

Influence of a Nanometer-Scale Surface Enhancement on De Novo Bone Formation on Titanium Implants: A Histomorphometric Study in Human Maxillae



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In this prospective randomized controlled clinical study, small titanium implants were placed in posterior maxillae for the purpose of assessing the rate and extent of new bone development. Nine pairs of site evaluation implants were placed in posterior areas of maxillae and retrieved with trephine drills after 4 or 8 weeks of unloaded healing. The amount of bone in linear contact (%) with the implant surface was used to determine the osteoconductive potential of the implant surface. Implant surfaces were dual acid etched (n = 9) (controls) or dual acid etched and further conditioned with nanometer-scale crystals of calcium phosphate (n = 9) (test implants), and the surfaces were compared. The implants and surrounding tissues were processed for histologic analysis. The mean bone-to-implant contact value for the test surface was significantly increased over that of the control implants at both time intervals (P < .01). For the implants/patients included in this study, the addition of a nanometer-scale calcium phosphate treatment to a dual acid-etched implant surface appeared to increase the extent of bone development after 4 and 8 weeks of healing. (Int J Periodontics Restorative Dent 2007;27:211-219.)

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Modification of turned or machined-surface titanium implants via treatment with acids to create microtopography has been shown to affect the manner in which titanium implants heal in bone.¹ The dual acid-etched (DAE) implant surface has been shown to develop significantly more adherent bone than a machined-surface implant during unloaded healing in the same bone conditions.² Recognizing that surface modifications can affect the rate and extent of new adherent bone formation, additional investigations have led to new surface developments.^{3,4} One such development is the modification of an implant surface using nanometer-scale-size crystals of hydroxyapatite.⁵

Bone-to-implant contact (BIC) was used as an indicator of differences in the surface healing capacity of two different implant surfaces where site evaluation implants (SEIs) were placed into posterior maxillae to determine whether the surface modification could increase the extent of adherent bone.² Similar approaches to evaluating human histology have been reported by Lazzara et al² and Trisi et al,^{6,7} where 5-mm-long SEIs were made with split

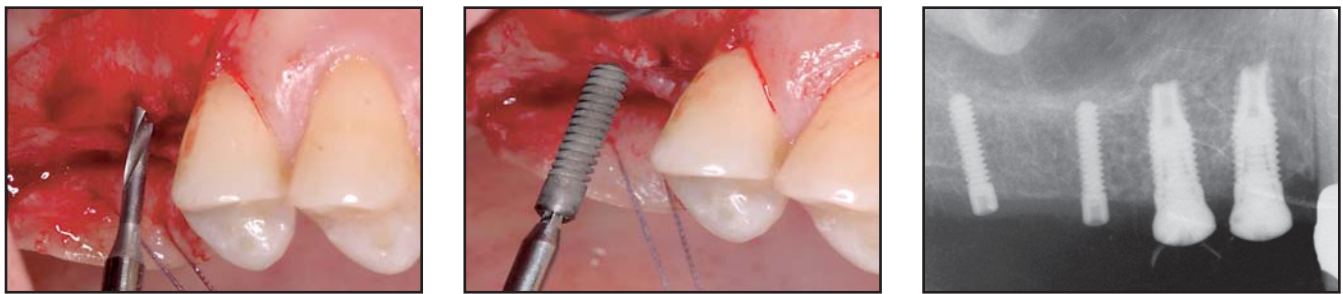


Fig 1 (left and center) Illustrations of SEI placement in the posterior maxilla. A 1.3-mm drill was used to prepare the osteotomy for placement of the 2-mm-diameter SEI. (right) Periapical radiograph taken immediately after implant placement.

