BONE RESPONSE TO NEW MODIFIED TITANIUM SURFACE IMPLANTS IN NONHUMAN PRIMATES (*PAPIO URSINUS*) AND HUMANS: HISTOLOGICAL EVALUATION

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The aim of this study is a comparative histological and histomorphometrical evaluation of the effect on early bone formation of 2 different implant surfaces: a machined and a new acid-etched implant surface (Leader, Milano, Italy). Ten screw-type microimplants were placed in 5 patients. Each patient received 2 microimplants (2 mm in diameter and 5 mm in length): 1 with a machined surface (control) and 1 with an acid-etched surface (test). The microimplants were retrieved after 60 days of healing with a 4-mm trephine bur and processed for histology. Moreover, 24 regular size implants—12 with a machined surface (control) and 12 with an acid-etched surface (test)—were placed in 2 adult nonhuman primates 3 months after the extraction of premolars and molars. Each animal received 3 machined implants (control) in the right hemimandible and 3 acid-etched implants (test) in the left hemimandible. The same animals received 3 control implants and 3 test implants in the rectus abdominis muscle. After 1 month, the implants were retrieved from the mandible and the rectus abdominis muscle and processed for histology. Histomorphometric evaluation demonstrated a higher bone-to-implant contact in the test implants compared with the controls in both primates (25.55% vs 15.8%) and humans (62% vs 45%). Moreover, in nonhuman primates after 1 month of healing, it was possible to observe a poor osseointegration in the control specimens, while newly formed bone in direct contact with test implants was evident. The rectus abdominis muscle specimens showed that the acid-etched surfaces can stimulate the formation and attachment of new connective and vascular tissues more than machined surfaces can. Implant surface geometry can speed up bone formation by the development of a special microenvironment that promotes angiogenesis. Long-term studies are needed to further test this new acid-etched implant surface.

Key Words: acid-etched surface; bone formation; implant surfaces; surface geometry; osseointegration

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Introduction

Endosseous dental implants have been shown to achieve high clinical success rates. Several implant surfaces have been used for the rehabilitation of the edentulous jaws: an understanding of their biological behavior is of relevant importance to improve surface geometries and implant designs to achieve predictable results.

In 1991, Buser et al[1] evaluated the bone-to-implant contact (BIC) using 5 different titanium surfaces in pigs. Histomorphometric analysis was performed after a relatively short healing period of 3 and 6 weeks, and implants with a surface sandblasted using a large grit and acid etched with hydrochloric and sulphuric acid resulted in the greatest BIC. More recent works supported these findings, reporting a higher BIC with rougher implant surfaces when compared with smoother surfaces. In the study by Wennerberg et al,[2] 3 different aluminum oxide–blasted surfaces with different grit sizes were compared with machined surfaces. After 12 weeks of healing, the largest amount of BIC occurred around the surfaces blasted with particles with a diameter of 75 μm.

Therefore, there are several studies demonstrating that roughened titanium surfaces presented a greater contact percentage than did smoother implant surfaces such as machined or polished titanium surfaces.[3–5]

Cochran et al[6] compared the bone response in the canine mandible to loaded titanium implants with sandblasted, acid-etched, and plasma-sprayed surfaces. The implants were allowed to heal for 3 months and then functionally loaded for up to 12 months. A greater bone apposition on the sandblasted implants after a healing period of 3 months and after 12 months of loading was reported, with no differences in bone apposition between sandblasted and acid-etched implants after a 3-month loading period. This led to the conclusion that sandblasted and acid-etched implants were more osteophilic and therefore promoted a higher BIC in the earlier phase of healing compared with plasma-sprayed implants.

