Bone Contact Around Acid-etched Implants: A Histological and Histomorphometrical Evaluation of Two Human-retrieved Implants

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The surface characteristics of dental implants play an important role in their clinical success. One of the most important surface characteristics of implants is their surface topography or roughness. Many techniques for preparing dental implant surfaces are in clinical use: turning, plasma spraying, coating, abrasive blasting, acid etching, and electropolishing. The Osseotite surface is prepared by a process of thermal dual etching with hydrochloric and sulfuric acid, which results in a clean, highly detailed surface texture devoid of entrapped foreign material and impurities. This seems to enhance fibrin attachment to the implant surface during the clotting process. The authors retrieved 2 Osseotite implants after 6 months to repair damage to the inferior alveolar nerve. Histologically, both implants appeared to be surrounded by newly formed bone. No gaps or fibrous tissues were present at the interface. The mean bone-implant contact percentage was 61.3% (±3.8%).

Introduction

Initial stability of an implant is important for successful tissue integration, and early osseointegration is important for long-term implant stability.1,2 Improved survival of dental implants in areas of poor bone quality and quantity is important, and this fact inspired a search for an improved surface that could enhance bone-implant contact percentages.3 These implant surface features have been demonstrated to be the only ones that influence the amount of bone-implant contact percentage and interface shear strength.3,4 Modifications of the implant surface features produce an increase of the retention between the implant and the bone by enlarging the contact surface, increasing the biomechanical interlocking between implant and bone, and by enhancing osteoblast activity with an earlier formation of bone at the interface.3,5

An increase of the bone-implant contact percentages with increasing roughness of the implant surface has been reported.6-10 Greater surface
roughness increases the implant surface area and increases the potential for interlocking of bone into the implant surface.\textsuperscript{11,12} However, surface roughness is not the only aspect of surface topography affecting osseointegration of dental implants; for example, the increased surface roughness does not explain the differences observed when comparing sandblasted and titanium plasma-sprayed surfaces.\textsuperscript{11} Other factors that probably have a role are ionic charge, surface energy, and surface tension.\textsuperscript{7-11}

Acid etching appears to greatly enhance the potential for osseointegration, especially in the earliest stages of peri-implant bone healing. Moreover, with this technique there is no need for external agents that could contaminate the implant surface.\textsuperscript{11} Acid treatment produces a clean, highly detailed surface texture that lacks entrapped surface material and impurities.\textsuperscript{2,11} This textured implant surface has been reported to have a positive affect on the biologic response of bone in terms of early bone apposition, a higher percentage of direct bone-to-implant contact, and strong implant anchorage.\textsuperscript{2,11} Acid etching creates an even distribution of very small (1 to 2 \( \mu \)m) peaks and valleys and large features of 6 to 10 \( \mu \)m.\textsuperscript{2,11}

Chehroudi et al\textsuperscript{13} showed that a modification of the implant surface topography influenced the frequency and the amount of bone deposited adjacent to implants, and the areas of mineralization were guided by the surface topography. Surface roughness alters the responsiveness of osteoblasts to systemic hormones.\textsuperscript{14} Only a few studies of in situ osseointegrated implants without complications and with an intact bone-implant interface have been reported in the literature.\textsuperscript{15-18} The analysis of human specimens is extremely useful to validate the experimental results obtained from animals. The aim of our study was a histologic and histomorphometric report of 2 implants with an acid-etched surface that were removed after 6 months because of damage to the inferior alveolar nerve in order to evaluate the healing events in the peri-implant tissues around this type of surface.

**CASE REPORT**

In another clinic, a 56-year-old female patient underwent the insertion of 2 threaded, acid-etched, titanium, screw-shaped implants (Osseotite, 3i, Implant Innovations, West Palm Beach, Fla) in the left mandible (Figure 1). Two months after the surgical procedure, the patient started to experience a dull pain in the left mandible; this pain increased in the following months, and 6 months after the surgical procedure the patient was referred to one of us.
The patient complained of increasing pain and paresthesia; it was then decided to remove both implants. A CT scan showed that the implants were in close contact with the inferior alveolar nerve (Figure 2). The implants were retrieved with a 5 mm trephine (Figure 3).

The specimens were immediately stored in 10% buffered formalin and processed to obtain thin ground sections with the Precise 1 Automated System (Assing, Rome, Italy). The specimens were dehydrated in an ascending series of alcohol rinses and embedded in a glycolmethacrylate resin (Technovit 7200 VLC, Kulzer, Wehrheim, Germany). After polymerization the specimens were sectioned longitudinally along the major axis of the implant with a high-precision diamond disc at about 150 μm and ground down to about 30 μm.