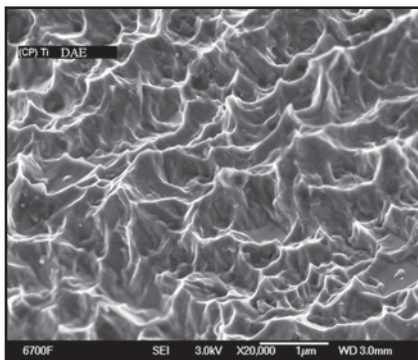
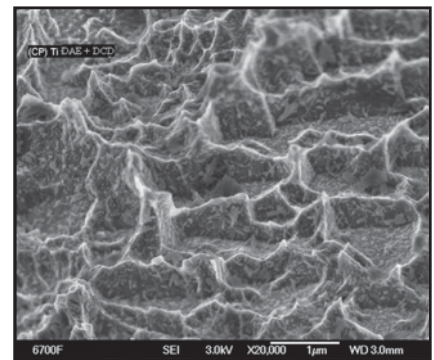


Fig 2 Scanning electron micrographs illustrating surface enhancement of (left) the DAE Osseotite surface and (right) the DAE surface with the addition of the Nanotite crystal depositions (magnification $\times 20,000$).



surfaces (one side with a microtextured [DAE] surface and the opposing with a machined surface). These studies evaluated the percent BIC after 8 weeks of unloaded healing. These implants were also placed in posterior maxillae. A similar implant design was also used in a study by Trisi et al.⁷ All of the above studies reported significant increases in the percent BIC for microtextured (Osseotite, BIOMET/3i) surfaces compared to machined surfaces after 8 weeks of unloaded healing.

The purpose of the present study was to evaluate, in humans, the extent of BIC with small SEIs that featured either a DAE surface or a DAE surface further modified with the addition of nanometer-scale hydroxyapatite (CaP) crystals.

Method and materials

Study implants

All SEIs were custom manufactured for this study and prepared with a DAE system (Osseotite). All SEIs were titanium alloy threaded implants measuring 2 mm in diameter and 9.5 mm in length. There was a 1.0-mm collar at the coronal aspect of each implant that did not have a modified surface (Fig 1). At the top of the implant was an internal hex capable of mating with a standard small-hex driver.

To create the test implants, the modified DAE implant surfaces were further modified by using a solution-based system whereby individual CaP crystals were bonded to the titanium



Fig 3 (left) Trephine and core samples, along with the 4.25-mm trephine drills used to recover the implants and tissue. (right) The extricated core sample shows the SEI in the middle of an intact bone core.



oxide surface that was previously acid etched (Nanotite, BIOMET/3i). Approximately half of the resulting surface of each implant was covered with crystals that ranged from 20 to 100 nm in length (Fig 2). The control implants did not receive the additional surface enhancement.

Study design

This was a randomized, controlled, double-blinded study in nine patients with partial or complete edentulism who were planning to use dental implants to restore their lost dentitions. Only patients in whom the SEIs could be placed at the site or in close proximity to a site where clinical implants would be placed were considered. Patients enrolled in the study exhibited no active infection or severe inflammation in the areas intended for implant placement. All patients provided written, informed consent.

One of each type of SEI (nine Osseotite [control], nine Nanotite [test]) was placed during or before the placement of definitive implants (ie, those that would support a prosthesis) in posterior maxillae. The SEIs were placed either on the same side or on contralateral sites, depending on available space on the ridge. Where space was plentiful, two SEIs were placed on the same side. A randomization scheme was followed to ensure balance in the placement of test and control implants in the most posterior positions of maxillary ridges so that not all the control or test SEIs would be at the most posterior locations. The SEIs were retrieved with 4-mm-diameter (interior diameter) trephine drills (Fig 3) at either 4 or 8 weeks after placement. The specimens were processed for histomorphometric analysis to measure the percent BIC.

Procedures

Enrolled patients were examined and interviewed at screening visits for collection of demographic information and medical and dental conditions as part of their clinical implant treatment planning.

Brasseler 1.3-mm-diameter twist drills were used to prepare the osteotomies. The SEIs were placed using a standard small-hex driver mounted on an implant drilling unit. Implants were placed so that approximately 1 mm of the coronal portions of the implants remained above the crestal bone margin. After placement of the SEIs, periapical or panoramic radiographs were obtained to document implant placement. The protocol for this study involved unloaded healing for all of the implants. Either 4 or 8 weeks after implant placement, the SEIs were retrieved by trephine drills with an internal diameter of 4.0 mm. A guide was mounted on the SEIs to establish the orientation of the trephine along the long axis of the implant.

Histologic processing of samples

Samples were removed from the trephine drills with care to avoid disturbing the bone material surrounding the implant (see Fig 3). The specimens were placed in 10% buffered formalin solution. Explanted specimens were packaged in labeled containers and shipped to the histologic processing laboratory by overnight courier. Specimen labels did not include the nature of the implant surface. Laboratory personnel were blinded to the implant groups (test vs control) during processing and analysis.

