

Comparative Evaluation of Microroughness created on Titanium Alloy for use in Dental Implants subjected to Two Different Acid Etching Techniques: An *in vitro* Study

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ABSTRACT

Aim: The aim of this study was to comparatively evaluate the microroughness created on titanium alloy for use in dental implants subjected to two different acid etching techniques.

Materials and methods: Commercially available grade 5 pure titanium plates were machine prepared into 26 plates measuring $3 \times 1 \text{ cm} \times 5 \text{ mm}$ for acid etching with hydrofluoric acid (HF) and dual acid etching technique using sulfuric acid (H₂SO₄) followed by hydrochloric acid (HCI). Twenty-five plates were divided into four groups based on the duration and sequence of acid etching. Upon completion of the acid etching procedure, the titanium plates were assessed for their surface characteristics by a surface profilometer. The average roughness parameters values Ra, Rq, Rz obtained for each titanium plate.

Results: The average roughness value Ra obtained was 0.480 μ m for untreated surface and 3.65 μ m maximum for the titanium plate etched for 72 hours in H₂SO₄ and 48 hours in HCl, which is about seven times the value of surface roughness on the unetched plates. The roughness values obtained after acid etching with HF for any duration were nonsignificant compared with the unetched plates.

Conclusion: The dual acid etching technique seems to be a simple method to develop a titanium implant surface, though evaluation of the biological response to this surface is necessary.

Clinical significance: The present study showed that by optimizing the parameters of acid etching, a rough titanium surface can be obtained similar to the various implant surfaces available commercially.

Keywords: Acid etching, Dental implant, Profilometer, Surface topography.

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INTRODUCTION

Osseointegration consists of a series of bone modeling and remodeling processes. It has actually been defined as the direct structural and functional connection between living bone and the surface of a load-bearing artificial implant. The success of osseointegration depends on the quality, distribution, and amount of bone present at the site of the dental implant.¹ The nature of the implant surface has been recognized to be a critical factor for osseointegration. The most important surface properties are topography, chemistry, surface charge, and wettability.² Endosseous dental implants are available with various surface characteristics ranging from relatively smooth-machine surfaces to more roughened surfaces by coating, blasting by various methods, by acid treatments or by a combination of the treatments.³ Response of the tissues to the implant is largely controlled by the nature and texture of the surface of the implant.⁴ Some of these have the ability to enhance and direct the growth of bone and achieve osseointegration when implanted in osseous sites.

Altering the surface topography of an implant can greatly improve its stability.⁵ Based on the scale of the features, the surface roughness of implants can be divided into macro-, micro-, and nano-sized topologies.⁶ Surface irregularities of an implant can be designed by making porous and/or by coating the implant surface with other suitable materials to increase bone–implant contact, as the anatomic surface of bone cannot be controlled.⁷

Surface irregularities can be produced through ablative/subtractive procedures or additive procedures.

Ablative procedures

• Grit blasting

- Acid etching
- Shot/Laser peening
- Sputter deposition

Additive procedures

Plasma spraying

• Electrophoretic

Sol Gel coating

deposition

- Anodizing
- Pulsed laser deposition
- Biomimetic precipitation

Modifications of the implant surface features an increase in 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 quicker formation of bone at the interface. ⁸

Acid etching appears to greatly enhance the potential for osseointegration especially in the earliest stages of periimplant bone healing. Also with this technique, there is no need for any external agent that contaminates the implant surface. Acid treatment produces a clean, highly detailed surface texture and lacks entrapped surface material and impurities. This has been reported to have a positive effect on the biologic response in terms of bone apposition, a higher percentage of direct bone to implant contact, and strong implant anchorage.9 Studies demonstrated¹⁰⁻¹³ that optimal surface roughness of particles of 75 µm made surface more resistant to torque and greater bone to metal contact than small (25 µm) or coarse (250 µm) particles. Also, precise acid selection and the sequence of processing played the main role in preparation of the rough titanium surface. The surfaces were poorer if they were etched with hydrochloric acid (HCl) than with sulfuric acid (H_2SO_4) . The sequence of H_2SO_4 followed by HCl showed the best results, and as the acid-etched texture is contiguous with the porous coating, there is no possibility of debonding or dissolution, thus avoiding concerns with third body wear particles or long-term fixation.¹⁰

Aim

The aim of this study was to comparatively evaluate the microroughness created on titanium alloy for use in dental implants subjected to two different acid etching techniques.

