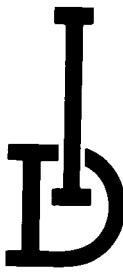


COUNTERTORQUE TESTING AND HISTOMORPHOMETRIC ANALYSIS OF VARIOUS IMPLANT SURFACES IN CANINES: A PILOT STUDY



Pablo Pebé Odont*
R. Barbot Odont*
J. Trinidad Odont*
A. Pesquera DVM*
Jose Lucente Odont, DDS**
Russell Nishimura DDS***
Hisham Nasr DDS, MSD†

As surface roughness may play a role in the mechanical attachment of an implant surface to bone, various implant surfaces have been prepared and analyzed by removal torque (countertorque) or push-out tests in a variety of animal model systems. Rougher surfaces generally have displayed higher mechanical testing values, indicating a stronger implant-bone interface. This pilot study was undertaken to test the countertorque values for integrated threaded implants with surfaces prepared by machining, blasting, and acid-etching, to compare the various implant surface types histomorphometrically for percentage of bone-implant contact under loaded and unloaded conditions, and to determine the degree of correlation between countertorque values and bone-implant contact with varying degrees of surface roughness. The results of this animal investigation suggest that the strength of the bone-implant interface, as determined by countertorque testing, is influenced by different surface characteristics. Acid-etched surfaces resisted countertorque forces more successfully as compared with blasted or machined surfaces. Histologic evaluation of bone contact with the various implant surfaces did not demonstrate a definite advantage for rougher surfaces in regard to percentage of bone contact at the light microscopic level. (Implant Dent 1997;6:259-265)

Surface roughness may play a role in the mechanical attachment of an implant surface to bone. Various implant surfaces have been prepared and analyzed by removal torque (countertorque) or push-out tests in a variety of animal model systems.¹⁻⁹ Surfaces have been tested that were altered by special plasma-sprayed coatings such as titanium⁹ or hydroxyapatite,^{4,6,7} acid-etching,³ blasting with various types and sizes of particles,^{5,7,8,9} and combinations of these methods.^{7,9} Rougher surfaces generally have displayed higher mechanical testing values, indicating a stronger implant-bone interface.

Histomorphometric analysis of interfacial bone-implant contact has also been conducted in several animal model systems using implant surfaces of varying surface topographies.^{1,7,8} Percentage bone contact was not always greatest for the roughest surface analyzed.^{1,8} This study was undertaken (1) to test the relative countertorque values for osseointegrated threaded implants with surfaces prepared by machin-

ing, by blasting with titanium dioxide particles, or by treating with acid-etching; (2) to compare the various implant surface types histomorphometrically for percentage of bone interfacial contact using the light microscope under loaded and unloaded conditions; and (3) to determine the degree of correlation between countertorque values and bone interfacial contact for the same implants with varying degrees of surface roughness.

MATERIALS AND METHODS

Five mongrel dogs, weighing approximately 30 kg each, had their posterior mandibles partially edentulated (premolars and first molars) at least 3 months before implant placement. At least one implant of each surface type (machined, blasted with 10- to 45-grit-size titanium dioxide particles, and blasted and acid-etched with hydrochloric and sulfuric acids) was placed in the edentulous premolar area, and two implants were placed in the first molar areas of each dog for support of two-unit fixed prostheses. One implant of each surface type was placed in each mandible for unloaded and loaded evaluation. All 30 intraoral implants (10 of each surface treatment) were threaded and made of commercially pure titanium with a diameter of 3.75 mm and a 10-mm length (3i Implant Innovations, Inc., Palm Beach Gardens, FL). At least one implant of each type was placed in the femur of

*University of Uruguay, Montevideo, Uruguay.

**Implant Innovations, Inc., Palm Beach Gardens, FL.

***University of California at Los Angeles, School of Dentistry, Los Angeles, CA.

†Louisiana State University School of Dentistry, New Orleans, LA.

each dog for subsequent surface analysis using scanning electron microscopy.

