Surface Analysis of Machined Versus Sandblasted and Acid-Etched Titanium Implants

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Initially, implant surface analyses were performed on 10 machined implants and on 10 sandblasted and acid-etched implants. Subsequently, sandblasted and acid-etched implant cytotoxicity (using L929 mouse fibroblasts), morphologic differences between cells (osteoblast-like cells MG63) adhering to the machined implant surfaces, and cell anchorage to sandblasted and acid-etched implant surfaces were evaluated. Results indicated that acid etching with 1% hydrofluoric acid/30% nitric acid after sandblasting eliminated residual alumina particles. The average roughness (R_a) of sandblasted and acid-etched surfaces was about 2.15 μm. Cytotoxicity tests showed that sandblasted and acid-etched implants had non-cytotoxic cellular effects and appeared to be biocompatible. Scanning electron microscopic examination showed that the surface roughness produced by sandblasting and acid etching could affect cell adhesion mechanisms. Osteoblast-like cells adhering to the machined implants presented a very flat configuration, while the same cells adhering to the sandblasted and acid-etched surfaces showed an irregular morphology and many pseudopodi. These morphologic irregularities could improve initial cell anchorage, providing better osseointegration for sandblasted and acid-etched implants. (INT J ORAL MAXILLOFAC IMPLANTS 2000;15:779–784)

Key words: *acid etching, cellular morphology, dental implants, immunologic cytotoxicity tests, surface properties*

Surface morphology and bone-implant interactions determine the predictability of endosseous dental implant/bone integration.[1,2] The influence of different surface characteristics on long-term implant survival has been demonstrated by several studies.[3–6] Morphometric analyses have shown differences in bone-implant contact percentages with the varying of surface characteristics, as well as a sensitivity of cells to surface microtopography.[4] Surface roughness has been shown to affect osteoblast proliferation and differentiation.[2,4,7] Alkaline phosphatase–specific activity was enhanced by surface roughness,[4,7–9] and cells grown on rougher surfaces produced a higher quantity of alkaline phosphatase activity than cells grown on smoother surfaces. Osteocalcin production, latent transforming growth factor β, and prostaglandin E_2 production—all of which are involved in bone formation—also increased with increasing surface roughness.[10]

Surface blasting and acid etching can increase the rate and amount of bone formation on the implant surface.[11] Moreover, one of the more effective bone-implant interfaces has been achieved with sandblasted and acid-attacked surfaces,[6] and no negative effects on cell adhesion have been shown.[3,10] The sandblasting procedure may be performed using either medium-grit[3,7,12] or large-grit[7,13] aluminum oxide (Al_2O_3) particles. Literature reports have shown that the acid-etching process can employ either a hydrochloric acid/sulfuric acid mixture (HCl/H_2SO_4)[1,7,13] or pickling in 2% hydrofluoric acid/10% nitric acid (HF/HNO_3).[6,10] In addition to increasing surface roughness, surface blasting and acid etching could remove surface contaminants and increase the surface reactivity of the metal. A significantly higher removal torque has been demonstrated for sandblasted and acid-etched implants.[13] Less loss of bone height at the preload evaluation, as well as after a loading period, has been demonstrated for sandblasted and acid-etched implants.[1]

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The purpose of the present study was to conduct an in vitro qualitative and quantitative evaluation of sandblasted and acid-etched implants using scanning electron microscopy (SEM) and electron spectroscopy for chemical analysis (ESCA) and to analyze cellular responsiveness to sandblasted and acid-etched surfaces.

MATERIALS AND METHODS

Implant Surface Analyses

Threaded machined and sandblasted/acid-etched commercially pure grade 4 titanium implants (Bone System, Milan, Italy) were used in this study. Ten sandblasted and acid-etched implants (test implants) and 10 machined implants (control implants) were analyzed.

