Histomorphometric Analysis of the Bone-Implant Contact Obtained with 4 Different Implant Surface Treatments Placed Side by Side in the Dog Mandible

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Purpose: *The different implant systems available today present several types of surface treatment, with the aim of optimization of bone-implant contact. This study compared 4 different types of implant surfaces.* ***Materials and Methods:*** *The first, second, third, and fourth mandibular premolars were extracted from 5 young adult mongrel male dogs. Ninety days after removal, four 3.75-mm-diameter, 10-mm-long screw-type implants (Paragon) were placed with different surface treatments in mandibular hemiarches. The dogs received 2 implants of each of the following surface treatments: smooth (machined), titanium plasma spray (TPS), hydroxyapatite coating (HA), and sandblasting with soluble particles (SBM). The implants were maintained unloaded for 90 days. After this period, the animals were sacrificed, and the hemimandibles were extracted and histologically processed to obtain non-decalcified sections. Two longitudinal ground sections were made for each implant and analyzed under light microscopy coupled to a computerized system for histomorphometry.* ***Results:*** *The following means were obtained for bone-implant contact percentage: machined = 41.7%, TPS = 48.9%, HA = 57.9%, and SBM = 68.5%.* ***Discussion:*** *The means for all treatments that added roughness to the implant surface were numerically superior to the mean found for the machined surface. However, this difference was statistically significant only between groups SBM and machined (Tukey test,* $P < .05$*).* ***Conclusions:*** *The SBM-treated surface provided a greater bone-implant contact than a machined surface after 90 days without loading in this model.* (INT J ORAL MAXILLOFAC IMPLANTS 2002;17:377–383)

Key words: *animal study, dental implants, histomorphometry, osseointegration, surface properties*

The different endosseous implant systems currently available use several types of surface treatment, aiming for optimal bone-implant contact percentage. Results of numerous investigations are not conclusive concerning the best implant surface for obtaining clinical success. The previous systems have used smooth, commercially pure (cp) titanium surfaces, with only a slight roughness resulting from the machining process. Extensive documentation can be found in the literature relative to the biocompatibility and clinical success of these surfaces[1,2] and the absence of toxic or unfavorable reactions to the organism.[3]

Notwithstanding, more recent studies have shown that the existence of a certain surface roughness increases bone-to-implant contact, mainly in the earlier phases of the integration process and in areas of low-quality bone.[4–7] The roughness seems to favor the migration of undifferentiated cells, which cover the implant surface and maximize new bone formation.[8] These circumstances would lead to greater mechanical encrusting, resulting in the need of higher torque for implant removal.

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Several types of treatment have been proposed for increasing implant roughness. These include acid etching of the machined surfaces, application of titanium plasma spray, incorporation of hydroxyapatite (HA), and blasting with different substances (in general followed by acid etching). Etching of the machined implant surface can be carried out using different acids, including hydrochloric acid, sulfuric acid, hydrofluoric acid, and nitric acid. The titanium plasma layer is obtained by heating the metal until a plasma state is achieved and applying this plasma to the implant surface. This process results in a sixfold increase in the crevices of the implant surface,[9] producing crevices 30 to 50 µm deep[4] and improving microretention. The incorporation of HA is another widely used industrial method to modulate the implant surface. HA ceramics show extensively proven biocompatibility, which provokes superficial topographic irregularities similar to those created by the titanium plasma-spray application. Several studies have shown an increase of new bone formation in the initial stages of osseointegration surrounding this material (though this is not a long-term effect[10,11]), with the development of an osteophylic surface.[12] Thus, HA is one of the surfaces selected for use where the intention is to obtain maximum bone formation in the earlier stages of healing; for instance, in low quality bone,[13] especially Type IV bone (Lekholm and Zarb's classification[14]), as well as for implants placed immediately after tooth extraction, in this case associated with guided bone regeneration.[15]

