World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

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Hydrophilicity effects of sandblasting Al_2O_3 and Acid-etching HCl 37% on implant surface to the blood adsorption

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World Journal of Advanced Research and Reviews, 2025, 28(03), 1689-1697

Publication history: Received 17 November 2025; revised on 23 December 2025; accepted on 25 December 2025

Article DOI: https://doi.org/10.30574/wjarr.2025.28.3.4268

Abstract

The clinical success of dental implants depends on several variables, including implant material, surface property, implant design, and patient bone quality. Among these variables, implant material and surface property are the most important parameters of in vivo reactivity, given that the implant surface is in direct contact with bone and biological fluid. Hydrophilicity is one of the surface properties that remarkably affects tissue attachment and cell adhesion, both are important factors for long-term implant survival. To evaluate the hydrophilicity effects of sandblasting Al2O3 50 µm, 100 µm, and acid-etching HCl 37% treatments on titanium dental implant surfaces based on blood adsorption. Titanium implant surfaces treated with sandblasting Al2O3 50 µm or sandblasting Al2O3 100 µm or acid-etched with HCl 37% were immersed in a blood reservoir. Blood adsorption heights were recorded every 30 seconds up to 600 seconds. Blood adsorption heights after 600 seconds were as follows: machined surface (0 mm), sandblasting Al2O3 50 µm (3.39 mm), sandblasting Al2O3 100 µm (4.01 mm), and acid-etching HCl 37% (1.73 mm). Statistical analysis indicated significant differences between the groups ($p < 0.05$). Sandblasting Al2O3 100 µm demonstrated the highest blood adsorption, suggesting superior hydrophilicity compared to sandblasting Al2O3 50 µm and acid-etching HCl 37%.

Keywords: W blood adsorption; Dental Implant; Sandblasting; Acid-etching.

1. Introduction

The clinical success of implants depends on several variables, including surface property, implant material, implant design, and patient bone quality. Among these variables, implant material and surface property are the most important parameters of biological reactivity, given that the implant is in direct contact with bone and biological fluids, which then induces osseointegration. (1) Hydrophilicity is one of the surface properties that remarkably affects tissue attachment and cell adhesion, both of which are important factors for long-term implant survival. (2)

Dental implants made of titanium alloy (Ti_6Al_4V) are one of the most used nowadays. (3) The problem is, by nature, titanium is a metal rather than a bioactive material. Osseointegration between the implant and the surrounding tissues can be hindered by titanium's and its alloys' intrinsic surface bio-inertness. Consequently, a significant amount of time is needed for the titanium implant and the surrounding tissues to fully integrate. (1)

As a way to resolve that issue, various surface treatment techniques have been developed, including SLA (sandblasted, large grit, acid-etching) technology, plasma spraying, hydroxyapatite coating, anodizing, and a lot more. SLA treatment can either be conducted individually or in a combination of them. (1)

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Sandblasting and acid-etching have been the most effective techniques to modify a metal surface. (4,5) Numerous research show that a range of 50 between 110 μm Al_2O_3 particles in the air was considered to be ideal blasting conditions. (6,7,8,9) According to a study by Spohr et al., sandblasting with 50 μm Al_2O_3 produced tensile bond strengths that were significantly higher than those obtained from sandblasting with 100 μm Al_2O_3. (10). However. recent findings suggest that sandblasting with larger particles like 100 μm Al_2O_3 may provide superior hydrophilicity effects due to increased surface roughness and enhanced blood adsorption capacity. (32)

