Bioactivity and Surface Characteristics of Titanium Implants Following Various Surface Treatments: An In Vitro Study

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This study compared the surface topography, hydrophilicity, and bioactivity of titanium implants after 3 different surface treatments (sandblasting and acid etching, modified sandblasting and acid etching, and thermal oxidation) with those of machined implants. One hundred indigenously manufactured threaded titanium implants were subjected to 3 methods of surface treatment. The surface roughness of the nontreated (Group A) and treated samples (Groups B through D) was evaluated with a scanning electron microscope (SEM) and profilometer. The wettability was visually examined using a colored dye solution. The calcium ions attached to the implant surface after immersing in simulated body fluid (SBF) were assessed on days 1, 2, and 7 with an atomic electron spectroscope. The data were analyzed statistically. The SBF test allowed the precipitation of a calcium phosphate layer on all surface-treated samples, as evidenced in the SEM analysis. A significantly higher amount of calcium ions and increased wettability were achieved in the thermally oxidized samples. The mean roughness was significantly lower in Group A (0.85 $\pm$ 0.07) compared to Group B (1.35 $\pm$ 0.17), Group C (1.40 $\pm$ 0.14), and Group D (1.36 $\pm$ 0.18). The observations from this in vitro study indicated that surface treatment of titanium improved the bioactivity. Moreover, results identified the implants that were sandblasted, acid etched, and then oxidized attracted more calcium ions.

Key Words: oral implants, titanium, surface treatment, bioactivity, osseointegration

INTRODUCTION

Dental implants have become a significant mode of tooth replacement and have revolutionized oral rehabilitation for managing partially and fully edentulous patients.[1–3] Osseointegration is crucial for determining the clinical success of dental implants. The implant-bone interface plays an important role in prolonging the longevity and effective function of the implant-supported prosthesis.[4] The rate and quality of osseointegration of titanium implants depend on several factors—including surface characteristics such as surface morphology, surface chemistry, and the surface energy—that significantly affect the initial bone cells' response to the implant at the bone-implant interface.[5–7]

Surface modification of titanium implants have been used to improve the physical, chemical, and morphological properties with the goal of enhancing clinical performance.[8] Techniques such as turned, blasted, acid-etched, porous-sintered, oxidized, plasma-sprayed, and hydroxyapatite-coated surfaces (as well as combinations of these procedures) are currently used.[9,10] A moderate roughness of 1–2 μm has been advocated for successful implant bio-integration, thereby reducing the risk of peri-implantitis and ionic exchange.[11] The concept of surface modification may be additive (such as coating layers of hydroxyapatite or tricalcium phosphate onto the implant surface[12] by plasma-spraying) or ablative in nature (whereby substrate materials are removed from the surface of the implant to create roughness).[13,14] The chemical composition or surface charge on the titanium implants aid in cell attachment, a property that varies depending on the bulk composition and surface treatments during the manufacturing process.[15] In view of their interactions with biological cells and

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tissues, hydrophilic surfaces seem more desirable than hydrophobic surfaces.[16]

Two commonly used surface modification methods are abrasive-blasting and acid-etching. Surface blasting is postulated to increase the reactivity of the implant surface and remove contaminants, while acid-etching results in a homogenous surface, removes impurities, and avoids possible contamination by materials used in blasting. Both methods are convenient for surface treatment and have widespread commercial implications. The advantage of the ablative technique over the additive technique is the lack of adverse effects caused by additive materials, which may be a product of dissolution into the systemic circulation due to fatigue failure of the implants.[16] Among the ablative methods, sandblasting and acid-etching (SLA) methods have been proven to be superior to machining or turning methods.[17–19] Studies have shown enhanced bone deposition with chemically modified SLA titanium surfaces when compared to standard SLA surfaces.[20]

Oxidation procedures, such as anodic oxidation or anodization and thermal oxidation, are surface treatments that produce modifications in the microstructure and the crystallinity of the titanium oxide layer.[21] Studies have compared oxidation procedures with machining procedures,[22] dual acid-etching procedures,[23] and sandblasting procedures.[24] However, no studies have compared SLA, modified SLA, and oxidation procedures in a single experimental study. It has been reported that formation of a calcium phosphate layer or bone-like apatite on the material's surface is vital for in vivo bone growth. This in vivo bone bonding bioactivity can be predicted with in vitro tests, such as immersion of synthetic materials into simulated body fluid (SBF).[25]

Hence, the aim of the present investigation was to compare the surface topography and hydrophilicity of titanium implants after different treatment procedures, such as SLA, modified SLA, and SLA with thermal oxidation. These surface-treated implants were also compared to machined implants, and the bioactivity of each type of surface was assessed.

