Early Bone Response to Dual Acid-Etched and Machined Dental Implants Placed in the Posterior Maxilla: A Histologic and Histomorphometric Human Study

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Nowadays, dental implants represent a predictable and effective solution for the rehabilitation of partially or totally edentulous patients, with satisfactory high survival and success rates, as confirmed by several clinical studies in the medium and long term.1–3 However, the survival and success rates of implants placed in areas of poor bone quality, such as the posterior maxilla, are still lower than those of implants placed in the anterior areas of the maxilla, or in the mandible, where the bone density is higher.4,5 The demand for improved dental implant survival at sites of lower bone density, such as the posterior maxilla, has stimulated researchers to introduce implant design alterations and therefore surface modifications, to increase the early bone response and accelerate osseointegration.6,7

In fact, the implant surface is the first part of the biomedical device to interact with the host: body fluids and cells interact with the implant surface, and micrometer-scale features (such as cavities, grooves, ridges, and wells) play an important role in determining molecular and cellular responses.6,7 Accordingly, in the last years, a variety of rough-surfaced implants have been introduced in the market.8–10

Acid-etching and sandblasting are 2 of the most commonly used methods for the preparation of rough implant surfaces.11–13

In the acid-etching procedure, dental implants are immersed in acidic solutions; the result obtained, namely the erosion of the surface with formation of peaks and cavities of various dimensions, depends on the concentration of the acidic solutions, the immersion time, and the temperature.14 In general, acid-etched surfaces are obtained by combined

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treatment with strong acids, such as hydrochloric acid (HCl) and sulfuric acid (H2SO4), or with hydrofluoric acid (HF) and nitric acid (HNO3). Dual acid-etched (DAE) surface implants are a good example of the application of these treatments, and the clinical application of these implants has been extensively documented, with high survival and success rates. DAE surfaces promote the organization of fibrin clot and the adhesion of platelets in the early healing phases. In several animal studies, DAE implants have shown improved histologic and histomorphometric bone response, compared with machined (MA) dental implants, and only in a few of them the implants were inserted and retrieved from different subjects, retrieved from native mature bone of the posterior maxilla.

**Study Design**

The present controlled histologic and histomorphometric study was to compare the early perimplant endosseous healing properties of DAE and MA implants, when placed in native mature bone of the posterior maxilla.

**MATERIALS AND METHODS**

**Study Design**

The present controlled histologic and histomorphometric study evaluated the early bone response to DAE surface implants and MA implants, inserted in the posterior human maxilla. Each patient received 2 transitional implants (n = 1 DAE implant: test; and n = 1 MA implant: control). These implants were left submerged for an undisturbed healing period of 2 months and finally retrieved for the histologic and histomorphometric evaluation. Bone-to-implant contact (BIC, defined as the amount of mineralized bone in direct contact with the implant surface), bone density in the threaded area (BDTA, defined as the fraction of mineralized bone tissue within the threaded area), and bone density (BD%), defined as bone density in a 500-μm-wide zone lateral to the implant surface) were the histomorphometric parameters evaluated in this study.

**Patient Selection**

A total of 14 subjects (6 men, 8 women; aged between 45 and 74 years, mean age 59.0 ± 8.5 years) who were referred to the Oral Implantology Clinic, Dental Research Division, Guarulhos University, SP, Brazil, for oral rehabilitation with dental implants, were included in the present study. Inclusion criteria were good systemic and oral health and sufficient native bone to place implants of 3.25 mm diameter and 10 mm length. Exclusion criteria were pregnancy, nursing, smoking, and any systemic condition that could affect bone healing. All participants received detailed explanations about the nature of the study and signed a written informed consent form. The Institutional Clinical Research Ethics Committee of Guarulhos University approved the protocol of the present study (CEP UnG #203/2013), which was conducted according to the principles outlined in the World Medical Association’s Declaration of Helsinki on experimentation involving human subjects, as revised in 2008.