While the size of red blood cells (6–8 μm) and proteins (~6 μm or smaller) is much smaller than the 50 μm let alone 100 μm sandblasting particles, it allows blood cells and proteins to physically enter and anchor within these features. Meanwhile, acid-etching creates a micro-roughness of 0.5 - 3 μm irregular pits with varying depths on the titanium surface. (11) Hydrochloric acid (HCl) is often used in acid etching for implant surface treatment due to its effectiveness in making significant surface alterations and elevating roughness value. (12) According to another study, etching using concentrated HCl provided superior surface modification effects in titanium compared to H_2SO_4. (13) As for temperatures of 60 and 90°C, a minimum of 15 minutes is required to obtain a surface that is classified as moderately rough. (14) Titanium discs etched with 37% HCl at 60 °C for 60 minutes showed optimal results because of the long exposure times. (15) The surface's enhanced roughness promotes osteoblast adherence and growth within the surface's curvature to generate stable focal adhesions. (16)

The first biological process that occurs upon implant placement is blood protein adsorption and the development of a blood clot on the biomaterial surface. Immediately after the implant is soaked in the patient's blood, within nanoseconds, a layer forms in the surrounding tissue, facilitating the adsorption of protein and other necessary molecules. (17) Albertini et al. stated that after exposing the dental implant surface to contact with blood, the adsorption time is around 5 seconds. Next, in 30 seconds to minutes and hours, the implant surface is then coated with intercellular matrix proteins, whose structure, composition, and inclination are determined by the type of surface. (18) Cell adhesion, migration, and differentiation are triggered by this protein layer, facilitating a multi-hour or multi-day contact between the cells and the implant surface. (19)

Given this background, this study can provide a fresh discovery to the dental implant world. This research aims to test and compare the hydrophilicity effects of sandblasting Al_2O_3 50 μm, 100 μm and acid-etching on titanium implant surfaces by using blood adsorption as the parameter. The implant surface after sandblasted Al_2O_3 50 μm, 100 μm or given acid-etching HCl 37% treatment, will be contacted with a blood reservoir, and the height of blood adsorption is observed.

2. Material and Methods

This study employed an in vitro experimental design with a post-test-only group design. The study conducted with four treatment groups: machined surface, sandblasting Al_2O_3 50 μm, sandblasting Al_2O_3 100 μm and acid-etching HCl 37% treatment, **Figure 1**. The blood adsorption is measured to represents how good is the hydrophilicity or wettability of the implant surface. The research is conducted at Research Center, Faculty of Dental Medicine, Universitas Airlangga (Jl. Prof. Dr. Moestopo 47, Surabaya, Jawa Timur, Indonesia) in November 2024. This study has been approved by Universitas Airlangga Faculty Of Dental Medicine Health Research Ethical Clearance Commission number 1196/HRECC.FODM/XII/2024. The sample size for this research was determined using the Federer formula for four treatment groups. From the formula, it was determined that the minimal sample size needed for each group was 8.5 samples. To ensure consistency and accuracy, each treatment group contained nine titanium dental implant samples (n = 36).

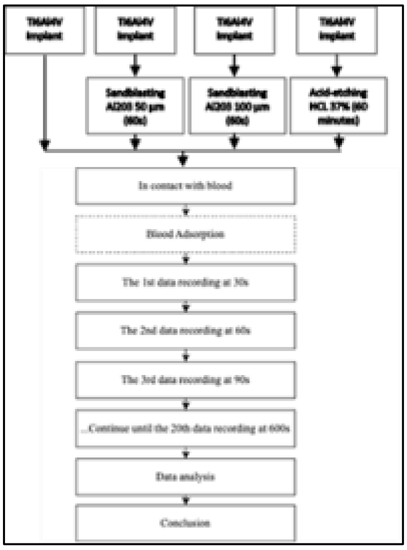

Figure 1 The impact of hydrophilicity research flow

2.1. Sample Preparation

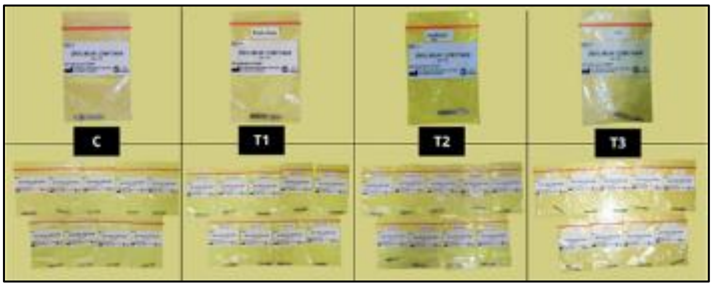

Figure 2 Research Sample Ti_6Al_4V screw type implant produced (PT. Marthys Orthopaedic Indonesia). C: Control, machined surface; T1: Sandblasted Al_2O_3 50 µm; T2: Sandblasted Al_2O_3 100 µm; T3: Acid-etched HCl 37%

