Histologic Comparison of a Thermal Dual-etched Implant Surface to Machined, TPS, and HA Surfaces: Bone Contact in Vivo in Rabbits

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Purpose: To evaluate the bone contact percentage around a proprietary high-temperature dual-etched (DE) implant surface (Osseotite) versus implants with machined, hydroxyapatite (HA), and titanium plasma-sprayed (TPS) surfaces. Materials and Methods: Each implant type was placed in rabbit tibiae of the same animal and assessed at 1 to 8 weeks. Histologic sections were prepared and analyzed histomorphometrically. Results: The DE implant surface achieved higher levels of bone contact percentage than the other surfaces. This enhanced contact level was apparent by 3 weeks and seen at all time intervals except 2 weeks, at which machined exceeded the DE mean. In evaluating which surface outscored the others in each individual rabbit, there was a statistically significant confidence for the DE surface (P < .001). The other 3 surfaces failed to show significance, although the numeric scores for the TPS surfaces were below random expectations and the machined scores were slightly above. There was no correlation between degree of roughness and bone contact percentage. Discussion: Arbitrarily roughening the implant surface may not result in a large change in bone conductivity. The specific texture of the DE process yielded more contact, possibly as the result of better fibrin clot retention and growth factor enhancement. Conclusions: There was no advantage demonstrated in this model to an HA surface over titanium. The bone contact to the rough HA surface scored similarly to that for the TPS surface of similar roughness, and well below that for the DE titanium surface. The DE surface appeared to have an advantage in bone contact percentage, particularly in early healing in a rabbit tibia model. (Int J Oral Maxillofac Implants 2002;17:369–376)

Key words: bone density, dental implants, histology, hydroxyapatites, rabbit, surface properties, titanium

For more than a decade, researchers have investigated implant surfaces possessing the property of enhanced bone-to-implant contact. This contact, and its rate of occurrence, may be key to implant function and clinical timing. Early “macro” rough surfaces such as plasma-sprayed hydroxyapatite (HA) have been seen to possess higher percentages of direct bone contact than smooth, sandblasted, and plasma-sprayed titanium surfaces. These macro-rough surfaces have also commonly been associated with rapid, severe bone destruction should exposure and contamination of the surface occur.1-3 Textured surfaces with a specific bone-promoting geometry may hold hope for improvement.

The specific texture resulting from various treatments can strongly influence bone contact and mechanical interface strength. A dual-etched (DE) surface treatment of machined titanium implants resulted in a 3.5-fold increase in mechanical pull-out force compared to the untreated machined surface implant controls.1 Wennerberg and associates1

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compared titanium surfaces sandblasted with 25-
and 75-µm particles (which resulted in actual sur-
face roughness of 1.1 and 1.5 µm, respectively) and
found higher pull-out strength and bone contact in
the 75-µm blasted surface. Additionally, Park and
Davies showed enhanced red blood cell agglomera-
tion and platelet aggregation on a DE surface com-
pared to a machined surface.

Various studies have evaluated the contact of
bone directly to implant surfaces in different mod-
els, both animal and human. In a pig leg model,
Buser and coworkers evaluated machined titanium,
plasma-sprayed titanium (TPS), plasma-sprayed
hydroxyapatite (HA), sandblasted, and blasted and
etched titanium surfaces. They showed that, among
the titanium surfaces, both blasted and TPS surfaces
showed a slight increase in bone con-
tact over the native machined surface. Etching with
hydrochloric acid/sulfuric acid after the blasting resulted in a more dramatic increase in the contact
level, approaching that of the HA-coated surface.
Hosseini reported that a DE surface allowed
increased fibrin stabilization in vitro. In an in vivo
study, Dziedzic and associates found that bone
growing into chambers maintained contact with the
titanium walls when etched to specific surface fea-
tures, whereas the control machined surfaces exhib-
it a pattern consistent with a lifting of the clot
from the wall, resulting in less bone contact. In a
large, 3-year multicenter study, HA-surfaced
implants were shown to have higher survival rates
than non-coated implants. Block and colleagues
showed greater bone-to-implant contact in HA-
coated implants than in grit-blasted titanium-surface
implants. Lazzara and coworkers, in a split-
implant study, evaluated the difference between
machined titanium and DE surfaces on either side
of the same implant in humans. They demonstrated
large differences, with the DE surface showing
increased bone contact when assessed at 6 months.