Treatments that roughen the implant surface through blasting with several types of particles represent a firm trend in industry, since they combine an efficient method with low cost for the manufacturer. When substances not biocompatible with bone regeneration (as for instance, aluminum oxide particles or bioglass particles) are used in blasting, the methods to remove debris frequently involve successive rinsing in baths containing various substances.[16,17] Depending on the nature of these substances, planing of the implant surface occurs, reducing peaks and valleys created by the blasting process; in theory, this would also reduce some of the benefits provided by the roughness. A possible solution to this problem would be blasting with substances that are biocompatible, thus demanding less radical treatment after the roughening process. A system currently on the market (Paragon, Paragon Implant Company, Encino, CA) claims these characteristics, with a Soluble Blasting Media (SBM) creating rough surfaces that would not be extensively planed by a subsequent nitric acid bath. According to the manufacturer, this surface would be 250% rougher than surfaces that were only machined or machined and acid-etched. The objective of this study was to conduct a comparative histologic analysis of 4 different implant surfaces placed side by side in the dog mandible.

MATERIALS AND METHODS

Five young adult mongrel male dogs weighing approximately 10 kg were used in this investigation. The animals selected had intact maxillae and mandibles and no generalized occlusal trauma. The animals also had no oral viral or fungal-related lesions and were in good general health, with no systemic disorders.

In the first surgery, the first, second, third, and fourth mandibular premolars (P1, P2, P3, and P4, respectively) on both sides were extracted. The animals had been fasting since the previous evening. They were sedated with an intramuscular injection of 2% Rompun (0.5 mL/10 kg in 20 mg/kg; Bayer Laboratories, Porto Alegre, RS, Brazil) and were then anesthetized with an endovenous injection of thiopental (1 mL/kg in 20 mg/kg solution; Laboratório Cristália, Itapira, SP, Brazil) diluted in 50 mL of saline. After full flaps were elevated, access to P1, P2, P3, and P4 was obtained bilaterally. The teeth were sectioned longitudinally at the bifurcation and extracted with the help of extractors and forceps, with maximum care to prevent fractures of the bone walls. Buccal flaps were displaced coronally to cover the alveoli and sutured with 4-0 Vicryl reabsorbable sutures (Ethicon, São José dos Campos, SP, Brazil). After surgery, the animals received periodic weekly prophylaxis for plaque control for a period of 90 days. After this period, radiographs were obtained for the edentulous areas to detect complete bone healing.

Ninety days later, a second surgery was carried out to place the implants. On the evening before, the animals received an intramuscular injection of 20,000 IU penicillin and streptomycin (Pentabiótico Veterinário, Wyeth Laboratories, São Bernardo do Campo, SP, Brazil) for small-sized animals in the 1 g/kg body weight dosage. Since 1 dose provided antibiotic protection for 4 days, another dose was administered after 4 days, for a total of 8 days antibiotic protection.[18] The anesthetic technique was as previously described. A horizontal incision was made in the middle of the ridge, from the distal of the canine to the mesial of the first molar on both sides of the mandible. Releasing incisions were also made on the buccal and lingual surfaces at the end of the horizontal incisions. Mucoperiosteal

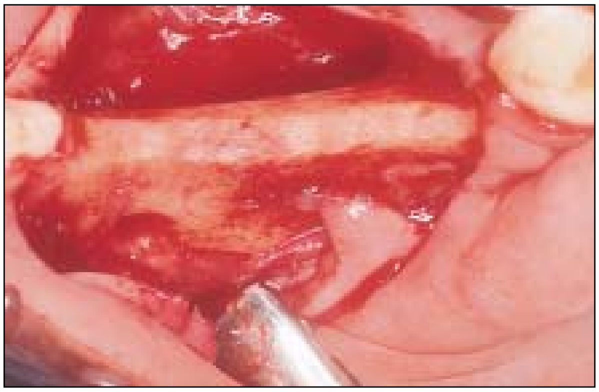

Fig 1a Mucoperiosteal flaps were elevated, and a superficial osteotomy was performed to improve alveolar ridge anatomy.