The dogs were tranquilized with acepromazine (0.3 mg/kg) and anesthetized with thiopental sodium (30 mg/kg) using an intravenous solution of Ringer's lactate. The surgical areas were infiltrated locally with 2 percent lidocaine containing 1:100,000 epinephrine. Preoperatively, the dogs received cephadrine (.15 mg/kg) intravenously and postoperatively, benzyl penicillin G (40,000 U/kg) intramuscularly.

The implants were allowed to integrate for approximately 4 months before abutment connection. After second-stage surgery, the molar implants were restored within 1 month and loaded for 4 months. The mandibular fixed prostheses occluded with the opposing natural first molars. The animals were fed soft dog chow for 1 week after the surgical procedures and sustained on a hard diet after the implants were loaded. The animals' teeth were brushed mechanically on a weekly basis.

The dogs were euthanized with thiopental sodium. Before removal of the posterior mandibles, sufficient bone was removed around each implant to allow attachment of an implant mount for connection to a digital countertorquing device (Mark-10 Corporation, Hicksville, NY) (Fig. 1). The implants were counter torqued to failure and precisely replaced into their alveolar housings with the aid of index marks on their midbuccal aspects. Hemimandibles were fixed in formalin and subsequently sectioned into blocks for histomorphometric analysis.

Histologic specimens were prepared according to the nondecalcified sectioning technique of Donath.¹⁰ The specimens were dehydrated in ethanol, infiltrated with a plastic medium, and subsequently embedded by light polymerization. The specimens were then mounted on slides and prepared for longitudinal buccolingual sections with an average thickness of 25 μm using a precision cutting and microgrinding technique. The slides were stained with a modified trichrome stain and preliminarily examined at original magnifications of $\times 1.3$, $\times 10$, $\times 20$, and $\times 40$.



Fig. 1. Simulated use of digital countertorquing assembly.

Interfacial Bone Contact

All specimens were analyzed for percent interfacial bone contact using an Olympus BH-2 light microscope with an attached MTV-3 video camera and Cue-2 image analysis software system version 1.7 (Olympus Corporation, Scientific Products Group, Lake Success, NY). Histologic sections were viewed at $\times 40$ magnification and transferred to the imaging system monitor via the video camera for analysis. A micrometer grid slide was projected at the same magnification and used to determine the magnification factor between the viewed images on the microscope and the analyzing monitor. This was calculated to be $\times 8.36$ lens magnification, producing a monitor image projected at $\times 334.4$ for interfacial analysis.

Bone interfacial contact was measured in microns along the longitudinal axis of each implant surface from the most coronal bone contact point. Marrow spaces and/or soft tissues that interfaced with implant threads were discounted as contact areas. Direct osseous tissue contact areas were summated to calculate a percent bone interfacial contact of the total possible implant surface available for integration. Results were averaged for buccal and lingual surfaces and statistically analyzed for each implant type.

Scanning Electron Microscopy

Femoral implants were removed by countertorquing, and their surfaces were enzymatically cleaned with EDTA, collagenases, and a proteinase. Scanning electron micrographs were taken at magnifications of $\times 2,000$ and $\times 20,000$ with an accelerating voltage of 30 Kv and visually compared for the degree of roughness.

Statistical Analysis

A three-way analysis of variance (ANOVA) was used to determine any differences in countertorque values and percent bone contact for the three different implant surfaces at a significance level of $P = .05$. The effects of loaded versus unloaded conditions and differences in outcome among animals were analyzed for countertorque values and bone interfacial contact, with follow-up comparisons using the Duncan Multiple Range Test with significance determined at the $P = .05$ level. The Pearson r correlation coefficient was used to compare individual and combined treatment groups for the relationship between countertorque value and mean percentage of bone contact.

RESULTS

Scanning electron micrographs showed a progressive roughness of the implant surfaces in the order of machined < blasted < acid-etched (Figs. 2 to 4). Of the 30 implants evaluated, two in the unloaded blasted group were mobile at the time of reverse



Fig. 2. Scanning electron micrograph of machined implant surface. Note regular surface (original magnification $\times 2000$).



