Plaque Accumulation on Exposed Titanium Surfaces and Peri-implant Tissue Behavior. A Preliminary 1-Year Clinical Study

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Purpose: The aim of this study was to evaluate plaque accumulation and peri-implant tissue response adjacent to machined and dual acid-etched (DAE) titanium implant surfaces. ***Materials and Methods:*** Two types of implants were used—control implants with a DAE surface in their apical portion and a machined coronal part, and test implants with a DAE surface throughout their entire length. A total of 10 sets of implants were placed in the posterior quadrants of eight patients, with at least 2 implants (1 control and 1 test implant) placed in each site. Machined healing abutments were placed on the control implants and DAE–surfaced healing abutments on the test implants. Plaque Index and bleeding on probing (BOP) were recorded together with histologic and microbiologic analyses of the peri-implant tissues. The healing abutments underwent a scanning electron microscope scan at 5 months postsurgery. Standardized radiographs were also taken at the time of implant placement and 3, 6, and 12 months postsurgery. ***Results:*** DAE surfaces accumulated more plaque than machined surfaces ($P < .0006$) and the plaque was assessed as more difficult to remove ($P < .0143$). No histologic abnormalities were seen and the test implants showed significantly lower crestal bone resorption than the control ($P < .0174$). ***Conclusion:*** DAE healing abutments showed an increased plaque accumulation, but no significant BOP differences or histologic analyses were found between test and control sites. The test implants showed less interproximal bone resorption than the control ones at the end of a 1-year follow-up evaluation. *Int J Prosthodont 2009;22:447–455.*

A substantial body of dental literature deals with the bone-implant interface.[1–4] However, fewer articles consider the response of peri-implant soft tissues. The latter may be of primary importance in protecting and maintaining implant health,[5,6] as well as contributing to the functional and esthetic success of prosthodontic treatment.[5] An ongoing evolution in the original machined titanium implant surface toward modified and bioengineered surfaces now demands an even better understanding of what happens at the soft tissues adjacent to implants with newer implant surfaces. Moreover, commercially available implants are now manufactured with a variety of surface treatments, such as acid-etched, grit-blasted, and plasma-sprayed, not only for the apical portions of the implants, but also for the entire length of the implants.

Osseotite and Full Osseotite (FOSS) implants (Biomet 3i) were used in this study. Osseotite implants are made of commercially pure titanium (grade IV) treated with a specific, proprietary dual acid-etching (DAE) protocol. The acid-etched surfaces do not include the coronal 3 mm, which have a machined surface. This design was developed on the premise that in the event of localized soft tissue recession, which would result in machined surface exposure to plaque formation, a less plaque-retentive surface would hopefully be present. This would presumably not be the case if a microscopically roughened implant surface was exposed. FOSS implants are similar to Osseotite implants except that the entire implant surface is treated with the company's DAE protocol.

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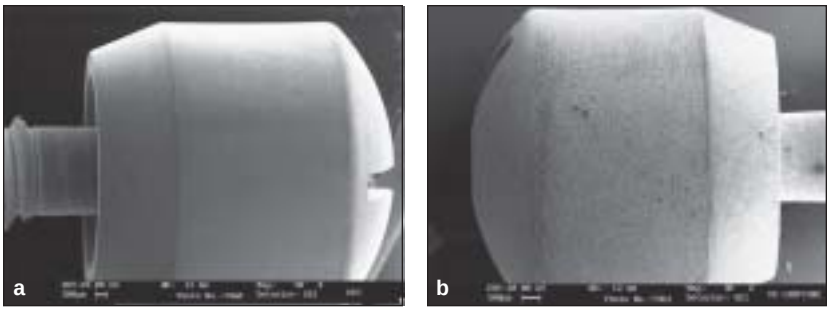


Figs 1a and 1b SEM photograph of **(a)** a machined abutment and **(b)** a DAE abutment; magnification ×50.

Fig 2 The machined healing abutments were connected to the Osseotite implants (control, *right*); the modified (DAE) surfaced healing abutments were connected to the FOSS implants (test, *left*).