Very rough implant surfaces have shown a
greater risk of long-term failure and morbidity.
Johnson first spoke of morbidity associated with
HA-coated implants in 1992. Block and associates
showed a non-morbid success rate of less than 65%
for HA-coated implants in a large prospective study
spanning 10 years. Wheeler reported survival rates
for HA-plasma sprayed implants of only 77.8%, ver-
sus 92.7% for TPS implants over a similar period of
time (up to 8 years). Assessing 16,935 implants in a
meta-analysis of 73 articles, Esposito and coworkers
found a higher rate of late failures because of pro-
gressive bone loss in TPS single-stage implants ver-
sus machined titanium, 2-stage implants. Addition-
ally, HA-coated implants have shown a tendency for
resorption or separation of the coating, with clinical
sequelae. Liao and colleagues demonstrated
detached HA particles and resultant inflammation
near the implant histologically. Rohrer and associ-
ates demonstrated histologically that the HA coat-
ing had separated from implants and was free within
surrounding connective tissue or surrounded by
invaginating epithelium.

The characteristics of HA may play a role in in-
vivo responses. Gross and coworkers evaluated sev-
eral manufacturers’ HA-coated implants. They
generally found 60% to 100% crystallinity, with 1 site as low as 40% and appearing molten and lamel-
lar. Chang and colleagues concluded that 50%,
70%, and 90% crystallinity resulted in no signifi-
cant difference in bone formation in a canine
model. While higher crystallinity may help stabilize
the implant surface, it appeared to show no benefit
in enhanced bone formation.

The ideal implant surface might exhibit the
enhanced bone contact seen with HA-coated
implants, combined with the long-term disease
resistance of machined titanium implants. It was
hypothesized that the proprietary DE process
would improve bone contact for titanium implants.
This histologic investigation of bone contact com-
pared the Osseotite high-temperature DE surface
(3i/Implant Innovations Inc, Palm Beach Gardens,
FL) to traditional, commonly used surfaces: TPS,
HA plasma-sprayed, and machined titanium.

MATERIALS AND METHODS

Implant Surfaces

The implants used were fabricated especially for
this study with 4 different surfaces, varying in com-
position and roughness (3i/Implant Innovations). The
specifications from the coating vendor used for
the HA-coated implants in this study included a
crystallinity of 75% to 85%, usually defined as
medium-high crystallinity. Mean profilometry data
(Fig 1) was gathered using an optical profiler–type
3-dimensional profilometer (RST Plus, WYCO,
Tucson, AZ) without filters, with vertical resolution
as low as 0.1 nm, a spot size of 80 by 60 µm, and
averaged for production implants. There is a
marked difference in surface roughness as measured
for roughness average (Ra, the mean height of
peaks); root-mean-square (RMS) roughness (Rq, the
3-dimensional volumetric average); and the maxi-
imum height of the profile (Rmax) from highest to low-
est point. The Ra is the most commonly compared
parameter, describing the mean departure of peaks
from a mean plane. The Rq is sensitive to extreme

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values because of the squaring in the RMS calculation. The R des describes the extremes of deviation, from lowest valley to highest peak. The DE and machined surfaces had much lower roughness than the TPS or HA surfaces. While DE increased the machined texture by 2 to 3 times, plasma spraying titanium or HA yielded about 30 times the roughness of the machined surface.

Surgical Procedure
Four non-threaded cylindrical implants were placed into the tibiae of 11 rabbits, 1 each of the DE, HA, TPS, and machined-surface implants, under anesthesia as described previously (Fig 2). The implants measured 3.3 mm in diameter by 4 mm in length. They were identical in placement parameters. The implant core diameters varied such that the final coated implants had the same diameter as the machined and etched implants. A 3.4-mm cover screw precisely controlled the depth of placement and provided a reference point for the original bone surface. Two implants were placed on each side, alternating right and left and proximal versus distal locations. Two animals were sacrificed at each interval of 1, 2, 3, 4, and 5 weeks and 1 animal at 8 weeks. The bone segments were retrieved, sectioned, and immediately fixed in formalin.

Histologic Preparation
Blocks were processed according to Akimoto and associates. Briefly, each block, with implant intact in bone, was fixed in 10% formalin, dehydrated, and infiltrated with embedding resin (Technovit 7200 VLC, Heraeus Kulzer, Wehrheim, Germany) for 30 days. Following infiltration the specimens were embedded and polymerized under temperature-controlled conditions. The specimens were then prepared by the Rohrer modification of the cutting/grinding method of Donath, which involved cutting the samples to 150 µm and polishing to 30 µm. The final slides were stained with Stevenel’s blue and van Gieson’s picro-fuchsin.

Histomorphometric Measurements
The section that was cut most perpendicular and central to the implant was selected for analysis. A photomicrograph was taken at 12:1 magnification using a light microscope (BX40, Olympus America, Melville, NY) fitted with a single-lens reflex camera (SC35, Olympus America) using slide film (Ektachrome 100, Eastman Kodak, Rochester, NY). The sections were photographed and the photographic slides were scanned at high-resolution (1,350 dpi) on a digital slide scanner (LS-1000, Nikon USA, Melville, NY).