The surface of the test implants was treated with a medium-grit corundum ruby sandblasting material (250 to 500 μm) and 1% HF/30% HNO_3. Chemical and physical characterizations were performed on the sandblasted titanium implants prior to and after the acid etching procedure, as well as on the machined implants. Qualitative surface analysis of the sandblasted implants before the specific etching process and after acid etching, and also of the machined implants, was conducted using a Cambridge 360 SEM (Cambridge Instruments, Cambridge, United Kingdom). The quantitative surface analysis of the same samples was obtained using ESCA, which could examine an implant surface area of 400 μm in diameter and 5 nm in depth, using the Perkin Elmer PHI 5500 ESCA System (Perkin Elmer, Norwalk, CT). Roughness measurements of the control and test surfaces were made with a Mitutoyo Surftest 211 Profilometer (Mitutoyo Instruments, Tokyo, Japan). Three readings were made for each surface and the results were averaged.

Cell Cultures and Cytotoxicity Tests

Three experimental studies were conducted.

Study I. Five test implants were used. L929 mouse fibroblasts (ECACC, NCTC clone 929) were tested by direct contact of extraction liquid of the test implant material. The extraction liquid resulted from the incubation at 37°C for 120 hours of the material in the extraction "vehicle" (minimum essential medium [MEM], without glutamine, phenol red) under specific conditions. A blank extraction was done using medium MEM under the same conditions, except for the absence of the material.

The positive control consisted of the culture medium at a concentration of 6.4 mg/mL. The negative control was culture medium with glutamine and serum that had not undergone any extraction process. Microscopic observation of the culture cells with the extraction liquid applied was carried out after 24 hours of incubation. The effect of the extract on cellular viability was analyzed using neutral red and the mitochondrial (MTT) stain test. The concentrations assayed of the extraction liquid of the blank and of the test substance were 100%, 50%, 10%, and 1%.

Concentrations of the blank extraction liquid and the extraction liquid of the test material were then evaluated. The cytotoxicity of the test material was evaluated by microscopic examination. A quantitative analysis was performed using a test based on the ability of viable cells to incorporate supravital dye, lysosomal-matrix binding of neutral red, and mitochondrial reduction of MTT. An alteration in cell surface or sensitive lysosomal membrane resulting from the cytotoxicity of the material provokes a decrease in staining with neutral red.

The results were evaluated as follows. The mean standard deviation (SD) and the standard error of the mean (SEM) of the readings for each concentration for each test were obtained. The result was expressed as a percentage of cytotoxicity for each concentration with respect to the blank group, according to the following formula:

$$\text{Percent cytotoxicity} = \frac{\text{Blank} - \text{extract (concentration)}}{\text{Blank}} \times 100$$

Study II. Five test and 5 control implants were used. The cells used were L929 mouse fibroblasts (ECACC, NCTC clone 929). A culture of 1×10^5 fibroblasts in modified Eagle's medium (Biochrom HG, Berlin, Germany) containing 10% fetal bovine serum (FBS), with additives glutamine, 1% penicillin, and streptomycin, was seeded in 6 multi-well plates (Corning Life Sciences, Acton, MA). Cells were incubated at 37°C with 5% carbon dioxide and a 98% humidified atmosphere during 72 hours in contact with samples of sterile pure titanium implants. Sterile machined pure titanium implants, 3.5 mm in diameter and 17 mm in length, which had been decontaminated using a washing process and with plasma in contact with the cell culture, were used as the control. The test implants were sterile, blasted and acid-etched, pure titanium implants, 3.5 mm × 17 mm (Bone System), in contact with the cell culture.

