Experimental Peri-implant Tissue Breakdown Around Different Dental Implant Surfaces: Clinical and Radiographic Evaluation in Dogs

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Purpose: Tissue reactions to 4 different implant surfaces were evaluated in regard to the development and progression of ligature-induced peri-implantitis. Materials and Methods: In 6 male mongrel dogs, a total of 36 dental implants with different surface coatings (9 titanium plasma-sprayed, 9 hydroxyapatite-coated, 9 with acid-etched surfaces, and 9 with commercially pure titanium surfaces) were placed 3 months [AU: 3 or 3.5? See Fig 1] after mandibular premolar extraction. After 3 months with optimal plaque control, abutment connection was performed. Forty-five days later, cotton ligatures were placed around the implants to induce peri-implantitis. At baseline and 20, 40, and 60 days after placement, the presence of plaque, peri-implant mucosal redness, bleeding on probing, probing depth, clinical attachment loss, mobility, vertical bone loss, and horizontal bone loss were assessed. Results: The results did not show significant differences among the surfaces for any parameter during the study (P > .05). All surfaces were equally susceptible to ligature-induced peri-implantitis over time (P < .001). Correlation analysis revealed a statistically significant relationship between width of keratinized tissue and vertical bone loss (r2 = 0.81; P = .014) and between mobility and vertical bone loss (r2 = 0.66; P = .04), both for the titanium plasma-sprayed surface. Discussion and Conclusions: The present data suggest that all surfaces were equally susceptible to experimental peri-implantitis after a 60-day period. Int J Oral Maxillofac Implants 2004;19:XXX–XXX

Key words: animal research, dental implants, digital radiography, implant surfaces, peri-implantitis, periodontal diseases

Dental implant therapy has been related with high success rates.1,2 Nevertheless, dental implant failures have also been reported.3,4 These failures can be classified on the basis of both chronologic (ie, early versus late) and etiologic aspects. Early implant failures have been attributed to surgical trauma, poor bone quality and quantity, lack of primary stability, and bacterial contamination of the recipient site.5 Late implant failures are commonly associated with the occurrence of peri-implantitis. Peri-implantitis has been described as a destructive inflammatory process affecting the soft and hard tissues around osseointegrated implants, leading to the formation of a peri-implant pocket and loss of supporting bone.6,7

The relationship between different dental implant surfaces and bacterial biofilm in peri-implantitis development has not been completely evaluated. In addition, studies seeking to determine which surface (microstructure) or implant coating is more favorable for progression of the peri-implantitis are scarce. Evaluations of peri-implantitis around uncoated,8-10 titanium plasma-sprayed (TPS), and hydroxyapatite (HA)–coated titanium dental implants have been reported.11-13

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The aim of this study was to evaluate ligature-induced peri-implantitis around implants with 4 different surfaces by means of clinical and radiographic evaluation in dogs.

MATERIALS AND METHODS

Implant Design
The experimental design for this study has been previously described. In brief, 36 dental implants with 4 different surfaces were used. Nine TPS implants (ITI Esthetic Plus; Straumann, Waldenburg, Switzerland), 9 HA-coated implants (Calcitek; Sulzer Medica, Carlsbad, CA), 9 implants with hybrid surfaces (machined titanium in the first 3 threads and acid-etched in other threads; Osseotite; 3i/Implant Innovations, Palm Beach Gardens, FL), and 9 commercially pure titanium (CPTi) implants (3i/Implant Innovations) were used. All implants had lengths of 10 mm and diameters of 3.75 mm (except the TPS implants, which had a diameter of 4.1 mm).

Animals
The Institute of Animal Care and Use Committee of the Dental School of Araraquara approved this protocol. Six adult male mongrel dogs were used. At the beginning of the study, the dogs were 2 years old, with an average weight of 18 kg.

