Purpose: The aim of this study was to histologically evaluate the crestal bone response to loaded and unloaded implants in beagle dogs.

Materials and Methods: Sandblasted and acid-etched implants (Bone System, Milano, Italy) were placed in the mandible of six beagle dogs. The two premolars and the first molars had been extracted 3 months previously. Each dog received 12 implants in the mandible, and a total of 72 implants were used in this study. Three months after implantation, second-stage surgeries were performed for placement of abutments or healing screws. Three dogs were killed after 6 months, and three dogs were killed after 12 months. All 72 implants were retrieved.

Results: No statistically significant differences were found in the amount of bone loss between test and control implants, both at 6 and 12 months. Statistically significant differences were found, in both groups, between the bone loss observed at 6 months and that found at 12 months.

Conclusion: Loading does not seem to be a relevant factor in the peri-implant bone resorption observed during the first year of function. Our results support previous findings that bone crest level changes could depend on the location of the microgap.

The ability to maintain a healthy bone-implant interface seems to be of great importance for the long-term survival of the implant. Crestal bone loss is present around different types of dental implants. It has been shown that a
crestral bone loss of about 2 mm will occur with the submerged, two-piece approach, dependent on the location of the microgap, and that minimal-to-no resorption will occur with non-submerged, one-piece implants. 4, 5 In these implants, no gaps exist at or below the alveolar crest. 4 The precise mechanisms of the bone resorption and deposition around dental implants is not yet completely known. 1 This bone loss may result from implant design, density of bone, surgical trauma at implant insertion or at second-stage surgery, occlusal overload, apical migration of crevicular epithelium in an attempt to isolate bacterial-induced infection or to establish a biological width, blood-supply interruption, or development of a pathogenic bacterial biofilm. 2-4, 6-8 Hooshaw et al 1 found that there was an increased bone resorption around the neck of the loaded implants, and suggested that crestal bone resorption is related to overload and to damage of the supporting interfacial bone. Other researchers have reported that bone loss around implants is often related to excessive stresses that overload the bone. 6, 9-11 In an experimental study using static and dynamic loading, Duyck et al 12 found that there was a significantly higher marginal bone loss around the dynamically loaded implants, and that bony craters and Howship's lacunae were signs of bone resorption in the neck area around the implants. However, the regions of lower stresses around the partially porouiscoated implants corresponded to the areas of crestal bone loss in animal studies, and in these cases the bone loss may be due to bone disuse atrophy. 2, 3 It must also be considered that a biologic width exists around implants. This biologic width will form at implant placement and is not correlated to implant loading. 13 It has been hypothesized that a certain width of the peri-implant mucosa is required to enable a proper epithelial-connective tissue attachment and, if this soft tissue dimension is not satisfied, bone resorption will occur to ensure the establishment of attachment with an appropriate biological width. 14, 15 This biological width is a physiologically formed and stable dimension as is found around teeth. 16-19 The significance of the existence and location of a microgap between implant components is not well studied or understood. The bone exposed to the oral cavity will cover itself with periostium and connective tissue, and the connective tissue will then be covered by epithelium. 13 Hermann et al 4 reported that their results clearly show that bone loss resulted from the creation of a microgap. The crestal bone will resorb and create a distance from the bacteria eventually present in the microgap. 13 Callan et al 7 found that approximately 4.2 years after prosthetic restoration, bone loss of more than 3 mm was observed in implants of different types (threaded, cylinder, basket) where the microgap was located in a subgingival position, whereas completely different results were obtained when the location of the microgap was at or above the gingival margin.

The aim of this study was to histologically evaluate the crestal bone response to loaded and unloaded implants and to the microgap in beagle dogs.

Materials and Methods **top**

Sandblasted and acid-etched implants (Bone System, Milano, Italy) were placed in the mandible of six beagle dogs (three males and three females) of at least 18 months of age. These are root form, threaded implants with an long pitch between spires; the connection between implant and abutment is made through an internal hexagon. The two premolars and the first molars had been extracted 3 months previously. Each dog received 12 implants in the mandible (six on each side), and a total of 72 implant were used in this study. The distance between the implants was at least 4 mm. All surgical procedures were performed using general anesthesia (premedication with 0.5-mg/kg acepromazine subcutaneously; anesthesia with 15-mg/kg Nembutal 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. All implants were placed in a submerged approach and the top of the implant (microgap) was located clinically at the alveolar crest. 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% (Peridex, Procter & Gamble, Cincinnati, OH) and the dogs were fed a soft-food diet. The sutures were removed after 1 week. An oral hygiene regimen consisting of plaque removal three times a week with a soft toothbrush and 0.2% chlorhexidine (Peridex, Procter & Gamble) was instituted. Three months after implantation, second-stage surgery was performed for abutment (36 implants) or healing screws (36 implants) connection. After a midcrestal incision, the peri-implant soft tissues were evaluated with exposure of the peri-implant bone crest and healing screws were inserted in the right mandible while abutments and prosthetic superstructures were placed in the left mandible. No postoperative complications or deaths occurred. Dogs were killed with an overdose of intravenous pentobarbital after 6 months (three dogs) or 12 months (three dogs). All 72 implants were retrieved. The implants were divided into four groups: implants with healing screws after 6 months (group I); implants with healing screws after 12 months (group II); implants with abutments after 6 months (group III); and implants with abutments after 12 months (group IV).