The negative control was a gold cylinder; the positive control was a copper-nickel-aluminum (Cu-Ni-Al) cylinder. Initially, microscopic examination of the culture cells with the samples after 72 hours

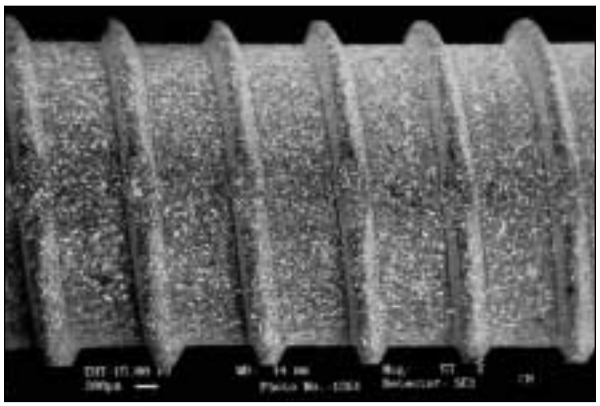

Fig 1 Sandblasted implant. Aluminum particles can be seen (*white dots*) (original magnification ×57).

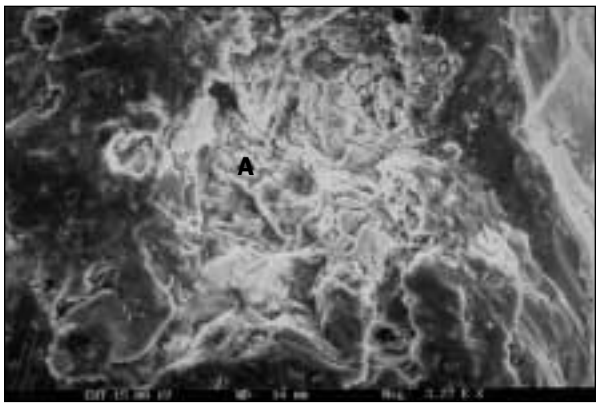

Fig 2 At high-power magnification (×5,000) an aluminum particle (A) is present on the sandblasted implant.

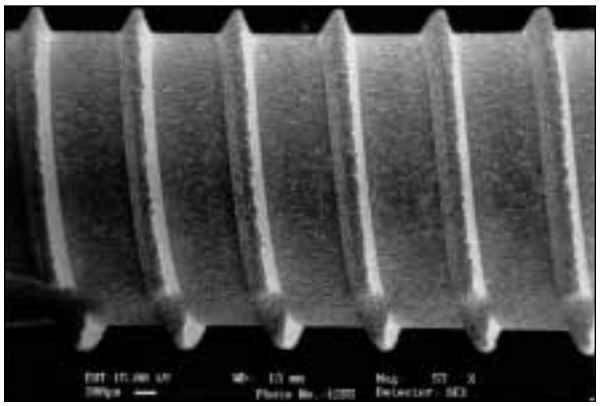

Fig 3 Sandblasted and acid-etched implant. No residual aluminum particles can be seen (original magnification ×57).

Fig 4 Sandblasted and acid-etched implant at high-power magnification (×5,000). Many surface irregularities are present.

of incubation was performed, and cytotoxicity was evaluated. Evaluation by SEM of the implant-cell interface for control and test implants was conducted. Mitochondrial analysis for evaluation of succinate dehydrogenase (SDH) enzyme activity was performed with a spectrophotometer at a 560-nm wavelength. Samples for the adhesion test were washed in phosphate-buffered saline (PBS), fixed with glutaraldehyde 4% in PBS, then dehydrated with ethanol and hexamethyldisilazane.

Study III. Five test and 5 control implants were used. MG63 osteoblast-like cells (American Type Culture Collection, Rockville, MD), originally isolated from a human osteosarcoma, were used for this experiment. A culture of 3×10^5 osteoblast-like cells in modified Eagle's medium (Biochrom HG) containing 10% FBS, with additives glutamine, 1% penicillin, and streptomycin, was seeded in 6 multiwell plates (Corning Life Sciences). Cells were incubated at 37°C with 5% carbon dioxide and a 98% humidified atmosphere for 24 hours. An adhesion test was performed in cell cultures in contact with samples of the control implants and in cell cultures in contact with samples of the test implants. Observations were carried out under an inverted microscope. Cell morphology and characteristics during the adhesion mechanism were examined under SEM.