Objectives

The objectives of this study are as follows:

- To evaluate the suitability and handling characteristics of hydrofluoric acid for obtaining a titanium surface suitable for use in dental implants.
- To evaluate the suitability of application of dual acid etching technique with H₂SO₄ and HCl at varying time intervals for obtaining a titanium surface suitable for use in dental implants.
- To identify the technique that yields minimum and maximum surface roughness as measured by a surface profilometer.
- To evaluate and compare the surface characteristics of etched and unetched titanium plates with the help of a surface profilometer.

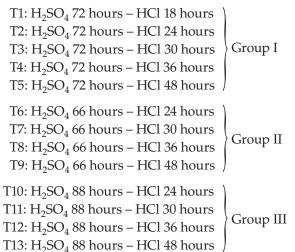
• To develop a technique of acid etching using a combination of two acids that yields a surface roughness similar to the commercially available dental implants.

MATERIALS AND METHODS

Acid Etching Procedure

Commercially available pure titanium grade 5 sheet was machine prepared to form 26 plates measuring 3×1 cm $\times 5$ mm each. One plate T₀ was left unetched; the rest of 25 plates that were to be subjected to acid etching were divided into four groups (group I – H₂SO₄ 72 hours, group II – H₂SO₄ 66 hours, group III – H₂SO₄ 88 hours, group IV – HF) based on the duration of exposure to H₂SO₄ and numbered from T1 to T13 based on the duration of exposure to HCl, which varied from 18 to 48 hours as follows:

T₀: Unetched plates



Based on the duration of exposure to HF acid (40%), the titanium plates were numbered from T14 to T25 as follows:

T14: HF 15 seconds T15: HF 30 seconds T16: HF 45 seconds T17: HF 60 seconds T18: HF 75 seconds T19: HF 90 seconds T20: HF 105 seconds T21: HF 120 seconds T22: HF 135 seconds T23: HF 150 seconds T24: HF 165 seconds T25: HF 180 seconds

A groove was prepared on one side of every plate with a straight fissure diamond bur and air-rotor to identify the side on which the roughness measurement will be made after acid etching. All procedures of acid etching were performed in a certified fume hood* available at MGM Central Research Laboratory.

*LabGuard technologies Fume Hood Maxima







Fig. 1: Acid etching of titanium plate

The plates numbered T1–T13 were kept angulated in 25 ml borosilicate glass beakers such that only top and bottom edge of the plate touched the beaker, and the acid H_2SO_4 (98%) (Fig. 1) was poured along the side of the beaker until the top edge of the titanium plate was completely immersed in the acid. These plates were then divided into three groups. Group 1 was exposed to H_2SO_4 for 72 hours, group 2 was exposed to H_2SO_4 for 66 hours, and group 3 was exposed to H_2SO_4 for 88 hours.

The beakers were kept untouched till the specified time as cited previously for each specimen. All T1–T13 plates were removed with tweezers washed in an ultrasonic bath with distilled water for 1 minute and kept in another beaker to be filled with HCl (35–38%). Plates in group 1 were subjected to HCl for 18, 24, 30, 36, and 48 hours, and plates in groups 2 and 3 were subjected to HCl for 24, 30, 36 and 48 hours.

The plates numbered T14–T25 were immersed in the previously filled teflon beaker containing HF (40%) for 15 to 180 seconds at every 15-second intervals.

Upon completion of the acid etching procedure, the titanium plates were held from the sides with a tweezer and rinsed with distilled water, dried and kept in air-tight plastic bags until further evaluation.

Topographical Evaluation of Titanium Plates

All 26 titanium plates were assessed for their surface characteristics by a Mitutoyo 178-561-02A Surftest SJ-210 surface profilometer** calibrated (Fig. 2) according to the manufacturer's instructions.

The marked surface of the plates was kept facing upwards on a flat surface parallel to the floor. The recording head of the surface profilometer was kept at a distance of 5 mm from the side edge and 1 cm from the top of the plate to record the roughness measurements in a linear distance of 5 mm (Fig. 3).



Fig. 2: Mitutoyo 178-561-02A Surftest SJ-210 surface profilometer

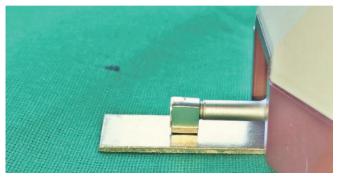


Fig. 3: Measuring the surface roughness of titanium plate using surface profilometer

The following variables of roughness were obtained for each plate:

- Roughness parameters (DIN EN ISO 4287:1998)
- Ra Arithmetic mean surface roughness: Arithmetical mean of the sums of all profile values in a given linear sample.
- Rq Root mean square of the Ra values; it is a value characteristic of a continuously varying quantity.
- Rz Average distance between the highest peak and lowest valley in each sampling length.