**Implant Design and Surface Treatment**

The transitional implants used in the present study (BT Konic; Biotec-BTK, Dueville, Vicenza, Italy) were made of titanium grade 4 (ASTM F67—ISO 5832-2). All these implants (test and control) were macroscopically identical, with a tapered design, 3.25 mm in diameter, and 10 mm in length. The test implants had the surface treated with a DAE procedure. A mix of strong inorganic acids (H2SO4, H3PO4, HCl, and HF) was used, in 2 different acid baths. After each acid bath, implants were rinsed and washed with distilled water, to neutralize and remove acid residuals. Finally, implants were taken to a cleaning room ISO 7 class to be decontaminated through a plasma spray decontamination process, in argon atmosphere. The DAE implant surface was studied with scanning electron microscopy (SEM) (Fig. 1). The following standard roughness parameters were measured: Ra (the arithmetic mean of the absolute height of all points), Rq (the square root of the sum of the squared mean difference of all points), and Rt (the difference between the highest and the lowest points). The SEM evaluation of DAE surface implants revealed a mean Ra of 1.12 (±0.41) μm, a mean Rq of 1.34 (±0.69) μm, and a mean Rt of 3.86 (±1.40) μm, respectively. The control implants had a MA surface.

**Surgical Protocol**

Twenty-eight transitional implants (n = 14 test implants and n = 14 control implants) were inserted in this study. All implants were placed under aseptic conditions. After local anesthesia, a crestal incision connected with 2 releasing vertical incisions was made. Mucoperiosteal flaps were raised and conventional implants were inserted, in accordance with the surgical and prosthetic plan prepared for each patient. Then, 2 transitional implants (n = 1 test implant and n = 1 control implant) were inserted in each patient. The transitional implants were placed in the posterior maxilla (in the areas of second premolars/first molars), distally to the most posterior conventional implant. The assignment of test and control implants (right posterior maxilla or left posterior maxilla) was random, as determined by a coin toss. The implant sites were prepared according to the manufacturer’s recommendations, under profuse irrigation with sterile saline. The stability of all implants was checked using a dedicated instrument (Osstell Mentor; Osstell, Sweden).
Goteborg, Sweden): if an implant showed insufficient primary stability (<35), a backup surgical site had to be prepared. The flaps were then sutured. Clindamycin 300 mg (Clindamycin C; Teuto, Anapolis, Goias, Brazil) was administered 3 times a day for a week, to avoid postsurgical infection. Postoperative pain was controlled with 600 mg of ibuprofen (Actron; Bayer Scherig Pharma, Berlin, Germany) every 12 hours for 2 days. To enable subjects to control postoperative dental biofilm, 0.12% chlorhexidine rinses (Chlorhexidine; OralB, Boston, MA) were prescribed, twice a day for 14 days. The sutures were removed after 10 days. All transitional implants were left submerged for an undisturbed healing period of 2 months. After this, during the 2-stage surgery to uncover the conventional implants, the 2 transitional implants (test and control) and the surrounding tissues were retrieved from each patient, using a 4.5-mm-wide trephine bur.

**Histologic and Histomorphometric Evaluation**

The biopsies were fixed by immediate immersion in 10% buffered formalin and processed (Precise 1 Automated System; Assing, Rome, Italy) to obtain thin ground sections, as previously described.26,27 The specimens were dehydrated in an ascending series of alcohol rinses and embedded in glycol-methacrylate resin (Technovit 7200 VLC; Kulzer, Wehrheim, Germany). After polymerization, the specimens were sectioned lengthwise along the larger axis of the implants with a high-precision diamond disk at about 150 μm, and ground down to about 30 μm. Two to 3 slides were obtained from each implant, stained with basic fuchsin and toluidine blue. The specimens were analyzed under a transmitted light microscope (Laborlux S; Leitz, Wetzlar, Germany) and a histometry software package with image-capture functionalities (Image-Pro Plus 4.5; Media Cybernetics, Immagini & Computer Snc, Milan, Italy). For the histomorphometric evaluation, the BIC%, defined as the amount of mineralized bone in direct contact with the implant threads, where active osteoblasts secreting osteoid matrix were present. In some areas, this matrix was undergoing mineralization. Preexisting bone, typical of the posterior maxilla, was also evident (histological staining: acid fuchsin-toluidine blue, ×18).

**Fig. 2.** Test implant (DAE surface). Newly formed trabecular bone with small marrow spaces was found along the implant perimeter, lacking only in the apical portion, maybe due to procedure of surgical removal. Low-density (D3-D4) preexisting bone, typical of the posterior maxilla, was also evident (histological staining: acid fuchsin-toluidine blue, ×18).