Materials and Methods

Implants

A total of 100 indigenously manufactured threaded implants of dimensions 4.3 × 12 mm and made from titanium alloy were used in this study. The diameters of the implants at the apex and platform were 3.3 and 4.7 mm, respectively. The remnants of coolant used for the milling process of the titanium implants were removed by repeated washing with detergent (Procter and Gamble Pvt Ltd, Mumbai, Maharashtra, India) and deionized water (Aqua Gold, Mano Agencies, Chennai, India) for 3 minutes. The samples were then subjected to ultrasonic cleaning with Milli Q water (Millipore India Ltd, Bengaluru, India) for 6 minutes, and renewing the water after the first 3 minutes. Milli Q water consists of double-distilled water filtered with a 0.2 μm-sized filter. This process was used to remove the cleansing detergent in the previously mentioned step as well as any residues from the milling process. The removal of moisture was performed by placing the samples in a burnout furnace (Techino, Kolkata, India) maintained at 100°C for 1 minute. The implants were handled using tweezer tips above the implant platform, taking care not to damage or contaminate the intraosseous fixture.

Surface treatments

The implants were divided into four groups of 25 implants, each according to the following methods of surface treatment:

Group A (25 implants). Machined control group with no surface treatment.

Group B (25 implants). Sandblasted and acid-etched group. The samples were attached to the fixture mount of the sandblasting motor (Sandy, MS Surgicals, Chennai, India) and sandblasted with 110 μm alumina (Delta, Vijai Dental Depot, Chennai, India) for 4 minutes at a pressure of 4 kg/m^2 and a speed of 20 rpm (1 revolution per 3 s). Before acid etching, the samples were ultrasonically cleaned twice for 3 minutes to remove any residual alumina on the surface. For acid etching, each sample was inverted with the abutment facing downward, and carefully dipped in molten modeling wax (Hindustan Dental Products, Hyderabad, India) to demarcate between the rough and smooth parts and protect the polished surfaces from subsequent acid attack. An orthodontic wire holder was then incorporated into the wax. The samples were subjected to sequential etching with mineral acids, namely, hydrofluoric acid (HF, 15% for 1 min), sulfuric acid (H_2SO_4, 96% for 3 min), and hydrochloric acid (HCl, 37% for 3 min), at room temperature. Neutralizing agents were deionized water, used for 2 minutes, and 20% sodium bicarbonate ($NaHCO_3$) used for 30 seconds, following each acid treatment, after which the samples were placed in boiling Milli Q water until all wax residue was eliminated. They were then dried in a 100°C furnace set for 1 minute.

Group C (25 implants). Modified sandblasted and acid-etched group. The samples were subjected to the same procedures described for Group B. The sandblasted and acid-etched samples were then flushed with nitrogen gas (N_2, NIIST, Trivandrum, India) at a pressure of 4 bar for 1 minute and then stored in isotonic saline solution (Marck Bioscience, Amdavad, Gujarat, India). Before evaluating the surface, the implants were treated again and dried with nitrogen gas.

Group D (25 implants). Sandblasted, acid-etched, and thermally oxidized group. The sandblasted and acid-etched samples were transferred into a furnace maintained at 450°C for a period of 1 hour and subsequently immersed in a glass tumbler containing 40 mL of 4% sodium fluoride solution (NaF) for 40 minutes to stabilize the film obtained through thermal oxidation.

Sterilization procedure

Normal sterilization protocol was performed on all samples to simulate clinical procedures. The samples were transferred into glass vials (Techino, Kolkata, India) that had been previously cleaned ultrasonically. These glass vials were closed with caps with minute perforations to effect sterilization by steam entering the vials. The vials were then autoclaved at 121°C for 20 minutes under 1.1 kgf/cm^{-2} of pressure. This procedure was repeated to ensure total sterilization.