After the samples were inspected for possible shipping artifacts, the implant specimens were infiltrated in Remacryl resin (Hoescht), from a starting solution of 50% ethanol/resin and progressing to a 100% resin solution. The implants were oriented longitudinally. Each step in this process required 24 hours. Photopolymerization was performed using 48-hour blue light exposure. After polymerization, the blocks were ground to remove excess resin and expose the bone-implant interface. The blocks were glued onto plastic slides using a methacrylate-based adhesive. A Remet Micromet high-speed rotating blade microtome was used to obtain a 250- μm -thick section from each block. To provide better contrast, a Remet LS-2 grinding machine equipped with waterproof grinding paper was used to grind sections to approximately 40 μm . After grinding, each section was polished with polishing paper and a 3- μm polishing cream. Two different staining procedures were used to process the

histologic sections. Toluidine blue was used to analyze the different healing periods and remodeling patterns of the bone, and basic fuchsin was used to distinguish fibrous tissue and improve contrast.

Histomorphometric analysis of ground samples

The microscopic images were digitized with a color video camera (TK-C1380, JVC Victor) and a frame grabber according to automated skeletal histomorphometry.⁸ The digitized images included the entire implant surface and were acquired at 25 \times . Subsequently the images were analyzed with image analysis software (Xres 2.0, Macro-media) for histomorphometric calculations. To determine the percent BIC for each section, the linear surfaces along the implants that were directly contacting bone were measured.

To determine bone volume (BV), the amount of bone matrix was measured over the entire microscopic field by outlining the bone islands and surfaces and calculating the surface areas of bone in each particular microscopic field. These two-dimensional measures of bone surface areas were expressed as three-dimensional percentages of the total volume using stereologic principles.⁹ For the purposes of the study, the authors considered this outcome to represent bone quality.

Statistical methods

In this study, the primary contributing independent variables were implant type (Nanotite vs Osseotite) and time (4 and 8+ weeks). Single and multiple analyses of variance were applied to test the influence on the dependent, continuous variables each of BIC and BV outcomes. In addition, a pairwise correlation was conducted between BIC and BV. All analyses were conducted using commercially available statistical software (JMP v5.0.1, SAS Institute).

Results

Enrollment and treatment took place between May and November 2006. Healing at all surgical sites was unremarkable and without complications or exposure of the SEIs. All trephine samples were obtained with a full core of intact material encompassing the SEIs. For all recovered SEIs, shipping to the histologic laboratory was successful without evidence of preservative leakage or damage to the core specimens. At the completion of histomorphometric analysis and data recording, the blinding codes were unsealed and the outcomes for the paired test and control implants were analyzed.

Histologic observations

Analysis of the stained sections found that, for the 18 specimens, the SEIs were placed within 1 mm of the crestal bone margin. For all but one SEI specimen, the surrounding tissue was com-

Table 1 Histomorphometric outcomes of SEI analyses

Pair	Weeks	% BIC		% BV	
		Nanotite	Osseotite	Nanotite	Osseotite
1	4	47.1	13.7	26.1	36.8
2	4	50.2	20.1	17.6	21.7
3	4	36.1	11.1	31.9	22.6
4	8	44.0	35.9	28.3	28.9
5	8	30.1	18.5	31.9	17.5
6	8	54.8	22.0	41.9	16.9
7	8	39.3	25.9	15.6	24.6
8	8	84.0	0.0	30.2	0.0
9	12	19.7	7.3	34.5	24.8
Overall		45.0 ± 18.1	17.2 ± 10.6	28.7 ± 8.2	21.5 ± 10.1
4-week mean		44.5 ± 7.4	15.5 ± 4.6	25.2 ± 7.2	27.0 ± 8.5
8+-week mean*		45.3 ± 22.4	18.3 ± 12.9	44.5 ± 7.4	18.8 ± 10.3

*Includes data from the SEIs retrieved at 12 weeks.

posed of bone. One implant was encapsulated only by dense fibrous tissue. All explant samples had the SEIs at or near the center of the specimens, with little evidence of cutting artifacts, indicating good alignment of the trephine along the long axis of the SEIs.