Sample criteria are Ti_6Al_4V "Screw Type" one-piece implant by PT. Marthys Orthopaedic Indonesia is made of grade 5 titanium alloy (Ti6Al4V), **Figure 2.** It comprises 90% titanium, 6% aluminum, and 4% vanadium, with trace amounts of other elements such as iron and oxygen. The implant dimensions include Ø 2.5 mm and a length of 22 mm.

- 9 Machined surface Ti_6Al_4V screw type implant produced by PT. Marthys Orthopaedic Indonesia (PT. Marthys Orthopaedic Indonesia, Surabaya, Indonesia).
- 9 Sandblasted Al_2O_3 50 µm 60s Ti_6Al_4V screw type implant by Marthys Orthopaedic Indonesia (PT. Marthys Orthopaedic Indonesia, Surabaya, Indonesia). Sandblasting is conducted with aluminum oxide (Al_2O_3), particle size 50 µm (Renfert GmbH, 78247, Hilzingen, Germany). Sandblasting lasts for 60 seconds with air pressure of 2.5 to 5 atm.
- 9 Sandblasted Al_2O_3 100 µm 60s Ti_6Al_4V screw type implant by Marthys Orthopaedic Indonesia (PT. Marthys Orthopaedic Indonesia, Surabaya, Indonesia). Sandblasting is conducted with aluminum oxide (Al_2O_3), particle size 100 µm (Renfert GmbH, 78247, Hilzingen, Germany). Sandblasting lasts for 60 seconds with air pressure of 2.5 to 5 atm.
- 9 Acid-etched HCl 37% Ti_6Al_4V screw type implant by Marthys Orthopaedic Indonesia (PT. Marthys Orthopaedic Indonesia, Surabaya, Indonesia). Dental implants were put in the oven at 40 ˚C for 1 hour and allowed to dry at room temperature. Acid-etching was done with a concentrated hydrochloric acid HCl 37% (PT. Smart Lab Indonesia, Tangerang Selatan, Indonesia) at 60 ˚C for 60 min. An electrical water bath was used to increase and sustain the temperature of the acids.

2.2. Tools Preparation

A custom-made tool was assembled for this type of research in the form of an iron railing, with a horizontal bar that can move up and down, **Figure 3**. This particular railing has been used previously in research related to blood adsorption in the saline-rehydrated membrane. (20)

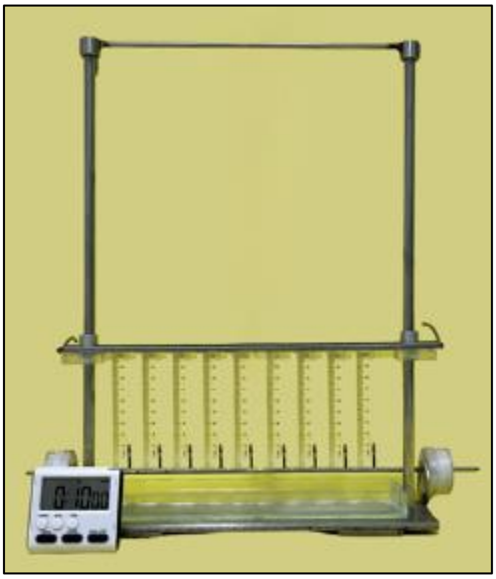

Figure 3 Iron railing with dental implants stick to the ruler

A yellow backdrop was installed. Then, each implant sample was attached to the ruler straight, in a normal installation position. Implants are attached to the ruler by giving a tip of glue at the implant's neck using Adhesive Araldite® Epoxy Rapid Setting (Pidilite Industries Ltd., Mumbai, India). The supporting rail was assembled with the ruler and the implant stick accordingly. Assembled tools and samples were placed on a flat surface. A digital camera Canon EOS M200 24.1 megapixel EF-M15-45 mm f/3.5-6.3 IS STM (Canon, Tokyo, Japan) was arrange in parallel, facing the samples.