Published papers have speculated on the inherent risks associated with exposed roughened implant surfaces secondary to peri-implant soft tissue recessions. Further complications associated with the so-called condition of peri-implantitis have been described,[7] since it is presumed that a rougher surface can provide a better potential matrix for bacteria to grow on.[8] This could then lead to the eventual loss of the implants, secondary to bone loss associated with a plaque-induced inflammatory process, although this has only been demonstrated in experimental animals and not in humans.[9–11] The other side of the argument would then be that the smoother an implant surface is, the less adherent the bacteria would be.[12]

The value of applying periodontal parameters in the monitoring of peri-implant tissue health remains unclear.[13,14] However, numerous clinicians continue to presume that the bleeding index indicates the level of gingival inflammation,[15] and the absence of bleeding on probing (BOP) around implants indicates healthy peri-implant tissues.[16] In regards to radiographic analysis,[17] DIB (distance from the implant shoulder to the alveolar bone crest) represents a reliable radiographic parameter for long-term monitoring in clinical practice,[17–20] even if minor changes in bone morphology in the crestal area may not be revealed until they reach a significant size and shape. Moreover, conventional periapical radiographs yield high specificity for the detection of peri-implant bone loss[17] and are believed to accurately evaluate crestal bone levels around implants clinically in a high percentage (89%) of cases.[21]

The aim of this preliminary study was to clinically, histologically, and radiographically compare short-term responses of peri-implant soft and hard tissues around a specific implant's machine-surfaced coronal margin with a comparable site on a similar implant that was entirely DAE. Additional observations included the evaluation of plaque indices and BOP at the implant sites, plus a scanning electron microscopic (SEM) analysis of two types of healing abutments in place for a 5-month period.

Materials and Methods

Osseotite and FOSS implants were used in this study. A minor and shorter part of the study involved the use of healing abutments with two different types of surfaces—standard machined ($Ra = 0.0263 \pm 0.0036$) (Fig 1a) and DAE–surfaced ($Ra = 0.489 \pm 0.079$). The latter were custom made by Biomet 3i for this study (Fig 1b). The machined healing abutments were connected to the Osseotite implants (control) and the DAE surfaced healing abutments to the FOSS implants (test) (Fig 2).

Eight patients (seven men, one woman) with a mean age of 59.75 years (range: 44 to 73 years) were arbitrarily selected for this study since they met the following inclusion criteria: systemically healthy and without any

Table 1 Study Protocol

	Visit											
	1 –1 wk	2 0 wk	3 3 wk	4 6 wk	5 2 mo	6 3 mo	7 4 mo	8 5 mo	9 21 wk	10 23 wk	11 6 mo	12 1 y
Presurgery screening and initial periodontal therapy	X											
Implant insertion		X										
Healing abutment connection		X										
Radiography		X				X					X	X
Bleeding on probing and Plaque Index			X	X	X	X	X					X
Professional oral hygiene					X	X	X					
Biopsy sample						X						
Microbiologic sample							X					
SEM analysis								X				
Impression for the provisional prosthesis								X				
Provisional prosthesis delivery									X			
Impression for the definitive prosthesis										X		
Definitive prosthesis delivery											X	

contraindications for undergoing oral surgery and the related prosthodontic protocols. No patient had any history of periodontal disease. All patients were partially edentulous (Kennedy Class I or II) and the opposing dentitions were natural teeth or fixed prostheses supported by natural teeth. Each patient had at least 2, but no more than 4 implants placed into an edentulous quadrant for a total of 10 implants placed in the mandible and 10 in the maxilla. In total, 10 pairs of implants were evaluated. In each edentulous quadrant, at least one Osseotite (control) and one FOSS (test) implant were alternately placed according to a specific format, with the choice for the most distal implant chosen on the basis of a draw. All implant diameters were 4 mm and lengths varied between 10 and 13 mm, depending on the amount of available host bone.

This research project was approved by the Scientific Ethical Committee of the University of Genoa, Genova, Italy. All patients provided written informed consent prior to the start of the study. All subjects agreed to return for the required recall appointments and their management schedule is listed in Table 1.

All implants were placed using a single-stage surgical protocol that required mucoperiosteal flaps at or slightly palatal to the ridge crest, with buccal relieving incisions. The osseous crests were flattened as needed prior to implant site preparation. Implant restorative platforms were placed at the level of the osseous crest and the respective healing abutments were connected according to the specific implant type (test or control).

All implants were assigned specific codes for blinding. The first number of the code (from 1 to 8) indicated the patient, while the second number in the code distinguished the implants inserted in a single patient. For each patient, the implants were numbered starting from the distal region of the first quadrant up to the distal region of the fourth quadrant, without distinction of test or control implant.