Surgery
Extraction. Extractions were carried out under general anesthesia and sterile conditions using 0.05 mg/kg of subcutaneous preanesthesia sedation (atropine sulphate) and intravenous injection of chlorpromazine (0.2 mL/kg body weight) and a 4% thiopental sodium solution (0.5 mL/kg body weight). The surgical site was disinfected with 0.12% chlorhexidine. Subsequently, 2% lidocaine hydrochloride with epinephrine 1:100,000 was administered as local anesthesia, and all 4 mandibular premolars were extracted, creating an edentulous ridge. To avoid occlusal trauma interference, the maxillary premolars were also extracted. Both the mandibular quadrants and the alveoli were allowed to heal for a period of 3 months.

Oral prophylaxis was performed for up to 2 weeks before tooth extraction. Plaque control during the healing period consisted of daily scrubbing with 0.12% chlorhexidine and scaling and root planing once a month until ligature placement (Fig 1).

Dental Implant Placement. Under aseptic surgical conditions, all dental implants were placed using a full thickness flap. Three implant sites were prepared per mandibular quadrant using original instruments for each dental implant system, according to the surgical techniques indicated by each implant manufacturer. A distance of approximately 10 mm between dental implant centers was maintained to avoid communication among the bone defects.

The implants were randomly distributed among the dogs so that each dental implant surface was represented at least once in each animal (Table 1). The implants were placed at the bone level, and a cover screw was screwed onto the implants, including the TPS implant (this was made possible by a modification in placement technique indicated by the manufacturer, ITI, for use with guided tissue regeneration, in which the implants are submerged) (Figs 2a and 2b). The flaps were sutured with single interrupted sutures to submerge all implants. Antibiotic therapy with potassium and sodium benzylpenicillicum (24,000 IU/kg) was started and continued once a week for 2 weeks to avoid postsurgical infection, and paracetamol was given for pain control. The sutures were removed after 10 days.

EXPERIMENTAL PERI-IMPLANTITIS

After a healing period of 3 months, healing abutments were connected, according to the indication of each dental implant system. After 45 days of peri-implant soft tissue healing, cotton floss ligatures were placed in a submarginal position around dental implants and sutured in peri-implant mucosa to hold the ligatures in position (Figs 3a to 3d). The positions of the ligatures were checked twice a week; further ligatures were placed at 20-day intervals for a period of 60 days, or until the implants had a loss of about 40% of radiographic bone.
height\textsuperscript{14,15} to accelerate peri-implant bone loss (Figs 4a to 4c).

### Clinical Evaluation

Clinical parameters were recorded at baseline and at 20, 40, and 60 days after ligatures had been placed. A single precalibrated examiner carried out the clinical exams. Presence of plaque, presence of peri-implant mucosal redness, and bleeding on probing (BOP) within 30 seconds of probe retraction were recorded at distobuccal, midbuccal, mesiobuccal, mesiolingual, midlingual, and distolingual aspects of each implant.

Probing depth (PD) and clinical attachment loss (CAL) were registered using a force-controlled calibrated periodontal probe (Florida Probe; Computerized Probe, Gainesville, FL) with a constant probing force of 0.20 N and a probe-tip diameter of 0.4 mm (Fig 5). PD and the distance between the gingival margin and a fixed point on the abutment surface were recorded. PD was then added to this distance to determine CAL. All measurements were performed at the same position with the aid of a dot marked in the abutment at baseline.

The width of keratinized mucosa at baseline was measured to the nearest 0.5 mm at the midbuccal and midlingual aspects of each implant.

### Mobility

Implant mobility was evaluated with the Periotest (Siemens, Bensheim, Germany) device. The implants were tapped with the Periotest rod perpendicular to the longitudinal axis of the implants. The Periotest handpiece was held parallel to the floor at a distance of about 2.0 mm from the abutment surface. The spot chosen for tapping was on the buccal aspect of the abutment. This spot was marked, and the measurement was always performed at the same place. The same Periotest device was used during the entire experiment. The Periotest was calibrated before each measurement, and all measurements were performed by the same investigator. Periotest

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**Table 1** Random Distribution of 4 Dental Implant Surfaces in 6 Dogs

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TPS = titanium plasma-sprayed; HA = hydroxyapatite-coated; AE = acid-etched surface; CPTi = commercially pure titanium; PMS, PM3, PM4 = mandibular premolars.

