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ORIGINAL ARTICLE

An *In Vitro* Study to Evaluate and Compare the Hemocompatibility of Titanium and Zirconia Implant Materials after Sandblasted and Acid Etched Surface Treatment

[1]Arjun Rajan, [2]Sujith Sivarajan, [3]Chitra G Vallabhan, [4]Anjana S Nair, [5]Shilpa Jayakumar, [6]Arya S Pillai

ABSTRACT

Aim: This study was aimed to investigate the hemocompatibility of zirconia and titanium implant materials after surface treatment with sandblasting and acid etching (SLA).

Materials and methods: Sixty specimens were procured from manufacturers of dimension 10mm x 3mm, thirty of each were prefabricated medical grade titanium (Ti-6Al-4V) and thirty of sintered zirconia. Silicon carbide grit papers of 240 to 1200µm, was used to polish the specimen surface. The surfaces were rinsed with water to remove any remnant particles after polishing. Later ultrasonic cleaning was done for 5 minutes using distilled water. The control specimens included 15 specimens each from titanium (groups A1) and zirconia (groups B1). The remaining 15 specimens (groups A2 and B2) were sandblasted using alumina particles of 150 microns particle size and using 20% hydrochloric acid, acid etching was done for 30 seconds. The specimens were scanned under electron microscope after surface treatment for analysis purpose and evaluated for surface characteristics. Before the exposure of specimens to blood, percentage hemolysis, prothrombin, platelet aggregation and activation, and thrombin time values were calculated. 1 ml of blood was added to each specimen for testing. The values before and after the exposure of specimens to blood were noted. Using a t-test, the values noted were statistically evaluated.

Results: A_1 (polished titanium) showed highest mean values after exposure, in platelet count (184.67 ± 1.29), Leucocyte count (7.27 ± 0.08), and Thrombin time (10.15 ± 0.34) while Prothrombin time's highest mean value after exposure were showed by A_2 (SLA treated titanium) with a mean value of 10.04 ± 0.24.

Conclusion: Surface treatment with sandblasting and acid etching (SLA) using 150 microns alumina particles and 20% hydrochloric acid increased the surface roughness of the titanium and zirconia implant materials and polished titanium showed maximum hemocompatibility.

Clinical significance: The implant's success depends on its biocompatibility and its property of osseointegration. The adverse interaction between blood and the artificial surface is detected by the hemocompatibility test for medical materials, to know if the surface can activate or destruct the blood components. The success of implant placement also depends on the interaction between the blood and the specimen.

Keywords: Acid etching, Hemocompatibility, Sandblasting, titanium, Zirconia.

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[1]Department of Prosthodontics, Coorg Institute of Dental Sciences, Virajpet, Kodagu, India

[2]Department of Orthodontics, PMS Dental College, Vattapara, Trivandrum, Kerala, India

[3,5]Department of Periodontics, Sree Mookambika Institute of Dental Sciences, Kulasekharam, Tamil Nadu, India

[4]Department of Orthodontics and Dento facial Orthopedics, Sree Mookambika Institute of Dental Sciences, Kulasekharam, Tamil Nadu, India

[6]Kakkanad, Ernakulam, Kerala, India

Corresponding Author : Arjun Rajan, Department of Prosthodontics, Coorg Institute of Dental Sciences, Virajpet, Kodagu, India, Mobile: +919900567556, Email: mnrarj@gmail.com

INTRODUCTION

The most widely acceptable and predictable treatment modality from past 30 years, for partial and fully edentulous patients,is the use of dental implants endosseous anchored directly with the bone-to-implant contact in the jaws.By following certain surgical principles, direct implant anchorage with bone can be achieved as shown in a few studies. This implant anchorage modality is termed often as functional ankylosis or osseointegration.[1]

Nowadays the necessity for implant supported long-duration prosthetic treatment is increasing in dental practice. The significant implant failure associated with the treatments holds as a drawback to resort to the treatment again. The experimental procedures that have been successfully used to improve the bone integration of dental implants have been limited to variations in design and to surface treatments.[2]

The implantation period for the induction of the bone using porous bioactive titanium is about 12 months which is longer compared to that of porous calcium-phosphate based biomaterials of 45 to 90 days.[3] the implant's success rate depends on its biocompatibility and osseointegration.[4]

Biocompatibility may be divided into hemocompatibility (blood) and cytocompatibility (cell and tissue). The biocompatibility depends on the survival of the cell affected by the material usage. The cascade of events occurred by the molecular interference with macromolecular synthesis, which causes unequivocal cellular, structural and functional damage is described as 'cytotoxicity'.[5]