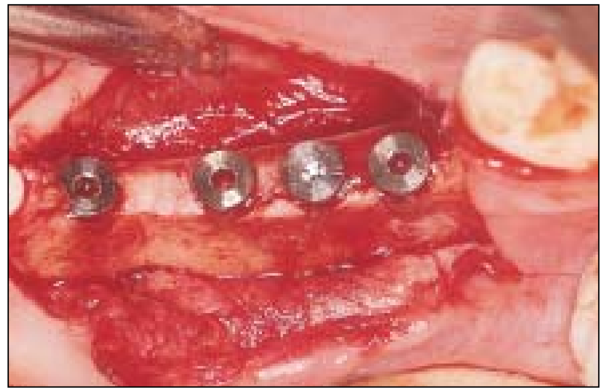

Fig 1b The implants were placed side by side from mesial to distal in the following order *(left to right)*: machined, HA, SBM, and TPS.

flaps were elevated, exposing the bone crest (Fig 1a), and according to the surgical protocol recommended by the manufacturer, 4 screw-type implants 3.75 mm in diameter and 10 mm in length (Paragon Implant Company, Encino, CA), each with a different surface treatment, were placed in each site (Fig 1b). The implants were placed from the mesial to distal of the mandible, without concern for implant position, especially since McMillan and coworkers[19] have shown that there are no significant differences in osseointegration caused by the position of the implant in the dog mandible, as follows:

1. Smooth implant (machined)
2. HA-coated implant
3. SBM-treated implant
4. TPS-treated implant

Forty implants were placed, 8 in each animal. After placement, the flap was returned to its original position and sutured with reabsorbable sutures (Vicryl 4-0) covering the implants. The animals received a soft diet for 14 days and were periodically evaluated to observe healing. During the healing period the teeth were cleaned weekly using ultrasonic points.

Twelve weeks after the surgical placement of the implants, the animals were sedated and sacrificed with a lethal dose of thiopental. The hemimandibles were removed, dissected, and fixed in a 4% buffered formaldehyde solution (pH 7) for 48 hours, and then transferred to a 70% ethanol solution until processing. The samples were dehydrated in increasing concentrations of alcohol (up to 100%), infiltrated in LR White resin (London Resin Company, London, England), sectioned with the technique described by Donath[20] in 50-µm-thick sections, and stained with Stevenel's blue and alizarin red 5.[21]

Histomorphometry was carried out by one investigator who was blinded to the study protocol. Two longitudinal histologic sections of each implant were analyzed using light microscopy (Axiophot, Zeiss-Jena, Oberkochen, Germany) coupled to a videocamera capture system ZVS47EC (Zeiss-Jena) and a Snappy Video Snapshot (Play, Rancho Cordova, CA). Measurements were made with Metamorph Software (Universal Imaging, West Chester, PA). The amount of contact between the bone and the implant surface at its middle one third (in the central 3.3 mm of the implant) was determined for all sections. The contact percentage between bone and the implant was obtained using the equation: $P_{ci} = (M_{ci} \times 100) / M_{tot}$, where P_{ci} = contact percentage between the bone and the middle third of the implant, M_{ci} = linear measure in micrometers of the contact between the bone and the middle third of the implant, and M_{tot} = linear measure in micrometers of the middle third surface of the implant.

The contact percentage was determined for each section. To obtain the implant's contact percentage, an arithmetic mean was calculated for the longitudinal sections for each implant. The arithmetic mean and the standard deviation for each of the 4 groups of implants (machined, HA, SBM, and TPS) were calculated. The significance of the statistical difference between the means for the groups was determined by the Kruskal-Wallis test ($P < .05$).

Table 1 Percentages of Bone-to-Implant Contact for All Implants

Implant no.	Machined	HA	TPS	SBM
1	32.2	71.9	39.8	70.6
2	53.3	36.4	26.4	29.4
3	39.7	46.4	33.8	47.6
4	34.7	73.9	34.5	87.6
5	45.0	44.9	—	75.4
6	29.0	49.0	44.8	84.3
7	48.6	33.6	54.8	—
8	41.7	86.1	39.5	78.9
9	44.5	69.1	84.2	64.8
10	48.5	67.7	82.6	77.7
Mean	41.7	57.9	48.9	68.5
SD	7.8	18.0	21.1	18.8

Overall mean and SD: 54.0 ± 19.2%.

