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RESEARCH ARTICLE

Titanium oxide coating and acid etching on platelet activation in dental implants

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ABSTRACT

Titanium is the most widely used dental implant material, but it requires surface treatment to improve osseointegration. While coating implants with TiO2, residue can arise, which may lead to contamination. To address this, 10% HF etching is added. This study investigated the effect of TiO2 coating using sputtering and HF etching on platelet activation in titanium dental implants. Laboratory experiments were conducted on titanium implants (Ø = 4 mm; length = 12 mm) treated with TiO2 coating and 10% HF etching. Twenty-four samples were divided into four groups (n = 6): Group I (TiO2), Group II (TiO2 + HF 5 min), Group III (TiO2 + HF 10 min), and Group IV (TiO2 + HF 20 min). Platelet activation was assessed using CD41 and CD62P expression markers. Data analysis employed one-way ANOVA and post hoc LSD test (p = 0.05). Platelet activation ranged from 20.31 ± 1.78% to 35.90 ± 3.81%. One-way ANOVA revealed a significant effect ($p < 0.05$) of both TiO2 coating and acid etching on activation. LSD post hoc test showed significant differences ($p < 0.05$) between all groups except for the 10 and 20-minute etching periods. TiO2 coating with 10 minutes of HF etching resulted in the optimal activation level.

Keywords: acid etching; dental implant; platelet activation; titanium oxide (TiO2) coating

INTRODUCTION

Tooth loss, caused by caries, periodontal disease, trauma, socio-demographic factors, and life style can lead to alveolar bone loss and neighboring tooth migration. Rehabilitative treatment with dentures addresses these consequences by improving comfort, mastication, oral health, and self-confidence.[1] An alternative option gaining popularity is dental implants. These replace missing teeth and restore essential functions like chewing, speaking, aesthetics, and optimal tooth-supporting tissue protection. Implanted surgically into the jawbone, dental implants function as replacement roots for artificial teeth, offering a better quality of life compared to conventional dentures.[2] Clinically, the success of implants is gauged by various parameters, including function, aesthetics, patient satisfaction, stable prostheses, absence of peri-implant infections, mobility, pain, and minimal bone loss on X-rays.[3]

Dental implants play a crucial role in replacing missing teeth, but selecting the right material is crucial for their success. The ideal material must exhibit good mechanical, chemical, and biocompatibility properties, readily integrating with the body while providing sufficient strength and prosthetic restorative functionality.[4] Among available options, titanium has gained popularity due to its biocompatibility, minimal toxicity, corrosion resistance, and favorable physical-mechanical properties.[5] These include a low density (around 4.5 g/cm),[3] low elastic modulus, light weight, high strength, and high resilience.[6] However, titanium exhibits limited osseointegration, with its success heavily reliant on the surface geometry, topography, and the entire osseointegration process itself.[7] Therefore, surface modification of titanium dental implants is necessary to enhance cell adhesion, proliferation, and osseointegration, ultimately

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accelerating healing and ensuring treatment success.[8]

Surface treatment can modify the surface characteristics of titanium dental implants, thereby increasing the interaction between the bone and the implant. One method for achieving this is through a coating process with titanium oxide (TiO2), which increases surface roughness. Sputtering is a reliable method for obtaining stable TiO2 coating, and it also produces strong adhesion between the coating material and the implant. Additionally, sputtering offers several other advantages, including protection against bacterial growth, good angiogenesis, corrosion resistance, and biocompatibility.[9] The sputtering process is applicable to a wide range of materials, including metals, nonmetals, alloys, oxides, carbides, nitrides, and polymers. Research has shown that the optimal thickness of the TiO2 layer formed on stainless steel using the sputtering method is ±5nm, which can be achieved with a coating duration of 90 minutes.[10]

