Peri-Implant Endosseous Healing Properties of Dual Acid-Etched Mini-Implants with a Nanometer-Sized Deposition of CaP: A Histological and Histomorphometric Human Study

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ABSTRACT

Purpose: The aim of this histological and histomorphometric study was to compare the early peri-implant endosseous healing properties of a dual acid-etched (DAE) surface (Osseotite®, Implant Innovations Inc., Palm Beach Gardens, FL, USA) with a DAE surface modified with nanometer-sized calcium phosphate (CaP) particles (NanoTite™, Implant Innovations Inc.) in grafted and mature maxillary bone.

Materials and Methods: Fifteen patients received two mini-implants, 1 with DAE surface (control) and 1 with a DAE + CaP surface (test), to fixate an iliac crest bone graft to the maxilla. A part of each mini-implant was in contact with the grafted bone and a part extended into the native maxillary bone. After a healing period of 3 months, the specimens were harvested and analyzed.

Results: Overall, a trend was seen for stronger bone response around the test mini-implants in the native bone of the maxilla. However, only the old bone particles measured by percentages of bone-to-implant contact and bone area were statistically significant ($p = .025$ and $p = .042$, respectively).

Conclusions: The NanoTite surface increases the peri-implant endosseous healing properties in the native bone of the maxilla compared with the Osseotite surface, while this difference was not visible in the bone graft area. This might be a result of the lower remodeling process of the graft.

KEY WORDS: CaP surface, dual acid-etched surface, endosseous implant, histology, histomorphometry, peri-implant healing properties

INTRODUCTION

Surface modifications of endosseous implants are of growing interest for their prospects of improving osseointegration. A complex surface microtopography or surface roughness[1,2] and, to a lesser extent, calcium phosphate (CaP) deposits on an implant surface are thought to accelerate early peri-implant bone healing by increasing activation of platelets.[3–5] These platelets would play an initiating role in the process called contact osteogenesis; they activate the osteogenetic cells to migrate to the surface of the implant. On the implant surface, these cells differentiate into osteoblasts and start to deposit new bone.[6,7] Especially in more challenging implant cases, such as immediate placement or loading of implants and insertion of implants in "poor" quality

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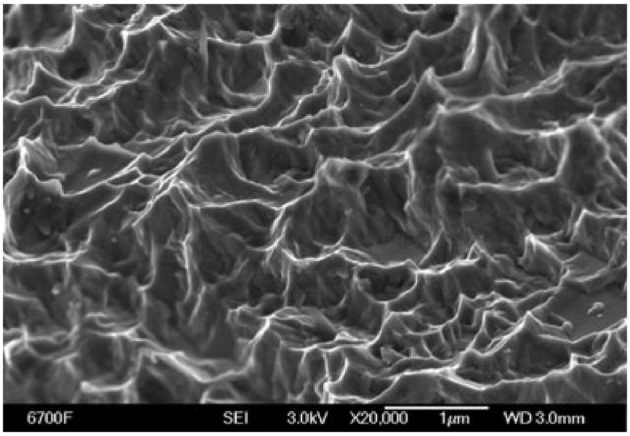

Figure 1 Scanning electron microscope (SEM) image of the dual acid-etched surface at ×20,000 magnification (Osseotite®, Implant Innovations Inc., Palm Beach Gardens, FL, USA).

bone, this acceleration of early peri-implant bone healing might be very useful.

Recently, a modification of the dual acid-etched (DAE) surface (Osseotite®, Implant Innovations Inc., Palm Beach Gardens, FL, USA) was introduced. This novel surface is created by discrete crystalline deposition of nanometer-sized CaP particles on the DAE surface (NanoTite™, Implant Innovations Inc.) (Figures 1–4). The CaP deposit on the DAE-implants does not form a confluent layer; the CaP particles (20–100 nm in size) are deposited in the peaks and valleys of the DAE surface microtopography and occupy approximately 50% of the surface. In this way, a more complex surface microtopography is developed.