Three measurements were performed for each specimen according to ISO 4287: 1997.

The arithmetic mean deviation of the profile (Ra) and the maximum height (Rz) were measured with a cut-off value of 0.8 mm, measurement length of 5 mm, measurement speed of 0.6 mm/s, and a Gaussian filter.

Statistical Analyses

Statistical analyses were performed using the Statistical Packages for the Social Science (SPSS)/ PC+ version 17.1 program (SPSS Inc., Chicago, Illinois, USA). The means and standard deviations of data were calculated. Shapiro-Wilk's test was done to find distribution of data. One-way analysis of variance (ANOVA) with Duncan's multiple comparisons test was performed to evaluate differences

**Mitutoyo 178-561-02A Surftest SJ-210 Surface Roughness Tester

between groups. Values of p less than 0.05 were considered statistically significant.

RESULTS

The average roughness values Ra, Rq, Rz evaluated, for untreated and acid etched samples, are reported in Tables 1 to 3 respectively.

The characterization of the implant surfaces carried out by roughness profilometer showed different aspects in the topographies of the surfaces of titanium plates due to the different duration and combination of acids used in the etching process.

From Table 1, it is clear that the roughness value of titanium surfaces increased after various surface treatments. From the experiment, the average roughness value Ra obtained is $0.480 \mu m$ for untreated surface and $3.65 \mu m$ maximum for the T5 titanium plate after 72 hours of H₂SO₄ and 48 hours of HCl etching, which is about seven times the base value of the unetched surface.

Test for normality of distribution of data was performed using Shapiro-Wilk's test. The data were found

Table 1: Ra values for T1-T13

Unetched	ТО	0.418	0.528	0.484	0.480
Groups	Groups	Ra-1	Ra-2	Ra-3	Average
72/18	T1	1.08	1.291	0.927	1.10
72/24	T2	0.847	0.843	0.843	0.84
72/30	Т3	3.328	3.244	2.918	3.16
72/36	T4	0.708	0.721	0.758	0.73
72/48	T5	3.732	3.729	3.489	3.65
66/24	T6	1.338	0.93	0.741	1.00
66/30	T7	0.632	0.794	0.74	0.72
66/36	T8	2.093	1.387	0.971	1.48
66/48	Т9	0.813	1.15	0.762	0.91
88/24	T10	0.915	0.903	0.809	0.88
88/30	T11	1.764	1.845	1.767	1.79
88/36	T12	2.743	2.158	2.874	2.59
88/48	T13	3.093	3.16	3.181	3.14

Table 2: Rq values for T1–T13

Unetched	ТО	0.609	0.622	0.601	0.612
Groups	Groups	Rq-1	Rq-2	Rq-3	Average
72/18	T1	1.361	1.648	1.162	1.39
72/24	T2	1.063	1.045	1.055	1.05
72/30	Т3	3.966	3.831	3.908	3.90
72/36	T4	0.896	0.911	0.973	0.93
72/48	T5	4.407	4.954	5.312	4.89
66/24	Т6	1.683	1.169	0.93	1.26
66/30	T7	0.802	0.999	0.93	0.91
66/36	Т8	2.611	1.735	1.213	1.85
66/48	Т9	1.012	1.438	0.96	1.14
88/24	T10	1.137	1.117	0.994	1.08
88/30	T11	2.278	2.355	2.225	2.29
88/36	T12	3.424	2.742	3.703	3.29
88/48	T13	3.882	3.81	3.977	3.89

Table 3: Rz values for T1–T13						
Unetched	ТО	3.336	3.342	3.426	3.394	
Groups	Groups	Rz-1	Rz-2	Rz-3	Average	
72/18	T1	7.851	9.471	6.849	8.06	
72/24	T2	6.278	6.255	6.321	6.28	
72/30	Т3	18.092	18.109	24.927	20.38	
72/36	T4	5.523	5.586	6.293	5.80	
72/48	T5	23.711	24	26.91	24.87	
66/24	Т6	10.038	5.996	5.426	7.15	
66/30	Τ7	5.319	5.818	5.519	5.55	
66/36	Т8	13.218	9.091	7.447	9.92	
66/48	Т9	6.151	7.578	5.982	6.57	
88/24	T10	6.305	6.107	5.396	5.94	
88/30	T11	11.894	14.267	12.673	12.94	
88/36	T12	19.238	16.784	20.011	18.68	
88/48	T13	19.875	19.813	20.305	20.00	