Kirsch and Donath[7] demonstrated that rough surfaces had a faster bone apposition than smooth surfaces because of their osteoconductivity. This has also been described in a review article by Schenk and Buser.[8] Several hypotheses have been proposed to explain the effect of implant surface geometry on peri-implant tissue formation. Many investigators have suggested that the greater bone apposition obtained with textured surfaces vs smooth surfaces is due to the ability of the surface features to modulate cellular activity. In vitro studies have shown that proliferation, differentiation, and production of proteins, growth factors, and cytokines by osteoblast-like cells are affected by the texture of titanium surfaces.[3,9,10]

The aim of the present experimental study was to compare histologically and histomorphometrically the effect of a new implant surface geometry (Leader, Milano, Italy) on early bone formation in humans and in nonhuman primates. The early healing phase after implant placement was evaluated because during this period, cell differentiation, tissue synthesis, and mineralization have been reported to take place.

Materials and Methods

Surface treatment

The test implants were treated as follows:

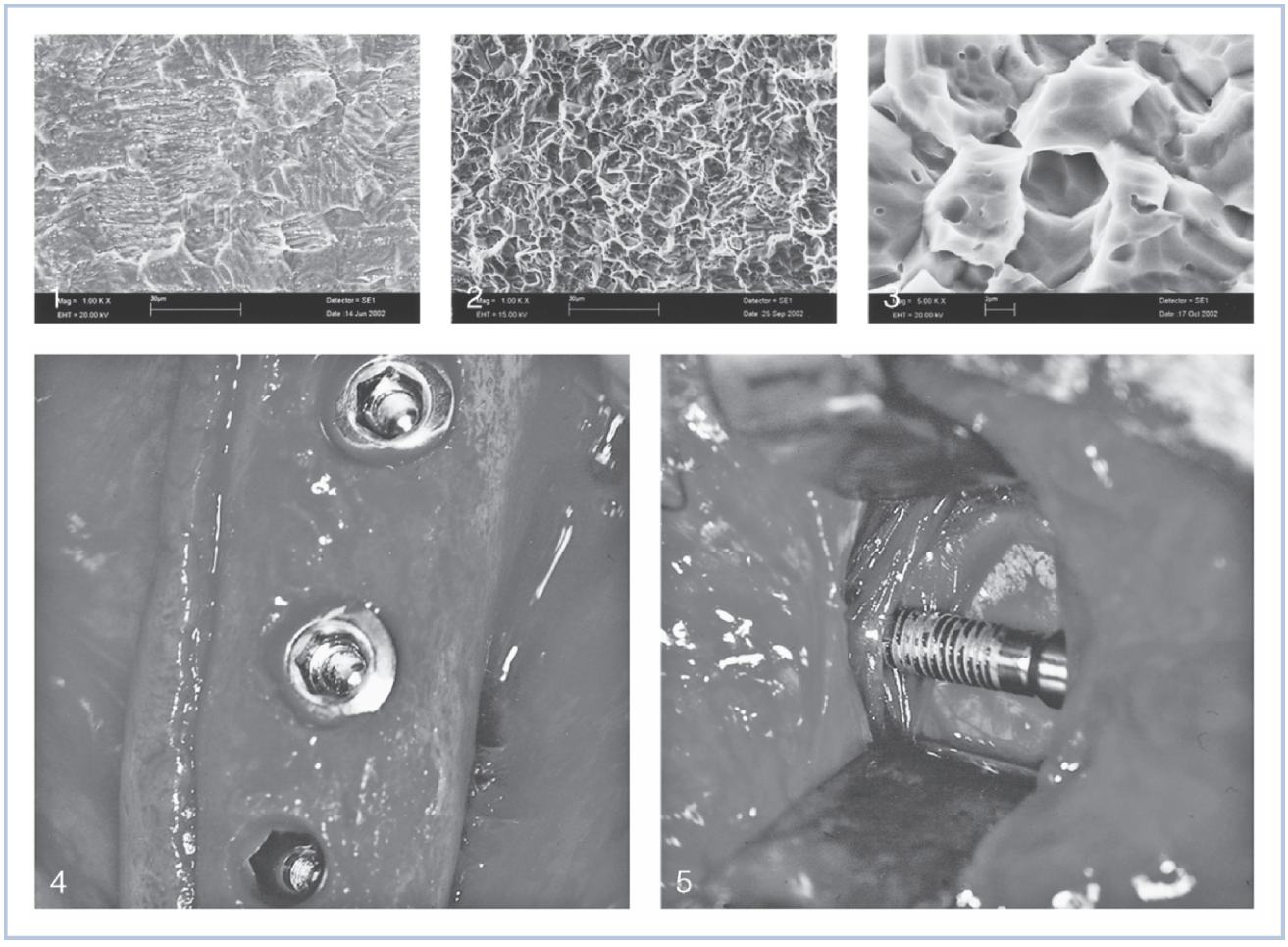
- sonic bath in distilled water at 25°C for 5 minutes to remove residuals deriving from manufacturing,
- immersion in NaOH (20 g/L) + H_2O_2 (20 g/L) at 80°C for 30 minutes,
- sonic bath in distilled water at 25°C for 5 minutes,
- acid etching in an organic mixture of 50% oxalic acid and 50% maleic acid at 80°C for 45 minutes,
- washing in distilled water and sonication for 5 minutes,
- immersion for 30 minutes in a solution of 65% nitric acid and distilled water with a volumetric range of 1 to 1 at 100°C, and
- washing in distilled water.