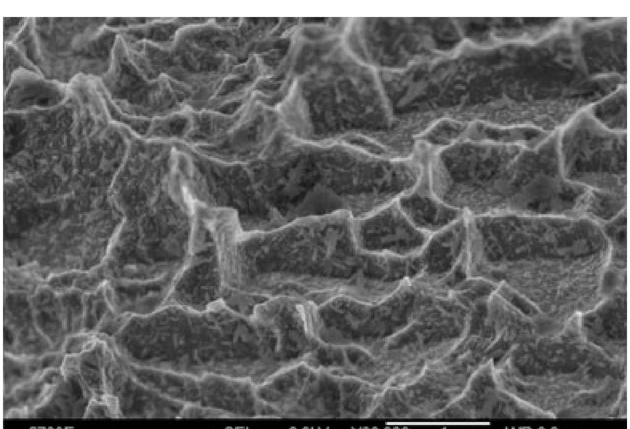

Figure 2 Scanning electron microscope (SEM) image of the dual acid-etched surface with a crystalline deposition of nanometer-sized calcium phosphate particles at ×20,000 magnification (NanoTite™, Implant Innovations Inc., Palm Beach Gardens, FL, USA).

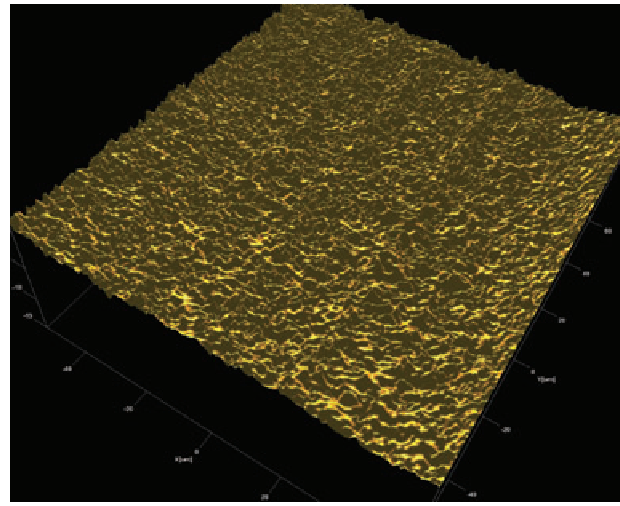

Figure 3 An optical three-dimensional topographical image of the DAE surface with a surface roughness measured by Wennerberg and colleagues (University of Göteborg, Sweden) of a mean height deviation (S_a) of 0.68 μm and a developed interfacial area ratio (S_{dr}) of 27%.[19,20] (DAE = dual acid-etched.)

This study was initiated to assess the early endosseous healing properties of both (DAE and DAE + CaP) surfaces in a model applying mini-implants to fixate monocorticocancellous anterior iliac crest grafts used for augmentation of a severely resorbed maxilla. This model was chosen to see whether the DAE surface + CaP has better healing properties than the DAE surface and to determine whether these properties are of particular

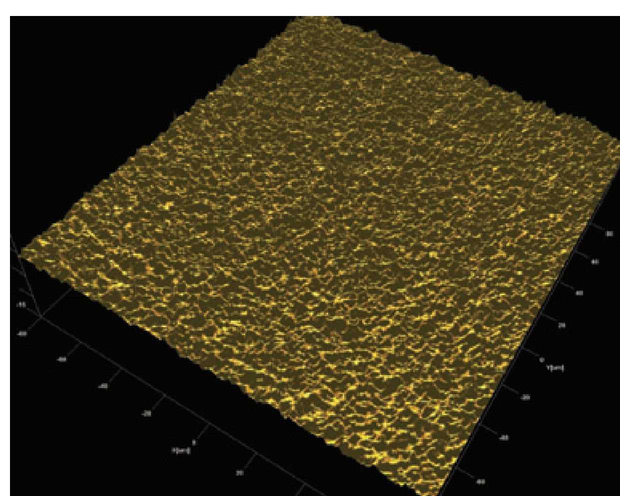

Figure 4 An optical three-dimensional topographical image of the DAE + CaP surface with a surface roughness measured by Wennerberg and colleagues (University of Göteborg, Sweden) of a mean height deviation (S_a) of 0.5 μm and a developed interfacial area ratio (S_{dr}) of 40%.[19,20] (CaP = calcium phosphate; DAE = dual acid-etched.)

benefit in areas with either high or low remodeling. Moreover, the remodeling process in the iliac crest graft and the osseointegration in the maxilla bone could be observed. Long-term results are known for the resorption of iliac crest onlay grafts[8] but, to our knowledge, are never compared with the endosseous healing properties of the native bone.