All patients were instructed to rinse twice daily for 10 days with chlorhexidine 0.2% solution (Curasept 0.2, Curaden Healthcare). Recall appointments for reevaluation and removal of any remaining sutures were scheduled for 7 days after surgery. The subjects were also instructed to perform personal oral hygiene at the investigated sites using a medium toothbrush, floss, and interdental cleaners of the adequate dimension.

A conventional loading protocol was performed since the implants were loaded at a 21-week healing interval when provisional prostheses were inserted.

Impressions for the provisional and definitive prostheses were made using the pick-up impression technique with polyvinyl siloxane (Express STD, 3M ESPE) impression material. Identical components, materials, and techniques were employed for each patient.

Radiographic Analysis of Interproximal Bone Levels

Standardized parallel periapical radiographs were taken to study interproximal bone levels at baseline (immediately after implant insertion), 3 months, 6 months, and 1 year post–implant placement. In order to guarantee the reproducibility of the radiographs over time, the radiographs were made using a long-cone paralleling technique with an individualized film holder (Rinn bite film holder for periapical radiographs, Dentsply) and a customized patient centric occlusion registration with a polyvinyl siloxane impression material putty (Express STD). Until insertion of the prostheses (at baseline and at 3 months), the radiographs were taken

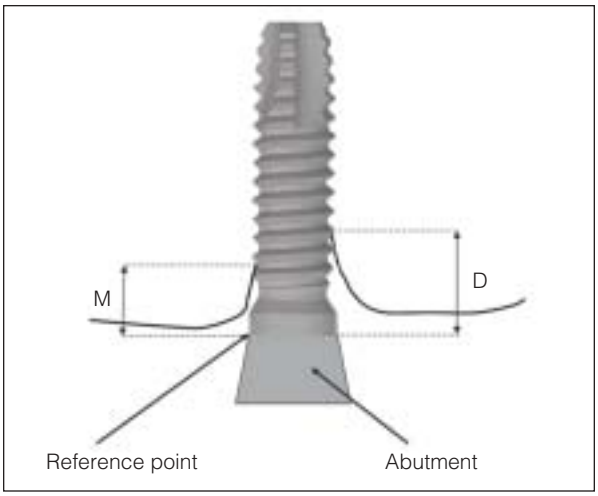


Fig 3 Interproximal crestal bone loss was measured from the implant-abutment junction to the most coronal bone level on the mesial (M) and distal (D) aspects of each implant.

using the registration of the healing abutments' positions. After insertion of the provisional prostheses (at 6 months and at 1 year), the subsequent radiographs were performed on the base of the prosthesis position. All of the radiographs were made using fast speed film (Ultra-Speed Kodak, Eastman Kodak). The threads on both sides of the implants were clearly seen in all of the radiographs. The implant-abutment interface was used as the reference point for interproximal bone level measurements.[22] Bone resorption over the length of the study was assessed from these reference points to the most coronal bone at the mesial and distal aspects of each implant, that is the DIB was measured mesially and distally (Fig 3). A blinded radiologist who was not otherwise involved in the study performed the radiographic measurements using a standard diaphanoscope and magnifying lens.[22] The measurements were repeated twice to minimize recording errors.

Plaque and Bleeding Index

The Plaque Index was recorded according to the O'Leary index[23] and identified by means of a plaque detector based on eritrosin (Red-Cote Liquid, Butler, Sunstar Americas). BOP (yes/no) was recorded with a Perio-Probe (Hawe Neos Dental). BOP and Plaque Index score were recorded around the healing abutments before the oral hygiene sessions.

The professional oral hygiene session, according to the protocol, consisted of manual instrumentation with a Universal Implant Deplaquer curette (Hawe Neos Dental) and lasted 2 minutes for each abutment. At 2 months, 3 months, 4 months, and at the end of each oral hygiene session, the Plaque Index was recorded again.

Histologic Analysis

Palatal or lingual peri-implant soft tissue biopsy specimens were obtained 3 months from implant insertion. The samples were immediately placed into 5% formalin solution and sent for histologic examination. The specimens were fixed in formaldehyde and dehydrated in ascending grades of ethanol. Histolemon (Carlo Erba Reagenti) was used as a diaphanizing agent. The specimens were then embedded in paraffin. The blocks were cut and dissected to a thickness of 5 µm. Sections were stained with hematoxylin and eosin. Polished sections were mounted on glass slides using Eukitt's mounting medium (American Histology Reagent) and glass cover slips. Every section was also photographed under a Leitz Dialux 20 EB microscope (LMS) for record purposes.