The blood used for this research is one bag of Whole Blood (WB) containing 402 ml type O+ with CPDA-1 (Citrate Phosphate Dextrose Adenine) anticoagulants (Batch No. 241305971). The blood was obtained from the Unit of Blood Transfusion, PMI, Surabaya (Unit Donor Darah, PMI, Surabaya, Indonesia) and had been screened as safe to use. It must be stored at 2 – 6 °C. CPDA-1 is an anticoagulant approved for storing whole blood or red blood, particularly in Indonesia. CPDA-1 only prevents blood clotting by inhibiting the coagulation cascade and improves red blood cell viability by delivering the adenine required to maintain red cell ATP levels. CPDA-1 does not does not chemically modify other blood components, such as proteins. (21) Therefore, even though the blood has an anticoagulant, the hydrophilicity still can be measured.

A clear glass box 32 x 6.5 x 4 cm was prepared and cleaned. The blood was transferred from the haemopack blood bag to the clear glass box using a 10 ml disposable syringe with needle (OneMed, Sidoarjo, Indonesia). The blood in the clear glass box was heated using a warmer pad until it reached the normal body temperature, 37 °C. The temperature was checked using an alcohol thermometer (GEA, Tangerang, Indonesia).

2.3. Hydrophilicity Testing

All the tools and iron railing were prepared well, with the rulers and implants stuck accordingly. Blood was already filled into the clear glass box, making it a full blood reservoir. Video recording by digital camera Canon EOS M200 24.1 megapixel EF-M15-45mm f/3.5-6.3 IS STM (Canon, Tokyo, Japan) started.

The bottom parts of the implants were dipped into the blood reservoir by slowly pulling the horizontal bar at the iron railing. By the time the bottom parts of the implants are in contact with blood, the 600 second (10 minutes) timer starts. The timer counts backward. Data recording started when the implant surfaces were in contact with blood. At 30 seconds, 60 seconds, 90 seconds, and multiples of 30, until 600 seconds: The data of blood adsorption is recorded by video and looking at the ruler. The stopwatch stopped after 10 minutes while pulling up the horizontal bar to stop the blood contact simultaneously. The recording was stopped and evaluated.

2.4. Hydrophilicity Measurement

Blood adsorption lengths were discovered by using Adobe Photoshop software (Adobe Photoshop CC 2014, Adobe Systems Inc., San Jose, CA, USA). Made a line along the adsorbed blood in each implant in every 30 seconds and every treatment group with "length measurement" feature. The length resulted in millimeter (mm) scale. The mean ($\bar{x}$) of blood adsorption from 9 samples in each treatment group was counted. The same steps were repeated for three different treatment groups (machined surface with no special treatment - control, sandblasting Al_2O_3 50 µm treatment, sandblasting Al_2O_3 100 µm treatment and acid etching treatment).

2.5. Wettability Analysis

Surface wettability was evaluated using a contact angle goniometer with the sessile drop technique. Scaffolds were placed on a glass substrate, and a 5 µL simulated body fluid (SBF) droplet was deposited on the surface. Droplet images were captured using a high-speed camera under visible light irradiation, and the contact angle was measured using image analysis software [12].

2.6. Statistical Analysis

Data were analyzed using the Shapiro–Wilk test for normality and Levene's test for homogeneity of variance. Differences in wettability among groups were evaluated using one-way ANOVA, followed by Tukey's HSD post-hoc test, with statistical significance set at $p < 0.05$.