The aim of this histological and histomorphometric study was to compare the early peri-implant endosseous healing properties of the DAE surface to the DAE surface with a discrete crystalline deposition of nanometer-sized CaP in an active remodeling (ie, grafted bone) and native (ie, mature bone) maxillary area.

MATERIALS AND METHODS

Patients

Fifteen consecutive patients (6 women, 9 men) with a mean age of 62.3 ± 7.1 years (range 48–69) fulfilling the inclusion criteria agreed to participate in this study. The patients were referred to the Department of Oral and Maxillofacial Surgery of the University Medical Center Groningen driven by an insufficient retention of their upper denture related to a severely resorbed maxilla. The patients had been edentulous in the maxilla for 3 to 21 years.

Patients were selected by using the following inclusion criteria:

- Severely resorbed maxilla (classes V and VI, Cawood & Howell)[9] with reduced stability and reduced retention of the upper denture
- Edentulous period of at least 1 year
- No history of radiotherapy in the head and neck region
- No history of bone-related diseases, autoimmune-related disorders and diabetes mellitus
- Patients either did not smoke or stopped smoking at least 6 weeks before surgery
- No history of reconstructive preprosthetic surgery or previous implant surgery

In all patients, overdentures were planned to be supported by 4 to 6 implants placed in the maxilla. Informed written consent to participate in this study was obtained from all patients.

Orthopantomograms, lateral cephalograms, and postero-anterior oblique radiographs were made to assess the height of the maxillary alveolar bone, the dimensions of the maxillary sinus, and the antero-posterior relationship of the maxilla to the mandible. The radiographs were also screened for sinus pathology. The mean vertical height of the alveolar bone on the orthopantomogram between the most caudal part of the maxillary sinus and the oral cavity under the maxillary sinus was 3 ± 2 mm (range 1–5 mm). The bone width in the anterior area was 2 ± 1 mm (range 2–5 mm).

Surgical Protocol

The maxilla of each patient was reconstructed with autologous anterior medial iliac crest bone grafts under general anesthesia. In all cases, bilaterally, a two-stage procedure (first stage, bone grafting; second stage, placement of implants) was performed because the height of the maxillary bone and/or the width of the alveolar crest were less than 5 mm. A bone height of 5 mm or more is a prerequisite for implant placement with sufficient primary stability.[10] In addition to elevation of the floor of the maxillary sinus, the width of the alveolar crest was reconstructed. An osteotomy was prepared in the lateral wall of the maxillary sinus by using the surgical procedure described by Raghoebar and colleagues.[10] After harvesting the bone grafts from the iliac crest, the floor of the maxillary sinus was augmented with bone blocks, and the remaining space was occupied by cancellous bone particles. The graft was ground in a bone mill (Stryker Leibinger GmbH, Freiburg, Germany). The monocorticocancellous bone blocks were then placed buccally of the cortex of the alveolar defect in order to increase the width of the superior alveolar process. The cancellous side of the bone graft was in contact with the maxillary bone, and, again, cancellous bone particles were used to fill the small gaps between the bone graft and the alveolar crest.

Randomly, on one side, the grafts were fixed to the alveolar bone with one titanium screw (Martin Medizin Technik, Germany) (diameter, 1.5 mm; length, 10 mm) and 1 custom-made mini-implant with a DAE surface (Osseotite) (diameter, 2.18 mm; length 10 mm). On the other side, the grafts were fixed to the alveolar bone with one titanium screw (similar to the screw used on the other side) and one test custom-made mini-implant with nanometer-sized CaP particles on the DAE surface (NanoTite) (diameter, 2.18 mm; length, 10 mm). The screws were inserted without tapping in a region where no endosseous implants were planned to be inserted. At least 3 mm of the mini-implants extended into the native maxillary bone to obtain adequate stability of the bone graft and a sufficient length of the mini-implant surface in contact with the native bone for histological and histomorphometric evaluation. The bone width was measured with a caliper. The bone grafts were covered by a membrane (Bio-Gide®, Geistlich Söhne AG, Wolhusen, Switzerland).[11]

Before the bone grafts were harvested, the patients received broad-spectrum antibiotics, starting 1 hour preoperatively (intravenously) and continued orally for 2 days after surgery. Postoperatively, the patients received an aqueous 0.2% chlorhexidine mouth rinse (1 minute, 3 times daily) for 2 weeks. One month postoperatively, the edentulous patients were allowed, if possible, to wear their denture in the operated areas, after relining the denture with a soft liner.