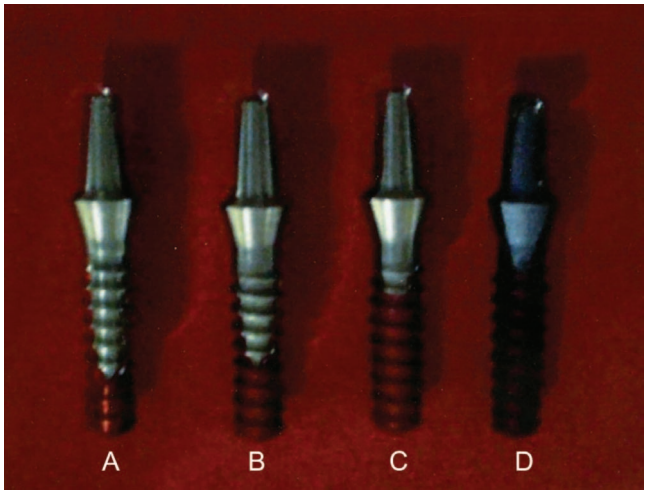

Figure 1. The method of assessment of the wettability of the implant surfaces. Note the difference in the area covered by the colored dye solution: (a) Machined control group. (b) Sandblasted and acid-etched group. (c) Modified sandblasted and acid-etched group. (d) Sandblasted, acid-etched, and thermally oxidized group.

Surface wettability evaluation using dye immersion method

The implants (1 from each group) were immersed in a petri dish (9 cm diameter, Schott, Czech Republic) containing 20 mL of Milli Q water and 1 mL safranin solution (SD Fine Chemicals, Mumbai, India) for 15 minutes (Figure 1). The amount of area (number of threads) covered by the bright red-colored dye was visually examined.

SBF test

The biological effect of the surface treatments, which involves the attachment of calcium (Ca) ions to the titanium surfaces, was studied using the SBF test. The SBF was prepared with the following analytical reagents in line with a previous study:[15] NaCl (1.5881 g), $NaHCO_3$ (0.0709 g), $NaHPO_4{\cdot}7H_2O$ (0.0492 g), $MgCl_2{\cdot}6H_2O$ (0.0617 g), KCl (0.0746 g), $CaSO_4{\cdot}2H_2O$ (0.0171 g), and $CaCl_2$ (0.0403 g). Six randomly selected samples per group (total of 72 implants) were immersed into 30 mL of SBF in a polypropylene tube (Cole-Parmer, Vernon Hills, Ill) at 37°C for 1, 2, and 7 days, respectively.

With the aid of tweezers, the implants were transferred into an Eppendorf tube (Eppendorf, Westbury, NY) containing 1 mL of 1% nitric acid solution. The calcium deposited on the samples dissolved in the nitric acid to form calcium nitrate after 24 hours, and the resultant solution was filled to 2 mL by adding Milli Q water. The quantitative determination of Ca content was performed by atomic electron microscopy.

Surface evaluation

Surface Morphology Evaluation Using Scanning Electron Microscopy (SEM)

One sample each from each group (4 implants) was selected for evaluation of surface morphology using SEM (JEOL, JSM-7100FT, JEOL Ltd, Peabody, Mass) at ×1000 magnification.

Surface Roughness Evaluation Using Surface Profilometer

Six samples from each group (24 implants) were subjected to quantitative evaluation using a surface profilometer and image analysis software (Talysurf CLI 1000, Taylor Hobson Inc, West Chicago, Ill). The laser gauge used for the measurement had a 10 mm vertical range with 1 μm resolution. Measurements were carried out with 500 μm linear spacing. The roughness parameters were estimated according to the international standard (ISO 11562), with 0.25 mm Gaussian filters that discriminate waviness from roughness without microroughness filtering. Three interthread regions at 3 different locations on the same sample were evaluated. The average roughness of the 3 interthread distances, considered as R_a, was taken for comparison.

Statistical analyses

The values obtained from the profilometer and the scanning electron microscope were analyzed using one-way ANOVA and the Mann-Whitney U test to determine the p value. Differences of $P \leq .05$ were considered statistically significant.