3. Data Analysis

Data analysis was conducted using SPSS Statistics for Windows, version 25 (IBM Corp., Armonk, NY). All data were tested for normality using Saphiro-Wilk (sample < 50) and homogeneity using Levene's test. In this study, if the results of normality test were normally distributed, the comparison test was continued using the statistical test One-Way ANOVA. Nevertheless, if the data were not normally distributed and not homogenous, the data needs to be processed to the Kruskal-Wallis test.

4. Results

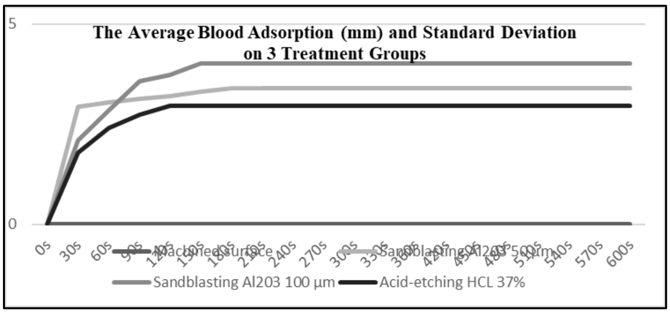

Figure 4 The Average Blood Adsorption (mm) and Standard Deviation on the Titanium Dental Implant with No Surface Treatment, Sandblasting Al_2O_3 50 µm, and Acid-etching HCl 37%