While depositing TiO2 coatings on titanium dental implants can enhance bone interaction, the process itself can leave residue on implant surface and irregularities that lead to contamination and hinder healing. Fortunately, subsequent acid etching with 10% hydrofluoric acid (HF) effectively cleanses the surface and creates uniform-sized roughness, promoting tissue integration, cell proliferation, and ultimately, better osseointegration.[11] This crucial step relies on several factors like acid type, concentration, and etching time for optimal results.[12]

For successful implantation, titanium dental implants coated with titanium oxide (TiO2) must be biocompatible and hemocompatible. Biocompatibility ensures the material is safe for human use and does not harm surrounding tissues. Hemocompatibility specifically focuses on compatibility with blood, especially crucial since the implant comes into contact with blood vessels. International standards like ISO 10993 mandate hemocompatibility testing for medical devices contacting blood circulation, either directly or not, including dental implants.[4] Non-hemocompatible materials can disrupt tissues and blood cells, causing issues like hemolysis and platelet activation. During implant placement, which causes bleeding, platelet activation is essential for stopping bleeding and initiating wound healing.[4,13] Therefore, the purpose of this present study was to investigate the effect of TiO2 coating using sputtering and HF etching on platelet activation in titanium dental implants.

MATERIALS AND METHODS

This research has received an ethical clearance from the Ethics Committee, Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada number KE/FK/0797/EC/2023. A laboratory experiment examined the effects of hydrofluoric acid (HF) etching on titanium dental implants coated with titanium oxide (TiO2). Twenty-four implants (Ø = 4 mm; l = 12 mm) were divided into four groups (n = 6): I (TiO2 only), II (TiO2 + 5 min HF 10%), III (TiO2 + 10 min HF 10%), and IV (TiO2 + 20 min HF 10%). Both coating and etching were performed at the National Nuclear Energy Agency (BATAN), Yogyakarta, using a sputtering method for 90 minutes with 70% argon and 30% nitrogen gas. Standby vacuum pressure was 2.5 × 10^{-4} mbar, and operating pressure was 2 × 10^{-2} mbar. Post-treatment, all implants were ultrasonically cleaned with 70% alcohol and NaCl for 10 minutes each.

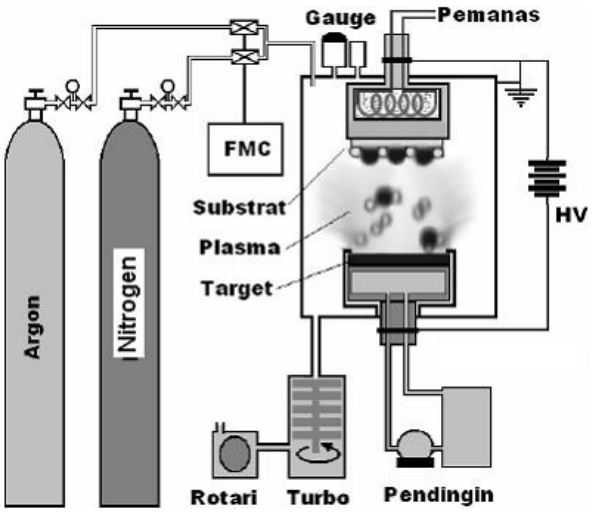

Figure 1. Schematic of the sputtering tool

The hemocompatibility test, specifically a platelet activation test, was carried out at the Clinical Pathology Laboratory, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada. Only one donor who met the inclusion criteria were fully informed about the procedure through an explanation and provided an informed consent form as approval to participate in the research. The inclusion criteria: A donor has a good general health (no routine medication), range age 17-60 years old, body weight at least 45 kg, hemoglobin (Hb) level at least 12.5 gr/dl and maximum 17.5 gr/dl; blood pressure 110/70 to 130/80 mmHg.