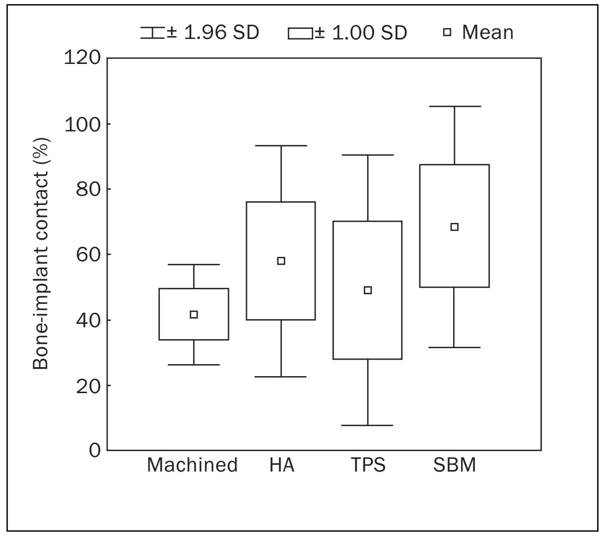

Fig 2 Mean percentages of bone-to-implant contact and standard deviations of the 4 implant surfaces examined.

RESULTS

Two implants were lost during the healing phase, 1 from the TPS group and the other from the SBM group; they presented extensive radiographic evidence of osseous reabsorption and/or clinical mobility. Thus, a total of 38 implants were analyzed histomorphometrically, 10 from the machined and HA groups and 9 from the TPS and SBM groups.

Table 1 shows the percentage of bone-to-implant contact for all the analyzed implants. The machined group presented values varying from 29.0% to 53.3%, with a variation amplitude between the maximum and minimum values of 24.3 (the lowest among all the groups). The HA group values ranged from 33.6% to 86.1%, showing greater amplitude value (52.5). For the TPS group, the lowest bone-to-implant contact percentage value was 26.4% (the lowest among all the groups), and the highest was 84.2%, providing a variation amplitude of 57.8. Finally, for the SBM group, the values ranged from 29.4% to 87.6% (the highest value among all the groups), with a variation amplitude of 58.2 (the highest among the analyzed groups).

The arithmetic means and the standard deviations for the bone to implant contact percentage of the groups are presented in Table 1. The mean of the machined group (41.7%) was numerically inferior to those of the other groups. Among the groups that received surface treatment to increase roughness, the SBM group presented the highest mean bone-implant contact percentage (68.5%), followed by the HA (57.9%) and TPS (48.9%) groups. Figure 2 shows the means for each group, as well as the values for standard deviation and 95% confidence interval for the standard deviation (1.96 × SD).

Figures 3a to 3d illustrate areas with high percentages of osseointegration in the 4 groups, observed with a 12.5× objective (original magnification ×100).

Analysis of variance among the groups was performed to detect statistical significance. The test results were as follows: degrees of freedom effect = 3; minimum square effect = 1259.04; degrees of freedom error = 34; minimum square error = 289.46; F = 4.35. The P value was .011 (lower than .05, the initially determined cutoff value), indicating that there was a statistically significant difference among the groups.

To compare the groups, the Tukey test for unequal samples was applied. Table 2 depicts the results considering significance of the differences among the groups. The comparisons between the machined and HA, machined and TPS, and TPS and HA groups were not statistically significant at the 5% level. The only statistically significant difference was between the machined and SBM groups (P = .017).

Fig 3a *(Left)* Bone-implant contact in a machined-surface sample (Stevenel's blue and alizarin red 5; original magnification ×100).

Fig 3b *(Right)* Bone-implant contact in an HA-coated sample (Stevenel's blue and alizarin red 5; original magnification ×100).

Fig 3c *(Left)* Bone-implant contact in a TPS sample (Stevenel's blue and alizarin red 5; original magnification ×100).

Fig 3d *(Right)* Bone-implant contact in an SBM sample (Stevenel's blue and alizarin red 5; original magnification ×100).