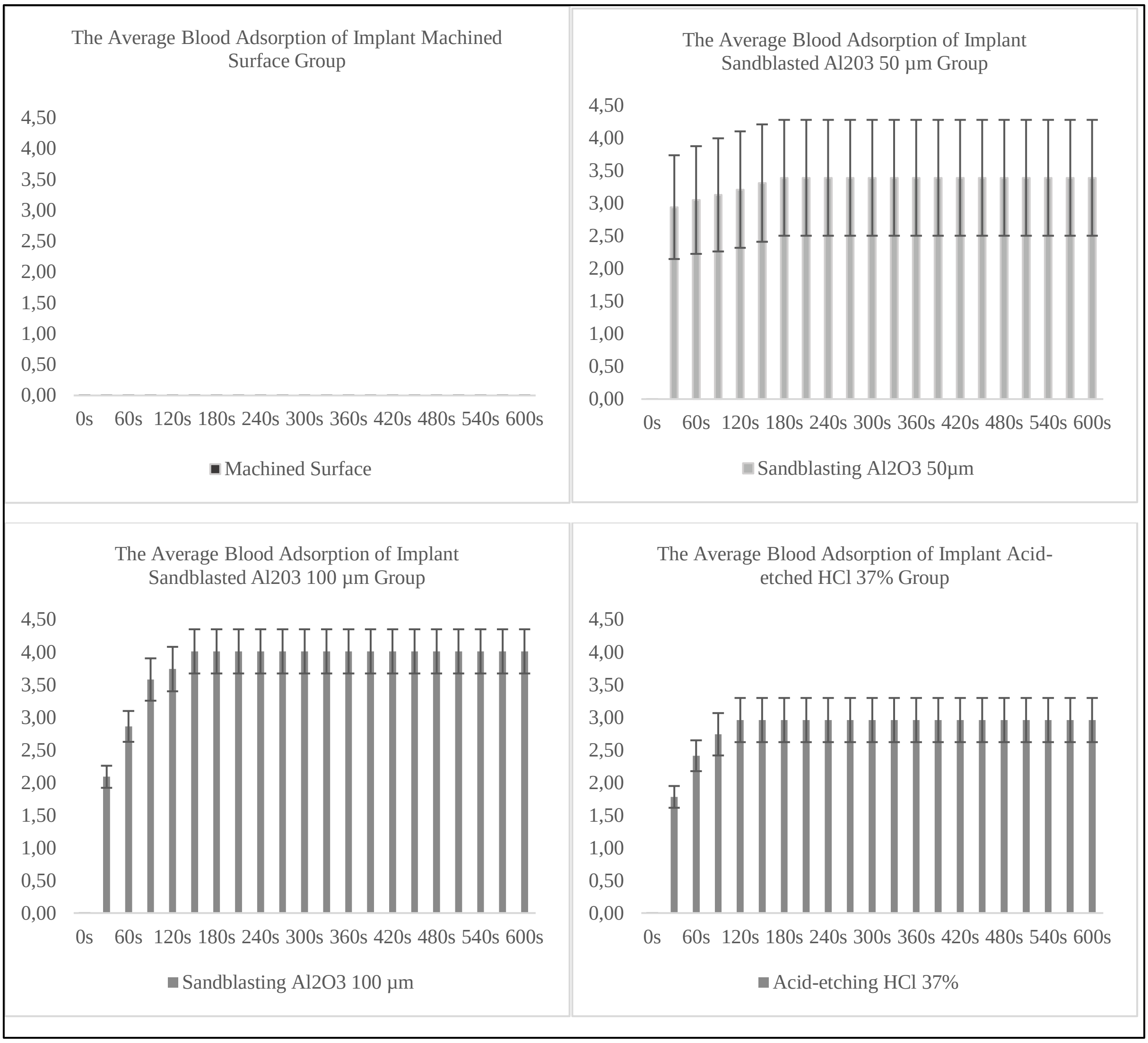

Figure 5 The Average Blood Adsorption (mm) and Standard Deviation in each treatment group

4.1. Analysis of Blood Adsorption

The study was conducted with three treatment groups: machined surface, sandblasting Al_2O_3 50 µm, sandblasting Al_2O_3 100 µm and acid-etching HCl 37%. Figure 4. Blood adsorption varied notably across surface treatments. The machined surface showed no adsorption, while sandblasting with 100 µm particles had the highest adsorption height (4.01 mm), stabilizing at 150 seconds. This was 18% higher than the 50 µm group (3.39 mm), likely due to its greater roughness (Ra = 2.1 µm) and deeper pits (10–15 µm). Acid-etching resulted in the lowest adsorption (1.73 mm), attributed to its smaller pit sizes (0.5–3 µm), which limited protein anchorage. **Figure 5.**

4.2. Analysis of Statistical Result

Statistical analysis indicated non-normal data distribution, as confirmed by the Shapiro-Wilk test ($p < 0.05$), and inhomogeneous variances based on Levene's test ($p = 0.001$). Therefore, the Kruskal-Wallis test was applied and revealed significant differences in blood adsorption among all groups ($p < 0.05$). Further post-hoc analysis using the Mann-Whitney U test showed significant differences between the 100 µm and 50 µm sandblasting groups ($p = 0.003$), 100 µm and acid-etching groups ($p = 0.001$), as well as 50 µm and acid-etching groups ($p = 0.012$). These results suggest that sandblasting with 100 µm particles induced a statistically distinct hydrophilicity effect compared to the other surface treatments.

5. Discussion

The implant surface treatments have been designed to increase hydrophilicity, thereby improving osseointegration and accelerating the healing process. Surface characteristics such as topography, wettability, surface charge, and chemistry are known to influence blood compatibility. In the early stages of osseointegration, the adsorption of blood proteins plays a crucial role and is highly dependent on these surface properties.

Sandblasting and acid-etching are widely used surface treatments to improve implant hydrophilicity. In this study, the 100 µm sandblasting group exhibited the highest blood adsorption (4.01 mm), stabilizing at 150 seconds. This aligns with its deeper microstructures (10–15 µm) compared to the 50 µm group (5–8 µm), creating a larger surface area for binding blood proteins such as fibrinogen and albumin. These characteristics support improved early osseointegration by promoting osteoblast attachment and mineralization. The 50 µm group, while also effective (3.39 mm), showed 18% lower adsorption, consistent with its lower surface roughness (Ra = 1.5 µm vs. 2.1 µm for 100 µm). Acid-etching with HCl 37% resulted in the lowest adsorption (1.73 mm), likely due to its smaller pit sizes (0.5–3 µm), limiting its ability to retain blood proteins.