Microbiologic Analysis

The microbiologic samples for aerobic and anaerobic bacteria were taken 4 months from implant insertion. Aerobic bacteria were collected using a swab (Venturi Transystem transport swab, Copan Diagnostic) placed onto the healing abutments before the professional oral hygiene session. The swabs were immediately sent to the laboratory for analysis. The samples were plated onto blood agar plates and cultured under an atmosphere of 5% carbon dioxide (for evaluation of the presence of Gram-positive and Gram-negative bacteria and fungi) and onto McConkey medium (for evaluation of the presence of Gram-negative bacteria). After 24 to 48 hours, the bacterial colonies collected from these plates were purified on the same media. After 24 hours of incubation, a Gram stain was carried out from each different colony and identification was obtained using biochemical systems (API System, bioMèrieux).

The microbiologic samples for anaerobic bacteria were taken with a smoothed needle in order to take plaque samples mixed with crevicular fluid and blood from the gingival sockets. The samples were immediately injected into a bacteria transport medium (Portagerm Flacons, bioMèrieux) and sent to the laboratory. The samples were plated onto blood agar, kanamycin vancomycin laked blood agar (KVLB) and josamycin vancomycin norfloxacinagar (JVN) plates, and were incubated for 7 days under anaerobic conditions. The bacteria were then identified thanks to the RapID ANA II System (Innovative Diagnostic Systems).

SEM Analysis

Five months following implant placement, the healing abutments were unscrewed and placed into 5% formalin solution. They were sent to the laboratory for SEM

Table 2 Mean Interproximal Bone Resorption at 3 Months, 6 Months, and 1 Year

	Mean (mm)	SD	Minimum (mm)	Maximum (mm)
FOSS T0	0.000	0.000	0.000	0.000
STD T0	0.111	0.220	0.000	0.500
FOSS 3 mo	0.306	0.391	0.000	1.000
STD 3 mo	1.139	0.801	0.000	2.000
FOSS 6 mo	0.444	0.370	0.000	1.000
STD 6 mo	1.444	0.682	0.000	2.000
FOSS 1 y	0.611	0.397	0.000	1.000
STD 1 y	1.472	0.667	0.000	2.000

SD = standard deviation; STD = control site; FOSS = test site; T0 = baseline.

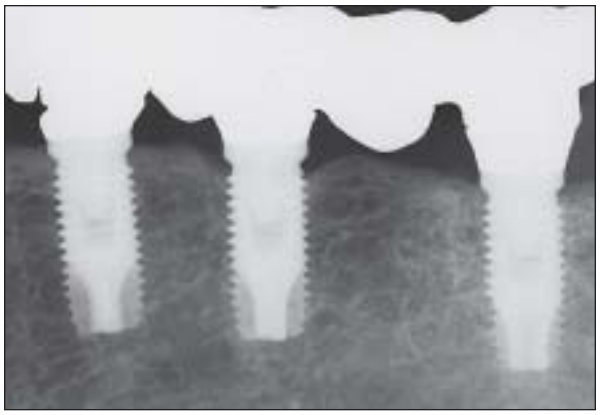


Fig 4 Periapical radiograph taken 1 year post–implant placement. The FOSS implants are mesial and distal to the Osseotite implant in the middle. Bone loss was noted to be at the level of the first thread for the Osseotite implant and 1 to 2 mm more coronal on the FOSS implants.

analysis (SEM LEO 420, LEO Electron Microscopy) where the samples were studied at a low magnification (×54) and then at ×1,000, ×5,000, and ×10,000 magnifications. Photographs were also taken at different parts of the abutment surfaces at these magnifications.

Statistical Analysis

The SPSS program (version 15.0, SPSS) was used for statistical analysis of the collected data. This included an evaluation of the amount of bone resorption at 3 months, 6 months, and 1 year post–implant placement together with plaque indices and BOP values. To compare the data with respect to the basal characteristics relative to the two different surfaces, the nonparametric Wilcoxon test was used.

