Histological and histomorphometric evaluation of immediate implant placement on a dog model with a new implant surface treatment

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Key words: conditioned implant surface, histomorphometric implant analysis, implant surface chemistry

Abstract

Purpose: The aim of this study was to evaluate crestal bone resorption and bone apposition resulting from immediate post-extraction implants in the canine mandible, comparing a conditioned sandblasted acid-etched implant surface with a non-conditioned standard sandblasted implant surface.

Material and methods: In this experimental study, third and fourth premolars and distal roots of first molars were extracted bilaterally from six Beagle dog mandibles. Each side of the mandible received three assigned dental implants, with the conditioned surface (CS) on the right side and the non-conditioned surface (NCS) on the left. The dogs were sacrificed at 2 (n = 2), 4 (n = 2) and 12 weeks (n = 2) after implant placement.

Results: The microscopic healing patterns at 2, 4 and 12 weeks for both implant types (CS and NCS) yielded similar qualitative bone findings. The mean crestal bone resorption was found to be greater for all implants with NCS (2.28 ± 1.9 mm) than CS (1.21 ± 1.05 mm) at 12 weeks. The mean percentage of newly formed bone in contact with implants was greater in implants CS (44.67 ± 0.19%) than with the NCS (36.6 ± 0.11%). There was less bone resorption with the CS than the NCS.

Conclusion: The data show significantly more bone apposition (8% more) and less crestal bone resorption (1.07 mm) with the CS than with the NCS after 12 weeks of healing. This CS can reduce the healing period and increase bone apposition in immediate implant placements.

The placement of implants in fresh extraction sockets has been cited by many authors as a means of reducing the number of surgical procedures, preserving the dimensions of the alveolar ridge and simplifying clinical procedures (Lazzara 1989; Schwartz-Arad & Chaushu 1997a, 1997b; Becker et al. 1998; Brägger et al. 1999; Grunder et al. 1999).

Botticelli et al. (2004a, 2004b, 2004c) monitored the hard tissue alterations occurring after implant placement in fresh extraction sockets in humans. Their results showed that marginal gaps in buccal and palatal/lingual locations were resolved through new bone formation from the inside of the defects and through substantial bone resorption from the outside of the ridge Botticelli et al. (2004c). Following tooth removal, the buccal and palatal portions of the ridge suffer minor vertical but major horizontal tissue loss Schropp et al. (2003).

One way of reducing the healing period may be to use new kinds of treated implant surfaces that accelerate and improve the osseointegration process. Albrektsson et al. (1981) recognized that among the factors

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influencing bone-to-implant contact [BIC] [topography, chemistry, wettability and surface energy], one of the most important is wettability Albrektsson et al. [1981]. Surface wettability is largely dependent on surface energy and influences the degree of contact with the physiological environment [Kilpadi & Lemons 1994; Zhao et al. 2005]. Several evaluations have demonstrated that implants with rough surfaces show better bone apposition and BIC than implants with smooth surfaces [Buser et al. 1999, Cochran et al. 2002]. Surface roughness also has a positive influence on cell migration and proliferation, which in turn leads to better BIC results, suggesting that the microstructure of the implant influences biomaterial–tissue interaction [Mat-suo et al. 2000; Plakco et al. 2000; Novaes et al. 2002].

Recently, a modified, sand-blasted, large grit and acid-etched [modSLA] titanium surface has been introduced with the aim of enhancing bone apposition [Buser et al. 2004; Zhao et al. 2005; Bornstein et al. 2008]. Observations made in clinical studies and animal experiments have also observed that following tooth extraction, the socket as well as the surrounding bone tissue undergoes substantial remodeling, remodeling and resorption. The socket will heal with woven bone formation, the establishment of a cortical ridge and the eventual replacement of woven bone with lamellar bone and marrow [Cardaropoli et al. 2003].

Findings from experiments in dogs, however, have failed to support this hypothesis [Botticelli et al. 2004a, 2004b, 2005; Ar-avero et al. 2005; Waldenburg, Switzerland). These teeth were hemi-sected along the mesial and distal aspects of the root according to a distribution pattern produced for each dog before surgery. Small sulcular flaps were adapted for tension-free wound closure with interrupted and horizontal mattress sutures. During the first week after surgery, the animals received Amoxicillin (300 mg, twice daily) and Ibuprofen 600 mg (three times a day) via the systemic route. The sutures were removed after 2 weeks. The animals were sacrificed at 2, 4 and 12 weeks after the implantation procedure by means of an overdose of Pentothal Sodium (Abbott Laboratories, Chicago, IL, USA) administered intravenously.