RESULTS

Implant Surface Analyses

Under SEM, characteristic machining grooves produced during manufacturing could be observed on the test implants. In contrast, sandblasted implants presented many alumina particles, which were not eliminated after commonly used ultrasonic cleaning processes (Fig 1). At high-power magnification, individual alumina particles were clearly visible (Fig 2). A cleaned and alumina-free implant surface was obtained after a specific etching process that used a solution containing 1% HF and 30% HNO_3 (Fig 3). High-power magnification of test implants revealed irregularities on the acid-etched surface (Fig 4).

Electron spectroscopy for chemical analysis was used to examine the outer part of the titanium surface of the implants. All the examined implants (test and control) presented different percentages of titanium and oxygen (TiO_2). Other elements, such as chlorine, calcium, silicon, phosphorus, and nitrogen, were present in small percentages. Sandblasted implants that had not undergone the acid etching procedure displayed residual alumina particles, while the surface of sandblasted and acid-etched implants appeared to be free of the particles (Fig 5). Surface roughness measurements (R_a) were 0.75 μm for the control implants and 2.15 μm for the test implants.

Cell Cultures and Cytotoxicity Tests

No differences in the morphology or cellular density of cells tested with the negative control, blank extraction liquid, and the test implant extraction liquid at the 4 concentrations assayed were detected on observation under an optical phase microscope. Semi-confluent cultures of fibroblasts, with intact membranes and attached, bipolar or multipolar-shaped, and spherical cells in the process of dividing, were always present. On the other hand, the positive control group presented spherical cells with altered membranes and zones of detachment of the cellular monolayer, along with fibroblasts that showed signs of intracytoplasmatic vesiculation.

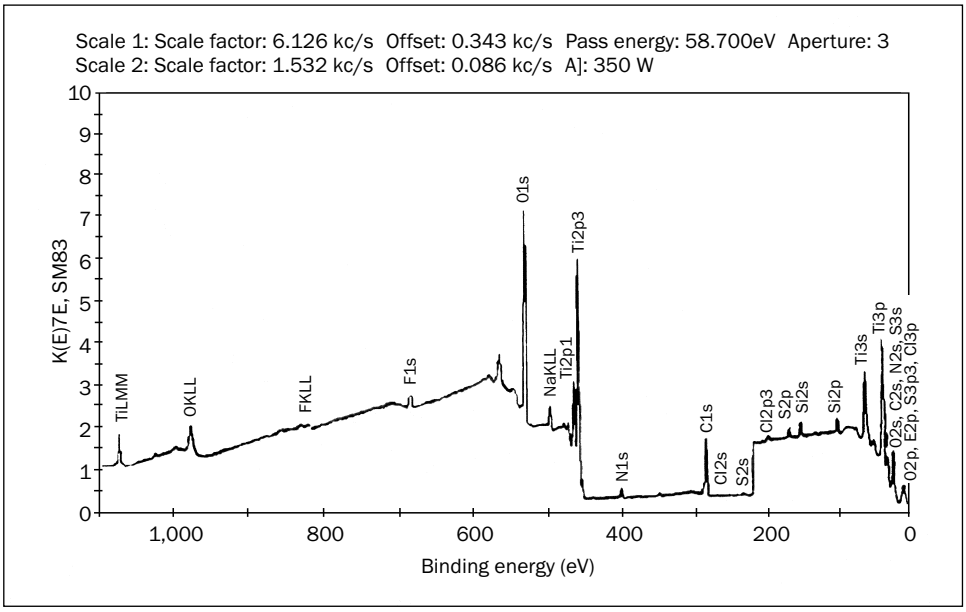

Fig 5 Electron spectroscopy for chemical analysis of sandblasted and acid-etched implant. No aluminum is present on the implant surface.