**Fig. 3.** Test implant (DAE surface). Newly formed bone could be observed inside the concavities of the implant threads, where active osteoblasts secreting osteoid matrix were present. In some areas, this matrix was undergoing mineralization. Preexisting bone, typical of the posterior maxilla, was also evident (histological staining: acid fuchsin-toluidine blue, ×40).

**Fig. 4.** Control implant (MA surface), only in few fields newly formed trabecular bone in contact with the implant surface was present (histological staining: acid fuchsin-toluidine blue, ×40).

**Fig. 5.** Control implant (MA surface), preexisting bone was in contact with the implant surface mainly in the coronal portion. Trabeculae of newly formed bone going toward the implant surface were observed in the middle and apical portions (histological staining: acid fuchsin-toluidine blue, ×18).

Matrix Vision GmbH, Oppenweiler, Germany) and a histometry software package with image-capture functionalities (Image-Pro Plus 4.5; Media Cybernetics, Immagini & Computer Snc, Milan, Italy). For the histomorphometric evaluation, the BIC%, defined as the amount of mineralized bone in direct contact with the implant threads, was measured around all implant surfaces. The BDTA%, defined as the fraction of mineralized bone tissue within the threaded area, and the BD% in a 500-μm-wide zone lateral to the implant surface were measured bilaterally, as previously reported.29

**Statistical Analysis**

All collected data were inserted in a sheet for statistical analysis (Excel 2003; Microsoft, Redmond, WA). Mean, SDs, median, and 95% confidence intervals of histomorphometric values (BIC%, DBTA%, and BD%) were calculated for each implant and then for each group of implants (test vs control implants). The Wilcoxon matched-pairs signed rank test was used to evaluate differences (BIC%, BDTA%, and BD%) between the implant surfaces. The level of significance was set at 0.05. All computations were carried out with a statistical analysis software (SPSS 17.0; SPSS Inc., Chicago, IL).
RESULTS

Clinical Observations

After 2 months of healing, a total of 28 transitional implants (n = 14 test implants and n = 14 control implants) were retrieved and evaluated. Three implants (one test implant and 2 control implants) were not clinically stable and showed no osseointegration, although they did not show any sign of infection. The remaining 25 implants were clinically stable at the time of retrieval.

Histologic and Histomorphometric Results

The bone surrounding both implant groups was healthy. Woven bone with several osteocyte lacunae and preexisting bone were present; the woven newly formed bone was separated from the preexisting bone by cement lines. Some bone remodeling was observed, at early stages, even in the coronal portions of the specimens.

In the test group (DAE surface), newly formed trabecular bone with small marrow spaces was found throughout the implant body, with the exception of the apical portion; this is because of the surgical removal. Newly formed bone was found also in the coronal part of the implant. Preexisting bone is also evident, with a quality comprised between D3 and D4 (Fig. 2). Inside of the implant threads, the concavities were colonized by new bone formation, with the presence of active osteoblasts secreting osteoid matrix; in some areas, this matrix was undergoing mineralization. Preexisting bone, not in contact with the implant surface, showed low quality and low affinity for fuchsin (Fig. 3).

In the control group (MA surface), the implant was in contact with the bone tissue mainly in the coronal portion, where preexisting bone could be detected. In the middle and apical portions, newly formed trabeculae coming from the old bone and going toward the implant surface could be observed (Fig. 4). Inside of the concavities, only in few fields newly formed trabecular bone in contact with the implant surface was found (Fig. 5).