	Ra			Rq	Rz	
	Standard		Standard			Standard
	Mean	deviation	Mean	deviation	Mean	deviation
T1	1.10	0.18	1.39	0.24	8.06	1.32
T2	0.84	0.00	1.05	0.01	6.28	0.03
Т3	3.16	0.22	3.90	0.07	20.38	3.94
T4	0.73	0.03	0.93	0.04	5.80	0.43
T5	3.65	0.14	4.89	0.46	24.87	1.77
T6	1.00	0.31	1.26	0.38	7.15	2.51
T7	0.72	0.08	.91	0.10	5.55	0.25
T8	1.48	0.57	1.85	0.71	9.92	2.97
Т9	0.91	0.21	1.14	0.26	6.57	0.88
T10	0.88	0.06	1.08	0.08	5.94	0.48
T11	1.79	0.05	2.29	0.07	12.94	1.21
T12	2.59	0.38	3.29	0.49	18.68	1.68
T13	3.14	0.05	3.89	0.08	20.00	0.27

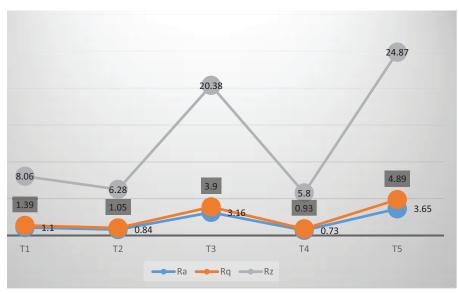
to be normally distributed; hence, we used ANOVA to compare in between the groups (Table 4).

Graph 1 shows the changes in roughness parameters in group 1 when H_2SO_4 was kept constant at 72 hours and duration in HCl was varied from 18, 24, 30, 36, and 48 hours. Analyzing the trend of Ra values, one can appreciate that the average depth of the grooves of the new roughened microsurface increased sharply when plates were etched for 36, and 48 hours in HCl.

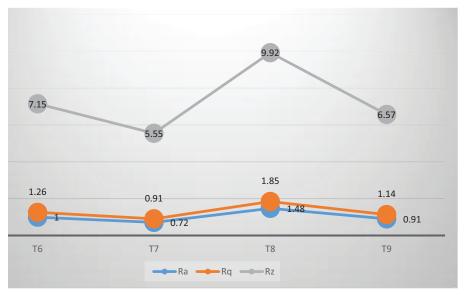
Graph 2 shows the roughness parameters in group 2 when H_2SO_4 was kept constant at 66 hours and duration in HCl varied from 24, 30, 36 and 48 hours. The roughness values in this group were lesser than group I, indicating that H_2SO_4 exposure at 66 hours was weaker and less significant for creating microroughness on the titanium surface.

Graph 3 shows the roughness values in group III when H_2SO_4 was kept at 88 hours and HCl was varied from 24, 30, 36, and 48 hours. Analyzing the trend of roughness parameter in this group indicated that as the

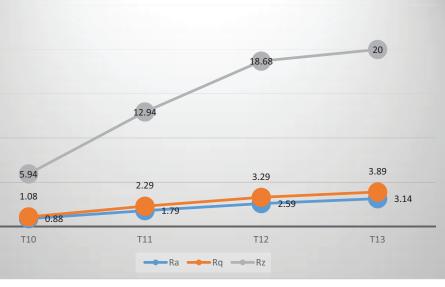




Graph 1: $H_2SO_4 - 72$ hours



Graph 2: $H_2SO_4 - 66$ hours



Graph 3: H₂SO₄ – 88 hours

Table 5: Ra, Rq, Rz values for T14–T25					
Unetched	ТО	0.418	0.528	0.484	
Groups	Groups	Ra	Rq	Rz	
15	T14	0.565	0.852	3.566	
30	T15	0.544	0.843	3.458	
45	T16	0.526	1.115	3.918	
60	T17	0.548	0.959	3.758	
75	T18	0.432	0.729	3.489	
90	T19	0.378	0.930	2.741	
105	T20	0.342	0.794	2.074	
120	T21	0.553	1.387	3.971	
135	T22	0.513	1.15	3.762	
150	T23	0.415	0.903	2.908	
165	T24	0.364	0.845	2.967	
180	T25	0.343	0.758	2.874	