Histomorphometric outcomes

BIC analysis

The quantitative histomorphometric outcomes for the SEIs are illustrated in Table 1. Of the nine pairs of explanted SEIs, three pairs were retrieved after 4 weeks of healing, five were retrieved after 8 weeks of healing, and one pair was not retrieved until 12 weeks because the patient was unexpectedly required to travel abroad. One control 8-week SEI was observed to have complete fibrous encapsulation on histologic analysis; therefore 0% BIC was included in the analyses. When this explant specimen's value was included

with the other control SEI values, the BIC values for the Nanotite and Osseotite SEIs were 45.0% and 17.2%, respectively ($P < .01$).

Bone volume analysis

Percent BV values for the individual test and control SEIs are included in Table 1. The 8-week SEI that was found to have a fibrous encapsulation had no BV value to record. The overall means for percent BV for the test and control SEIs were 28.7% and 21.5%, respectively. The difference trended toward but did not reach statistical significance ($P = .19$).

Discussion

This study was done to assess BIC and the extent of proximal de novo bone formation along the long axis of small-diameter implants during the early stages of unloaded healing in posterior maxillae of human subjects. A specific

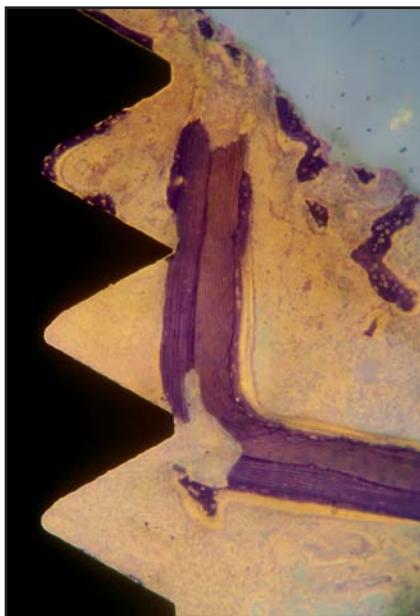


Fig 4 (left) Control DAE surface at 4 weeks. New lamellar bone is forming on the surfaces of fractured trabeculae. The fractured trabeculae are being repaired by layers of new bone covered by osteoid layers and osteoclastic remodeling. New woven bone and osteoid layers were also found at the interface with the implants. The implant surface is not completely covered by new bone (toluidine blue; $\times 50$).

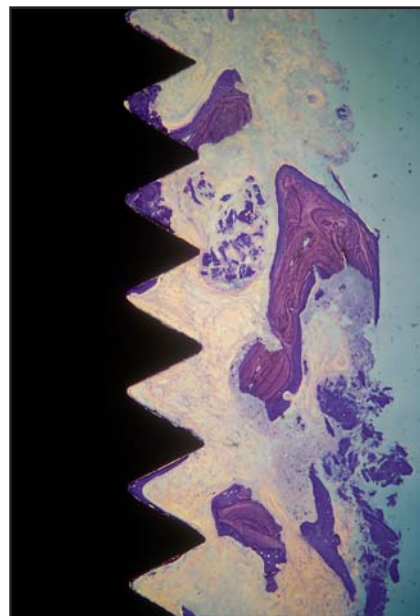


Fig 5 (right) Control surface at 8 weeks. On the implant surface, the new bone is formed in a typical flowing pattern, from the nearest trabeculae onto the Osseotite surface. Large portions of the implant are covered by marrow tissue (toluidine blue; $\times 25$).



Fig 6 (left) General overview of a control sample at 8 weeks. Bone does not extend over the entire implant surface but is limited to the threads in close proximity to the peri-implant trabeculae. In this area, a continuous layer of bone covers the implant, but where the trabeculae are distant from the implant, only marrow tissue is observed at the interface (toluidine blue; $\times 12$).