Processing of Specimens **top**

The specimens were retrieved and stored immediately in 10% buffered formalin and processed to obtain thin ground sections with image-capturing capabilities (Image-Pro Plus 4.5, Media Cybernetics Inc., Immagini & Computer Snc Milano, Italy).
**Statistical Evaluation**

The differences in the crestal bone remodeling in the groups were evaluated with the analysis of variance, and significance was evaluated with Student-Newman-Keuls test for multiple comparisons. The percentage of bone-implant contact is expressed as a mean ± standard deviation and standard error. Statistically significant differences were set at $P < 0.05$.

**Results**

**Unloaded Implants**

*Group I (healing screws: 6 months).*

The implant surfaces were in close contact with mature bone with large marrow spaces. Many multinucleated osteoclasts were observed at the level of the crestal bone actively resorbing bone (Fig. 1). In the area of the cover healing-implant microgap, many lymphocytes and plasma cells were present. The dimensions of sulcular epithelium, junctional epithelium, and connective tissue contact area were 0.6, 1.2, and 1.2 mm, respectively. The mean distance between the top of the implant and the crestal bone (bone loss) was 1.24 (SD 0.32) mm. Connective tissue fibers were located around the sandblasted and acid-etched surface and, under polarized light, they ran perpendicular to the implant surface.
Fig. 1. Unloaded implant (6 months). Vertical bone resorption is present in crestal bone. Osteoclasts and Howship's lacunae are present (arrows). Toluidine blue and basic fuchsin 30X. Fig. 4. Loaded implant (12 months). Vertical bone loss is present.

Group II (healing screws: 12 months). TOP

The bone in contact with the implant surface was more mature and with large marrow spaces. A 2.09 ± 0.42 vertical bone loss in the cortical bone in coronal area was present (Fig. 2). The dimensions of sulcular epithelium, junctional epithelium, and connective tissue contact area were 1.0, 1.1, and 1.3 mm, respectively.

Loaded Implants TOP

Group III (abutment: 6 months). TOP

At low magnification, it was possible to observe that bone was in close contact with the implant surface, and no gaps were present at the bone-implant interface. In the most coronal area, it was possible to observe a vertical bone loss with the presence of a few osteoclasts actively resorbing bone (Fig. 3). The dimensions of sulcular epithelium, junctional epithelium, and connective tissue contact area were 1.2, 1.1, and 1.2 mm, respectively. The mean distance between the top of the implant and the crestal bone (bone loss) was 1.32 (SD 0.35) mm.

Group IV (abutment: 12 months). TOP

In the apical area, the presence of bone in close contact with the implant surface was observed. No connective tissues were present in the apical area. In the coronal area, it was possible to observe osteoclasts actively resorbing bone (Figs. 4 and 5). The dimensions of sulcular epithelium, junctional epithelium, and connective tissue contact area were 1.1, 0.9, and 1.2 mm, respectively. The mean distance between the top of the implant and the crestal bone (bone loss) was 2.21 (SD 0.35) mm.

Statistical Evaluation TOP

No statistically significant differences were found in the amount of bone loss between test and control implants both at 6 and 12 months (Tables 1 and 2). However, statistically significant differences were found, in both groups, between bone loss observed at 6 months and that found at 12 months (Tables 1 and 2).

Table 1. Statistical Evaluation of Crestal Bone Loss
Table 1. Statistical Evaluation of Crestal Bone Loss

<table>
<thead>
<tr>
<th></th>
<th>Mean Bone Loss (mm)</th>
<th>Std. I</th>
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<tbody>
<tr>
<td>6 months (Group 1)</td>
<td>1.24</td>
<td>0.3</td>
</tr>
<tr>
<td>12 months (Group 2)</td>
<td>2.09</td>
<td>0.4</td>
</tr>
<tr>
<td>6 months (Group 3)</td>
<td>1.32</td>
<td>0.3</td>
</tr>
<tr>
<td>12 months (Group 4)</td>
<td>2.21</td>
<td>0.3</td>
</tr>
</tbody>
</table>

Values measured in mm of bone loss.
* Significant at 95% (according to the ANOVA test).