The modified surfaces obtained with this procedure were examined under a Cambridge stereoscan with Link EDS AN 10 000 (Figures 1 through 3).

Nonhuman primates study

Two adult, nonhuman primates of the genus *Papio* (*Papio ursinus*) were selected for the present study. The study protocol was approved by the Ethics Committee of the University of Witwatersrand, Johannesburg, South Africa. Premolars and molars were extracted to create edentulous ridges in the adult baboons. After 3 months of healing, the edentulous mandibular ridges were exposed by elevating mucoperiosteal flaps, and 3 control (machined) and 3 test (acid-etched) implants were inserted in the hemimandible of each animal (for a total of 12 implants). The same animals received 3 control and 3 test implants in the rectus abdominis muscle (Figures 4 and 5).

Thirty days after surgery, the animals were killed, and the mandibular and rectus abdominis specimens



FIGURES 1–5. FIGURE 1. Implant surface before acid-etched treatment. SEM ×1000. FIGURE 2. Implant surface obtained after organic acid mixture treatment. SEM ×1000. FIGURE 3. High magnification of the treated surface showing the microconcavities' appearance. SEM ×5000. FIGURE 4. Clinical image showing implants inserted in the hemimandible of nonhuman primates. FIGURE 5. Clinical image showing a test implant inserted in the rectus adbominus muscle of nonhuman primates.

were harvested. They were immediately fixed in 70% ethanol and embedded in Technovit 7200 VLC resin (Kulzer, Wehrheim, Germany). From each block section, 2 serial sections were cut using diamond blades mounted on the Grinding and Cutting Exact Apparatus (Nordenstedt, Germany) to produce undecalcified bone-titanium sections that were stained with Goldner trichrome stain to assess the ratio of mineralized bone vs osteoid matrix. Undecalcified titanium implants and surrounding bone and/or soft tissues were analyzed histomorphometrically to determine the BIC percentages.

Human study

Five partially edentulous patients (3 women and 2 men) with a mean age of 59 years (range, 54 to 67 years) were included in this study. The protocol was approved by the Ethics Committee of the University of Witwatersrand, Johannesburg, South Africa, and all patients signed a written informed consent form. The surgical area underwent local anesthesia with 2% lidocaine with epinefrine (Xylocain, Astra Zeneca, Sweden). After a crestal incision, a mucoperiosteal flap was elevated. The implant site was prepared using a twist drill, 3 mm in diameter, with adequate irrigation. Each patient received 2 screw-type microimplants (2 mm × 5 mm), 1 control (machined), and 1 test (acid etched) (for a total of 10 microimplants) in the posterior region of the mandible. After a healing period of 60 days, the control and test microimplants were retrieved with a 4-mm trephine bur. The specimens were rinsed in sterile saline solution, fixed in 10% neutral buffered formaldehyde solution, and processed for histology.