The experiment used the third, fourth premolar and first molar distal sockets in both quadrants of the mandible [iP3, iP4 and iM1]. These teeth were hemi-sectioned with a tungsten–carbide bur and the distal roots were removed using forceps. Sulcular incisions were made along the buccal and lingual aspects of the teeth to disclose the ridge’s buccal and lingual hard tissue crestal wall. The root canals of the remaining roots were filled with hydroxylapatite and the coronal pulp chambers were sealed with light curing cement. The apical portion of the socket was prepared using a conventional drill before receiving the implants. These were SPI Implants (Thommen Medical, Waldenburg, Switzerland). Thirty-six implants with a diameter of 4 and a length of 9.5 mm, with a solid screw SPI element, were divided into two groups: 18 with a standard surface made up the control group and 18 with a CS comprised the test implants. Test implants were installed on the right side of the mandible and control implants on the left. Implant position in relation to bone walls was related to the shape and volume of the alveolar process, in turn determined by the form and size of each tooth’s root.

All the implants were submerged into extraction sockets with the smooth collar slightly apical of the buccal and lingual bone crest in order to improve anchorage during the early phases following implant placement [Fig. 1].

Implants were placed under the same surgical conditions as the tooth extractions (in terms of sterility, operating room and anesthesia). Six implants were placed into each mandible [three in the control group on the left side and three in the test group on the right side] according to a distribution pattern produced for each dog before surgery. The small sulcular flaps were adapted for tension-free wound closure with interrupted and horizontal mattress sutures.

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Material and methods

This experimental animal study was designed in order to evaluate bone apposition to a new implant surface [Thommen Medical, Waldenburg, Switzerland] in the canine mandible. The surface was produced using the usual acid etching and sandblasting but prepared with hydroxide ions to provide high surface energy.

Six beagle dogs about 1 year old and weighing approximately 12–13 kg each were used in the experiment. The animals were fed a daily pellet diet. The Ethics Committee for Animal Research at the University of Murcia, Spain, approved the study protocol following guidelines laid down by the European Union Council Directive of November 24, 1986 [86/609/ EEC].

Surgical procedure

During surgical procedures, the dogs were anesthetized with Pentothal Natrium® (30 mg/ml; Abbot Laboratories, Chicago, IL, USA) administered intravenously.

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Histologic preparation and histomorphometric analysis: histological examination

The specimens were prepared for histological examination as described by Schenk et al. [1984].

The samples were dehydrated in increasing grades of ethanol, infiltrated with
methacrylate, polymerized and sectioned at the buccal-lingual plane using a diamond saw [Exakt, Apparatebau, Norderstedt, Hamburg, Germany]. Two sections were cut from each biopsy unit. The first was cut from the center of the implant and the second from the surrounding bone. Each block was sectioned with a high-precision diamond disk at about 100 μm thickness and ground to approximately 40 μm final thickness by an Exakt 400 CS grinding device [Exakt, Apparatebau]. Each section surface was stained using toluidine blue stain according to Schenk et al. (1984) and a semi-quantitative evaluation of BIC was performed. For this evaluation, the percentage of direct contact between mineralized bone and the titanium surface was determined by intersection counting within the thread area. Six thread pitches were counted per sample. BIC was estimated as percentages in steps of 10%. A mean evaluation of thread counts per implant was carried out.

Evaluations were performed using calibrated digital pictures at ×10 magnification (Leica microscope Q500Mc, Leica DFC320°, 3088 × 2550 pixels, Leica Microsystems, Barcelona, Germany).

The most central sagittal section of each implant was used for histomorphometric analysis using MIP 4.5 (Microm® Image Processing Software, CID, Consulting Image Digital, Barcelona, Spain) connected to a Sony DXC-151* 2/3-CCD RGB Color Video Camera.

To improve the differentiation between native and newly formed bone, blue and light blue chromaticity were enhanced by processing the images digitally. Lastly, the contact interface length between bone and implant surface (BIC) was determined.