A blood sample of approximately 50 mL was then collected from each donor. Venipuncture, a method of collecting blood by puncturing a vein, was used for this purpose. While various suitable veins exist, the median cubital vein located on the anterior arm's inner side (at the elbow crease) was chosen due to its ease of access, size, and minimal presence of large nerves, thus minimizing pain for the donors.[14]

As part of the hemocompatibility test, 10 ml of blood was first drawn and analyzed using hematology analyzer for routine blood values to confirm donor eligibility. Subsequently, 1 ml of blood was mixed with a titanium implant in a test tube, rotated at 37 °C for 3 hours at 10 rotations per minute. After the incubation period, 10 microliters (µL) of blood that had been in contact with the titanium dental implant were extracted to determine the hemoglobin (Hb) value using a hematology analyzer. From the remaining blood sample, 40 µL was centrifuged to separate cellular components (platelets) from plasma. Then, 20 µL of the cellular components were incubated twice, each time with 20 µL of both CD41 and CD62P markers. These markers bind to activated platelets, and their expression level, measured as a percentage, indicates the degree of platelet activation. Statistical analysis using one-way ANOVA (95% significance) with an LSD post hoc test assessed the data.

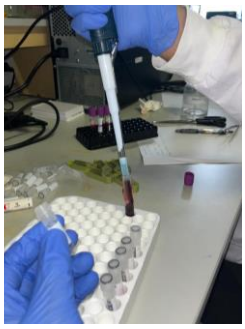

Figure 2. Implant contact with blood

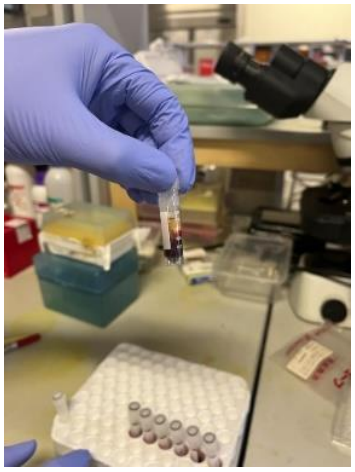

Figure 3. Results of centrifugation of cellular components and blood plasma

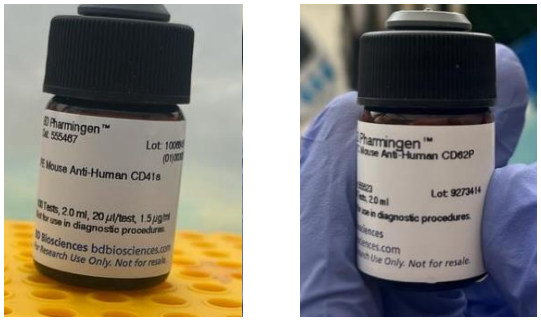

Figure 4. CD41 and CD62 markers

RESULTS

The donor's initial hemoglobin (Hb) level measured 13.1 gr/dL, falling within the normal range for women (12-16 gr/dL).[14] Platelet activation interpretation relies on the diagram in Figure 5 showcasing CD41 and CD62P expression: Q1 (CD41 negative, CD62P positive), Q2 (CD41 positive, CD62P positive), Q3 (CD41 negative,

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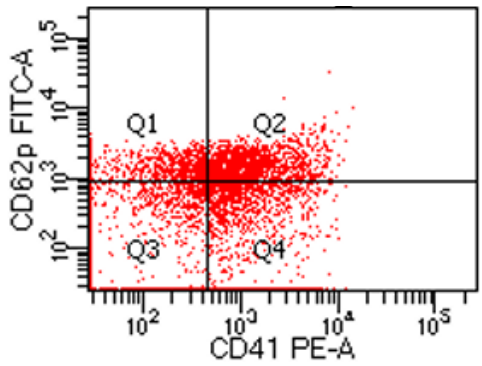

Figure 5. Interpretation example of platelet activation values

CD62P negative), and Q4 (CD41 positive, CD62P negative). This study calculated platelet activation values (in percentages) based on the Q2 diagram based on percentage. Among dental implant groups, the highest mean platelet activation percentage (35.90 ± 3.81%) was observed in the group treated with 10% TiO2 + HF coating for 10 minutes (Group III). Conversely, the lowest mean value (20.31 ± 1.78%) belonged to the titanium implant group with only TiO2 coating (Group I). The detailed results are presented in Table 1.