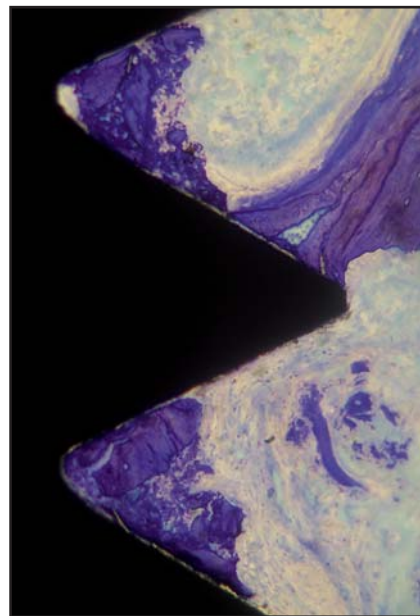


Fig 7 (right) Control surface at 8 weeks. Small bone debris are found packed at the bottom of some implant threads. These minute bony fragments are being resorbed and possibly substituted by new bone (toluidine blue; $\times 100$).

objective was to determine whether a difference could be observed between SEIs treated with the Nanotite surface and SEIs with the Osseotite DAE surface. With both test and control SEIs placed in the same patients, the study benefited from a paired analysis with

an optimal similarity of bone conditions. The present study is unique among human histologic evaluations in that some samples were obtained 4 weeks after implant placement. At this early period in the healing process, the observation of a substantial extent

of new bone formation suggested that new bone generation takes place sooner after implant placement than originally thought.

For the Osseotite implants, woven bone formation was almost complete, and new lamellar bone was observed

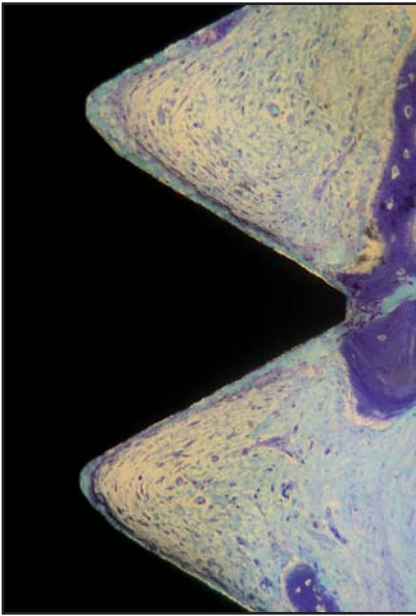


Fig 8 Control surface at 4 weeks. In some threads, particulate condensation of the collagen fibers and osteoblastic precursor cells and small capillaries were found. This pattern represents the prelude to new bone formation into the thread (toluidine blue; $\times 100$).

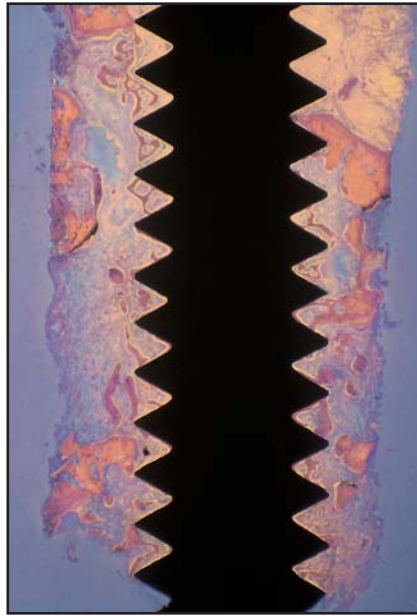


Fig 9 Overview of a Nanotite (test) surface at 4 weeks. The stage of bone healing is similar to that seen in the implants of the control group. A larger portion of the implant is covered by new bone (toluidine blue; $\times 10$).

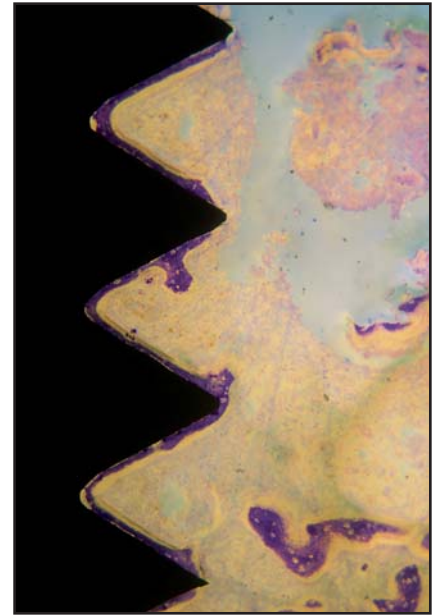


Fig 10 Nanotite surface at 4 weeks. An almost continuous layer of thin bone covers the surface in a flowing pattern typical of osteoconduction (toluidine blue; $\times 50$).