After a healing period of 3 months, the control mini-implants with DAE surface and the test mini-implant with DAE surface modified with the nanometer-sized CaP particles were removed, and the implant placement procedure was performed.[10] This second-stage surgery was performed under general anesthesia in the day clinic. Shortly after the mucoperiosteal flap was reflected, the width of the reconstructed alveolar crest was measured, and the titanium screws were removed. The test and the control mini-implants were removed by using a trephine (internal diameter 4.25 mm). Surgical and prosthetic procedures were then followed to construct an implant-supported overdenture.

Clinical Evaluation

Clinically, all patients were evaluated according to a standardized protocol 1, 3, 6, and 12 weeks after surgery. The clinical protocol included assessment of complications during surgery and postoperative healing (inflammation, redness of the mucosa, wound dehiscence, sequestration, and loss of bone particles).

Histological Examination

The harvested specimens were immersed in 10% formalin and sent to the laboratories of the Biomaterials department, Sahlgrenska Academy, Göteborg. The laboratory staff was blinded for test and control specimens. The aberrant surface texture of the nanometer-sized CaP particles is not visible when using magnification as used for light microscopy. The sample preparation followed the internal guidelines of the laboratories, and, in brief, this involved change to fresh 4% neutral buffered formaldehyde upon arrival in the laboratories and further immersed in the solution for 1 week. After being rinsed in tap water, the specimens were dehydrated in ethanol, from 70% to absolute ethanol. Following this, the samples were immersed in diluted resins and finally infiltrated in pure resin (Technovit® 7200 VLC, Heraeus Kulzer GmbH & Co., Wehrheim, Germany). All these steps were carried out under stirring and vacuum conditions. Embedment in pure resin with polymerization in ultraviolet light was the final step. The bloc samples were divided in the long axis of the implant in a band saw. A supporting plexiglass was glued onto the surface, and a thick central section (200 μm) was prepared from each biopsy. These sections were further ground to approximately 10 to 20 μm. The preparation of undecalcified cut and ground sections involved the usage of the EXAKT® equipment (EXAKT Apparatebau GmbH & Co., Norderstedt, Germany) and followed the recommendations by Donath,[12] Donath and Breuner[13], and Johansson and Morberg.[14,15] The sections were stained with Toluidine blue mixed in pyronin G prior to coverslipping and investigations in the light microscope. The histological staining differentiates between pale-purple-stained bone and darker-purple-stained new-formed bone. Soft tissue as well as cellular nuclei is blue stained.

All samples were investigated in a Leitz Aristoplan® light microscope (Ernst Leitz GmbH, Wetzlar, Germany) coupled to a computer-based Microvid unit enabling quantitations of the bone-to-implant contact (BIC) and bone area (BA) inside the threads.[16]

Histomorphometric Examination

Histomorphometric examination was performed to quantitate peri-implant endosseous bone healing related to the type of implant surface and the quality of bone. Interindividual comparisons of data performed by two independent observers, on blinded sections, revealed similar qualitative judgments. One person performed all histomorphometrical measurements.

The percentages of BIC and BA of eight different implant threads were determined, namely, the four uppermost threads (these threads were all in contact with grafted bone) and the four lowest threads (these threads were all in contact with native maxillary bone).

To determine the process of osseointegration of the mini-implants in the maxilla and the remodeling process around the mini-implants in the grafted area, a