A histomorphometric analysis was carried out utilizing image analysis software (ImageTool, University of Texas Health Science Center, San Antonio, TX) on a personal computer (Fig 3). Scoring was done in a single-blind manner, with the operator unaware of the surface. To assess contact percentage, the total linear distance of the implant below the bone crest was measured. After this, each segment of bone contact was measured and summed. The total distance of...
contact (TDC) divided by the total linear distance (TLD) yielded the percentage contact ([TDC/TLD] × 100%).

**Statistical Analysis**
The samples were compared using a pairwise Wilcoxon signed rank test with Bonferroni correction and a Friedman test for randomized block design. The Friedman test is a nonparametric test that uses the ranks of the implant contact within each animal. All statistical analysis and linear regression lines were calculated utilizing statistical software (SPSS 10.0, Chicago, IL) on a personal computer, setting significance at \( P = .05 \).

**RESULTS**

**Observations by Surface**

**Variability.** Mean contact, averaging each data point for the duration of the study, was calculated by surface. In the histologic sections from each animal (Fig 4), bone-to-implant contact ranged from 3% (Week 1, TPS) to 60% (Week 4, DE). The mean bone contact percentages (± SD) were: DE, 38.9 ± 3.9%; MACH, 31.0 ± 4.3%; TPS, 24.0 ± 4.6%; and HA, 22.5 ± 3.9%. The DE surface demonstrated a significantly \( (P < .05) \) higher overall mean bone contact than the HA implants, averaged over the 8 weeks. The machined and TPS surfaces, while showing a tendency toward lesser overall mean contact than DE, were not significantly different from any of the surfaces tested. The percentage bone contact at all time points is shown in Fig 5, which also provides the range seen for each surface.

**Ranked Implant Analysis.** Since clinically one might wonder which surface would give the highest contact percentage in a given subject, the top-ranked in each surface comparison was determined for each individual animal. Each surface was ranked as higher or lower in bone contact than the other surfaces, in that same animal. When surface contact percentage was ranked in this manner within the same animal, the results were assessed with a nonparametric blocked statistical comparison. The rankings of bone contact in each animal are apparent in Fig 5. The Friedman test showed significant \( (P < .001) \) overall differences in the ranked bone

**Fig 3** Histomorphometry. *(Left, A)* To assess contact percentage, the total linear distance of implant placed below the bone crest was measured. *(Right, B)* After this, each segment of bone contact *(inset)* was measured and summed. The total distance of contact *(B)* divided by the total linear distance *(A)* yielded the percentage contact.

**Fig 4** Histology. Sections analyzed for percent bone contact. Shown here is half of each implant from one animal, to allow an appreciation of the differences in bone conduction. Yellow line = bone level at placement; MACH = machined implant; DE = dual-etched implant; TPS = titanium plasma-sprayed implant; HA = HA-coated implant.
contact scores, with the ranking order of DE > machined > TPS > HA.

In examining each of the implant surface pairings in a given animal, the DE surface exhibited significantly higher bone contact than each of the other surfaces: versus machined ($P = .035$), versus TPS ($P = .028$), and versus HA ($P = .004$). None of the other surface pairings demonstrated significant differences. There was no linear relationship between surface roughness and contact percentage (data not shown). In fact, the rough surfaces (TPS and HA) ranked lower than the smoother surfaces. The DE surface ranked higher in bone contact than the other surfaces 30 of 33 possible times. Thus, the DE surface showed the highest ranking per animal more frequently than the other surfaces.

Observations by Time
Each of the implant surfaces showed increases in bone contact percentage over time. Linear regression (trend) lines made comparisons easier to follow (Fig 6). At every time interval except 2 weeks, the DE surface averaged a higher percentage of bone contact. The initial elevation of the DE trend line shows a trend toward higher bone contact earlier than the other surfaces. Its slope shows a rate of gain in contact percentage that parallels the other surfaces, while remaining greater. The machined implant trend line slope indicated a slower rate of bone contact gain over time than the other surfaces. While the single 8-week specimen showed a drop, this was deemed to be a variation in healing in the particular animal. Three of the 4 surfaces (DE, machined, and TPS) converged toward similar bone contact levels in the week 8 animal. Despite this decrease, the DE trend line still gained at the steepest rate, although no further inferences were made.