The null hypotheses (stating that no differences existed between the soft and hard tissues surrounding the two different surfaces) were tested with the bilateral method; the alpha risk of error was contained into the 5% and the beta contained into the 20%.

The type of surface investigated (test or control) was revealed only at the end of the statistical analysis.

Results

During the 1-year follow-up, no superstructure or implant was lost and all implants were judged as being clinically stable.

Radiographic Analysis of Interproximal Bone Levels

When the implants were inserted, the interproximal bone levels were usually at the implant-abutment interface. There was little if any difference in regards to distance between the bone crest and implant shoulder for both implant surface types at the start (Table 2).

At 6 months, a significant difference in bone loss between the two implant surface types was noted ($P < .0146$). At 1 year post–implant placement, the difference in bone resorption between the test and control implants was also significant ($P < .0174$) (Fig 4).

Plaque Accumulation Evaluation

Greater plaque accumulation was found and a greater difficulty in plaque removal was noted for the modified (DAE) healing abutment surfaces. In fact, as reported in Table 3, greater plaque indices were recorded both before and after the oral hygiene session for the DAE healing abutment surfaces.

With regards to plaque accumulation around the healing abutments, the difference between the two surfaces was significant both for the plaque indices recorded before ($P < .0006$) and after the 2-minute oral hygiene sessions ($P < .0143$).

Bleeding on Probing Evaluation

The majority of the patients did not show bleeding on probing, which was considered to indicate an absence of tissue inflammation (Table 4). In regards to the first 4 months of data, in one patient the machined abutment showed one bleeding surface during two different sessions, while the tissues surrounding the DAE healing abutment never showed bleeding on probing in the same patient.

In another clinical case for the DAE healing abutment, a greater number of bleeding surfaces were

Table 3 Number of Healing Abutments Investigated and Relative Plaque Index Scores[23]

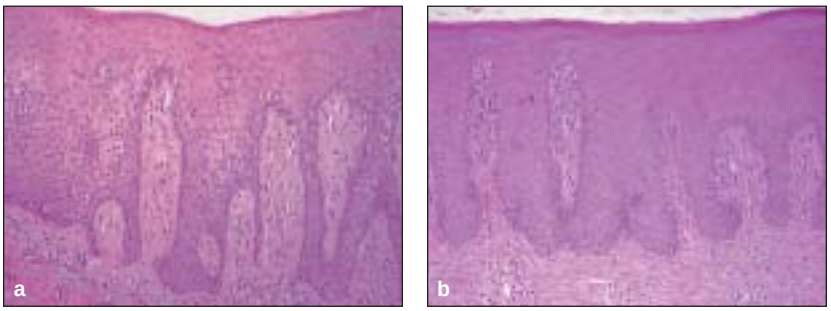
			2 mo		3 mo		4 mo	
PI score	3 wk	6 wk	B	A	B	A	B	A
FOSS								
0	5	1	1	9	1	6	1	8
1	3	–	4	1	1	3	2	1
2	1	4	1	–	3	1	3	1
3	1	–	2	–	2	–	1	–
4	–	5	2	–	3	–	3	–
STD								
0	6	1	1	10	3	9	4	10
1	3	3	3	–	–	1	5	–
2	1	3	3	–	4	–	–	–
3	–	2	3	–	3	–	–	–
4	–	1	–	–	–	–	1	–

PI = Plaque Index; B = before professional oral hygiene session; A = after professional oral hygiene session; STD = control site; FOSS = test site.

Table 4 Number of Implant Sites Evaluated and Relative Bleeding on Probing Values

BOP	3 wk	6 wk	2 mo	3 mo	4 mo	1 y
FOSS						
0	10	8	8	10	9	8
1	–	2	1	–	–	2
2	–	–	1	–	1	–
3	–	–	–	–	–	–
4	–	–	–	–	–	–
STD						
0	9	10	9	9	8	4
1	1	–	1	1	2	4
2	–	–	–	–	–	1
3	–	–	–	–	–	–
4	–	–	–	–	–	–

PI = Paque Index; B = before professional oral hygiene session; A = after professional oral hygiene session; STD = control site; FOSS = test site.