TABLE 1

Primates: bone-to-implant contact after 30 days

Control Surface (%)	Test Surface (%)
15.8 ± 3.4	25.5 ± 4.3

Histological processing

The specimens were dehydrated in a graded series of ethanols, embedded in methylmethacrylate resin (Technovit 9100 VLC, Kulzer, Wehrheim, Germany), and polymerized. Ground sections were obtained according to the Donath protocol.[11] The samples were cut and ground to 10 μm in an Exakt apparatus (Exakt, Nordenstedt, Germany). A total of 3 slides were obtained for each implant. The samples were then stained with the Goldner trichrome stain. The sections were examined under a Leitz microscope (Leitz, Wetzlar, Germany). The microscope was equipped with a Microvid System (Leitz, Wetzlar, Germany) that permitted direct histomorphometric measurements.

Data analysis

The differences in the percentage of BIC between control and test implants were evaluated. The BIC percentage values were expressed as the means ± standard deviation. The differences between the primate and human groups were analyzed by analysis of variance, and the statistical significance of multiple comparisons was evaluated using the Fisher protected least significant difference and Scheffe *F* tests. Significance was set at $P \leq .05$.

RESULTS

Nonhuman primates

A histomorphometrical analysis of the BIC percentage was performed in control (machined) and test (acid-etched) implants.

For the machined implants (control), the surface showed a low percentage of BIC (15.8% ± 3.4%). For the acid-etched implants (test), there were osteoblasts actively secreting new bone directly on the implant surface (Table 1). The BIC was higher (25.5% ± 4.3%) than in the control implants. Microscopically, in test implants, newly formed bone without gaps at the interface was evident. Specifically, the new bone formation apparently started within the concavities between the implant threads. In the same areas, an intense neoangiogenesis with the presence of newly formed small vessels was observed.

The rectus abdominis muscle specimens showed clearly that test implants, when placed in an extraskeletal location, could promote the formation and attachment of new connective and vascular tissues on the acid-etched titanium surface. The tests implants showed a greater amount of tissues attached to their surface when compared with control (Figures 6 through 11).