Table 2. Significance of the Differences of Bone Loss Between with Bonferroni Test for Multiple Comparisons ($P < 0.5$)

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Significance</th>
</tr>
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<tbody>
<tr>
<td>1 vs 2:</td>
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</tr>
<tr>
<td>1 vs 4:</td>
<td>Yes</td>
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<tr>
<td>1 vs 3:</td>
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<td>3 vs 2:</td>
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<tr>
<td>3 vs 4:</td>
<td>Yes</td>
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<tr>
<td>4 vs 2:</td>
<td>No</td>
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</tbody>
</table>

Table 2. Significance of the Differences of Bone Loss Between with Bonferroni Test for Multiple Comparisons ($P < 0.5$)

Discussion TOP

The precise mechanism responsible for the crestal bone remodeling in two-piece implants is not known. At present, little is known about the impact of loading on the peri-implant bone. Excessive load can trigger bone resorption due to the production of bone microdamage with the creation of craterlike bone defects lateral to the implants. In our specimens, no statistically significant differences were found in the vertical bone resorption in loaded and unloaded implants at 6 months (1.32 versus 1.24 mm) and at 12 months (2.21 versus 2.09 mm). The vertical bone loss increased from 6 to 12 months only as a function of time. These results support previous studies that found that for all two-part implants, the bone crest level changes seem to be dependent on the location of the microgap, with a distance between the microgap and the most coronal bone-implant contact being approximately 2.0 mm. The significance of the existence and location of a microgap between implant components is not well studied or understood. The reason for the reaction to the presence of a microgap is not known, but, as we have already pointed out, it could be related to the presence of a contamination by bacteria present at the implant-abutment interface or to micromovements of the implant abutment interface. Gaps and cavities have been described in two-piece implants, even where good marginal fit of implant components is present. These hollow spaces can be a trap for bacteria, which might cause inflammation in the peri-implant soft tissues. The existence of bacterial leakage both at the junction between the abutment and the fixture as well as along the abutment screw has been reported. The microorganisms found inside the implants might be associated with the bone loss observed during the first
This resorption is not observed around sleeping implants where both exposure to microbial colonization and loading are absent. The size of this microgap could be significant, as well as the presence of movements between implant and abutments. In a recent study, Hermann et al demonstrated that no significant difference in the amount of the crestal bone could be attributed to the size of the microgap, but that welding the abutment to the implant (with a laser) resulted in significantly lower bone resorption. The movements between abutment and implants, in addition to microbial contamination, also seemed to influence crestal bone resorption. It has been shown that an inflammation results if the abutment loosens on the implants placed in a submerged approach, with possible fistula formation. Tightening the abutment eliminates this fistula. Moreover, in cases of movement of the abutment, the epithelium could attach to the stable implant rather than to the abutment.

In previous studies, the epithelial attachment was more apical and always located below the microgap in submerged implants. The epithelium could migrate beyond the bacteria and the microgap in an attempt to isolate the infection. This proliferation of the epithelium and subsequent response to reestablish the dimension of the biological width could be responsible for the approximately 2 mm of distance that is present apical to the microgap.

Conclusion

Our data do not seem to support a role of loading in the peri-implant bone resorption that is observed during the first year of function. No statistically significant differences were found in the amount of bone loss in loaded and unloaded implants. On the contrary, the amount of bone loss found at 12 months was strikingly similar (2.21 and 2.09) to the 2 mm reportedly found between the microgap and the most coronal bone-implant contact. Additional studies must be performed to better understand the mechanisms underlying peri-implant bone resorption.

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Disclosure

The authors claim to have no financial interest in any company or product mentioned in this article.

References


Eine histologische Studie zur Wiederherstellung des Knochengewebes im Leistenbereich bei belasteten und unbelasteten Implantaten in Zusammenhang mit dem Minimalspalt


SCHLÜSSELWÖRTER: Knochengewebsumformung, Belastung, Minimalspalt, Osteoklasten

Remodelado do hueso crestal em implantes cargos e sans cargar e o micro espaço: Un estudio histológico