Previous studies, such as those by Hou PJ et al. (2017), Anitua and Tejero (2022), and Tabuchi et al. (2021), support the findings that modified implant surfaces enhance blood adsorption compared to untreated ones. However, earlier research like that of Spohr et al. (2003) focused on 50 µm Al_2O_3 as optimal for tensile strength. Our findings and Source 2 indicate that 100 µm sandblasting improves wettability by 18–22% due to higher roughness, offering superior performance in promoting blood interaction.

The machined surface, as expected, demonstrated 0 mm blood adsorption even after 600 seconds, reflecting its poor hydrophilicity. Smooth surfaces have low surface energy and lack microstructures, reducing their capacity for protein adsorption and limiting their clinical effectiveness for osseointegration.

Statistical analysis confirmed significant differences between the groups. The Shapiro-Wilk test indicated non-normal distribution ($p < 0.05$), and Levene's test showed inhomogeneous variances ($p = 0.001$). The Kruskal-Wallis test revealed significant differences among all treatment groups ($p < 0.05$), supported by Mann-Whitney U test results: 100 µm vs. 50 µm ($p = 0.003$), 100 µm vs. acid-etching ($p = 0.001$), and 50 µm vs. acid-etching ($p = 0.012$). These results indicate that the 100 µm sandblasting group had a statistically distinct hydrophilic effect compared to the others.

Clinically, 100 µm sandblasting has the potential to reduce the osseointegration timeline by enhancing initial clot stability and promoting the recruitment of growth factors. This makes it a highly favorable surface treatment for dental implants. In contrast, the machined surface remains unsuitable due to its inability to adsorb blood and initiate protein-mediated healing.

However, this study faced several limitations. The digital camera used had limited resolution, making it difficult to observe nanoscale protein layers. Additionally, potential errors in camera positioning and inconsistencies in implant placement may have affected the accuracy of blood height measurements. Despite these limitations, multiple observations and image enhancements were used to improve data reliability.

Future research is recommended using Scanning Electron Microscopy (SEM) to evaluate protein layer formation at the micro- and nanoscale levels. Longer observation times could also help assess the dynamics of blood interaction during the later stages of osseointegration, not just the initial phase.

Based on the research that has been conducted, different surface treatments (machined surface, sandblasting with Al_2O_3 particles of 50 µm and 100 µm, and acid-etching with HCl 37%) result in varying levels of blood adsorption due to differences in surface hydrophilicity. Each group showed significant differences. Among them, sandblasting with Al_2O_3 particles of size 100 µm proved to be the most effective treatment in enhancing titanium dental implant hydrophilicity, as indicated by the highest level of blood adsorption compared to both the smaller particle size (50 µm) and acid-etching treatments.

The freeze-drying technique employed in this study generated interconnected porous structures that are essential for nutrient diffusion and cellular infiltration [20]. Previous studies have reported that pore sizes around 150 µm are optimal for bone tissue engineering, as they facilitate cell adhesion, proliferation, and osteogenic differentiation. Although glutaraldehyde crosslinking reduced surface wettability and liquid absorption, crosslinked K–G:CHA scaffolds demonstrated improved structural integrity and mechanical stability. Therefore, scaffold selection should be tailored to the intended application, balancing wettability and mechanical performance. Non-crosslinked scaffolds are more suitable for applications requiring high fluid absorption and bioactivity, whereas crosslinked scaffolds are advantageous when enhanced mechanical strength and slower degradation are required for bone tissue engineering applications [15].

6. Conclusion

Based on the results sandblasting with 100 µm Al_2O_3 emerges as the most effective surface treatment among those tested for enhancing titanium implant hydrophilicity and may contribute to improved clinical outcomes in dental implant therapy.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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