Hemocompatibility is a prerequisite for materials used in biomedical products like cardiovascular implants, catheters and medical membranes as otherwise surface initiated coagulation processes and immune reactions occur. This interaction can destruct or activate the blood components. Blood is a complex tissue consists of cells and plasma. Blood plasma is an electrolytic isotonic solution, consisting of sodium chloride of 0.9% with high protein content relatively 40–60 g/L. Albumin makes more than half of total plasma proteins, but many low level constituents are critical for the function of whole organism e.g. transporter proteins, clotting factors, antiproteases, or immunoglobulins.[6]

The surface property of a titanium implant is largely determined by a thin oxide film covered as a protective layer.[7] there are no such studies to prove the hemocompatibility of the zirconia and the effect of surface treatment on it, as it is a newly introduced implant material. And there is no available literature with a combination of 20% HCL acid etchant and 150 microns (average particle size). So this study was conducted to investigate hemocompatibility of commercially available materials (zirconia and titanium) after sandblasting and acid etching (SLA).

MATERIALS AND METHODS

The present *in vitro* study was conducted in the Department of Prosthodontics, Coorg Institute of Dental Sciences, Virajpet.

Sample Size

Thirty specimens (10 mm × 3 mm) each of both titanium and zirconia disks were categorized under 2 groups (A and B). Group A and B were subdivided as 1 and 2, wherein smooth polish disks were categorized under 1 and sand blasted with acid etched (SLA) disks were categorized under 2.

- *Group A_1*: Smoothly polished titanium disks (Fig. 1)
- *Group A_2*: Sandblasted, and acid-etched (SLA) treated titanium disks (Fig. 2)
- *Group B_1*: Smooth polished zirconia disks (Fig. 1)
- *Group B_2*: Sandblasted, and acid-etched (SLA) treated zirconia disks (Fig. 2)

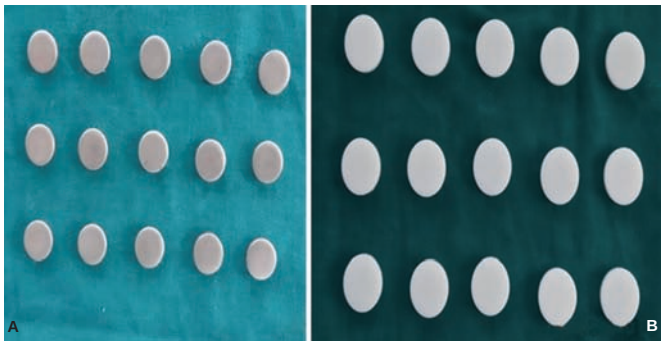
Procedure

Thirty specimens of Grade 5 titanium (Ti-6Al-4V), prefabricated medical grade with 10mm x 3mm dimension and thirty specimens of the same dimension of sintered zirconia were procured from manufacturers. The specimen surfaces were polished using grit papers of silicon carbide (240 to 1200 µm) and later washed with water to remove any particles created while polishing. Using distilled water, ultrasonic cleaning was done for 5 minutes. Fifteen specimens each from titanium (Group A1) and zirconia (Group B1) were used as control specimens. The remaining titanium (Group A2) and zirconia (Group B2) were subjected to surface treatments.

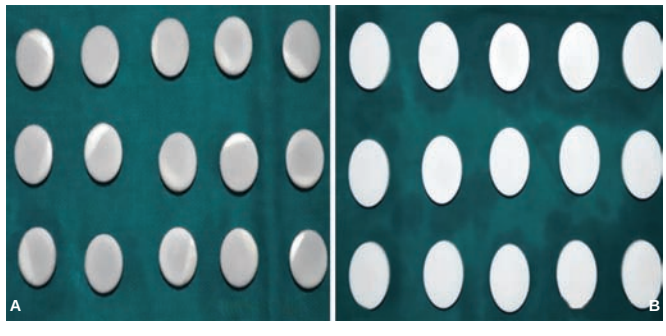
SLA Treatment of Specimens

Sandblasting (Fig. 3)

Fifteen specimens of both titanium (Group A2) and zirconia (Group B2) were placed inside the sandblasting



Figs 1A and B: (A) Polished titanium specimens (A_1), (B) Polished zirconia specimens (B_1)