forming on the surfaces of fractured trabeculae and onto the surfaces of the woven bone trabeculae at 4 weeks (Fig 4). The fractured trabeculae were undergoing repair by new bone covered by osteoid layers. New woven bone and osteoid layers were also found at the interface with the implants. Osteoid was associated with bone resorption pits and with osteoclasts, which are typical of bone remodeling. On the implant surfaces, new bone was formed in a typical pattern, where it grew from the nearest trabeculae onto the implant surface at 8 weeks (Fig 5). Nevertheless, this

osteoconduction did not completely extend over all implant surfaces; it was limited to the threads in close proximity to the trabeculae facing the implant at 8 weeks (Fig 6). Certain regions of the interface showed a condensation of minute bone debris undergoing resorption at 8 weeks (Fig 7). In some regions, where bone was not covering the implant surface, a condensation of collagen fibers and cell precursors of new bone formation was observed at 4 weeks (Fig 8). This pattern indicated that, at a later stage of implant healing, a larger portion of the Osseotite surface would be covered by new bone.

The BV values recorded for the explanted Osseotite samples indicated that all the SEI recipient sites had low-density bone and showed few bony trabeculae over the total bone volume, including the marrow spaces.¹⁰

For the Nanotite implants, bone resorption was coupled to bone formation as in bone remodeling at 4 weeks (Fig 9). On the Nanotite surfaces, an almost continuous layer of thin bone covered the implant surfaces and was observed in a flowing pattern at 4 weeks (Fig 10). This bony layer was not related to the vicinity of the

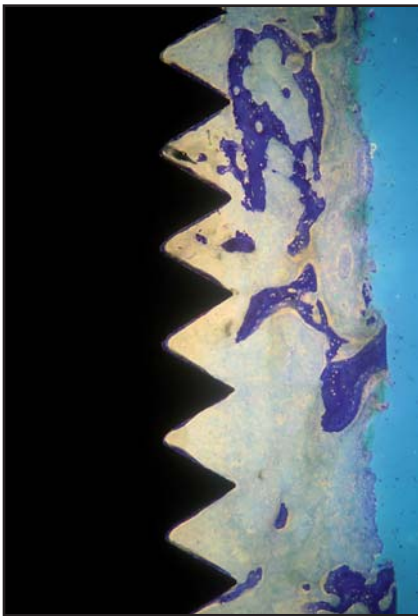


Fig 11 Nanotite surface at 4 weeks. The layer of bone covering the implant surface does not seem to be related to the vicinity of the bony trabeculae. Note how the bone penetrates deeply into the bottom of the threads (toluidine blue; $\times 25$).

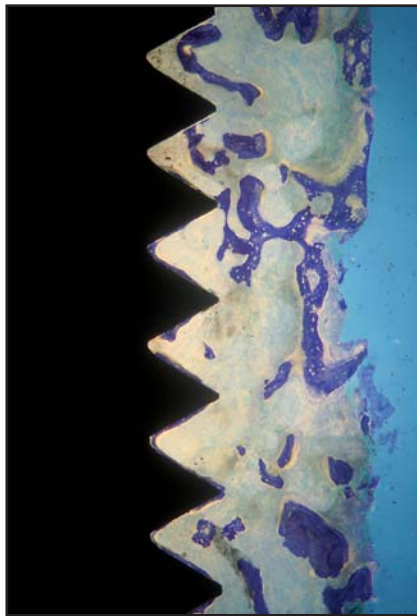


Fig 12 Nanotite surface at 4 weeks. A very thin layer of bone uniformly covers the implant surface (toluidine blue; $\times 25$).

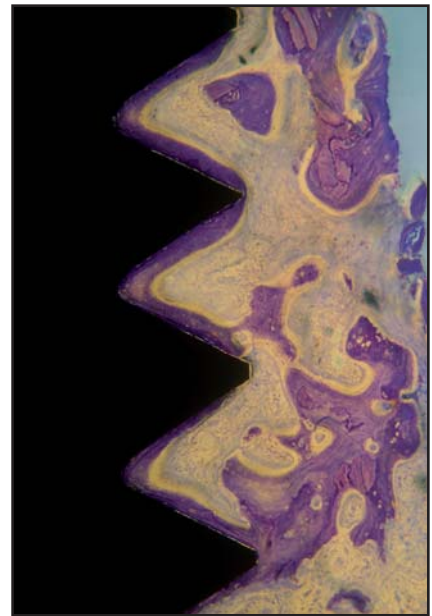


Fig 13 Nanotite surface at 8 weeks. A layer of bone about 100 μm thick covers the implant surface with osteoid and osteoblasts, suggesting active bone healing (toluidine blue; $\times 50$).