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**Fig 2a** Clinical view of the implants placed at the level bone. Note the TPS implant (arrow).

**Fig 2b** Diagram showing (a) the submerged and (b) nonsubmerged implants at level bone as well as the landmarks used to measure (1) VBL and (2) HBL.
Clinical view of experimental implants at baseline.

Cotton floss ligature sutured in peri-implant mucosa (arrow).

Clinical view of the same implants after a 60-day period of ligature-induced peri-implantitis.

Detail of the peri-implant tissue breakdown (arrow) after ligature removal.

Radiographic view at baseline of (left to right) a TPS implant, an HA-coated implant, and an acid-etched implant. Note the absence of radiolucency around all 3 experimental implants.

Radiographic view after a 20-day period of ligature-induced peri-implantitis.

Radiographic view of the implants after a 60-day period of ligature-induced peri-implantitis.
value (PTV) mean variations were assessed for each surface to avoid differences among the different implant surfaces used in this study.

**Radiographic Analysis**

Standardized periapical radiographs were taken with a digital image system (Computed Dental Radiography; Schick Technologies, Long Island City, NY) in order to measure the relative peri-implant vertical bone loss (VBL) and horizontal bone loss (HBL). A film holder system was affixed in a silicone bite block made of polyvinylsiloxane putty impression material also used to standardize the placement of the sensor in relationship to the implants and the x-ray source. Radiographs were obtained at baseline and 20, 40, and 60 days after ligature placement.

A dental x-ray machine equipped with a 35-cm-long cone was used to expose the periapical intraoral sensor. Exposure parameters were 70 kV (peak), 15 mA, and ¼ second at a focus-to-sensor distance of 37 cm. The linear distance between a fixed point on the abutment and the first visible bone-to-implant contact (VBL) on the digital image was determined on the mesial and distal sides of each implant. The mesial and distal values were averaged to obtain a mean VBL for each implant. The distance between a fixed point on the implant shoulder and the crestal bone margins in the horizontal aspect on the digital image was measured to determine HBL.

Two examiners made all radiographic measurements independently. If there were a discrepancy of 0.5 mm or less, the mean value of the 2 measurements was used. In situations with greater discrepancies, the images were analyzed again and discussed until consensus was reached.

**Statistical Analysis**

Data management and calculation were done using statistical software (SPSS version 10.1, Chicago, IL). Analysis of variance, using comparison of several proportions (contingency table), was used to compare the distribution of percentage of plaque, redness, and BOP for each type of implant and for different locations.

PD, CALs, VBL, and HBL were compared by means of 2-tailed paired t tests. To determine the correlations between keratinized tissue and clinical and radiographic features as well as the correlations between PTVs and VBL and HBL, $R^2$ correlation was determined. All tests were stratified according to dog (unit of analysis), ie, n = 6. Level of significance was set at .05.

**RESULTS**

**Clinical Parameters**

At baseline, following mechanical and chemical plaque control, the percentages of positive sites for plaque, peri-implant marginal redness, and BOP are presented in Table 2. There were no significant differences among the dental implant surfaces at baseline. After ligature placement and plaque accumulation, all indices increased significantly over time ($P < .001$). Some implants (2 CPTi, 1 HA, and 2 acid-etched) did not receive a ligature after 40 days of induced peri-implantitis because 40% of the peri-implant bone had already been lost.

The mean PD ranged from 1.49 ± 0.55 mm for acid-etched surfaces to 1.97 ± 0.79 mm for HA-coated surfaces at baseline (Table 3). After tissue breakdown, the CAL was associated with continuous increase. The mean CAL for the 60-day period ranged from 3.87 ± 1.69 mm for the TPS surface to 5.16 ± 1.53 mm for the CPTi surface. Statistically significant differences were not observed among the surfaces ($P > .05$); however, statistically significant differences were observed in regard to the baseline ($P < .05$).
At baseline, the mean width of keratinized mucosa in the buccal and lingual aspects was 2.04 ± 0.84 mm for TPS implants, 1.91 ± 0.91 mm for HA-coated implants, 2.20 ± 0.71 mm for acid-etched implants, and 1.95 ± 1.24 mm for CPTi implants. Statistical differences among implant surfaces were not found at baseline ($P > .05$).