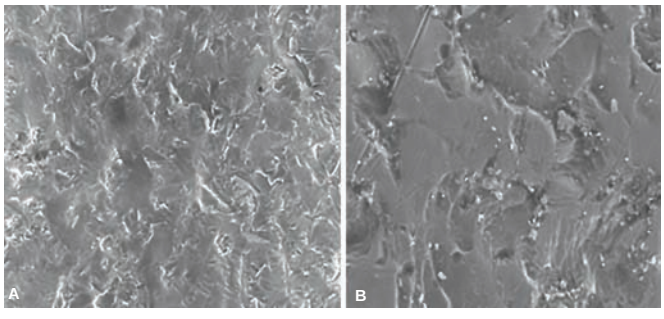
Figs. 2A and B: SLA treated (A) titanium specimen (A_2), (B) zirconia specimen (B_2)

machine at a standard distance of 10 mm from the nozzle and sandblasted using alumina (average particle size 150 microns) for 10 seconds. The sandblasted specimens were washed for 30 seconds using an ultrasonic cleaner to eliminate any remaining alumina particles.

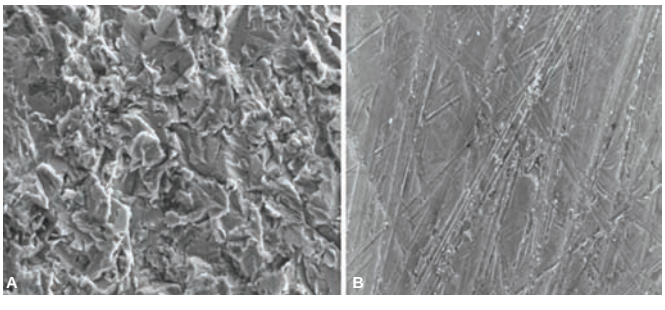
Acid etching

The specimens were kept in a petri dish for 10 seconds, under 20% dilute solutions of hydrochloric acid, subjecting to acid etch. The specimens were washed again placing under ultrasonic cleaner to eliminate the remaining solution for 30 seconds.

Fig. 3: Sand blaster



Figs. 4A and B: SEM image of (A) SLA treated titanium specimen; (B) zirconia specimen



Figs. 5A and B: SEM image of (A) polished titanium specimens; (B) polished Zirconia specimens

Scanning Electron Microscopy

The test specimens were analyzed for surface characteristics after surface treatment using a scanning electron microscope (SEM) for evaluation purpose. Using the mounting plate, all the specimens were mounted and for analysis purpose, they were loaded on the SEM machine. For cleaning the surface, the specimens were sprayed with 90% ethanol. Afterward, a vacuum is created inside the chamber. To focus the lens inside the SEM machine exactly at the center of the specimen, the camera inside the chamber is used. All the specimen's images were recorded at 3000x magnification (Figs 4 and 5).

Exposure of Materials to Blood

Volunteer healthy adult human's blood was collected in the collection tube (total of 60 ml) with anticoagulant [sodium citrate, Ethylenediaminetetraacetic acid (EDTA)] and for the uniform mix of blood with the coagulant, thecollection tube is kept for 10 minutes on blood roller. Before exposing blood to specimens, leucocyte and platelet count were recorded. Both group specimens were agitated for 5 minutes with phosphate buffer saline to clean the surface before exposing it to blood. All the specimens from each group were kept in separate petri dishes. About 1 ml of blood from the collection tube was added to the specimens kept in the Petri dish for 30 minutes.

Analysis of Blood Parameter (Fig. 6)

"Automated hematology analyzer" was used to record the leucocyte and platelet count, before and after exposing specimens to blood for 30 minutes.