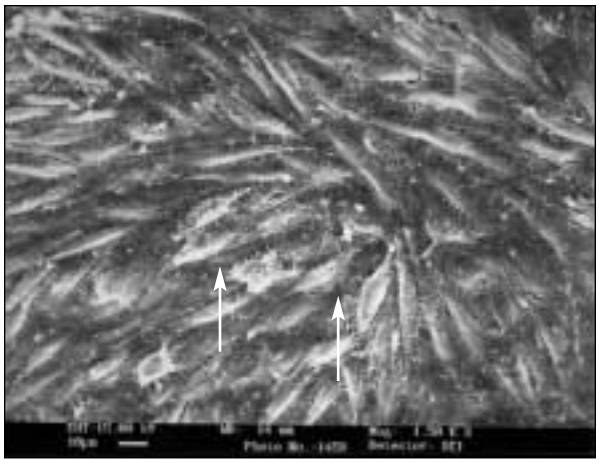

Fig 6a Normal fibroblast (*arrows*) adhesion to a machined implant (original magnification ×1,500).

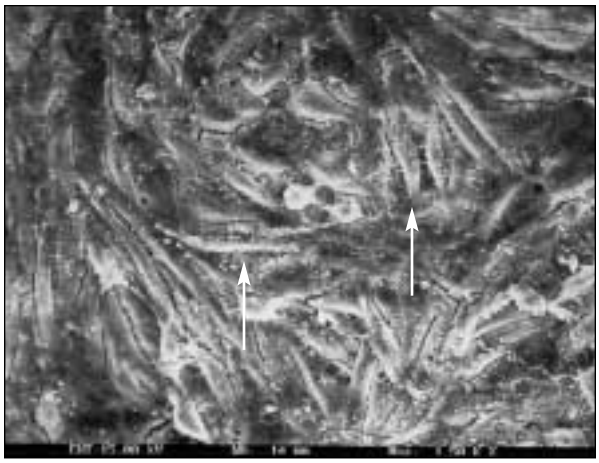

Fig 6b Normal fibroblast (*arrows*) adhesion to a sandblasted and acid-etched implant (original magnification ×1,500).

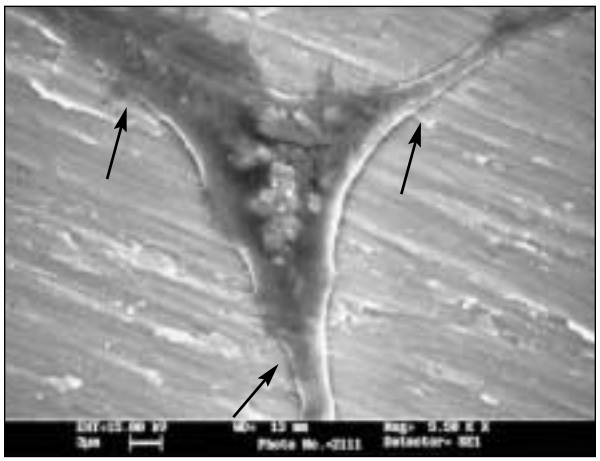

Fig 7 Flat morphology of the osteoblast-like cells (*arrows*) adhering to machined implants (original magnification ×5,500).

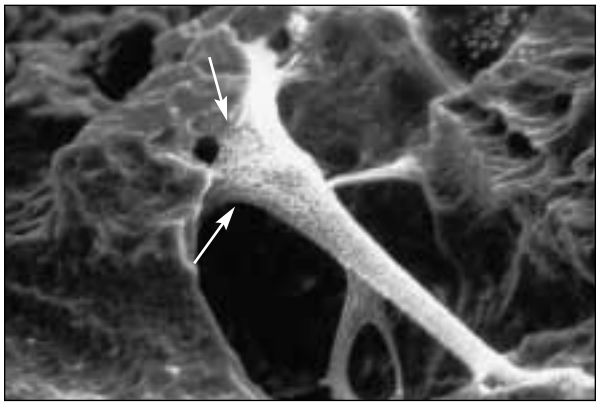

Fig 8 Adhesion of the osteoblast-like cells to sandblasted and acid-etched surfaces. Many pseudopodi (*arrows*) are present (magnification ×6,500).