Table 6: Ra comparison with initial value = 0.48

	t	df	р	Mean difference	Interpretation
T1	5.869	2	0.028	0.61933	S
T2	273.250	2	0.00001	0.36433	S
Т3	21.460	2	0.002	2.68333	S
T4	16.625	2	0.004	0.24900	S
T5	39.377	2	0.001	3.17000	S
T6	2.969	2	0.097	0.52300	NS
Τ7	5.082	2	0.037	0.24200	S
T8	3.065	2	0.092	1.00367	NS
Т9	3.519	2	0.072	0.42833	NS
T10	11.806	2	0.007	0.39567	S
T11	49.483	2	0.00041	1.31200	S
T12	9.594	2	0.011	2.11167	S
T13	100.420	2	0.00010	2.66467	S

S: Significant; NS: Not significant

Interpretation criteria:

• p value less than that of 0.05 indicates significance of difference between the group mean and initial value, i.e., 0.48

· Lower the p value, more is the significance

duration of exposure was increased with HCl, the roughness increased considerably.

Table 5 summarizes the Ra, Rq, and Rz values of titanium plates numbered T14–T25, which were subjected to HF acid etching. These plates did not show any significant increase in the roughness values compared with the unetched titanium plates and dual etched plates.

Plates T_6 , T_8 , and T_9 did not show any significant change in roughness parameter Ra (Table 1) when compared with unetched plates. All three being from group II indicating that exposure of titanium for 66 hours in H_2SO_4 did not yield a significantly rough surface (Table 6).

DISCUSSION

Osseointegration consists of a series of bone modeling and remodeling processes, which causes direct structural and functional connection between living bone and the surface of a load-bearing artificial implant. Barier et al¹⁴ discussed the features that play the most significant role in early osseointegration and immobilization of the implant in the tissue bed. Texture, charge, and chemistry of the surface as well as cleanliness were considered to be the most important requirements for the implant material.¹² Predecki et al¹⁶ observed rapid bone growth and good mechanical adherence with an implant that had an irregular surface. Based on the fact that the quality of osseointegration is directly related to the topography of dental implant surfaces, many techniques related to the modifications carried out on implant surfaces have been tested during the last 30 years. These tests take into account the principle that the topography of a rough surface presents an area for bone anchorage that is much larger than a smooth surface does.¹⁴ Characteristics of titanium implant surfaces have been modified by additive methods (e.g., titanium plasma spray) or subtractive methods (e.g., blasting, acid etching) to increase the surface area and to alter its microtopography or texture.¹⁵

Buser et al¹⁹ showed that implants with sandblasted and acid-etched surfaces had higher bone to implant contact percentages than implants with titanium plasma sprayed surfaces. However, it should be emphasized that this titanium surface was gained using two methods of processing-sandblasting and acid etching.

A new surface treatment that produces a microroughness similar to the blasted/etched surface but uses only special dual acid etching without grit blasting has been developed. The purpose of this dual etching is to produce a micro-rough surface that provides rapid osseointegration, while maintaining the long-term success associated with a machined implant surface.¹⁷

In the present study, two methods of surface treatments, namely, dual acid ethcing that was performed using H₂SO₄ (98%) and HCl (35–38%), in sequence, for time durations ranging from 66, 72, 88 hours for H₂SO₄ and 18, 24, 36, 48 hours for HCl, and etching with HF acid for time duration ranging from 15 to 180 seconds, to create a surface topography on titanium that encorporates all the surface features in the aforementioned studies, and comparatively analyzed the resultant surface with the unetched titanium plates, using a surface profilometer. This was done to minimize the cost of surface treatment and to simplify the process.

The present study showed that precise acid selection, time, and the sequence of processing was the most crucial step in obtaining roughened titanium surface, which was in accordance with the study carried out by David Baker and co-workers,¹⁸ who determined that the dual etched surfaces using H_2SO_4 and HCl achieved higher roughness values and higher level of bone implant contact percentages. In a study by Buser et al, comparing influence



of different surface characteristics on bone integration of titanium implants found highest extent of bone-implant interface in sandblasted (large grit) and acid etched HCl + $\rm H_2SO_4$ group with mean values of 50 to 60%.¹⁹

Profilometer readings were used to determine the surface irregularities of unetched and etched titanium plates in this study. Generally, the surface characteristics of implant surfaces are compared using scanning electron microscopic examination or profilometer reading. Scanning electron microscopic examination studies are qualitative tests that reveal scratches produced on a surface.²¹ Profilometer results provide quantitative recording of surface irregularities. The profilometer is a device that uses a diamond stylus of precise dimensions to trace a fixed linear distance over the surface of the prepared sample. The profilometer produces a tracing and, using digital and analog hardware and software, also calculates the average surface roughness (Ra value) for the resultant tracing.