Figs 5a and 5b Section of a biopsy specimen from peri-implant mucosa surrounding **(a)** a machined healing abutment and **(b)** a modified (DAE) healing abutment at 3 months.

recorded when compared to the machined healing abutment, with a cumulative value equal to five in the five recordings. The tissues in this instance were noted to bleed on probing at one surface on one occasion. Regarding BOP values in the first 4 months of healing, the difference between the two surfaces was not significant. At 1 year post–implant placement, no significant differences were found in BOP values between the two surfaces.

Histologic Analysis

No differences were found relative to the two healing abutment surface treatments in the 10 clinical cases histologically analyzed (Figs 5a and 5b). In all patients, the histologic findings were similar: The samples were composed of a pluristratified squamous epithelial tissue with a fibrous stroma below the basement membrane. Granulation tissue with a poor inflammatory infiltrate was also noted. This was thought to be consistent with normal healing.

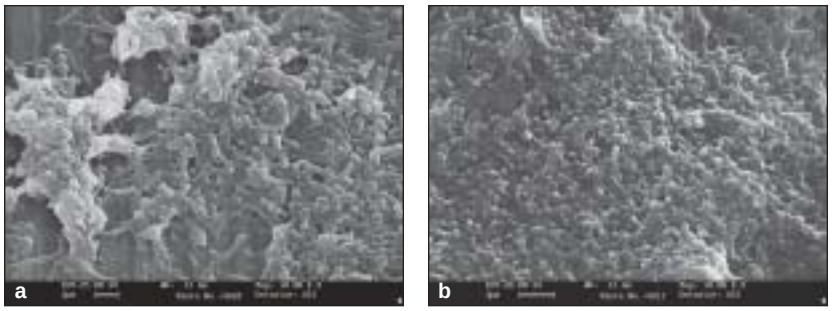
No differences were noticed in cellular composition or in inflammatory infiltrate of the tissues surrounding the two different surfaces.

Microbiologic Analysis

The microbiologic tests confirmed the results of the plaque indices: The plaque on the machined surface is numerically scarcer with respect to the DAE abutments. Nevertheless, the bacterial flora was not part of a pathogenic condition in any of the cases presented. This explains the anathomo-pathologic results, which did not find a greater inflammatory infiltrate next to the modified surface abutment.

SEM Analysis

The abutment analysis with the SEM confirmed what the Plaque Index and the microbiologic analysis had already demonstrated. For the DAE surface healing abutments, plaque was present in greater quantities than visualized on the machined healing abutment surfaces (Figs 6a and 6b).



Figs 6a and 6b SEM photographs of **(a)** a machined abutment and **(b)** a DAE abutment removed from the same patient. Some areas free from plaque are still visible on the machined abutment. There are more accumulations of plaque on the DAE abutment, which is completely covered with plaque; magnification ×10,000.

Discussion

This preliminary and mainly clinical study sought to lay down the groundwork for expanded studies of a longer duration in the future, hence the attempt to introduce a protocol for assessing plaque accumulation around different surfaced implant components and consequent soft tissue reactions. Furthermore, preliminary responses of bone resorption next to abutments and implants with different surface treatments could also be assessed.

Several short-term publications endorse the observation that peri-implant soft tissues are not negatively influenced by the type of titanium implant surface selected—whether machined or indeed modified.[24–28] Other studies show that implant surface roughness could facilitate the creation of a longer connective tissue seal and inhibit epithelial down-growth into the bone-implant interface.[29–32]

Numerous animal studies are available that may be interpreted to suggest possible trends for peri-implant soft tissue behavior in humans.[24–28,30,32] However, results from animal studies cannot be extrapolated to humans. The complexity of the phenomena (both chemical and physical) that implants are exposed to in the human oral cavity is significantly different than the typical animal study in a very broad context that includes diet, oral hygiene, and occlusal function, hence the importance of designing pilot or preliminary clinical studies, such as the one described in this paper, in an effort to pose more relevant clinical research questions.

The results obtained in this short-term investigation suggest that in the first 4 months of healing, a greater plaque accumulation occurred around modified (DAE) healing abutments when compared to the amount of plaque found on machined ones. This was also shown in the SEM analyses and the plaque indices with a significant difference between the two surfaces noted ($P < .0006$).

Another observation was that plaque was more difficult to remove from the modified (DAE) surfaces than from the machined surfaces. In fact, the plaque indices recorded after 2 minutes of professional oral hygiene treatment demonstrated a significant difference between the two surfaces, with $P < .0143$. Nevertheless, the plaque accumulated on the two types of abutments was not associated with the sort of pathogenic characteristics that might lead to alterations in a wound healing process or development of inflammatory lesions. Additional long-term and more comprehensive studies are necessary to determine if the DAE surfaces and the machined surfaces favor the adhesion of different bacterial species.