**Mobility**

All dental implants remained immobile during the study. Mobility values were lower for the HA surface at baseline, but not significantly lower ($P > .05$). After ligature placement the PTV scores increased (Table 5). No significant differences were observed among implant surfaces. After ligature-induced peri-implantitis had developed, the TPS surface had smallest difference between baseline and day 60.
(1.67 ± 0.51), followed by the CPTi surface (3.83 ± 3.54), the acid-etched surface (4.00 ± 2.00), and the HA-coated surface (4.33 ± 3.01). No statistically significant differences were observed among the surfaces tested, although significant differences were observed in regard to the baseline measurements (P < .05).

**Radiographic Parameters**

At baseline, no dental implant exhibited peri-implant radiolucency. Relative VBL for all surfaces is presented in Table 6. Mean VBL was highest for implants with HA-coated surfaces (4.20 ± 0.47 mm) and lowest for implants with TPS surfaces (3.50 ± 0.97 mm), although no significant differences were observed among implant surfaces (P > .05). When the VBL was compared between baseline and 60 days after ligature placement, statistically significant differences were found (P = .001 for all surfaces).

The mean HBLs are presented in Table 7. Mean HBL was highest for implants with acid-etched surfaces (3.27 ± 1.29 mm) and lowest for CPTi implants (2.65 ± 0.50 mm). However, no significant differences were observed among implant surfaces (P > .05). When HBL was compared between baseline and 60 days after ligature placement, statistically significant differences were found (P = .002 for the AE surface and P < .001 for all other surfaces).

**Correlation**

Correlation analysis revealed a strong correlation between width of keratinized tissue and VBL for the TPS surface (r² = 0.81; P = .014) (Fig 6a). A significant correlation was also noted for PTV and VBL for the TPS surface (r² = 0.66; P = .04) (Fig 6b).

**DISCUSSION**

This study suggests that ligature-induced peri-implantitis around different dental implant surfaces results in rapid peri-implant tissue breakdown in dogs. Significant attachment loss and bone loss were established within 60 days (P < .05). The implants seem to depend on a functioning tissue barrier provided by the peri-implant mucosa in close contact with the implant surface. When plaque accumulation occurs, this barrier collapses, leading to an inflammatory infiltrate which leads to 2 distinct events: peri-implant mucositis, a lesion confined to the superficial soft tissues, and peri-implantitis, which involves the deeper soft tissues as well as the peri-implant bone.

At baseline all parameters confirmed the healthy status of all implants, with no differences among the dental implant surfaces (Table 2). At this time plaque control was suspended and the ligatures were placed. During the following observation period the presence of plaque, redness, and BOP increased significantly for all dental implant surfaces (P < .001).
Based on the available literature, it seems meaningful to use several clinical parameters to evaluate the health of dental implants.\textsuperscript{20–24} The percentage of BOP was relatively high at baseline, but it was not associated with plaque and mucosal status.\textsuperscript{[AU: Change OK?] In periodontal disease the BOP parameter is not a useful predictor of disease activity,\textsuperscript{25,26} although the absence of BOP is useful as a clinical indicator of periodontal stability.\textsuperscript{27} Peri-implant probing could provoke bleeding unrelated to the amount of inflammation in peri-implant soft tissues. These findings were in agreement with published animal\textsuperscript{28,29} and human studies.\textsuperscript{30,31} In addition, the higher BOP scores suggested that the junctional epithelium around implants might be more fragile than that found around teeth.\textsuperscript{32,33} All implant surfaces presented an increase of PD and CAL after a short time period (20 days) (Tables 2 to 4). These increases were similar to results achieved by Lang and coworkers,\textsuperscript{9} Schou and associates,\textsuperscript{10} and Nociti and colleagues.\textsuperscript{34} Increase of PD contributed to initial CAL, while other studies have shown gingival recession to be responsible for continued attachment loss.\textsuperscript{9,11–13}