In this study, the following parameters for quantifying surface roughness were obtained for each plate:

- Roughness parameters (DIN EN ISO 4287:1998)²²
- Ra–Arithmetic mean surface roughness: Arithmetical mean of the sums of all profile values in a given linear sample.
- Rq–Root mean square of the Ra values; it is a value characteristic of a continuously varying quantity.
- Rz–Average distance between the highest peak and lowest valley in each sampling length.

In the present study, the surface characterization of the titanium surfaces evaluated by surface profilometer showed different aspects in the topographies of the surfaces of titanium plates due to the different duration of acids used in the etching process. The average roughness value Ra obtained is 0.480 µm for untreated surface and $3.65 \ \mu m$ maximum for the T5 titanium plate after 72 hours of H₂SO₄ and 48 hours of HCl etching, which is about seven times the base value of the unetched surface. The average roughness values Ra for etched titanium plates ranging from 0.73 to 3.65 shows that acid etching was able to create micro-textured pits and waviness on the titanium surface, which was in accordance with the observations by Davies¹¹ who also showed that acid etching of titanium creates a micro-textured surface (fine rough surface with micropits of 1-3 µm and larger pits of approximately 6–10 µm) that appears to enhance early endosseous integration and stability of the implant. This may be related to a change in surface roughness and/or chemical composition.²³

Another very important variable for determining the surface topography of titanium surface is Rz, which reveals the average distance between the highest peak and lowest valley in each sampling length. In this study, we found a wide range for the Rz values maximum in plate T5 (Rz = 24.87), which indicates that this surface had a very uneven waviness and distance between the crests and troughs was quite large, implying that the pits created after this sequence protocol were deepest compared with other plates. While comparing the surface of the etched titanium plates with the untetched titanium plates, we found that the surface roughness values for all plates were statistically significant except plate nos. T6, T8, T9, which belonged to the group II (H_2SO_4 for 66 hours). Based on this observation, one can conclude that exposure of titanium surface to H_2SO_4 for 66 hours was not enough to significanlty alter the tianium surface from baseline.

The roughness values obtained after acid etching with HF acid did not show any significant improvement in surface characteristics compared with the unteched titanium plates. Moreover, the handling of HF was found to be cumbersome and caused time-dependent erosion of the titanium plates leading to severe loss in surface texture and weight at longer time durations above 60 seconds. These findings are in accordance with the results found by A. Thirugnanam et al also found nonsignificant results and loss of an average of 14% weight after acid etching of titanium surface with HF acid alone.²⁴ Thus from the findings of this study, we conclude that HF acid, used alone, was unsuitable at a concentration of 40%, to be used for acid etching of titanium plates, although microroughness obtained by the same is similar to the semipolished unetched titanium plates and could be superimposed on macro roughness obtained by other means.

A study conducted to comparatively analyze the surface of four etched implants used the profile roughness measurements to characterize the surface of the four tested implants–DPS Frialit II, Osseotite, SLA-ITI, HaTi. The analyses showed that each implant surface displayed a distinct surface topography with Ra values ranging from 0.589 μ m of osseotite implant to 2.455 μ m of SLA-ITI implant, though all of them were found to be equally successful in osseointegration in clinical studies.²⁵ This observation implies that various acid etching techniques for obtaining surface roughness, though appear to be comparatively significant in *in vitro* studies, may not be superior clinically, and further biological, histomorphometric, and human trials are required to establish its effectiveness.

CONCLUSION

The specific dual acid etching technique using H_2SO_4 (98%) followed by HCl (35–38%) proposed in this experiment yields roughness parameters similar to the various implant surfaces available commercially when studied

in vitro. Etching of titanum plates with HF acid was found to be cumbersome and hazardous in the proposed setting and did not yield any significant enhancement of surface roughness compared with machine-prepared unetched plates. The present study showed that by optimizing the parameters of acid etching a rough titanium surface can be obtained similar to the various implant surfaces available commercially. Thus, dual acid etching seems to be a simple technique to develop a titanium implant surface, though evaluation of the biological response to this surface is necessary.

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