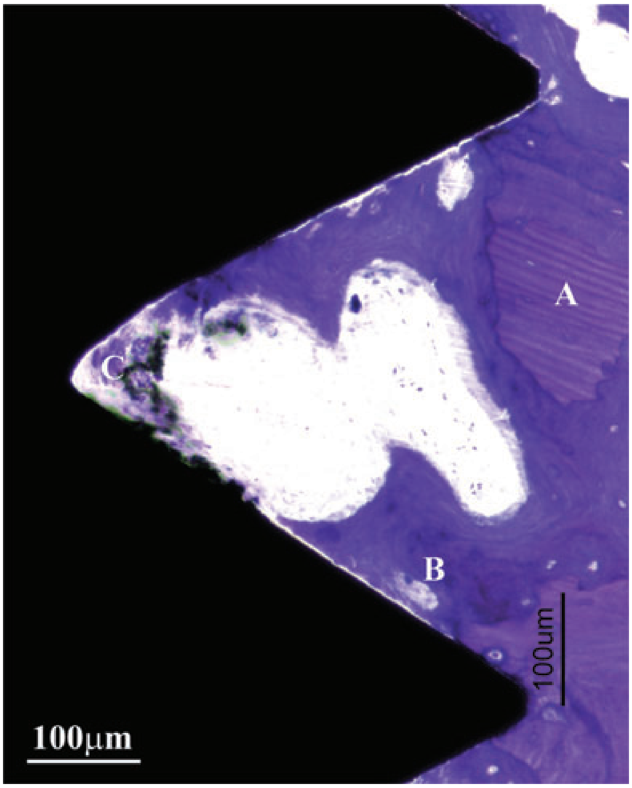

Figure 5 A thread of a mini-implant with a DAE + CaP surface (Toluidine blue). (A = old bone; B = newly formed bone; C = bone dust; CaP = calcium phosphate; DAE = dual acid-etched.)

distinction in percentages of BIC and BA was made between the old bone and the newly formed bone particles. For each thread, the total percentage of BIC and BA was measured and for the old bone and the newly formed bone separately. For some bone particles, it was very difficult to identify whether the particle was an old piece of bone that was on its way to dissolve (from a graft or a result of initial drilling) or new bone; these minor areas were classified as unidentified areas, or "bone dust" (Figure 5). Such particles are to be expected in the bone remodeling process of the iliac crest graft. The unidentified areas were not counted for in the assessment of BIC and BA.

Statistical Analysis

The histomorphometric data were collected and subjected to statistical analysis by using a statistical program (SPSS 14.0, SPSS Inc., Chicago, IL, USA). The mean value of the eight (4 + 4) threads per mini-implant was filed; also, when there was no bone seen in a thread, these so-called "zero-values" were included. The data were analyzed with descriptive statistics to see whether there was a standard distribution. To compare the means between the control and the test implants and between the graft and the native bone, paired samples *t*-test were performed. *p* Values $< .05$ were considered statistically significant.

RESULTS

Clinical Results

No complications were observed during the surgery or during the postoperative healing period. At the time of reentry surgery, all mini-implants (test and control) were immobile and surrounded by bone.

Histology

All mini-implants were available for histological examination. Most of the surface of the mini-implants was covered with bone. However, because of physiologic resorption at the screw head, some mini-implants were lacking in coverage by bone, and, because of the harvesting process with the trephine bur, the bottom section of some specimens showed small artifacts. Nevertheless, at all mini-implants, areas of at least four threads covered by the monocorticocancellous bone of the bone graft derived from the anterior iliac crest, and areas of at least four threads covered by native maxillary bone were distinguished (Figures 6 and 7).

Histomorphometry

An overview of the quantitative histomorphometric results is presented in Tables 1 and 2. In Table 1, the percentages of BIC and BA of the control (DAE surface) and test (DAE + CaP surface) per patient are given. In Table 2, an overview is provided of the level of significance of the BIC and BA percentages of the upper (graft bone) and lower parts (native bone) and the old and newly formed bone of the control and test mini-implants.

Overall, a trend can be seen for a stronger bone response around the lower threads of the test mini-implants in the native bone of the maxilla (*p* value of BA = .100), but only the old bone particles measured by percentages of BIC and BA were statistically significant ($p = .025$ and $p = .042$, respectively). Furthermore, when the overall results of the upper part of the mini-implant (into the iliac crest bone graft) were compared with those of the lower part (into the maxillary bone), both for the control and test mini-implants, BIC and BA were