Fig. 3. Scanning electron micrograph of blasted implant surface. Macroirregularities are evident (original magnification $\times 2000$).

torque testing. Individual animal mean counter-torque values and percent bone-implant contact for loaded implants in fixed prostheses and nonloaded implants in the same dogs are listed in Table 1. No significant differences among the animals were discerned.

Loaded and unloaded implants performed at the same level with regard to counter-torque values with no statistically significant difference (Table 2). Duncan Multiple Range comparison revealed a significant advantage for percent bone contact for loaded as compared with unloaded implants, especially for loaded implants with acid-etched surfaces.

ANOVA testing demonstrated an overall significant difference in counter-torque values among the three surface groups at $P = .01$ (Table 3). The acid-etched group exhibited significantly higher values as compared with machined and blasted implants ($P =$

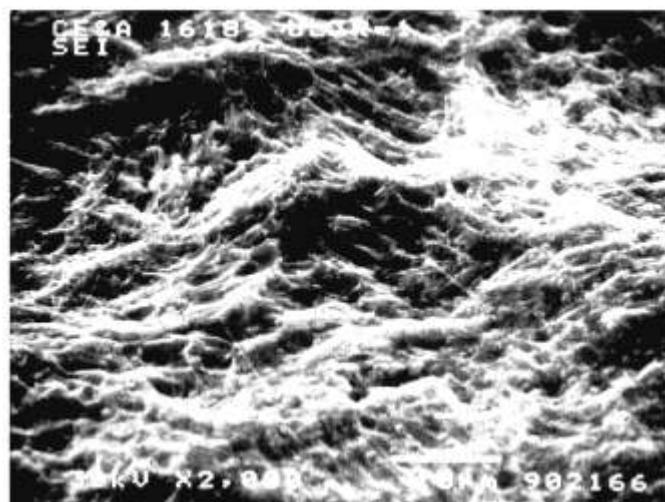


Fig. 4. Scanning electron micrograph of acid-etched implant surface. Macroirregularities and microirregularities are evident (original magnification $\times 2000$).

Table 1. Mean Countertorque Values and Bone-Implant Contact (No Significant Differences at $P = .05$)

Animal	Number of implants	Countertorque (N-cm)	Bone-implant contact (percent)
1	6	46.2 ± 20.4	46.7 ± 19.8
2	6	64.3 ± 12.7	56.8 ± 8.9
3	5	52.2 ± 32.0	56.1 ± 17.7
4	5	55.9 ± 18.2	53.6 ± 18.4
5	6	46.8 ± 19.9	46.5 ± 14.4

Table 2. Mean Values for Loaded and Unloaded Implants

Condition	Number of implants	Countertorque (N-cm)	Bone-implant contact (percent)
Loaded	15	56.3 ± 22.2	57.6 ± 15.02
Unloaded	13	49.2 ± 19.0	44.9 ± 13.9

Table 3. Mean Values for Implant Surface Type

Surface	Number of implants	Countertorque (N-cm)	Bone-implant contact (percent)
Machined	10	50.6 ± 21.3	55.0 ± 13.2
Blasted	8	39.8 ± 14.6	38.5 ± 15.8
Acid-etched	10	66.0 ± 17.9	59.0 ± 11.6

.05) according to the Duncan Multiple Range Test. There was also a significant difference for percentage bone contact among the implant surface groups at $P = .05$ (Figs. 5 to 7). A Duncan Multiple Range comparison revealed a significant disadvantage for the blasted surface group.