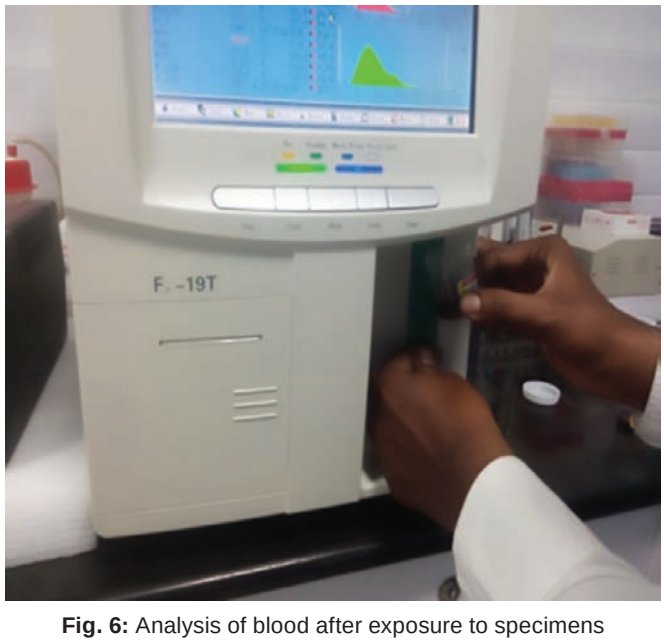


Fig. 6: Analysis of blood after exposure to specimens

Percentage Hemolysis

The platelet count after and before the exposure of blood to specimens was calculated and the change in the count was noted.

Thrombin time (TT) and Prothrombin time (PT) measurement

Using centrifugation method at 3000 rpm for 15 minutes, the platelet-rich plasma was separated from the blood stored in anticoagulant (sodium citrate) collected in collection tube collected from the same volunteer. The supernatant 2 mLof platelet poor plasma was kept in contact with the test specimens for 10 minutes at a temperature of 37°C kept in a petri dish inside an incubator. The measurements of PT and TT were performed by adding respective PT and TT reagents into the test tube containing 200 mL of platelet poor plasma and clotting time were evaluated.

Platelet Adhesion and Activation

Before the exposure of the specimens to the blood, leucocyte count was noted. Specimens were later exposed to the blood in the test tube containing anticoagulant (sodium citrate). After 30 minutes of exposure, the leukocyte count is calculated. The before and after difference in the leukocyte count of the blood exposed specimens will be directly proportional to the number of lysed cells.

Data Analysis

The statistical analysis was done using t-test and the values were obtained form all the groups followed by p-values.

RESULTS

The results from Table 1 shows the control group values before exposure of specimens to the blood; the platelet count was 188 x 10^3/µL, leucocyte count was 7.4 x 10^3/µL, prothrombin time value was 12 seconds and thrombin time value was 13 seconds.

The comparative difference values in the mean platelet count before and after the specimen exposure to blood has been revealed in Table 2. A_1 (polished titanium) has shown the highest mean value of 184.67 ± 1.29 after exposure, followed by A_2 (SLA treated titanium) with 183.40 ± 2.74, B_2 (SLA treated zirconia) with 182.20 ± 2.75 and least was seen in B_1 (polished zirconia) with 182.13 ± 2.23 specimens.

Table 1: Platelet count, leucocyte count, prothrombin and thrombin time values before exposure of specimens to blood (control group)

Sl. No.	*Control group*	*Values*
1	Platelet count	188 x 10^3/µL
2	Leucocyte count	7.4 x 10^3/µL
3	Prothrombin time (PT)	12 seconds
4	Thrombin time (TT)	seconds

Table 3 describes the leucocyte count after the specimen exposure to blood. The highest mean value was seen in A_1 (polished titanium) with 7.27 ± 0.08, followed by A_2 (SLA treated titanium) with 7.23 ± 0.07 and least was seen in both B_1 (polished zirconia) and B_2 (SLA treated zirconia) with same mean of 7.22 ± 0.07.

The prothrombin value after the specimen exposure to blood as shown in Table 4, was highest in A_2 (SLA treated titanium) with the highest mean of 10.04 ± 0.24, followed by A_1 (polished titanium) with 9.97 ± 0.26, B_2 (SLA treated zirconia) with 9.90 ± 0.29 and least was seen in B_1 (polished zirconia) with 9.88 ± 0.32.

Table 5 describes the thrombin time after the specimen exposure to blood. The highest mean value was seen in A_1 (polished titanium) with 10.15 ± 0.34, followed by A_2 (SLA treated titanium) with 10.006 ± 0.41), B_2 (SLA treated zirconia) with 10.00 ± 0.25 and least was seen in B_1 (polished zirconia) with 9.95 ± 0.32.

The SEM image shows (Figs 2 and 4) that the SLA treated titanium and zirconia has more surface roughness followed by sand blasted and untreated titanium, zirconia specimens.