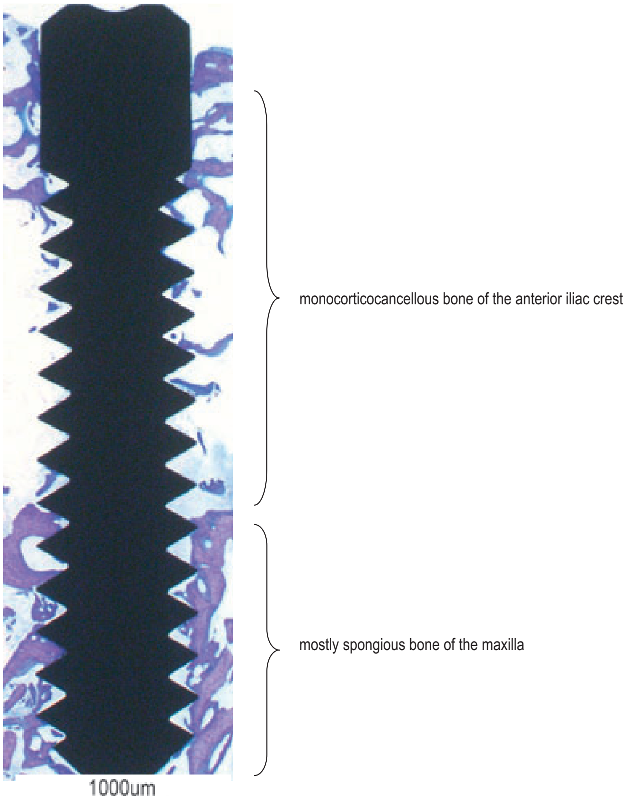

Figure 6 Histological representation of a harvested specimen with a DAE surface (Toluidine blue). (DAE = dual acid-etched.)

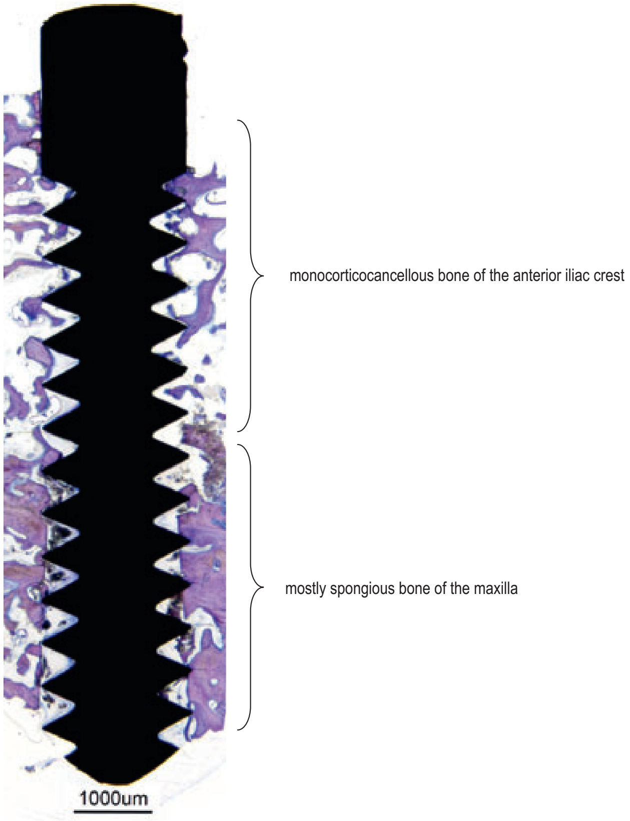

Figure 7 Histological representation of a harvested specimen with a DAE + CaP surface (Toluidine blue). (CaP = calcium phosphate; DAE = dual acid-etched.)

TABLE 1 BIC (%) and BA (%) of the Control (DAE) and Test (DAE + CaP) Mini-Implant per Patient

	Control (DAE)		Test (DAE + CaP)	
Patient	BIC %	BA %	BIC %	BA %
1	2.52	9.09	9.06	12.86
2	7.29	13.56	12.45	22.90
3	4.86	3.19	11.53	7.69
4	16.40	23.13	7.34	11.27
5	14.47	19.04	15.54	25.22
6	0	0	5.50	11.14
7	3.16	7.16	10.29	10.56
8	8.15	9.78	8.70	12.00
9	5.42	12.65	8.53	12.00
10	4.66	6.69	6.63	10.94
11	1.36	2.20	4.11	9.44
12	17.11	12.23	14.68	20.80
13	12.78	9.55	6.39	15.80
14	11.51	14.10	8.76	11.09
15	11.78	8.51	6.53	5.00
Mean ± SD	8.68 (±5.29)	10.78 (±5.66)	9.07 (±3.30)	13.25 (±5.62)

BA = bone area; BIC = bone-to-implant contact; CaP = calcium phosphate; DAE = dual acid-etched.