From the sections, linear measurements [×10 magnification] were made between the following points [Fig. 2]:

A zone: smooth collar.
B zone: base of the smooth collar up to the middle of the second thread.
C zone: middle second thread up to the last thread in the biopsy.

The heights of the buccal and lingual crestal bone walls were determined by measuring the distance between the buccal or the lingual surface of the implant body from the top to the base of the smooth collar [A] and the base of the smooth collar up to the first coronal contact between bone and implant [B].

A final analysis was performed by batch processing the prepared images. Two types of bone in direct contact with the implant surface were differentiated: newly formed bone and native bone. The total amount of bone in contact with the implant was calculated as the sum of native bone and newly formed bone.

The percentages of BIC were calculated around the entire implant perimeter. CRESTAL bone resorption was measured from the base of the implant shoulder up to the first coronal BIC. Newly formed bone was situated in the peri-implant area and between the implant threads.

Statistical analysis
Mean values and standard deviations were calculated using a descriptive test for BIC and bone resorption (depth) measurements. The Wilcoxon–Mann–Whitney, a non-parametric test, was applied for all the groups (n = 18 control group and n = 18 test group). All histomorphometric parameters were analyzed using descriptive methods [SPSS 15.0 for Windows]. For all the tests performed, the confidence level 5% was chosen.

Results
The buccal, lingual, mesial and distal dimensions of the entrance to the fresh extraction socket were measured using a sliding caliper before implant placement. The extraction sockets’ mean alveolar ridge measurements were 3.8 ± 0.5 mm [P3], 3.9 ± 1 mm [P4] and 5.8 ± 0.3 mm [M1].

Histological and histomorphometric examination
Following tooth extraction and immediate implant placement, the overall post-operative wound healing was excellent. No tissue dehiscences or visible infections were observed during the study period, and all 36 placed implants were available for histological and histomorphometric analysis.

Because microscopic healing patterns at 2, 4 and 12 weeks for both implant types (NCS and CS) yielded similar quantitative findings, the descriptive histology was combined at the three times of evaluation.
**Week 2**

Sockets’ buccal bone walls were thinner than their lingual wall. Bundle bone was present only in the marginal portion of the buccal and lingual walls.

Large amounts of newly formed bone had occurred in the apical and lateral portions of the extraction sockets and had already established direct contact, more with the test than the control surface. Newly formed trabecular woven bone could be observed around multiple vascular structures, often extending from the native bone both toward and reaching the implant surfaces (Figs 1 and 2).

Osteoblasts lined the woven bone trabeculae, and in some areas osteocytes were present in the newly formed bone. The woven bone surfaces were lined with densely packed osteoblasts and included primitive bone marrow (Figs 3 and 4).

The mean crestal bone resorption at 2 weeks was $2.15 \pm 1.3 \text{ mm SD}$ for the test surface (CS) and $1.74 \pm 1.1 \text{ mm SD}$ for the control surface (NCS). The mean of BIC was $23.35 \pm 1.5\% \text{ SD}$ for the NCS and $29.46 \pm 1.4\% \text{ SD}$ for the CS (Tables 1 and 2).

**Week 4**

The center of the buccal and lingual bone walls comprised varying amounts of old lamellar bone surrounded by a newly formed BIC surface. The surface of the woven bone was lined with densely packed osteoblasts and included primitive bone marrow.

Newly formed woven bone extended from the cut region of the native bone to the implant surface, and also projected parallel to the implant along both the control and the test surfaces. Large portions of bundle bone had been replaced by lamellar bone and marrow (Figs 5 and 6).

The mean crestal bone resorption at 4 weeks was $1.42 \pm 1.1 \text{ mm SD}$ for the test surface (CS) and $2.31 \pm 0.7 \text{ mm SD}$ for the control surface (NCS). The mean of BIC was $33.64 \pm 1.2\% \text{ SD}$ for the NCS and $41.64 \pm 0.9\% \text{ SD}$ for the CS.

**Week 12**

In several specimens, it was observed that islands or a thin continuous layer of woven bone lined a portion of the implant surface coronal to the buccal bone crest (Figs 7 and 8). Scattered osteoclasts were found in the corresponding locations of the lingual bone wall. The internal portion of the socket region was occupied by bone marrow but included trabeculae of mineralized tissue made up of woven bone and lamellar bone (Figs 7 and 8).