DISCUSSION

Protein adsorption is the first event which occurs when blood contacts any artificial surface. The denaturing of the adsorbed protein such as fibrinogen activates the platelet or the coagulation factors causing blood coagulation cascade reaction. Formation of thrombosis is the final step. The effective blood-compatible material should help in prohibiting the adsorption of globulin or fibrinogen, which are known as harmful protein on the material surface and to be favorable for albumin (good protein) to adsorb on the surface. Secondly, the protein adsorption to the artificial surface can be prevented from becoming denatured. The fibrinogen denaturation has been proved to be related to the transfer of charges from fibrinogen to the material and the decomposition of the fibrinogen into fibrin peptides and fibrin monomer.[8]

To test the thrombogenicity, a most commonly in-vivo method is used. For the unsuited devices to this first method, ISO 10993-4 requires tests to be conducted in four categories each: hematology, coagulation, complements system, and platelets. Testing the complement activation is most recommended for implant devices that contact the circulatory blood. This in-vitro assay measures the human plasma complement activation indicating the plasma exposure to the

Table 2: Comparative values of difference in mean platelet count before and after exposure of specimens to blood using t-test

Groups		*N*	*Mean*	*Std. deviation*	*T*	*p-value*
A1	Preplat	15	188.00	0.000	10.000	0.000
	Case	15	184.67	1.291	10.000	0.000
A2	Preplat	15	188.00	0.000	6.487	0.000
	Case	15	183.40	2.746	6.487	0.000
B1	Preplat	15	188.00	0.000	10.181	0.000
	Case	15	182.13	2.232	10.181	0.000
B2	Preplat	15	188.00	0.000	8.148	0.000
	Case	15	182.20	2.757	8.148	0.000

PREPLAT–Platelet count before exposure of specimens to blood, CASE–Mean platelet count after exposure of specimens to blood

Table 3: Comparative values of difference in mean Leucocyte count before and after exposure of specimens to blood using t- test

Groups		*N*	*Mean*	*Std. Deviation*	*T*	*p-value*
A1	Preleuco	15	7.400	0.0000		0.001
	Casegroup	15	7.273	0.0884	5.551	
A2	Preleuco	15	7.400	0.0000	8.919	0.001
	Casegroup	15	7.233	0.0724	8.919	
B1	Preleuco	15	7.400	0.0000	8.404	0.001
	Casegroup	15	7.227	0.0799	8.404	
B2	Preleuco	15	7.400	0.0000	8.404	0.001
	Casegroup	15	7.227	0.0799	8.404	

PRELEUCO–Leucocyte count before exposure of specimens to blood, CASEGROUP–Leucocyte count after exposure of specimens to blood

Table 4: Comparative values of difference in mean prothrombin time values before and after exposure of specimens to blood using t-test and p-values

Groups		*N*	*Mean*	*Std. Deviation*	*T*	*p-value*
A1	Prept	15	12.000000	0.00	29.233	0.000
	Casegroup	15	9.973333	0.2685056		
A2	Prept	15	12.000000	0.00	31.064	0.000
	Casegroup	15	10.040000	0.2443651		
B1	Prept	15	12.000000	0.00	24.886	0.000
	Casegroup	15	9.880000	0.3299351		
B2	Prept	15	12.000000	0.00	27.360	0.000
	Casegroup	15	9.906667	0.2963267		

PREPT–Prothrombin time values before exposure of specimens to blood, CASEGROUP–Prothrombin time values after exposure of specimens to blood

Table 5: Comparative values of difference in mean thrombin time values before and after exposure of specimens to blood using t- test and p-values

Groups		*N*	*Mean*	*Std. deviation*	*t-value*	*p-value*
A1	PreTT	15	13.000000	0	31.852	0.001
	Testgroup	15	10.153333	0.3461351		
A2	PreTT	15	13.000000	0	27.717	0.001
	Testgroup	15	10.006667	0.4182731		
B1	PreTT	15	13.000000	0	36.324	0.001
	Testgroup	15	9.953333	0.3248443		
B2	PreTT	15	13.000000	0	44.840	0.001
	Testgroup	15	10.000000	0.2591194		

PreTT–Thrombin time values before exposure of specimens to blood, Testgroup–Thrombin time values after exposure of specimens to blood

test article or an extract. The complement activation measurement indicates if the test article is capable of inducing an immune response as a complement-induced inflammatory response in humans.

Materials used in this study are titanium and zirconia. The study conducted by Gahlert et al.[9] have used the same implant materials (titanium and zirconia), to study the osseointegration. Titanium materials are commonly used because of its well-documented beneficial results, favorable mechanical properties, and excellent biocompatibility. Microscale roughness of zirconia is more preferred than the conventional surfaces, such as machined surfaces. Titanium with micro-roughened surfaces can be attained by the process of particle blasting, Ti plasma spraying, machining, chemical/ electrochemical etching, or particle blasting and by chemical etching.