The importance of the PD around dental implants has not received much attention. Several authors have evaluated relationship between PD and microbiologic and immunologic factors\textsuperscript{14–36} and have shown a positive association between deeper peri-implant pockets and the detection of periodontal pathogens. However, the clinical impact of probing measurements around dental implants is not clear. Some authors\textsuperscript{10,29} have examined probe penetration around teeth and implants and concluded that probes penetrate deeper in peri-implant tissue. Recently, Schou and colleagues\textsuperscript{28} evaluated probing measurements around implants and teeth in monkeys. The authors compared the PD in monkeys with healthy tissue, gingivitis/mucositis, and periodontitis/peri-implantitis. They observed that marginal inflammation was associated with deeper probe penetration around dental implants in comparison to teeth. The authors also suggested that differences between peri-implant and periodontal PD might be explained by the different marginal connective tissue fiber configurations.\textsuperscript{9} The data obtained from the present study showed PD increase as well as CAL over time; however, the study design (clinical evaluation) did not allow direct conclusions about the influence of connective tissue fiber [AU: configurations on?] soft peri-implant tissue.

Mobility is regarded as an important indicator of implant success or failure.\textsuperscript{1–12} Mobility was highest for CPTi surfaces and lowest for TPS surfaces, although no statistically significant differences were observed among implant surfaces. Differences between mechanisms of osseointegration of the implant surface and bone, different macrostructures among the implant surfaces, and difference available surface areas (ie, different microstructures) may somewhat explain the different PTVs observed. In addition, the greater diameter of the TPS implants used (4.1 mm for TPS implants versus 3.75 mm for other implants) may somewhat explain and validate these data. However, all implant surfaces presented statistically significant differences from the baseline PTV after 60 days of experimental peri-implant tissue breakdown. These results are in agreement with Tillmanns and associates\textsuperscript{11} and Ericsson and coworkers.\textsuperscript{37}
All 36 implants placed achieved successful tissue integration at baseline, demonstrating ankylosic stability without clinical signs of early failure. Crestal bone changes were observed around all implant surfaces at 60 days, although there were no statistically significant differences among implant surfaces. VBL was initially lowest for the HA-coated surface, but at the end of the experimental peri-implantitis period, the HA-coated surface presented the highest VBL, followed by the CPTi, acid-etched, and TPS surfaces. In a previous study, VBL was measured by means of periapical intraoral radiography. The results obtained from that study and the present investigation showed that both radiographic techniques (conventional and digital) were able to assess the progression of bone loss after experimental peri-implantitis.

In contrast, the HBL average was higher for the acid-etched surface and lower for the CPTi surface. It can be speculated that different mechanisms are instrumental in VBL and HBL. The parallel collagen fiber orientation observed around dental implants could influence this bone resorption mechanism.

Implant surface characteristics can influence bone response during the healing period. The sandblasted acid-etched surface has better osteoconductive properties compared to the TPS surface. When the 4 different dental implant surfaces were compared, no clinically statistical difference could be found. However, when the results for each dental implant surface were compared with baseline recordings, statistical differences were found for all clinical and radiographic parameters in this investigation. These findings suggest, in the short term (60 days), that the analyzed dental implant surfaces are similarly susceptible to and respond similarly to ligature-induced peri-implantitis.

The finding concerning which implant surface is more susceptible to peri-implant infection is still controversial. Studies have shown that TPS and HA surfaces are most affected by peri-implant disease. In the present investigation, contrasts were found with regard to bone loss. The TPS surface presented the lowest means for VBL while the CPTi surface presented a lower range for HBL. The HA-coated surface exhibited both the highest VBL over time and the greatest HBL. Some authors have suggested that the HA-coated surface may be reabsorbed and consequently may lose osseointegration in the presence of periodontal pathogens. However, the present data must be analyzed with caution because of the short period utilized for peri-implant tissue-breakdown, the small sample size, the mechanical production of peri-implantitis, and the use of an animal model. Further studies evaluating these surfaces for longer periods are needed.

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