Results

Ca-SBF-nitric acid analysis

This analysis helped to determine the in vitro bioactivity of the implants with the different surface treatments. There was a steady increase in the Ca content in the solution from day 1 to day 7 for all treatments, with a maximum increasing tendency observed in samples from Group D. There was significant difference in the Ca content with time between each experimental group and Group A ($P \leq .05$), but there was no significant difference in the Ca content on days 2 and 7 for Group D. Furthermore, there was no significant difference between the treated samples from Groups B and C on days 1 and 2. Table 1 and Figure 2 show the Ca content in the solution from days 1–7 for all implant groups.

SEM observations

Minor differences in surface morphology were identified among all surface treatments at ×1000 magnification when compared to the machined implants, as shown in Figure 3. Group A showed parallel grooves with relatively smooth surfaces, and irregular and randomly distributed micropits (Figure 3a). This distinct orientation of the grooves was not evident among the surface-treated samples. The elevations and depressions were more pronounced in the SLA-treated (Figure 3b) and modified SLA-treated implants (Figure 3c). The implants from Group D showed a relatively smoother surface with irregularities distributed more evenly (Figure 3d). SEM analysis also revealed small white spots spread over the surface, suggesting the presence of Ca from the SBF test after 7 days of soaking time.

Surface roughness

A comparison of the mean roughness or R_a value among the 4 experimental groups obtained by profilometry is given in Table

TABLE 1

Comparisons of the mean differences in the Ca concentrations obtained from the different surface treatments with regard to soaking time in simulated body fluid (SBF)†

Experimental Group (n = 18 in Each Group)	Soaking Time (Days) (n = 6)	Ca Content in SBF (ppm) (Mean ± SD)	P Value
Group A (n = 18)	1	8.86 ± 0.67	0.007*
	2	10.80 ± 0.05	
	7	18.67 ± 0.58	
Group B (n = 18)	1	10.86 ± 0.45	0.007*
	2	13.23 ± 0.60	
	7	24.06 ± 0.97	
Group C (n = 18)	1	10.94 ± 0.41	0.007*
	2	13.25 ± 0.38	
	7	28.15 ± 0.49	
Group D (n = 18)	1	25.96 ± 0.55	0.010*
	2	31.32 ± 1.83	
	7	34.39 ± 1.22	

*$P \leq .05$, statistically significant.

†Group A: Machined control group. Group B: Sandblasted and acid-etched group. Group C: Modified sandblasted and acid-etched group. Group D: Sandblasted, acid-etched and thermally oxidized group.

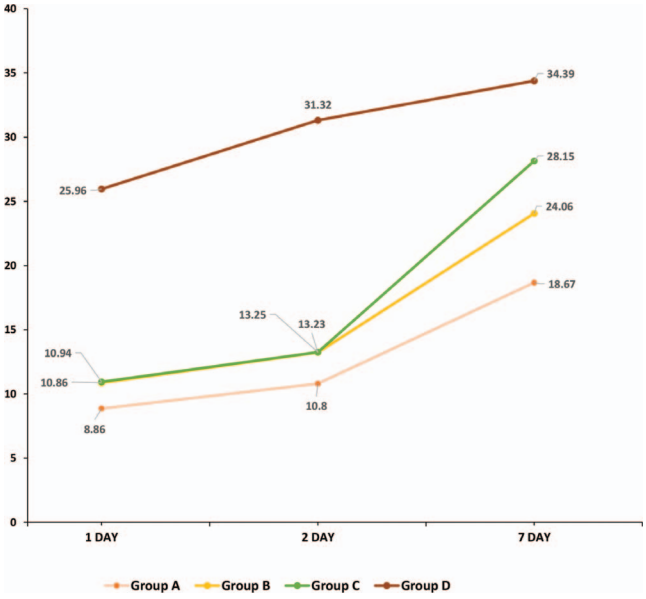

FIGURE 2. The Ca concentrations obtained from the different surface treatments after soaking time in simulated body fluid for 1, 2, and 7 days. (a) Machined control group. (b) Sandblasted and acid-etched group. (c) Modified sandblasted and acid-etched group. (d) Sandblasted, acid-etched and thermally oxidized group.