In the MA implants, the histomorphometric analysis revealed mean (±SD) BIC% = 37.43 (±12.79), BDTA%, and BD% of 28.45 (±16.91), and 21.76 (±11.67), respectively. In the DAE implants, the histomorphometric analysis revealed mean (±SD) BIC% = 44.21 (±29.51), BDTA%, and BD% of 37.49 (±21.78), and 31.60 (±18.06), respectively (Table 1). For the MA implants, the BIC% ranged from 0 to 54.51; for the DAE implants, the BIC% ranged from 0 to 78.08. Although the mean BIC% of DAE implants value was almost double than that of MA implants, the Wilcoxon matched-pairs signed rank test found no significant differences between the 2 groups of implants, with regard to BIC% (P = 0.198). The BDTA% was similar in the 2 groups, as it ranged from 0 to 54.51 for the MA implants, and from 0 to 60.5 for the DAE implants. Again, the Wilcoxon matched-pairs signed rank test failed to find a significant difference between the 2 groups of implants, with regard to BDTA% (P = 0.778). Finally, for the MA implants, the BD% ranged from 0 to 41.54; for the DAE implants, the BD% ranged from 0 to 55.9. Although BD% was higher in the test group than in the control group, this difference was not statistically significant (P = 0.124).

DISCUSSION

At present, the relationship between surface topography and osseointegration is well recognized.8–10 In fact, the nature of the implant surface is known to influence the rate of osteoblast proliferation, matrix synthesis, and local autocrine factor production, which all, ultimately, influence the rate of osseointegration.8–10 Rough surfaces have demonstrated better adsorption of biomolecules from biological fluids, which has the potential to alter the cascade of events that leads to bone healing and intimate apposition with the device.7,9,12 In vitro reports indicate that rough surfaces improve the initial cellular response, including cytoskeletal organization and cellular differentiation with matrix deposition.6,7,9,12,19 Histologically, it has been demonstrated that rough surfaces can effectively promote better and faster osseointegration when compared with MA surfaces.20–22,25–29 From a clinical point of view, several studies have reported excellent long-term survival/success rates for rough surface implants.2,16–18

In challenging implant cases, such as immediate loading, immediate implant placement in postextraction sockets, and placement of implants in “poor” quality bone, the acceleration of early perimplant bone healing might be very useful; 4,5 however, the precise nature of surface characteristics needed for optimal osseointegration remains to be elucidated.6,8,12,19 Among different surface treatments, acid etching seems to be one of the most popular, and DAE implants have been used for several years, with satisfactory high survival and success rates.16–18

At present, histologic and histomorphometric assessments are the most accurate methods to investigate the bone healing processes and the morphological characteristics of the bone-implant interface.30 Unfortunately, only a few studies in the present literature have dealt with histologic

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Table 1. Mean, SD, Range, and Confidence Interval of BIC%, BDTA%, and BD% of DAE and MA Implants Placed in the Posterior Maxilla (n = 14 Subjects)

<table>
<thead>
<tr>
<th></th>
<th>DAE Surface (Test Implants)</th>
<th>MA Surface (Control Implants)</th>
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<tbody>
<tr>
<td>%</td>
<td>Mean (SD)</td>
<td>Range</td>
</tr>
<tr>
<td>BIC</td>
<td>37.49 (29.51)</td>
<td>0–78.08</td>
</tr>
<tr>
<td>BDTA</td>
<td>30.59 (21.78)</td>
<td>0–60.5</td>
</tr>
<tr>
<td>BD</td>
<td>31.60 (18.06)</td>
<td>0–55.9</td>
</tr>
</tbody>
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Wilcoxon matched-pairs signed rank test (level of significance set at 0.05). CI indicates confidence interval.
and histomorphometric evaluation of human-retrieved DAE implants: this is because of ethical issues related to implant retrieval from human subjects.25–29 Lazzara et al25 conducted a human histologic/histomorphometric study to compare the percentage of BIC% at 6 months for DAE and MA titanium implant surfaces. Eleven patients were selected for installation of 1 DAE and 1 MA mini-implants (2 mm diameter × 5 mm length), in the posterior maxilla (type III and type IV bone), during conventional dental implant surgery.25 After 6 months of undisturbed healing, the mini-implants and surrounding hard tissue were removed.25 Histomorphometric analysis indicated that the mean BIC% value for the DAE surfaces (72.96 ± 25.13) was significantly higher than the mean BIC% value for the MA surfaces (33.98 ± 31.04).25 The authors concluded that in poorer quality bone (posterior maxilla), implants with DAE surface can guarantee a faster bone healing when compared with implants with MA surface.25 In a more recent histological study, the authors retrieved 2 DAE implants from the mandible, to repair damage to the inferior alveolar nerve.26 After 6 months of healing, both implants seemed to be surrounded by newly formed bone.26 No gaps or fibrous tissues were present at the bone-implant interface.26 The mean BIC% (61.3 ± 3.8) was high.26 In another study, the authors documented the osseointegration of 2 DAE implants after 2 months of healing, with different loading conditions.28 A completely edentulous patient received a total of 11 DAE implants in the mandible.28 Six implants were immediately loaded to support a provisional fixed partial denture and 5 were left submerged. After 2 months, 2 submerged and 1 immediately loaded implants were retrieved for histologic/histomorphometric analysis.28 The BIC% was 38.9 for the submerged implants and 64.2 for the immediately loaded one. The authors concluded that osseointegration can be achieved after 2 months by DAE implants placed in the mandible, either when immediately loaded or when submerged and unloaded.28 Finally, in a recent work, DAE surface was compared with bioceramic molecular-impregnated surface.29 Ten subjects received 2 transitional mini-implants (1 of each surface) during conventional implant surgery in the posterior maxilla.29 After an undisturbed healing period of 2 months, the implants and the surrounding tissue were removed by means of a trephine and were nondecalcified processed for ground sectioning and analysis of BIC%, BDTA%, and osteocyte index (Oi).29 At the end, histometric evaluation showed significantly higher BIC% and Oi for bioceramic molecular-impregnated implants (P < 0.05), whereas BA% was not significantly different between groups. The authors concluded that bioceramic molecular-impregnated surface can positively modulate bone healing at early implantation times compared with the DAE surface.29