Non-cytotoxic cellular effects were observed in the control and test implants. Neither dead cells nor multinucleated giant cells were seen. The positive control revealed a large area of dead cells surrounding the Cu-Ni-Al cylinder. The mean of the values for the SDH enzyme activity of the negative control was 0.340, the mean for the test specimens was 0.344, and the mean enzyme activity for the positive control was 0.101. The cellular adhesion test revealed a basically normal adhesion mechanism of the fibroblasts, which seemed to enable rapid adherence to the control (Fig 6a) and test (Fig 6b) implant surfaces by means of several long pseudopodi.

Neither cell atypia nor cell damage and cytotoxic effects were observed around either control or test implants. Scanning electron microscopic examination revealed a morphologic difference between osteoblast-like cells adhering to the control surface and cells adhering to the test surface. After 24 hours of incubation, osteoblast-like MG63 cells adhering to the control surface showed a very flat morphology, with few small cytoskeletal processes or pseudopodi (Fig 7). Cells adhering to the test surface showed irregular cellular morphology and many pseudopodi that assisted osteoblast attachment to the surface (Fig 8).

DISCUSSION

Scanning electron microscopic observations and ESCA revealed that decontamination of pure titanium sandblasted implants apparently could be obtained with acid etching. The sandblasting procedure produced macrorough "valleys," onto which the acid etching process superimposed microrough "micropits."[13] The roughness measurement results in test implants showed a R_a of approximately 2.15 μm.

The results suggest no cytotoxicity of the test titanium surfaces. The test implant extraction liquid gave a non-cytotoxic cellular response at the 4 concentrations evaluated in L929 mouse fibroblasts during a 24-hour period. Light microscopic and SEM evaluation and MTT testing revealed biocompatibility of both the control and the test implants; in fact, a sharp decrease in SDH enzyme activity (0.101) was present in the positive control, while the test specimen values were closer to those seen for the negative control (0.344 vs 0.340). This enzyme is extremely sensitive to the presence of toxic effects. Sandblasting and acid etching apparently produced alumina-free implant surfaces that had no negative effects on fibroblast adhesion. However, no interferences in the cell adhesion mechanism were observed for surfaces undergoing only the sandblasting procedure, most probably because only a very limited and transient release of aluminum ions was present.[3]

Geometric surface properties seem to affect the components of the cell cytoskeleton that are involved in cell spreading and locomotion.[14] Surface roughness can also enhance osteoblast-like cell adhesion and seems to have an effect on the configuration and conformation of cellular pseudopodi, which are important in cell adhesion. In addition, cells on rougher surfaces have been shown to release higher levels of factors involved in the regulation of bone formation.[10] Cochran and coworkers[1,15] found significantly less coronal bone loss in arches in which sandblasted and acid-etched implants had been placed, and this may be the result of the higher osteoconductive properties of the sandblasted and acid-etched surface. Bowers and coworkers[2] found

that the largest quantity of attached cells was found on rough, irregular, sandblasted surfaces.

Optimum surface microroughness, along with a better understanding of the relationship between the cytoskeletal arrangement of the cells and the surface micromorphology,[2] can have a significant impact on the anchorage of dental implants in bone. The fact that some cells can orient in the grooves of micromachined surfaces supports the concept that cells are sensitive to microtopography.[7] Bowers and coworkers[2] concluded that sandblasted implants provided a unique environment and opportunity for initial cell attachment. Morphometric analysis showed a relationship between the increase in bone-implant contact and surface roughness.[5] Wennerberg and colleagues[3,16–19] observed no untoward effects resulting from the aluminum ions found on the implant surface following the sandblasting procedure. In the sandblasting and acid etching procedure used in the present study, all residual alumina particles were eliminated by the specific etching process that was used.

CONCLUSION

It was found that sandblasting and acid etching are safe and predictable procedures that can increase implant roughness and can improve cellular adhesion and proliferation. More research in vitro and in vivo is certainly needed in the search for a surface that can offer the best anchorage for dental implants.

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