The mean crestal bone resorption at 12 weeks was $1.21 \pm 0.49 \text{ SD mm}$ for the test surface (CS) and $2.28 \pm 1.9 \text{ SD mm}$ for the control surface (NCS). The mean BIC was $36.6 \pm 0.11 \text{ SD\%}$ for the NCS and $44.67 \pm 0.19 \text{ SD\%}$ for the CS.

Of the 36 mandibular implants, all 36 central sections were prepared and analyzed. BIC values for native bone alone revealed no statistically significant differences between the three different healing periods.

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**Table 1. Crestal bone resorption measurements (mm) at 2, 4 and 12 weeks evaluation time**

<table>
<thead>
<tr>
<th>Time period-surface</th>
<th>Depth 2 weeks (mm)</th>
<th>Depth 4 weeks (mm)</th>
<th>Depth 12 weeks (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CS</td>
<td>2.15 ± 1.2</td>
<td>1.42 ± 1.1</td>
<td>1.21 ± 0.49</td>
</tr>
<tr>
<td>NCS</td>
<td>1.74 ± 1.1</td>
<td>2.31 ± 0.7</td>
<td>2.28 ± 1.9</td>
</tr>
</tbody>
</table>

**Table 2. Bone-to-implant contact (BIC) measurements at different time periods**

<table>
<thead>
<tr>
<th>Time period-surface</th>
<th>BIC 2 weeks (%)</th>
<th>BIC 4 weeks (%)</th>
<th>BIC 12 weeks (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CS</td>
<td>29.46 ± 1.4</td>
<td>41.64 ± 0.9</td>
<td>44.67 ± 0.19</td>
</tr>
<tr>
<td>NCS</td>
<td>23.35 ± 1.5</td>
<td>33.64 ± 1.2</td>
<td>33.6 ± 0.11</td>
</tr>
</tbody>
</table>

CS, conditioned surface or test surface; NCS, non-conditioned surface or control surface.
Implants with the CS showed more BIC at 2, 4 and 12 weeks than implants with the NCS, increasing only 8% more than BIC.

The CS showed less crestal bone resorption (1.07 mm) than NCS, at the 12-week healing period (Tables 3 and 4).

**Discussion**

The present investigation demonstrated that in fresh extraction sockets marked hard tissue alterations occurring during the 12-week healing period following tooth extraction and implant installation related more to the lingual plate than to the buccal plate. Surfaces combining grit blasting and acid etching with a microporous topography have shown significantly enhanced cell spread rates in comparison with other surfaces. A recent study examined the promotion of osteoblast attachment and differentiation on various implant surfaces (Masaki et al. 2003; Sammons et al. 2005). The CS tested demonstrated excellent BIC and favored the maintenance of the alveolar buccal plate after immediate implant placement, which was related to preservation of the periosteal vascular network and accelerated BIC due to the different surface treatment. Several previous studies have examined the effect of implant surface on bone healing and bone apposition. Micro-rough titanium surfaces have often been observed to produce a significantly greater percentage of BIC when compared with machined or polished titanium surfaces; this can be observed for as long as 5 years after implant placement and such implants show good survival/success rates of approximately 99% (Roccuzzo et al. 2001; Cochran et al. 2002; Bornstein et al. 2003, 2005).

Descriptive histologic analysis of standard SLA and modified SLA surfaces in the present study confirms the findings of Berglundh et al. [2003]. In their study, the authors described a standardized wound chamber model (integrated in the threads of an experimental implant device), which was used to investigate the different phases of wound healing and the process of osseointegration. In the first 4 days of healing, they observed that the initially empty wound chamber became filled with a coagulum and granulation tissue that were gradually replaced by a provisional matrix Berglundh et al. [2003]. The process of new bone formation started during the first week. Likewise, in the present study, with standard SLA and conditioned SLA surfaces this process was clearly underway after 2 weeks of healing. Our control implants had the same sandblasted surface as that used in the standard implants in...
previous studies [Cochran et al. 2002; Bornstein et al. 2003, 2005, 2008], and the BIC results found in our study are in accordance with other immediate implant studies, which report 10% > BIC with grit blasted/acid-etched surfaces than with standard surfaces [Novaes et al. 2002].