In our present study, we have decided to evaluate DAE (test) and MA implants (control) with an intra-individual comparison to overcome the possible anticipated variability between individuals. This represents a clear advantage of our present study, as an intra-individual comparison between different surface is a rarity in the literature.25,29,30 In our study, in the MA implants, the histomorphometric evaluation revealed mean (±SD) BIC%, BDTA%, and BD% of 21.76 (±12.79), 28.58 (±16.91), and 21.54 (±11.67), respectively. In the DAE implants, the histomorphometric analysis revealed mean (±SD) BIC%, BDTA%, and BD% of 37.49 (±29.51), 30.59 (±21.78) and 31.60 (±18.06), respectively. Although the mean BIC% of DAE implants value was almost double than that of MA implants, the statistical analysis found no significant differences between the 2 groups, with regard to BIC% (P = 0.198). These statistical results may be influenced by the fact that also nonosseointegrated samples (3) have been included in our analysis, and most of all, the overall number of samples (14 per type) was low; if the sample had been larger, we would probably have expected a statistically significant difference between the 2 groups in the BIC%. No significant differences were found in our study between DAE and MA implants with regard to BDTA% (P = 0.778) and BD% (P = 0.124). Anyhow, our results indicate that DAE surface can potentiate healing process and new bone apposition, compared with MA surface.

It should be noted that most of the human histologic/histomorphometric studies that are currently available in the literature have focused on hard and soft tissue reactions around experimental implants with smaller dimensions than those of regular dental implants.25,29,31 To date, only a few studies have evaluated the histological response around standard-diameter implants,13,30 and most of these were based on implants removed for fracture.13,32 In our present study, we have used transitional implants of standard dimensions (3.25 mm diameter × 10 mm length). This may represent an advantage of our study because we have evaluated how the healing processes take place in a situation closer to the real one. However, the retrieval of our transitional implants was carried out in such a way that the resulting prepared canal in bone could be used for dental implants with a larger diameter.30

CONCLUSIONS

At present, only a few histologic and histomorphometric studies have evaluated the bone healing around dental implants in humans; however, the histological data from the retrieved human implants are absolutely necessary to obtain useful information about the bone healing processes around dental implants, as well as the bone-implant interface. Within its limits (such as the small sample size), the present study reports that the DAE surface improved the perimplant early healing processes in the native bone of the maxilla when compared with the MA surface. Further studies on a larger sample of patients are needed to confirm these results.

DISCLOSURE

The authors claim to have no financial interest, either directly or
indirectly, in the products or information listed in the article.

**APPROVAL**

The present study was approved by the Institutional Clinical Research Ethics Committee of Guarulhos University (number of approval: CEP UnG #203/2013).

**REFERENCES**