Human study

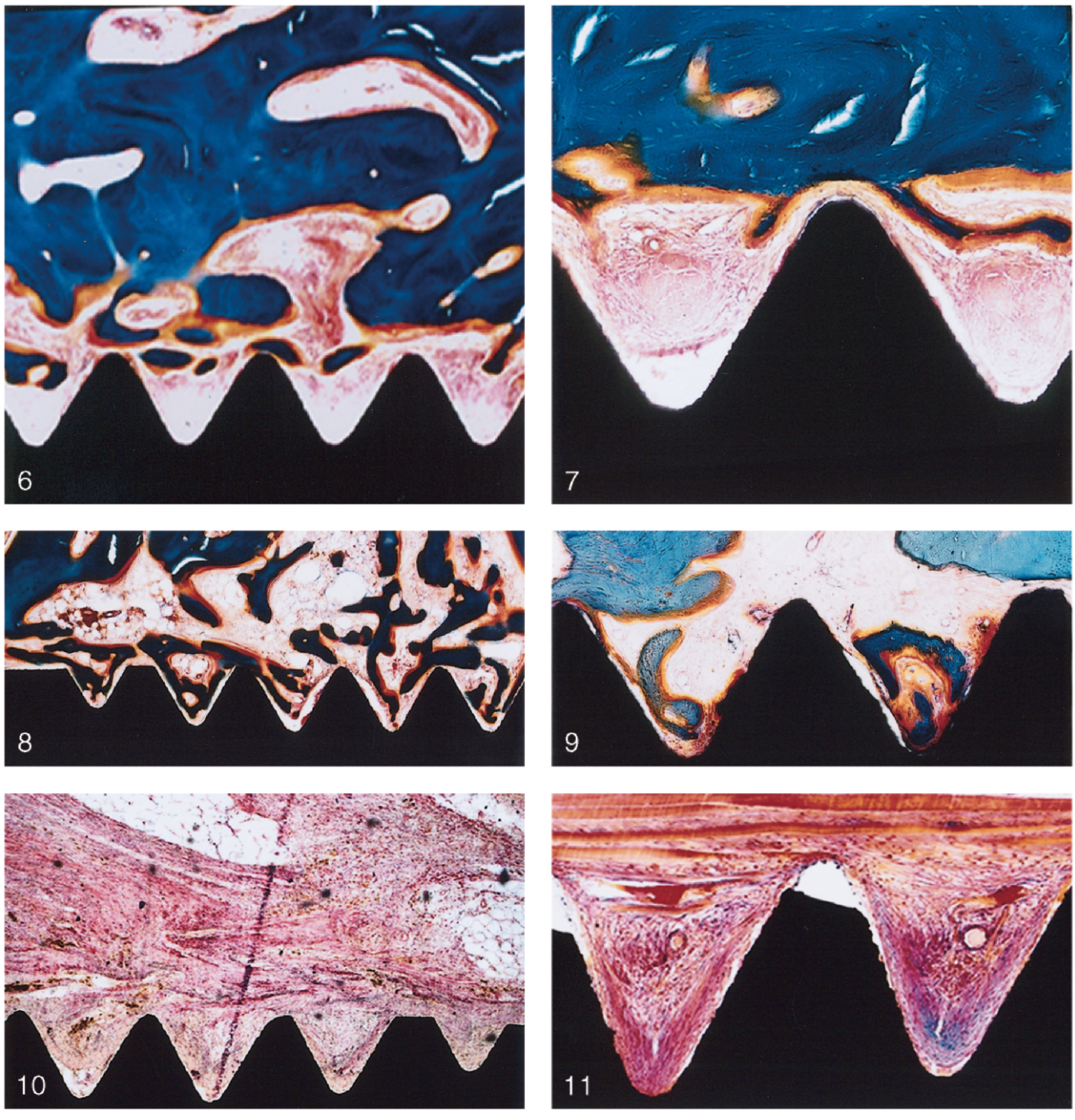
Histomorphometrical evaluation showed differences in the BIC percentages between control and test sections. Two months after the placement of the implants, acid-etched implants (test) showed a higher percentage of bone contact (62% ± 5.2%) when compared with control surfaces (45% ± 4.6%) (Table 2). In the machined implants, it was possible to observe a lower amount of ongoing remodeling areas when compared with test implants, which, on the contrary, showed a good osteoconductivity, especially in the microcavity of the implant surface. This was due to the presence of osteoblasts that were actively secreting new bone directly on the implant surface. Furthermore, in the acid-etched implants, it was possible to observe a good osseointegration along with the presence of small vessels, osteoid matrix, and new bone formation (Figures 12 through 14).

Statistical evaluation

Statistically significant differences in the BIC values between control and test implants in primates ($P = .001$) as well as in humans could be detected ($P = .0003$).