TABLE 2 Levels of Significance of the Control (DAE) and Test (DAE + CaP) Mini-Implants

	Control (DAE) (Mean ± SD)	Test (DAE + CaP) (Mean ± SD)	Significance
BIC (%) upper threads (graft)	**8.13 ± 8.35**	**8.27 ± 8.85**	**0.968**
New BIC (%) upper threads	3.27 ± 7.27	5.57 ± 7.70	0.390
Old BIC (%) upper threads	4.86 ± 6.08	2.70 ± 2.48	0.294
BIC (%) lower threads (maxilla)	**27.02 ± 19.73**	**29.01 ± 15.19**	**0.746**
New BIC (%) lower threads	16.54 ± 18.21	9.62 ± 9.79	0.187
Old BIC (%) lower threads	10.48 ± 7.62	19.39 ± 13.27	0.025*
BA (%) upper threads (graft)	**14.73 ± 14.66**	**13.85 ± 7.34**	**0.848**
New BA (%) upper threads	4.24 ± 4.80	5.66 ± 5.77	0.299
Old BA (%) upper threads	10.45 ± 15.12	8.18 ± 5.98	0.639
BA (%) lower threads (maxilla)	**29.97 ± 16.32**	**39.17 ± 21.22**	**0.100**
New BA (%) lower threads	15.50 ± 13.14	13.14 ± 14.64	0.593
Old BA (%) lower threads	14.46 ± 12.44	26.03 ± 19.68	0.042*

*$p < .05$.
BA = bone area; BIC = bone-to-implant contact; CaP = calcium phosphate; DAE = dual acid-etched.

significantly higher for the native maxillary bone ($p = .009$ and $p = .006$), for the controls ($p = .000$ and $= .003$), and for the test mini-implants, respectively.

DISCUSSION

This study showed better results at the level of the native bone for the test mini-implants (DAE + CaP surface) than for the control (DAE surface) after 3 months in service of fixating an iliac crest bone graft to the maxilla bone, but only the results of the old bone particles were statistically significant. This would mean that, if there are already old bone particles in contact with the surface of the mini-implants, a more active osteogenesis process is going on and that the osseointegration process is accelerated by the more complex microtopography and/or CaP deposits.

When the BIC and BA percentages of the bone graft of both the control and the test mini-implants were compared with the native bone of the maxilla, significantly more bone was observed in the maxillary bone. As is obvious from the results of our study, the remodeling process in the iliac crest graft was still ongoing 3 months after grafting. This result raises the question whether it is premature to insert an implant 3 months after augmentation. More histological and histomorphometric research is needed to clarify when this remodeling process of the augmented areas is in such a stage that implantation is predictable.

Similar histological and histomorphometric investigations comparing the DAE surface with the DAE + CaP surface in the posterior maxillae showed results like ours. Furthermore, to our knowledge, no other study has compared the endosseous healing properties of the augmented bone with those of the native bone so far. Goené and colleagues[17] placed 18 unloaded site evaluation implants (SEIs) in the posterior maxillae of nine patients and compared the two surfaces we studied. After 4 to 12 weeks, the DAE + CaP surface showed a statistically significant higher percentage of BIC when a zero contact value in the control group was included ($p < .01$), but bone volume (BV) percentages were comparable. The discrepancy between BIC and BV percentages could be explained from their observation that, on the DAE + CaP surface, an almost continuous layer of thin bone was seen. Orsini and colleagues also placed 32 unloaded SEIs in the posterior maxillae of 15 patients.[18] They observed no difference in BIC and BV percentages when zero contact values were included ($p = .20$). However, by excluding the zero contact values, a trend toward statistical significance was seen in favor of the test SEI. In our study, all the zero values were included because all the harvested specimens were surrounded by bone, and no artifacts or fibrous encapsulation was seen.

The model used in the present study was chosen for the “poor” bone quality of the iliac crest bone graft.

The question was whether this new surface could also accelerate the remodeling process in a bone graft. Presumably because of the poor vascularization of the grafted area at the time of placement of the control and test mini-implants, the platelet activation did not take place as expected on this surface with complex microtopography and CaP deposits. The results of the native bone of the maxilla were significantly better for the test mini-implants; the new DAE surface with CaP particles might prove to be a more reliable implant in cases of immediate placement and immediate loading of implants.

From this study, it can be concluded that the DAE surface with CaP particles improved the peri-implant endosseous healing properties in the native bone of the maxilla when compared with the DAE surface but did not improve the healing properties in the bone-graft area. We assume that this might be a result of the lower remodeling process of the bone graft area, which is still in progress 3 months after grafting.

ACKNOWLEDGMENTS

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