The Pearson r Correlation Coefficient demonstrated a significant correlation between mean counter-torque value and mean percentage bone contact for all treatment groups combined (Table 4). For the in-

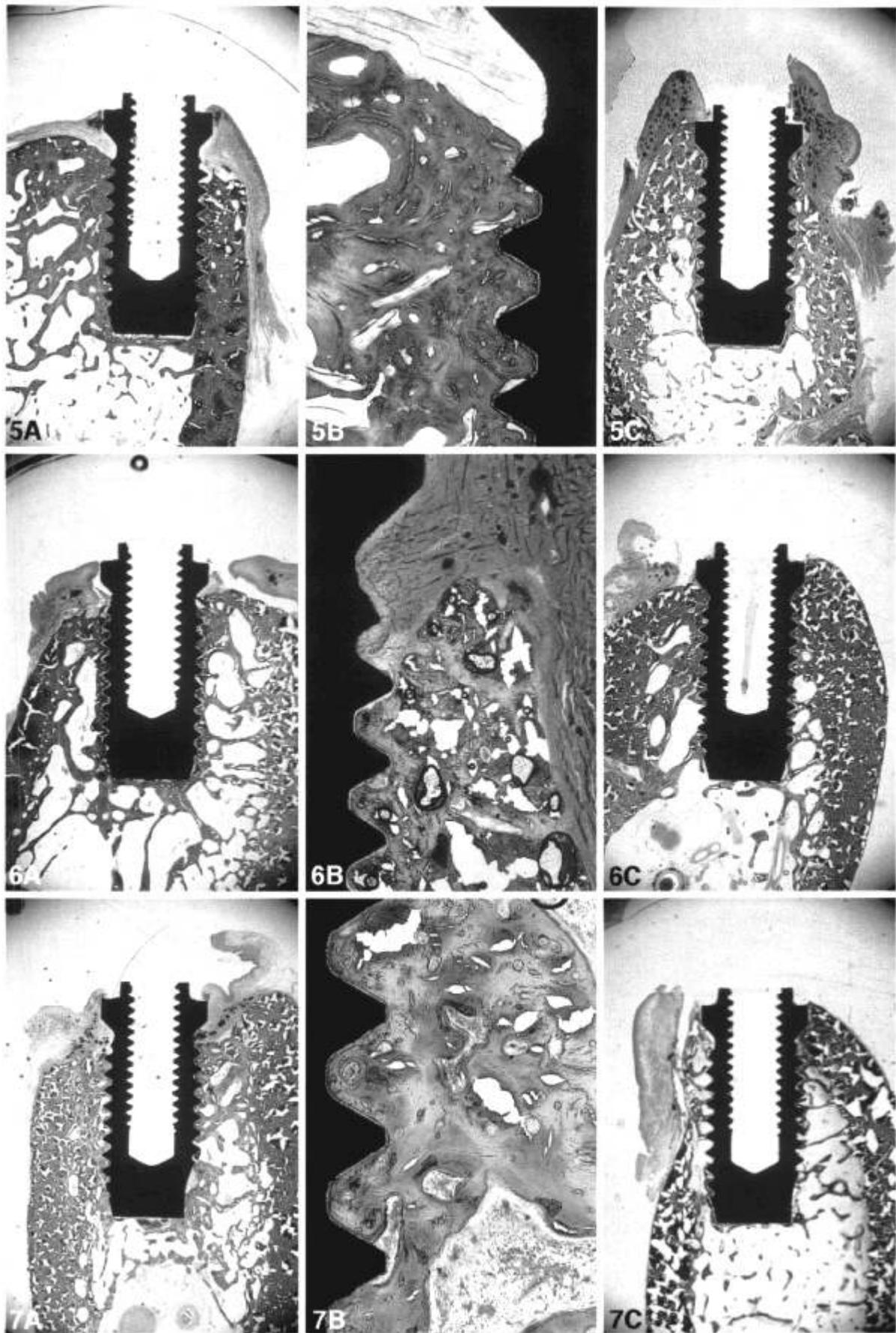


Fig. 5. A, Loaded machined implant well anchored in cortical bone. Note functional adaption of bone apically. The bone-implant contact is 74.6 percent; the countertorque value is 80.3 N-cm (original magnification $\times 1.3$). B, Implant pictured in 5A with a higher magnification showing fair adaption of bone to implant threads (original magnification $\times 10$). C, Unloaded machined implant in loose cancellous bone. Note thin seam of bone surrounding implant. The bone-implant contact is 62.0 percent; the countertorque value is 52.5 N-cm (original magnification $\times 1.3$).

dividual group correlations, the blasted group showed the strongest correlation between countertorque values and bone-implant contact.