DISCUSSION

During implant placement, the development of a microenvironment favorable to osteoblast activity is of utmost importance. The interaction of cells of mesenchymal origin with the implant surface via integrins is mediated through focal attachments. The number and distribution of these attachments can produce changes in cell shape and can influence their gene expression.[12–14] Osteoblasts may exhibit several shapes depending on their functional status. When an implant is placed into the bone, the surface is immediately coated with a layer of proteins, salts, sugars, and lipids, thus constituting a biosurface. The presence and the proportion of these components is in turn influenced by the implant surface characteristics.[15] Furthermore, the cells attached on this biosurface can be affected by a variety of signals, which lead



FIGURES 6–11. FIGURE 6. Control (machined) implant. After a 1-month healing period, scarce bone formation, especially within the concavities, was detected. Goldner trichrome stain ×10. FIGURE 7. Control (machined) implant. Higher magnification of the bone-implant interface. No bone formation within the concavities was detected. Goldner trichrome stain ×30. FIGURE 8. Test (acid-etched) implant. After 1 month of healing, newly formed bone (both osteoid and mineralized) filled the spaces between the old bone and the implant surface. New bone formation directly on the implant surface and within the concavities was observed. Goldner trichrome stain ×10. FIGURE 9. High magnification of the bone-implant interface. Newly formed bone was evident within the concavities along with the presence of newly formed vessels and osteoblast activity. Goldner trichrome stain ×30. FIGURE 10. Control (machined) implants harvested from the extraskeletal site (muscle) after 1 month. Only a scarce amount of connective tissue was attached to the implant surface. Goldner trichrome stain ×10. FIGURE 11. Test (acid-etched) implant. It was possible to observe the presence of new connective and vascular tissue. Goldner trichrome stain ×30.

Table 2

Human: bone-to-implant contact after 60 days

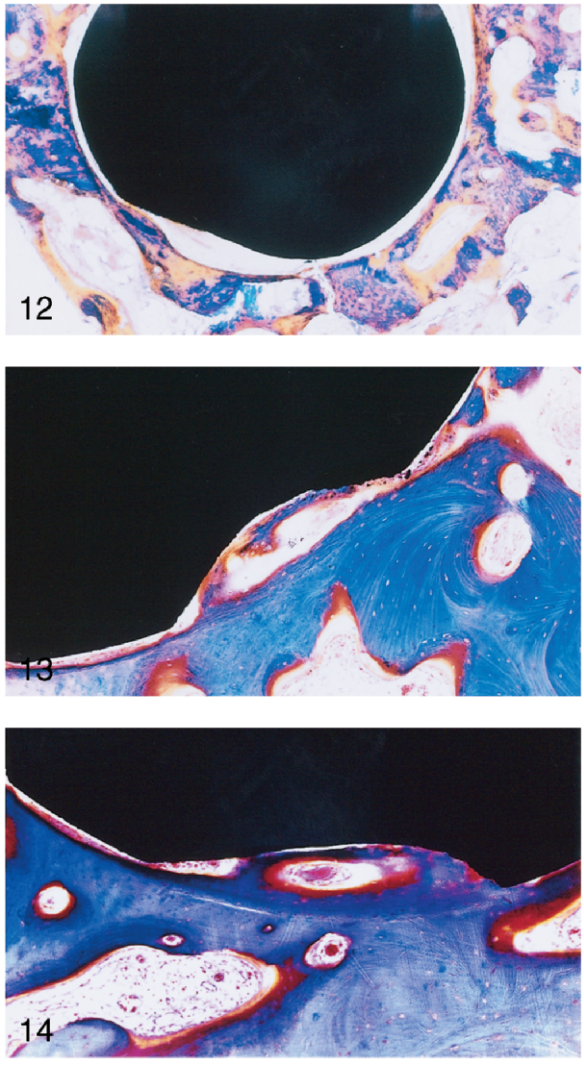
Control Surface (%)	Test Surface (%)
45 ± 4.6	62 ± 5.2

to their differentiation and, eventually, activation.[16–18] Cell differentiation is also regulated by the release of intrinsic local factors during bone healing after implant insertion.[17] There are several factors released by platelets that can contribute to wound healing; specifically, they have been shown to be necessary for cell recruitment and differentiation.[19,20] Furthermore, chemical composition and topographical aspects of the implant surface play a very important role during wound healing because they can determine which cell line will be stimulated and, consequently, express its genetically planned activity.