One-way ANOVA analysis revealed a significant difference in platelet activation percentage across groups ($F(3.16) = 30.888$, $p = 0.000$), indicating statistically meaningful variations between treatment groups. The significance level ($p = 0.000$) is well below the commonly accepted threshold of 0.05, strongly suggesting that observed differences are not due to chance. Details of the mean platelet activation percentage and standard deviation for each group are presented in Table 2.

LSD post hoc test revealed significantly different platelet activation values among the groups (Table 3). Notably, no statistically significant difference was observed between groups II (TiO2 + HF 10%, 5 minutes) and IV (TiO2 + HF 10%, 20 minutes).

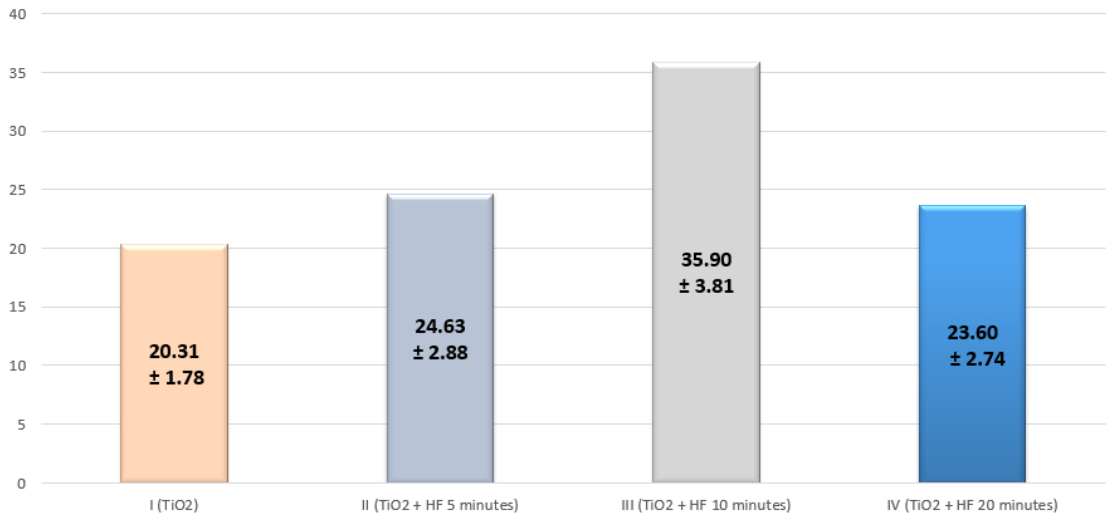

Figure 6. Mean and standard deviation of platelet activation values (%)

Table 1. One way ANOVA test results

	Sum of squares	df	Mean square	F value	p
Between groups	777.375	3	259.125	30.888	0.000*
Within groups	167.782	20	8.389		
Total	945.156	23			

Significant at $p < 0.05$

Table 2. Post Hoc LSD test results in each group

Mean Difference				
Groups	I	II	III	IV
I	-	-4.31*	-15.58*	-6.28*
II		-	-11.27*	-1.97
III			-	9.30*
IV				-

Note: *Significant difference at $p < 0.05$

DISCUSSION

This study assessed platelet activation in titanium dental implants coated with titanium dioxide and treated with 10% hydrofluoric acid etching. To ensure reliable results, freshly drawn blood from human donor was immediately used in the experiments. This is crucial for hemocompatibility research, as stored blood cell components possess increased sensitivity to hemolysis, potentially affecting the experimental outcomes.[15]