ABSTRACT: PROPOSIÓ: El objetivo de este estudio fue realizar una evaluación histológica en perros sabuesos de la respuesta del hueso crestal a implantes cargados y sin cargar. MÉTODOS Y MATERIALES: Implantes pulidos con chorro de arena y con ácido (Bone System, Milán, Italia) se colocaron en la mandíbula de seis perros sabuesos. Los dos premolares y los primeros molares habían sido extraídos tres meses antes. Cada perro recibió 12 implantes en la mandíbula. Se usaron un total de 72 implantes en este estudio. Tres meses luego del implante, se realizaron cirugías de segunda etapa para la colocación de pilares o tomillos de curación. Tres perros fueron sacrificados después de 6 meses y tres meses luego de 12 meses. Se recuperaron un total de 72 implantes. RESULTADOS: No se encontraron diferencias estadísticamente significativas en la cantidad de pérdida de hueso entre los implantes de prueba y de control, a los 6 y a los 12 meses. Se encontraron diferencias estadísticamente significativas, en ambos grupos, entre la pérdida de hueso encontrada a los 6 meses y a los 12 meses. CONCLUSIÓN: La carga no parece ser un factor relevante en la reabsorción perimplante del hueso observado durante el primer año de función. Nuestros resultados apoyan conclusiones anteriores que los cambios en el nivel de la cresta del hueso dependen del lugar del microespacio.

PALABRAS CLAVES: remodelado del hueso, carga, microespacio, osteoclastos

Reconstrucción da Crista Óssea em Implantes Carregados e Sem Carregar e a Micro-Abertura: Un Estudo Histológico


SUMÁRIO: PROPÓSITO: O objetivo deste estudo foi uma avaliação histológica em cachorros da raça beagle da resposta da crista óssea em resposta a implantes carregados e não carregados. MÉTODOS E MATERIAIS: Implantes com superfície trabalhadas com jato de areia e corrosão ácida (Sistema Ósseo, Milão, Itália) foram colocados nas mandíbulas de seis cães da raça beagle. Os dois pré-molares e os primeiros molares foram extraídos 3 meses antes. Cada cachorro recebeu 12 implantes na mandíbula. Um total de 72 implantes foram usados neste estudo. Três meses após o implante, cirurgias de segundo estágio foram realizadas para a colocação de suportes ou parafusos de cura. Três cachorros foram sacrificados depois de 6 meses, e três depois de 12 meses. Um total de 72 implantes foram recuperados. RESULTADOS: Nenhuma diferença estatística significativa foi encontrada na...
quantidade de perda óssea entre os implantes de teste e controle, ambos aos 6 e 12 meses. Estatisticamente diferenças significativas foram encontradas em ambos grupos, entre as perdas ósseas encontradas aos 6 meses e aquelas encontradas aos 12 meses. **CONCLUSÃO:** A carga não parece ser um fator relevante na ressorção do osso peri-implantado observada durante o primeiro ano de função. Nossos resultados apoiaram descobertas anteriores que as mudanças no nível da crista óssea poderiam depender na localização da micro-abertura.

**PALAVRAS-CHAVE:** reconstrução óssea, carga, micro-abertura, osteoclastos

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**Loaded**または**Unloaded**インプラントにおける**Crestal Bone Remodeling**とマイクロ組織学的研究

著者：パルトロメオ・アッセンザ、MD、DDS*、アントニオ・スカラーノ、DDS**、ペトローネ、DDS、PhD***、ジョバンナ・レッジ、DDS****、ウルフ・タン、DDS*****、フィデル・サン・ロマン、MD******、アドリアーノ・ビアッテーリ、MD、

要約：

目的：本研究の目的は、ビーグル犬におけるloadedまたはunloadedインプラントcrestal boneの反応の組織学的評価を行うことにある。

方法と素材：砂ブラストと酸処理されたインプラント（Bone System、ミラノア）が6匹のビーグル犬の上顎に設定された。小臼歯2本と第1臼歯は術後3か月前れた。イスには上顎にそれぞれ12のインプラントが設置された。本研究では、インプラントが用いられた。インプラント設置3か月後に、アパラメントまたscrewsの設置のための第2次手術が行われた。イスは3匹が6か月後に、残りの3匹後に安楽死させられた。合わせて72のインプラントが回収された。

結果：6か月後と12か月後のどちらの場合も、骨損失量については検体群・対比間に統計的に有意差は認められなかった。両方の群において、6か月後と12か月後の間に骨損失量について統計的に有意差が認められた。

結論：Loadingが、機能開始1年間におけるインプラント周辺骨の吸収に関連しているとは思われない。本研究の結果は、bone crest高さの変化はマイクロギャップに関連するという過去の研究結果を支持するものとなった。

キーワード：bone remodeling、loading、マイクロギャップ、破骨細胞
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Figure. No caption available.

Keywords:
bone remodeling; loading; microgap; osteoclasts

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