2. The mean roughness was significantly lower in the control Group A compared to the other experimental groups ($P = .004$). However, there were no significant differences in the mean roughness among the surface-treated groups.

Wettability

The wettability of the implant surfaces was determined by the amount of the area covered by the colored dye solution (Figure 1). The control group had the minimum wettability, with only 2 threads (3.3 ± 0.48) in the apical region covered by the dye solution. The Group B sample had 4 (4.1 ± 0.57) threads covered, while Group C had 8 threads (7.7 ± 0.48) covered by the dye solution. The wettability in Group D was found to be significantly higher, with all 10 threads (9.9 ± 0.32) covered and the meniscus of the liquid extending to the neck of the implant.

DISCUSSION

Surface properties—such as topography, chemical composition, and hydrophilicity—play important roles in implant-tissue interaction and osseointegration.[16] The quality of osseointegration and biomechanical fixation can be affected by the surface roughness profile of titanium implants.[26,27] Increased surface roughness is known to enhance cell adhesion, proliferation, and differentiation.[28]

The present in vitro study was undertaken to evaluate the bioactivity and surface characteristics produced by 3 different surface treatment procedures through an assessment of calcium ions and the resulting surface wettability. It was observed that the Ca deposition on the surface showed a steady increase with time in all groups, with the maximum deposition in the sandblasted, acid-etched, and thermally oxidized group. Earlier studies have shown an increase in Ca deposition and wettability in treated titanium implants.[29–31] The SEM images showed vivid bioactivity in the form of white Ca-P deposits coating all treated titanium surfaces. This is considered essential for in vivo bone formation on a synthetic material, and the use of SBF in vitro helps in predicting bone bioactivity.[25] Clinical and animal studies have proven better bone healing with surface-treated and modified implants.[32–34]

The higher wettability of the thermally oxidized samples may be attributed to the hydrophilic nature of the modified oxide layer on the titanium implants, as supported by the current DCA measurements. The oxidation procedures performed on the SLA surfaces were found to reduce the carbon concentration and consequently increase the oxygen concentration, which is related to the increased amount of hydroxylated groups bound to the surface.[20] The significance of increased wettability is its influence on the interaction between the implant and the surrounding bone.[35] By maintaining the hydroxylated oxide layer, thermally oxidized surfaces could enhance the surface reactivity to surrounding ions in the tissue fluid. In the present study, this was proven when the samples were placed in SBF; the largest amounts of Ca ions were present on the thermally oxidized samples at all time points, thereby altering the chemical composition of the oxide layer.

The profilometry results revealed a higher R_a value among the test samples, which was statistically significant. Although a comparatively lower R_a value among the test samples was obtained for the thermally oxidized group, this was not statistically significant. The reason for this low R_a value and the homogeneous surface in the SEM analysis when compared to the other test groups may be due to the isotropic surface