This study revealed the highest mean platelet activation percentage in the dental implant group treated with 10% TiO2 + HF coating for 10 minutes (Group III, 35.90 ± 3.81%), and the lowest in the group without acid etching (Group I, 20.31 ± 1.78%). This variation likely stems from changes in the implant surface topography induced by the treatment. The resulting surface irregularities increase available area for bone attachment, enhance surface energy (related to hydrophilicity), and promote cell adhesion, including platelet activation, essential for osseointegration.[13,16] The employed sputtering method effectively coats the implant surface by breaking bonds within the coating material, allowing atoms to arrange and form a rough layer. This process ensures strong adhesion between the TiO2 coating and the implant. Sputtering offers several advantages, including protection against bacterial growth, improved angiogenesis, enhanced corrosion resistance, and good biocompatibility.[9]

Modifying the surface of titanium dental implants with TiO2 particles and subsequent 10% HF etching alters the surface topography, chemical composition, and wettability. This creates an irregular surface, increasing its area and roughness. Additionally, the chemical composition and wettability induce a hydrophilic nature to the implant surface. When blood interacts with the implant, friction occurs between blood cells and the surface. These combined effects can enhance platelet activation, blood clotting, wound healing, and osseointegration.[15,16]

Titanium dental implants directly interact with blood, cells, and tissues, requiring hemocompatibility to maintain blood component balance, prevent cell damage, and avoid altering plasma protein. Surface topography plays a crucial role in this hemocompatibility, [17] with platelet activation being a key parameter. Therefore, both the TiO2 coating and 10% HF etching applied in this study must uphold hemocompatibility standards due to their direct blood contact during implantation. During implant installation, bleeding occurs, triggering platelets to initiate clotting and healing at the implant site.[15] The first cell types that arrive at the implant site are platelets, which then release a variety of proteins with well-known impacts on the healing process. Platelet attachment and activation can modulate the inflammatory response, bone wound healing process, and ultimately, the osseointegration. To assess platelet activation, the CD41 marker serves as a specific identifier, present only in platelets and absent in other blood cells. Additionally, the CD62P (P-selectin) marker specifically indicates platelet activation, as it's normally stored within their granules and only exposed upon activation.[18] The presence of CD41 dan CD62P in this study, play an important role in platelet activation value that modulate early bone healing process and osseointegration.[19]

LSD Post Hoc analysis revealed statistically significant differences in platelet activation values across all groups, except for Groups II (TiO2 + HF 10%, 5 minutes) and IV (TiO2 + HF 10%, 20 minutes). A 5-minute etching duration (Group II) might have been insufficient, leaving residual sputtering material that could hinder osseointegration, affect platelet interactions, and

alter red blood cell membrane permeability due to incomplete removal of the previous TiO2 coating, ultimately influencing the observed activation value of permeability and blood homeostasis.[20] Conversely, excessive etching (20 minutes) could remove excessive material, reducing surface roughness and potentially inducing weaker bonds between coating, etching material, and the implant itself, ultimately impacting wettability and hindering platelet activation and cell attachment.[17] Notably, the 10-minute etching duration employed in Group III yielded the highest activation value, possibly due to its creation of a homogenous rough surface promoting blood compatibility through ideal wettability, adhesion, stability, and hydrophilicity, all crucial factors for smooth blood flow and activation of various blood components during implant placement, ultimately contributing to successful osseointegration and implant success.[11,21] The limitation of this study was how the surface topography and surface roughness after coating with TiO_2 and HF acid etched processes could not be known. Therefore, further research is needed to determine the surface roughness, contact angle, and pore size formed after the surface treatment.

CONCLUSION

This study confirms that applying titanium oxide (TiO2) coating using the sputtering method and hydrofluoric acid (HF) etching positively impacts platelet activation on titanium dental implants. Notably, the optimal etching duration for this effect was identified as 10 minutes.

CONFLICT OF INTEREST

The authors declare no conflict of interest with the data contained in the manuscript.

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