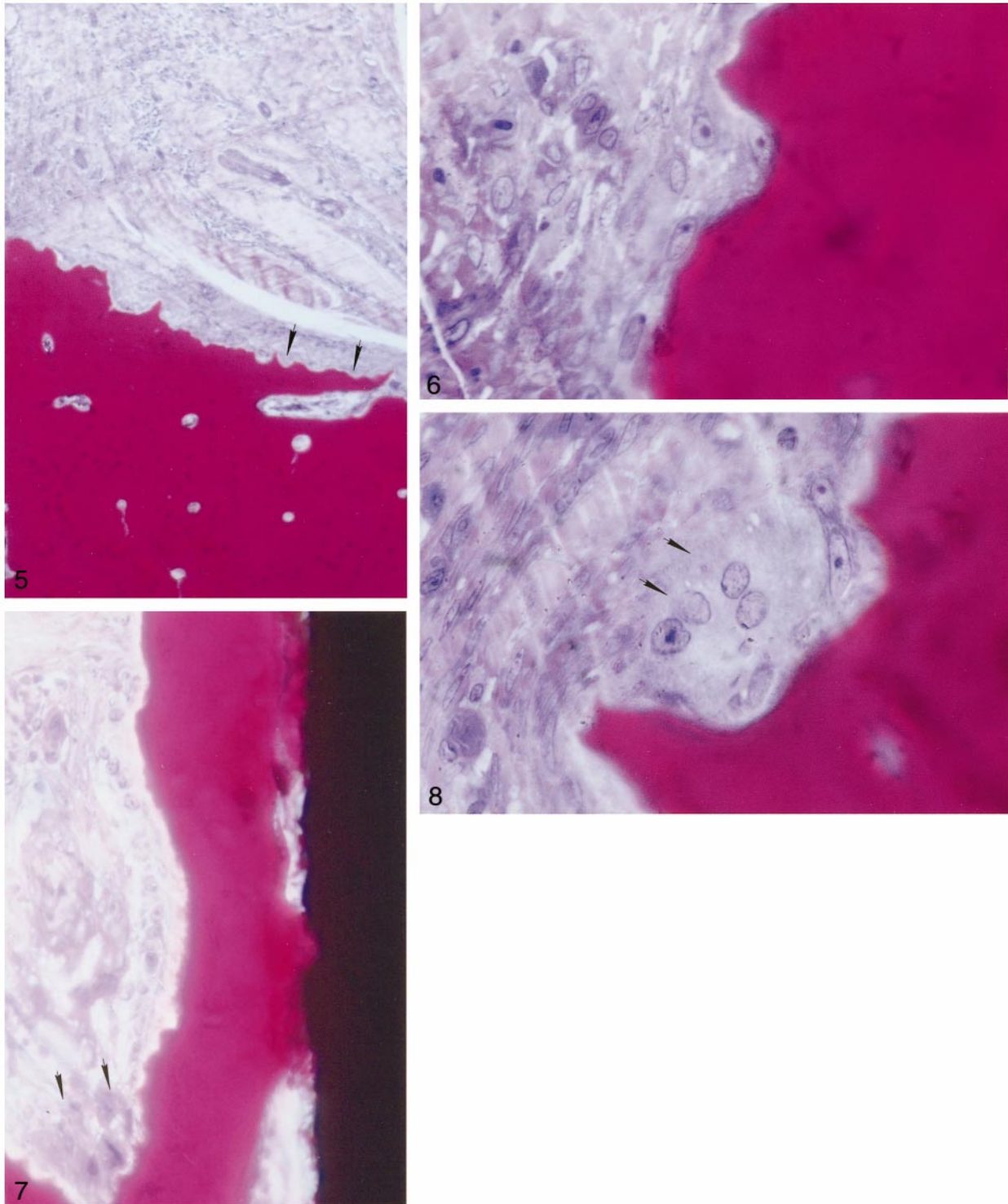
month specimens. According to our data, loading does not seem to have a relevant importance on the osteoclast activation in the peri-implant bone with subsequent bone resorption.