DISCUSSION

Some authors have noted greater values for histologic bone contact with loaded as compared with unloaded implants in the same canine model system. Evans et al¹¹ found such differences with commercially pure titanium threaded implants at time periods of 3 and 6 months after prosthetic loading with mandibular fixed prostheses in dogs. A similar finding was reported by Kohri et al.¹² This investigation found a significant overall difference between loaded and unloaded implants of all surface types for histologic bone contact but not for countertorque values.

Countertorquing threaded implants at certain threshold forces has been advocated as a means of determining successful integration or the relative strength of the implant-bone interface by Sullivan et al.¹³ Wilke et al⁹ placed threaded implants with varying surface treatments in sheep tibiae and determined removal torque values at different time periods. The investigators found that blasting with large grit particles combined with attack by strong acids produced an implant surface with the highest removal torque values. This was in contrast to electropolished, plasma sprayed, or surfaces sandblasted and/or treated with weaker acids. In this study, acid-etched implants also produced the highest countertorque values.

Mechanical pushout tests have been conducted to measure the interfacial strength between bone and cylindrical implants with various surface features. Wong et al⁷ implanted cylinders of various metals into trabecular bone sites in mature miniature pigs with surfaces that had been fine-blasted with glass beads of 150- to 250- μm diameter, rough-blasted with alumina particles of 300- to 400- μm diameter, or rough-blasted and etched with hydrochloric and sulfuric acids. They found a correlation between implant surface roughness and pushout failure load, which after hydroxyapatite-coated implants, was highest for rough-surfaced, commercially pure titanium implants etched with hydrochloric and sulfuric acids.

In this investigation, implants with machined or titanium dioxide blasted surfaces, under loaded and nonloaded conditions, were not different in regard to

Table 4. Correlations Among Mean Countertorque Values and Bone-Implant Contact (Pearson r Correlation Coefficient)

Surface	Number of implants	Correlation value
Machined	10	0.61
Blasted	8	0.82
Acid-etched	10	0.43
All surfaces combined	28	0.68

countertorque values. These findings are in contrast to a canine study by Gotfredsen et al⁸ of unloaded machined as compared with TiO_2 -blasted implants placed immediately in extraction sockets and subjected to removal torque forces 12 weeks after placement. The investigators computed the total torsion moment necessary to unscrew the implants and found over twice the resistance to removal torques in blasted as compared with machined commercially pure titanium implants. Possible reasons for the differences in outcome between the findings of Gotfredsen et al⁸ and this investigation might include differences in surface topographies of the specimens and a possible advantage for blasted implants placed immediately in extraction sockets (especially at earlier observation times) due to greater surface area for improved clot retention and increased bone-implant interfacial contact.

Wennerberg et al¹⁴ tested commercially pure titanium implants with different surface topographies in rabbits and found a short-term disadvantage for highly increased surface roughness as compared with a moderately increased surface roughness using mechanical and histologic evaluations. The authors offered the possible explanations of ionic release, potentially a negative factor for osteogenesis, and alterations in implant thread geometry by the blasting procedure.

Buser et al¹⁵ using a miniature pig model reported the greatest histologic bone-implant contact in surfaces sandblasted with large grit particles and further roughened with hydrochloric and sulfuric acids, second only to hydroxyapatite surfaces. In this study, histomorphometric comparison of the various surfaces at the light microscopic level demonstrated no statistical differences between machined and acid-etched surfaces. Both surface types were statistically

Fig. 6. A, Loaded blasted implant in loose trabecular bone. The bone-implant contact is 58.2 percent; the countertorque value is 51.9 N-cm (original magnification $\times 1.3$). B, Loaded blasted implant exhibiting poor bone adaptation to implant threads. The bone-implant contact is 22.7 percent; the countertorque value is 21.5 N-cm (original magnification $\times 10$). C, Unloaded blasted implant in loose cancellous bone (apical half). The bone-implant contact is 41.4 percent; the countertorque value is 57.5 N-cm (original magnification $\times 1.3$).