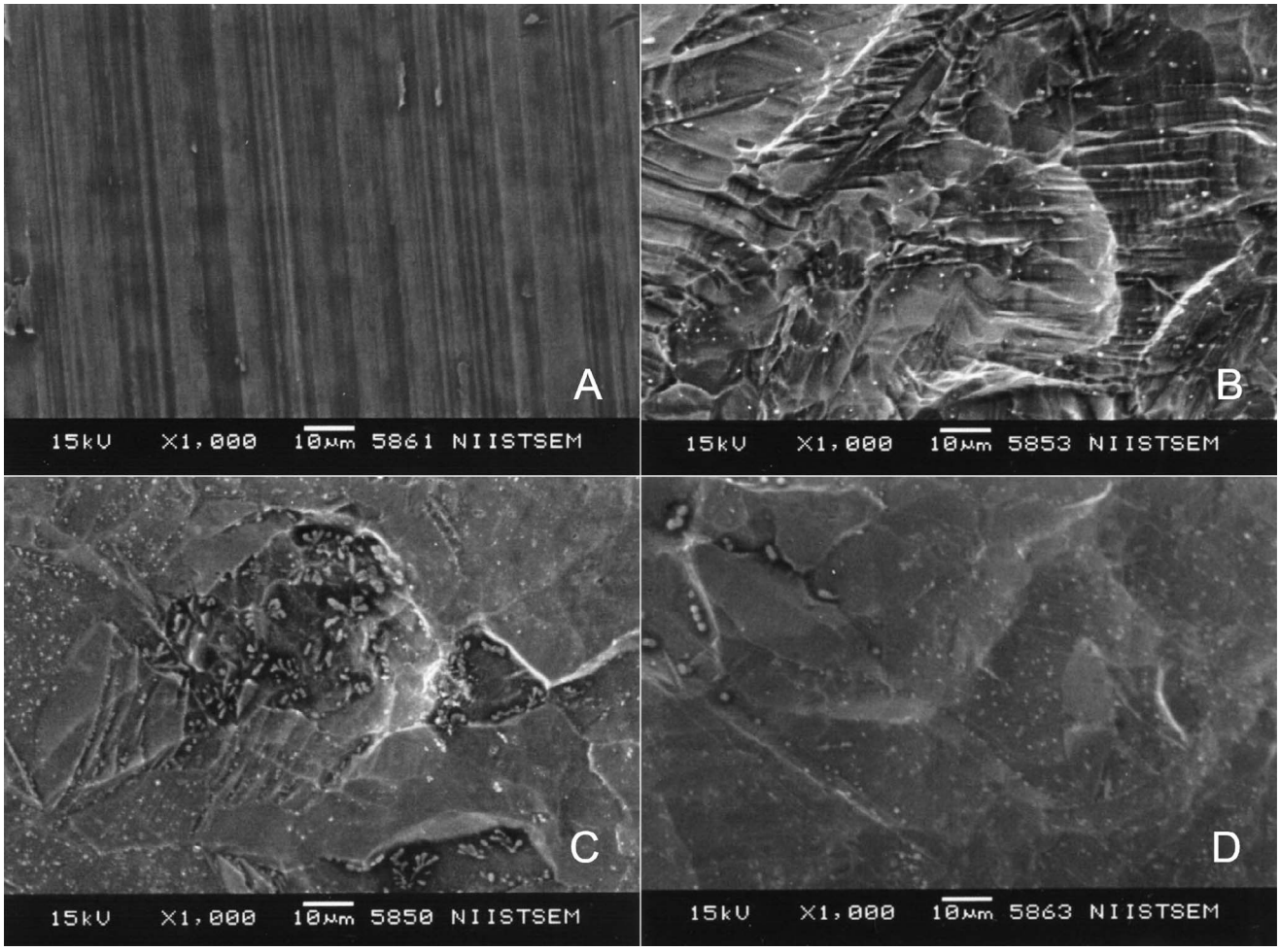

Figure 3. SEM images of the surface-treated titanium implant samples at ×1000 magnification (a) Machined control group. (b) Sandblasted and acid-etched group. (c) Modified sandblasted and acid-etched group. (d) Sandblasted, acid-etched and thermally oxidized group.

obtained during the heat treatment. This finding is in agreement with the observations of Vanzillotta et al,[15] who reported an improvement in titanium bioactivity subsequent to surface treatment.

Conclusions

Within the limitations of this in vitro study, it can be concluded that the thermally oxidized implant surface had better early bioactivity compared to machined, sandblasted and acid-etched, and modified sandblasted and acid-etched surfaces. The increased wetting of the oxidized surfaces was proven to facilitate the rapid and higher adsorption of the calcium ions, which improved the bioactivity.

Abbreviations

SBF: simulated body fluid
SEM: scanning electron microscope
SLA: sandblasting and acid-etching

Table 2

Variations in surface roughness obtained from the profilometer*

Experimental Group (n = 24)	Roughness (R_a Value in µm) Mean ± SD	*P* Value	Significant Group at 5% Level
Group A (n = 6)	0.85 ± 0.07	.004	Group A had a significantly lower value than the other three groups.
Group B (n = 6)	1.35 ± 0.17		
Group C (n = 6)	1.40 ± 0.14		
Group D (n = 6)	1.36 ± 0.18		

*Group A: Machined control group. Group B: Sandblasted and acid-etched group. Group C: Modified sandblasted and acid-etched group. Group D: Sandblasted, acid-etched and thermally oxidized group.

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