The present results agree with the histological data reported in a study of 2 plasma-sprayed, nonsubmerged implants, 1 loaded for 3 months and the other left unloaded and retrieved 6 months after placement, where we found osteoclast resorption activity in both.<sup>20</sup> In that study, we concluded that the hypothesis of an osteoclast activation correlated to an excessive load of the implant must be discarded because bone resorption was observed also in the unloaded implant.<sup>20</sup> This osteoclast activation could then be related in part to the presence of bacteria found inside the microgap between the implant and abutment.<sup>21-29</sup> It has been reported that some bacteria or their products may be involved in periodontal bone loss.<sup>30</sup> This bone loss has been established to be closely related to osteoclast activation.<sup>30</sup> This activation seems to be controlled by parathyroid hormone, IL-1, IL-6, PGE<sub>2</sub>, TNF-alpha, and also by periodontopathogens like *Actinomyces actinomycetemcomitans*, *Porphyromonas gingivalis*, and *Treponema lecithinolyticum*.<sup>30-32</sup> In particular, TNF-alpha upregulates IL-1, IL-6, and granulocyte-macrophage colony-stimulating factor.<sup>31</sup>

The release of polyethylene debris from hip implants induces a macrophage activation in the joint space.<sup>33</sup> These macrophages release humoral factors into the joint fluid, and these factors may stimulate the differentia-



FIGURES 1–4. FIGURE 1. Unloaded implant (6 months). Resorption lacunae with osteoclasts are present (arrows; Toluidine blue and basic fuchsin, original magnification  $\times 50$ ). FIGURE 2. Unloaded implant (6 months). At higher magnification it is possible to observe an osteoclast resorbing bone (arrow; Toluidine blue and basic fuchsin, original magnification  $\times 400$ ). FIGURE 3. Unloaded implant (12 months). Many areas of remodeling are present in the crestal bone (Toluidine blue and basic fuchsin, original magnification  $\times 40$ ). FIGURE 4. Unloaded implant (12 months). At higher magnification it is possible to observe an osteoclast resorbing crestal bone (arrows; Toluidine blue and basic fuchsin, original magnification  $\times 400$ ).



FIGURES 5–8. FIGURE 5. Loaded implant (6 months). Many Howship's lacunae are present in the crestal bone (arrows; Toluidine blue and basic fuchsin, original magnification  $\times 100$ ). FIGURE 6. Loaded implant (6 months). At higher magnification, an osteoclast is active in crestal bone resorption (Toluidine blue and basic fuchsin, original magnification  $\times 400$ ). FIGURE 7. Loaded implant (12 months). Vertical bone loss extending in an apical direction. In the apical portion of the pocket a few osteoblasts and osteoclasts (arrows) are present (Toluidine blue and basic fuchsin, original magnification  $\times 100$ ). FIGURE 8. Loaded implant (12 months). At higher magnification it is possible to observe an osteoclast (arrows; Toluidine blue and basic fuchsin, original magnification  $\times 400$ ).

tion of the bone marrow cells into osteoclasts, which begin to resorb bone at the prosthesis-bone interface.<sup>34-39</sup> According to Hermann et al,<sup>26</sup> the peri-implant bone loss is determined by the creation of a microgap between the implant and abutment. The bone will resorb with the creation of a distance from the bacteria present in the microgap.<sup>27</sup> In a retrospective study, Callan et al<sup>27</sup> found that the peri-implant bone loss was in part related to the position of the implant-abutment microgap. These authors reported that a bone loss equal or higher than 3 mm was observed in all implants where this microgap had been positioned in a subgingival position. The penetration of bacteria or their products through the microgap may constitute a risk of loss of supporting bone.<sup>21</sup> Future studies need to be done to try to better understand the mechanism of peri-implant bone resorption.

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