Three slides were obtained for each implant and stained with acid fuchsin and toluidine blue. A double staining with von Kossa and acid fuchsin was also done to evaluate the degree of bone mineralization, and one slide per implant, after polishing, was im-

Figure 4. At low-power magnification, newly formed bone can be observed at the implant-bone interface (Toluidine blue and acid fuchsin, original magnification ×12). Figure 5. Newly formed bone is present at the bone-implant interface. Figure 6. No gaps are present at the interface (Toluidine blue and acid fuchsin, original magnification ×200). Figure 7. Newly formed bone and marrow spaces are present at the interface with the implant (Toluidine blue and acid fuchsin, original magnification ×100).
mersed in AgNO₃ for 30 minutes and exposed to sunlight; the slides were then washed under tap water, dried and immersed in basic fuchsin for 5 minutes, and then washed and mounted.

The percentage of bone contact was calculated using a light microscope (Laborlux S, Leitz, Wetzlar, Germany) connected to a high-resolution video camera (3CCD, JVC KY-F55B, JVC Professional Products, Milan, Italy) and interfaced to a monitor and PC (Intel Pentium III 1200 MMX, Intel Ireland Ltd, Kildare, Ireland). This optical system was associated with a digitizing pad (Matrix Vision GmbH, Oppenweiler, Germany) and a histometry software package with image-capturing capabilities (Image-Pro Plus 4.5, Media Cybernetics Inc, Immagini and Computer Snc, Milan, Italy).

**RESULTS**

Radiographically and clinically, both implants appeared to be osseointegrated. At low-power magnification both implants appeared to be surrounded by newly formed bone (Figures 4 and 5). At higher magnification, the bone was compact and mature with small marrow spaces (Figure 6). Remodeling areas were present. In some portions of the interface the bone appeared in close contact with the implant surface, whereas in other areas narrow spaces were present (Figure 7). The bone-implant contact percentage for both implants was a mean 61.3% (±3.8%). No inflammatory or multinucleated cells were present. No foreign body reaction was found at the bone-implant interface. The newly formed bone showed many viable osteocytes. No epithelial downgrowth was observed at the interface.

**DISCUSSION**

Implant surface modifications are important because direct bone-implant contact is created between the molecules of the bone tissue and the titanium dioxide layer at the implant surface. The geometric properties of the surface produce mechanical restrictions on the cytoskeletal cell components, which are involved in the spreading and locomotion of the cells. Cellular adhesion is related to surface-free energy of the substratum, and surfaces with a low surface-free energy are reported to be less adhesive. Surface roughness seems to have an effect on the osteoblast differentiation and the formation of a differentiated matrix. Surface roughness may be one of the most important factors determining long-term implant success, especially in low bone quality and quantity areas.

The proliferation and differentiation of cells has been reported to be enhanced by surface roughness, and Mustafa et al demonstrated that DNA synthesis appeared to be dependent on surface roughness. Osteoblast matrix formation and mineralization, in a multilayering culture system, is modified by surface topography. The Osseotite surface is created by a process of thermal dual acid etching with hydrochloric and sulfuric acids; this treatment produces a clean surface with no embedded materials or impurities on the surface.

It has been reported that acid etching enhanced early endosseous integration to a level similar to that observed around the more complex titanium plasma-sprayed surface. The acid treatment seems to have an additional stimulation influence on bone apposition. This acid attack helps in producing a more stable adhesion of the blood clot, and this fact could help obtain intimate and earlier bone contact. Osteoblast-like cells respond to increasing surface roughness with a decreased proliferation and an increased differentiation of the osteoblasts: alkaline phosphatase, osteocalcin, Transforming Growth Factor beta, and PGE₂ production is increased. Abrahamsson et al found that the proportion of mineralized bone between threads and outside the threads was nearly identical in turned and Osseotite implants; this fact could indicate that the surface characteristics of an implant may influence tissue reactions only in a narrow area near the implant surface. Klokkevold et al compared the resistance to removal torque forces in the femur of rabbits. They used two surfaces: acid-etched (Osseotite) and machined. It was found that after a 2-month healing period the force necessary to remove the acid-etched implants was 4 times greater than that needed in the machined implants. The osteoconductive nature of the microtextured Osseotite surface may increase the rate at which new bone forms on the implant. The higher and earlier bone contact reported for thermal dual-etched surfaces has been attributed to the fixation of fibrin to the surface, to an enhancing of bone growth through enhancing levels of bone growth factors, and to an increased activation of platelets that leads up to an up-regulation of osteogenic responses.

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