Fig. 7. A, Loaded acid-etched implant in bone of variable density. The bone-implant contact is 79.0 percent; the countertorque value is 92.7 N-cm (original magnification $\times 1.3$). B, Loaded acid-etched implant displaying intimate bone adaptation to implant threads. The bone-implant contact is 63.2 percent; the countertorque value is 61.3 N-cm (original magnification $\times 20$). C, Unloaded acid-etched implant in extremely loose trabecular bone (note crestal bone to neck of implant). The bone-implant contact is 41.8 percent; the countertorque value is 77.8 N-cm (original magnification $\times 1.3$).

superior to blasted surfaces when analyzed for bone interfacial contact. These results are in contrast to those reported by Gotfredsen et al,⁸ who demonstrated no histomorphometric differences between implants with blasted or machined surfaces, although differences in removal torque values were found.

Although blasted and machined implant surfaces were not found to be significantly different in regard to countertorque values in this investigation, a significant difference was observed with the addition of acid-etching. Acid-treatment produces microirregularities on the machined implant surface, and the mechanical advantages can exceed those provided only by machining or blasting.

Johansson et al² found a progressive increase of bone in direct contact with commercially pure titanium implant surfaces and a similar increase in removal torque forces over healing times ranging from 3 weeks to 12 months. The investigators reported that when implants from each time group were compared for removal torque values as compared with percentage of bone contact, there was a high correlation between a high removal force in one rabbit tibial metaphysis and a high percentage of implant-bone contact in the opposite limb. A significant overall correlation between these two variables was found in this study.

Due to the limited sample size, it is difficult to draw any definite conclusions regarding the statistical significance suggesting superiority of acid-treated surfaces over machined or blasted surfaces with regard to removal torque forces. Certainly a larger sample size would be appropriate for studying the biologic performance of different implant surfaces and to clarify the correlation between mechanical testing and histomorphometric measurements. Light microscopic histomorphometry of one longitudinal section for a single replaced implant may be misleading for interpretation of bone contact at the cellular level. Without more definitive histologic interpretations, countertorquing may constitute the most practical test for adequacy of the implant-bone interface.

Klokkevold et al³ found that chemical etching of the titanium implant surface in rabbits increased the strength of integration fourfold, as determined by resistance to reverse torque testing. Some manufacturers are developing acid-etched implant surfaces with microirregularities, which may enhance interfacial strength without the risk of incorporating blasting particles and/or their attendant contaminants on the implant surface. Further testing is indicated for clinical evaluation of the efficacy of such surfaces. Studies using larger groups of animals and analyses of implant-bone interfacial contact at the cellular level will provide more information concerning the relationship between implant surface topography and bone interfacial strength.

CONCLUSION

The results of this pilot animal investigation suggest that the strength of the bone-implant interface,

as determined by countertorque testing, is influenced by different surface characteristics. Acid-etched surfaces resisted countertorque forces more successfully as compared with blasted or machined surfaces. Histologic evaluation of bone contact with the various implant surfaces did not demonstrate a definite advantage for rougher surfaces in regard to percentage of bone contact at the light microscopic level.

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Reprint requests to:

Dr. Russell D. Nishimura
Room AO - 156 CHS
10833 LeConte Avenue
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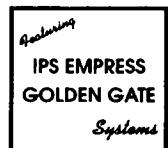
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Gary R. Gann CDT, MAAIP is a Certified Dental Technician and holds a Mastership in implants from the American Academy of Implant Prosthodontics. He has completed the Misch Implant Prosthetics course and has extensive experience in implant dentistry. Gary is a member of the ICOI, AAIP and Pinnacle Study Club.

Steven A. DeMatos CDT is a Certified Dental Technician. He is a Master Ceramist with extensive training in dental ceramics and implants. He has completed many courses including The Peter Dawson Occlusion Series. Steve is a member of The Pinnacle Study Club.

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