DISCUSSION
Extrapolating a possible application in humans, the DE surface showed the highest percentage of early contact, when greater contact is most valuable for rapid application of load or resistance to transmucosal loading. For single-stage surgical applications, this early gain in contact percentage may provide opportunity for enhanced support to resist
inadvertent loading. This early bone contact may in part explain the higher clinical success rates of this implant's surface in humans compared to the other surfaces.26-30

When the clinician is considering which surface to select for a particular application, the most relevant information is demonstrated success in humans. In this study, the DE surface showed a high propensity, in each individual animal, to achieve higher contact percentages than any of the other examined surfaces. The extent of clinical significance of this is unknown. It is not clear how much contact percentage or total surface is necessary to achieve integration and clinical stability. As all of the surfaces tested have had some degree of success in humans, clearly they can achieve adequate contact. Assuming some minimum threshold of bone contact is required, the greater the typical contact, the more frequently the implant will reach that threshold.

When implants are placed into low-quality bone, greater bone contact may compensate in part for the fewer trabeculae. In grafting situations, where a non-absorbable or non-vital bone graft material may be occupying a large percentage of the bone volume, a larger contact percentage with the areas of vital bone could allow the implant to stay above that threshold.

The specific character of implant surface roughness has greater significance than the measured degree of roughness. In this study, the DE surface displayed greater bone contact than surfaces of both lesser and greater roughness. Cordioli and coworkers demonstrated that DE implants yielded greater bone-to-implant contact than grit-blasted implants of similar roughness.31 While Ivanoff and associates showed no difference between grit-blasted and machined titanium micro implants in the human maxilla,32 Lazzara and colleagues found considerable difference between the DE surface and machined surface on a 2-surfaced micro implant in the human maxilla.17 The former blasted and latter DE surfaces measured similar roughness values (Ra of 1 to 2 µm), yet the DE appeared to yield greater bone in a similar clinical setting.

Buser and coworkers12 and Wong and associates13 found a marked difference in the scanning electron micrographic appearance between grit-blasted versus blasted-then-dual-etched titanium surfaces; yet to the naked eye, the clinician may perceive the corresponding implants as identical. In both of these pig models, the addition of DE resulted in significantly greater bone contact than grit blasting alone.

In addition to the aforementioned fixation of fibrin by the DE surface studied here, the surface may enhance bone growth via enhancing levels of bone growth factors. Park and Davies illustrated greater numbers of platelets on DE than machined titanium disks in vitro.6 Park and coworkers showed greater P-selectin expression and platelet microparticle formation with DE and blasted implant surfaces than with machined or polished titanium surfaces.34 By fixing the clot to the surface and by favoring bone growth factors, the DE surface appeared to have a synergistic increase in osteoconduction along its surface.

The implants used in this study were short enough to allow for a clean comparison, with the surface as the only variable. In one 5-week HA specimen, bone contact spread from the opposing cortex. This was scored as contact and could positively influence the outcomes for such implants. The small implant size prevented other incidents of this, thus not affecting outcomes while allowing the use of a reproducible, inexpensive model. The rabbit model is limited in that the proximal tibiae are devoid of significant trabeculation. Most bone contact appeared to migrate apically from the cortex. As long as variables are highly controlled, this appears to be a valid comparative model. The proximal-distal sectioning plane, and the recording of bone contact measurements as a percentage of the total surface, prevented the influence of nearby bone walls from affecting the results, even if sections were sliced somewhat eccentrically.

CONCLUSIONS

A proprietary DE implant surface (Osseotite) achieved higher levels of bone contact percentage than machined, HA, and TPS surfaces in rabbit tibiae. This enhanced contact level was seen at all time intervals, except at 2 weeks, where the machined-surface implant exceeded the DE implant mean. In evaluating which surface outscored the others in each individual rabbit, there was a statistically significant confidence for the DE surface to exceed the level of the other surfaces. The other 3 surfaces compared to each other failed to show significance, high or low, although the numeric scores for the sprayed surfaces were below random expectations, and the machined-surface scores were slightly above random limits.

There was no observed correlation between the profilometry data and bone contact percentage. There would appear to be something else dictating bone apposition in direct contact with the implant surface than merely roughness. Simply roughening
the surface may not result in a large change in bone conductivity. Grit-blasted and machined implant surfaces have demonstrated less bone contact than when etched with hydrochloric acid/sulfuric acid. The specific texture of the DE titanium yielded more contact, possibly the result of better fibrin clot retention, as speculated by Dziedzic and associates.14 Similarly, there was no advantage demonstrated in this model to an HA surface over titanium. The rough HA surface scored similarly to the TPS surface of similar roughness and well below the DE titanium surface. The DE surface appeared to have an advantage in bone contact percentage, particularly early in healing in a rabbit tibia model. It would appear to offer bone advantages over HA, with surface roughness closer to a machined surface. This may offer enhanced maintainability without sacrificing high bone contact.

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