These results match those of Quirynen et al[8] and Quirynen,[33] who examined sandblasted and machined surface abutments placed in vivo and found that the amount of plaque harbored on sites with rough surface abutments was larger than the amount formed on abutments with smooth, polished surfaces. The composition of the plaque samples was similar in the two abutment groups. The presence and density of periodontal pathogens subgingivally were, however, more related to the patient's dental status than to the surface characteristics of the abutments.[8]

Finally, the radiographic measurements demonstrated that in 9 out of 10 cases, the modified (DAE) implant surface appeared to be associated with bone growth along the implants, in spite of a greater plaque accumulation. In fact, at 1 year the peri-implant bone levels were found at the level of the first threads (mean bone resorption: 1.47 mm) next to the Osseotite implants. At the same time, interproximal bone levels were found at the coronal margins of the FOSS implants (mean bone resorption: 0.61 mm). This difference between the two surfaces was significant ($P < .0174$) and may very well have happened in the short-term since DAE surfaces are reported to promote osseointegration.[34–37] Moreover, the absence of pathogenic bacteria in the accumulated plaque would have been unlikely to influence peri-implant tissues in an adverse manner. Nevertheless, both at the control and test sites, measured bone loss was acceptable and consistent with the criteria of success proposed by Albrektsson et al.[38]

It must be conceded that the conventional radiographic technique used provides only two-dimensional information. Therefore, tissue breakdown on buccal or lingual aspects may be missed.[39]

While no differences were noted in comparing modified (DAE) healing abutments and machined healing abutments, it must be emphasized that healing abutments do not have threads and therefore only partially simulate the likely effects of microroughness of an implant surface (as a result of surface treatment). The threads of any implant's macrostructure will certainly influence plaque retention, but this aspect of the implant design has not been investigated.

The potential effect of the DAE surface of the healing abutment with increased plaque formation was given up to the 21st week of treatment, when healing abutments were removed and provisionals were placed. Any further changes after the fifth month post–implant insertion may therefore be attributed to the different surface modalities in the coronal portion of the implants.

The DAE healing abutments tested revealed a tendency to increase plaque accumulation (see Table 3). The DAE implant surface (in the upper compartment), however, facilitated less bone resorption during the first year after implant placement (see Table 2). This observation is in agreement with the clinical findings with less BOP positive sites around test implants at 1 year (see Table 4). It is appropriate to note that all of the patients in this study had high motivation levels and underwent a strict follow-up protocol. It is not possible to predict what would happen in patients with fewer oral hygiene sessions or longer observation periods. Furthermore, soft and hard tissue reactions to exposed surfaces of the DAE type have not been evaluated for a period longer than 5 months. Increased titanium roughness leads to increased plaque accumulation and insufficient plaque removal may lead to peri-implant tissue disease with bone loss.[40–42]

It is also interesting to note that a retrospective radiographic evaluation in humans that compared two nonsubmerged implant designs with different machined collar lengths reported that crestal bone loss for implants placed in patients with poor oral hygiene was significantly higher than in patients with adequate or good plaque control ($P < .005$). Moreover, the implant design with the shorter, smooth coronal collar had no additional bone loss at 3 years. The authors suggested that this approach may help to reduce the risk of an exposed metal implant margin, especially in areas of esthetic concern.[43] It is therefore tempting to suggest that patients with poor oral hygiene may benefit from the use of components with smoother surfaces.

Conclusions

The findings in this preliminary and short-term clinical study suggest that implants and healing abutments with DAE surfaces show greater plaque accumulation ($P < .0006$). Additionally, this plaque is more difficult to remove when compared to machined surfaces ($P < .0143$). Nevertheless, specific periodontally borrowed parameters suggest no significant differences between the tissues surrounding the two surfaces. This was also consistent with the microbiologic analysis, which found similar microbiota harbored on the two surfaces. The modified (DAE) implant surfaces appeared to promote better bone healing with less interproximal bone loss at 1 year post–implant placement ($P < .0174$). Further comprehensive and long-term clinical research is needed to provide valid support for this short-term study's preliminary observations.

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