After 4 weeks, it was observed that the void that had occurred at the time of surgery between the marginal portions of the implant and the walls of the fresh socket had become filled with a coagulum at greater speed with the CS than the NCS. Marginal gaps in the premolar areas between the implant and the socket walls, which were present at the time of implantation, disappeared as a result of newly formed bone filling. Barros et al. [2009] described a flapless approach for immediate post-extraction implants, which was found to reduce the buccal bone height loss.

A study involving an immunohistochemical analysis of the initial angiogenesis revealed that the organization of blood clots seemed to have been initiated within 24 h after implant placement [Schwarz et al. 2007]. After a month, this coagulum had been replaced by newly formed, immature bone, which also made contact with the rough surface of the implant in the marginal gap region. This observation is in agreement with previously reported findings [Sennerby et al. 1993; Berglundh et al. 2003; Botticelli et al. 2003a; Abramhamsson et al. 2004; Araújo et al. 2005]. In our study, the smaller [0.2 mm wide] gap at the premolar sites had already been resolved after 4 weeks and the larger [1–1.3 mm] horizontal and vertical defects at the molar sites were completely resolved after 12 weeks. These findings are in agreement with data presented by Botticelli et al. [2003b]; Araújo et al. [2005] in dog experiments. The process of bone modeling and remodeling around an implant placed in a fresh extraction socket differs from the resolution of marginal defects that may occur following implant installation in a healed ridge [Botticelli et al. 2006]. Our findings are consistent with earlier data gathered by other authors who showed that marked dimensional alterations to the ridge occur following tooth extraction and that these take place during the first 12 weeks of healing [Cardaropoli et al. 2003, 2005; Schropp et al. 2003; Araújo & Lindhe 2005].

Despite this, implant diameter has a significant influence over crestal bone resorption. All the implants used in the study were 4 mm in diameter and 9.5 mm in length, placed in the middle of the extraction socket, and this is one of the keys to improved maintenance of periimplant crestal bone. We differ from Araújo et al. [2005], who carried out non-submerged placement of 4.1-mm-diameter implants in sockets of reduced dimension in order to achieve initial buccal bone resorption [Araújo et al. 2005]. All the implants in our study were placed submerged to improve implant anchorage in the initial phase immediately after placement and this was similar to the procedures carried out by Choi et al. [2008].

Chung et al. [2008] also revealed that implant geometry and surface treatment affect the rate of crestal bone resorption and bone healing surrounding dental implants. This suggests that the tissue alterations that occur between 2 and 12 weeks are related to the functional adaptation of the alveolar ridge that occurred after the loss of the teeth.

The present investigation revealed greater depth of crestal bone resorption at the lingual crest than at the buccal plate; this bone dehiscence following implant placement corroborates findings reported...
in previous dog experiments [Spray et al. 2000; Araújo & Lindhe 2005; Araújo et al. 2005; Cardaropoli et al. 2006].

In our study, the amount of buccal resorption was less pronounced than resorption at the lingual aspect, and resorption in the molar region was more substantial than in the premolar region.

We agree with Rupp et al. (2006), who described how the hydrophilic surface properties observed for hydroxylated/hydrated modified SLA when compared with NCS. Furthermore, findings taken after 12 weeks suggest that in canine mandibles the conditioned titanium surface may promote more early bone osteointegration in premolar and molar sites than a non-conditioned titanium surface and reduced crestal bone resorption.

Conclusions

In our study, the biopsy evaluations were performed after 12 weeks of healing when the inner surfaces of the bone walls exhibited more direct bone apposition to the CS than with the NCS. The BIC of newly formed bone was greater with the CS (44.67 ± 0.19%) than with the NCS (36.6 ± 1.1%), inducing 8% more bone formation at the 12-week follow-up. Furthermore, the height of the crestal bone walls with the NCS (2.28 ± 1.9 mm) was reduced by about 1.07 mm compared with the CS (1.21 ± 0.49 mm) at 12 weeks after implantation. The hydrophilic surface properties of the hydroxylated/ionized CS result in greater wettability when compared with the NCS (control implants).

There are reasons to suggest that either the loss or the preservation of crestal bone was partly due to surgical trauma resulting from the flapless approach, the coronal design of implants, the CS of implants, subcrestal implant position and the variable width of the alveolar ridge.

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