One of the most versatile instruments used to examine and to analyze the morphological microstructure and characterization of chemical composition is scanning electron microscope. To evaluate the surface characteristics before the surface treatment and after the surface treatment, the specimens were analyzed under a scanning electron microscope (SEM). Through SEM images, it was proved that the Ti and Zr after SLA treatment have a rougher surface. This indicates that there is an increase in the surface area for osseointegration, with an increase in the bone to implant surface ratio which leads to the success of the implant.

An *in vitro* study was conducted by Bhavanchand et al.[4] to evaluate the titanium's hemocompatibility after surface treatment. The hemocompatibility test showed a reduction in the platelet count, but was under ISO standards, after exposing to titanium samples. It was concluded that hemocompatibility does not vary in medical grade titanium after different surface modification. The increase in the surface roughness was observed, which increased the implant to bone ratio. Similar results were seen in the present study in both titanium and zirconia polished and SLA treated implant materials.

The present study showed that there were increased surface irregularities in SLA treated titanium compared to that of polished titanium from the SEM image. These results were similar to the biocompatibility study conducted by Kim et al.[10] on SLA treated implants of titanium. The surface of the titanium implants was sandblasted (using large grits) and acid etched (SLA). It increased the surface of the implants for osseointegration. The topographic surface of the titanium was scanned for investigation with a profilometer and SEM. A small uniform micro pits measuring 1 to 2 µm diameter were demonstrated on SLA treated implants. The average roughness (Ra) was 1.19 µm with a maximum height (Rt) of 10.53 µm after SLA.

The study on titanium osteoinductive porous implants was conducted by Takemoto et.al.[3] The diluted HCL treatment gave topographic (etching) and chemical (titania formation and sodium removal) effects on the surface of the titanium, though predominant factor cannot be determined. In the present study, surface treatment was done with 20% HCL on zirconia and titanium after sandblasting using alumina particles (150 microns). From the above study, it can be suggested that osteoinductivity will be better after the surface treatment.

Thrombogenicity depends on the relative adsorption of plasma proteins such as albumin and fibrinogen to the surface as suggested by the study conducted by Packham[11] and Kang et al.[12] The blood clot formation is mediated by fibrinogen (factor I) glycoprotein.

To access the hemocompatibility between the materials and arterial blood flow, Sanak et al[6] conducted a study. The hemocompatibility test for medical materials to detect the interaction between the blood material and artificial surface, to notice the adverse reaction, which can destruct or activate the blood component.

The *in vitro* study conducted by Schreiber et.al[13] on surface modified dental implants showed that titanium nitride (TiN) coated on titanium implant material decreased the colonization of the bacteria compared to other clinically used implant surfaces. The surface of the titanium material which has an antimicrobial role is also examined for the support of fibroblast growth within a TiN surface.

The study on the zirconia implant's osseointegration was conducted by Depprich et.al[14], a bone to implant interface was done through SEM observation. The observation showed the bone attachment seen already after a week, which is remarkable and increased further for intimate contact with the bone after 4 weeks, which is observed on the implant surfaces of both titanium and zirconia. Osseointegration was seen after 12 weeks, without the interposition of an interfacial layer. A study was conducted by Li et al.[15] to know the effect of hydrofluoric acid treatment on osseointegration of titanium implant. The results showed improvement in the osseointegration of titanium implant proved the effectiveness of the HF treatment on Ti surface. An in-vivo study to know the zirconia surface characteristics of the implant material was conducted by Zinelis et.al.[16] Differences were found between the implants which are in the monoclinic to tetragonal ZrO_2 phase transformation,extent of contamination of carbon, residual alumina content, and 3D-roughness parameters contributing to a substantial differentiation in the tissue and cellular response in bone.

Currently, there were no available studies on zirconia hemocompatibility after surface treatment with SLA. Therefore, the present study was conducted to know the zirconia's hemocompatibility after surface treatment with SLA. The SEM imaging showed an increase in the surface irregularities in SLA treated specimens when compared to polished zirconia and titanium specimens. Before and after the surface treatment, both implant materials (titanium and zirconia) showed hemocompatibility. The maximum hemocompatibility was seen with polished titanium compared to other groups.

CONCLUSION

In conclusion, surface treatment with sandblasting and acid etching (SLA) using 150 microns alumina particles and 20% hydrochloric acid increased the

surface roughness of the titanium and zirconia implant materials and polished titanium showed maximum hemocompatibility.

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