DISCUSSION

Endosseous implants have appeared in the implant literature as a highly predictable technique for replacement of lost teeth. Recognized initially as a treatment for complete edentulism, the osseointegration concept was soon extrapolated for use in other restorative applications. Thus, implants previously used for treating edentulous mandibles and maxillae are today a feasible alternative, with high rates of success, for the rehabilitation of single teeth and of partial edentulism.

Extrapolation of the technique in different situations found in the oral environment was paralleled by

Table 2 Multiple Comparison of Samples

Comparison	*P* value
Machined versus HA	.165
Machined versus TPS	.806
Machined versus SBM	.011*
HA versus TPS	.680
HA versus SBM	.559
TPS versus SBM	.089

*Statistically significant ($P < .05$), Tukey test for unequal samples.

a significant development in the industry of the methods and materials used to manufacture implants and prosthetic rehabilitation components. There are now predictable protocols for placing implants immediately after tooth extraction[18,22] as well as in association with bone grafts in block or particle form.

In recent years, important alterations in the commercially available implants have been made. Several in vitro studies,[23,24] histologic studies in animals[23] and in humans,[25] and clinical investigations[7] have demonstrated some superiority of rough surfaces over smooth surfaces in relation to early bone-to-implant contact percentages, mainly in less-than-ideal situations, eg, areas of poor bone quality.

Superficial roughness on implants can be obtained using several methods such as acid etching, application of TPS or HA, and blasting with several substances. Blasted surfaces have shown good results in increasing bone-to-implant contact.[5,17] The use of a soluble, easy-to-remove substance in blasting that produces irregularities of adequate size is quite useful in the manufacturing of implants.

Paragon implants with a SBM surface have acid-etched apical (2.5-mm extension) and coronal (1.5-mm extension) extremities, and the rest of the implant is blasted with tricalcium phosphate subsequently removed with nitric acid. According to the manufacturer, this treatment produces a surface that is 2 to 3 times rougher than the machined surface. Thus, it is important to histologically analyze its behavior in comparison with other types of surfaces previously described in the literature, for example, the machined, the HA-coated, or the TPS.

The histomorphometric analysis was performed at the middle one third of the implants, as suggested by Evans and associates[10] and Novaes Jr and coworkers.[18] The coronal one third was avoided, because of the risk of misinterpreting the loss of crestal bone and epithelium downgrowth adjacent to the polished collar of implants that is commonly seen in dogs[26–28] as was the apical one third, since the implants approximated or slightly penetrated the superior wall of the inferior alveolar canal.[18,24]

The present results showed a statistically significant difference between the machined and SBM implants. The mean bone-to-implant contact percentage on machined implants was numerically the lowest among the 4 tested groups, presenting the smallest standard deviation; this indicated that this group of samples was the most homogeneous. These results support other investigations stating that formation of new bone on machined surfaces is less than roughened surfaces[4,29] in the short term.

The HA and TPS groups presented higher mean bone-implant contact percentages than the machined group; however, this difference was not statistically significant. These results are similar to those reported by Weinlaender and colleagues,[30] who compared cp titanium, TPS, and HA-coated implants 12 weeks after implantation in dogs. The authors found a significantly greater amount of bone associated with HA-coated surfaces versus TPS or cp titanium. In the present study, although there were similar differences (HA > TPS > machined), they were not statistically significant.

The SBM surface, which is blasted with tricalcium phosphate and subsequently dissolved in nitric acid, presented results statistically superior to the machined surface and numerically superior to the other 2 surfaces. It is interesting to observe that, although variation was large, this was caused only by 1 specimen with a bone-to-implant contact rate below 30%. In this group, 7 of the 9 implants presented bone-to-implant contact percentages above 60% (6 above 70%), a high value when compared to other studies.[30,31]

CONCLUSIONS

Considering these results, the SBM surface analyzed in relation to bone-implant contact was statistically superior to the machined surface and showed a response numerically superior to HA and TPS surfaces after 90 days in place without loading in the dog model. A recent study has shown that under certain conditions the histologic difference may be transferred to the clinical situation.[32] Thus, well-controlled human clinical studies should be conducted, placing implants in low-quality bone, to analyze the behavior of the SBM surface in that situation.

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