bony trabeculae. It was present almost anywhere on the implant surface, penetrating deeply into the bottom of the threads at 4 weeks (Fig 11). Over large portions of this thin bone an osteoid layer was present with osteoblasts. In some implants, this layer was 10 to 20 μm thick at 4 weeks (Fig 12), whereas at other sites it was thicker, at approximately 100 μm at 8 weeks (Fig 13).

With respect to time, the differences between the mean test and control values for BIC were 29% and 27% for the 4-week and 8-week groups, respectively, representing 197% and 148% increases, respectively, over the control values. There was a slight correlation but no statistical significance for the variables of time and BIC development in these outcomes. When

grouped by time, the differences between the test and control mean BV values were -6.8% and 61.7% for the 4-week and 8-week groups, respectively. There was a slight correlation for the variables of time and percent BIC development in these outcomes, but this did not reach statistical significance ($P = .13$).

Limitations of the study

There were several limitations to this study. First and foremost was the limited sample size, which included only nine pairs of implants for BIC analysis. Also, insertional torque values could not be quantified because of the poor-quality bone encountered and the small size of the implants. Determination of primary stability at implant placement was qualitative, not quantitative. However, the significant differences between test and control outcomes provided sufficient power to achieve statistical significance.

Conclusions

In this study, the addition of nanometer-scale CaP crystals to the DAE surface of the implants appeared to have a significant effect on the development of new bone at 4 and 8 weeks after implant placement. This may have significant clinical implications in terms of implant placement, with accelerated healing in areas of poor-quality bone. Additional long-term prospective clinical trials are warranted and in progress.

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References

1. Baker D, London RM, O'Neal R. Rate of pull-out strength gain of dual-etched titanium implants: A comparative study in rabbits. *Int J Oral Maxillofac Implants* 1999; 14:722–728.
2. Lazzara RJ, Testori T, Trisi P, Porter SS, Weinstein RL. A human histologic analysis of Osseotite and machined surfaces using implants with two opposing surfaces. *Int J Periodontics Restorative Dent* 1999;19: 117–129.
3. Block MS, Finger IM, Fontenot MG, Kent JN. Loaded hydroxylapatite-coated and grit-blasted titanium implants in dogs. *Int J Oral Maxillofac Implants* 1989;4: 219–225.
4. Johansson CB, Albrektsson T. A removal torque and histomorphometric study of commercially pure niobium and titanium implants in rabbit bone. *Clin Oral Implants Res* 1991;2:24–29.
5. Kenealy JN, Berckmans B, Stach RS. Nanometer-scale CaP enhances early implant-bone fixation in an animal model [abstract 302]. *Clin Oral Implants Res* 2006;17:cxxi.
6. Trisi P, Lazzara R, Rao W, Rebaudi A. Bone-implant contact and bone quality: Evaluation of expected and actual bone contact on machined and Osseotite implant surfaces. *Int J Periodontics Restorative Dent* 2002;22:535–545.
7. Trisi P, Lazzara R, Rebaudi A, Rao W, Testori T, Porter SS. Bone-implant contact on machined and Osseotite surfaces after 2 months of healing in the human maxilla. *J Periodontol* 2003;74:945–956.
8. Smith JM, Jee WSS. Automated skeletal histomorphometry. In: Recker RR (ed). *Bone Histomorphometry, Techniques and Interpretation*. Boca Raton, FL: CRC Press, 1983:53–87.
9. Parfitt AM. Stereologic basis of bone histomorphometry: Theory of quantitative microscopy and reconstruction of the third dimension. In: Recker RR (ed). *Bone Histomorphometry, Techniques and Interpretation*. Boca Raton, FL: CRC Press, 1983:75–91.
10. Trisi P, Rao W. Bone classification: Clinical-histomorphometric comparison. *Clin Oral Implants Res* 1999;10:1–7.