It has been suggested that surface texture may also dictate the mechanism of osseointegration by influencing the stability of the fibrin scaffold, which is formed shortly after implantation.[4,5] A stable attachment of fibrin to the implant should be better achieved by a rough surface because of its greater surface area for protein adsorption. Several studies have demonstrated that nonfunctional, porous-surfaced implants become osseointegrated more rapidly than do plasma-sprayed implants; thus, porous-surfaced implants may be functionally loaded earlier.[21] Cochran et al[6] suggested similar advantages in sandblasted and acid-etched implants over plasma-sprayed implants.

Davies[22] described several mechanisms of healing around endosseous implants with a machined and dual acid-etched titanium surface depending on their surface topography. This author emphasized the importance of fibrin retention on the implant surface as the key mechanism to direct bone matrix deposition on the implant surface due to the migration of progenitor cells through the fibrin matrix, which determined their location, and direct bone matrix deposition directly on the implant surface. The present study demonstrated that osteoconductive surfaces showed bone deposition directly on the implant surface as a result of a constant migration of progenitor cells toward such surfaces.[23–27]

Likewise, Ripamonti[28–30] reported the importance of surface geometry to promote new bone formation also in extraskeletal sites. His studies showed that because their surface geometry, dental implants can adsorb, store, and release endogenous bone morphogenic proteins that stimulate bone formation. Specifically, the author demonstrated that a concavity with a diameter ranging from 400 to 1600 μm can induce and regulate the differentiation of osteoblasts and thus the bone tissue formation by the development of a microenvironment where angiogenesis is stimulated.[31] Histological, immunohistochemical, and biochemical studies have suggested that osteogenetic vessels, as defined by Trueta,[32] can provide a regulated flow of cells expressing an osteogenetic



Figures 12–14. Figure 12. Control (machined) implant harvested from human after 2 months. No bone formation on the implant surface was detected. Goldner trichrome stain ×30. Figure 13. Test (acid-etched) implant. It was possible to observe newly formed bone directly on the implant surface and especially within the concavities. Goldner trichrome stain ×30. Figure 14. Test (acid-etched) implants. Axial section showing new bone formation within the concavities. Osteoid matrix and newly formed small vessels were present. Goldner trichrome stain ×20.

phenotype. All of this evidence confirmed the role of implant surface topography to optimize the interaction with the host tissue during the healing period after implant placement. The acid-etched implants tested in the present study gave excellent results in term of osseointegration. This is possibly because of their specific surface topography. These implants undergo an organic acid mixture treatment, which creates a surface with a defined geometry, consisting of homogeneous and uniform micro- and macroconcavities able to promote and regulate differentiation of osteoblasts and thus induce new bone formation. The importance of concavities rather than convexities in attracting bone cells is undoubtedly demonstrated during the physiological phenomenon of bone remodeling. Previous studies have shown that osteoblasts are stimulated to migrate, attach, and proliferate on surfaces with a certain pore size (200–400 μm diameter).[33,34] This may be due to the curvature of these pores, which provides optimal compression and tension forces on the cells' mechanoreceptors.[35,36] Mechanical stretching of osteoblasts in vitro has been shown to activate synthesis of mRNA for the proto-oncogenes c-fos, c-jun, and zif/268, suggesting a role of these genes in the signal transduction of mechanical stimuli in osteoblasts.[37]

Finally, concavities can promote bone formation through the creation of a microenvironment favorable to angiogenesis, which in turn plays a pivotal role for osteogenesis. Osteogenetic vessels, in fact, provide for a regulated flow of cells and growth factors, which are fundamental in new bone formation.[32]

The present study demonstrated in nonhuman primates and in humans that this specific acid-treated surface can induce a greater and faster BIC percentage when compared with a machined surface, demonstrating the importance of implant surface configuration as a key factor for early and successful osseointegration.

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