Bio-Functional Coating on Ti6Al4V Surface Produced by Using Plasma Electrolytic Oxidation

Aqmar Zakaria,1,* Hamdi Shukor,2,3 Masahiro Todoh,4 and Kamaruzaman Juso5

1 Graduate School of Engineering, Hokkaido University, 8 Chome Kita 13 Jonishi, Kita Ward, Sapporo, Hokkaido 060-8628, Japan
2 Centre of Advanced Manufacturing and Material Processing, University of Malaya, Kuala Lumpur 50603, Malaysia; hamdi.shukor@ukm.edu.my or hamdi@um.edu.my
3 Chancellory Office, Universiti Kebangsaan Malaysia, Bangi 43600, Selangor, Malaysia
4 Faculty of Engineering, Hokkaido University, 8 Chome Kita 13 Jonishi, Kita Ward, Sapporo, Hokkaido 060-8628, Japan; todoh@eng.hokudai.ac.jp
5 UKM Press, Universiti Kebangsaan Malaysia, Bangi 43600, Selangor, Malaysia; kjuso@yahoo.com or kjuso@ukm.edu.my

* Correspondence: aqmar.zakaria@gmail.com or aqmar_zakaria@eis.hokudai.ac.jp

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Abstract: One way to improve the biofunctionality of titanium alloys is by implementing plasma electrolytic oxidation (PEO) to incorporate bioactive elements such as fluoridated hydroxyapatite, into surface coatings of orthopaedic and dental implants. Hydroxyapatite (HAp) is known as a bioactive coating while fluorapatite (FAp) has an antibacterial effect that would enhance the bio-functionality and reduce the failure rate of orthopaedic and dental implants. The purpose of this study was to develop fluoridated hydroxyapatite as a bio-functional coating on Ti6Al4V with electrolyte containing trisodium orthophosphate, potassium hydroxide, and calcium fluoride. The coating surface and cross-section morphologies were evaluated, and the species in the electrolyte solution were found, and irregular micropores shapes were observed by field emission scanning electron microscopy (FESEM) and energy dispersive spectrometer (EDS). The phase composition of the coating surface containing TiO2 (anatase and rutile), tricalcium orthophosphate, HAp, and FAp was characterized by X-ray diffractometer (XRD). The adhesive strength of the coating was analysed by a micro-scratch test. Simulated body fluid (SBF) immersion test was performed to investigate the bioactivity of the coating. In this study, we demonstrated that the PEO technique has a good potential to develop bio-functional surface modifications that can affect the chemical composition and roughness of the coating surface. The FAp coating may provide insights for subsequent bioactive coatings while improving the antibacterial properties for orthopaedic and dental implants. Future work shall investigate the optimal amount of fluoride in the coating layer that obtains excellent results without causing adverse effects on adjacent tissue.

Keywords: hydroxyapatite; fluorapatite; plasma electrolytic oxidation; Ti6Al4V; bioactive coating

1. Introduction

Titanium and its alloys have been widely used for medical applications. In particular, titanium is a feasible material for dental and orthopaedic implants owing to its excellent properties, including high strength-to-weight ratio, corrosion resistance, and bioinert properties [1]. Despite those advantages, titanium always exhibits weak biological bonding with adjacent tissues [2]. Therefore, the incorporation of bioactive materials at the titanium/bone tissue interface would enhance the functionality and bioactivity of titanium substrates. Calcium phosphate, or hydroxyapatite (HAp), is among the main
natural elements of the bone, and has the potential to endow bone formation on the implant surface [3]. Fluoride is a sought after element for its antibacterial effects [4]. It is able to disrupt bacterial metabolism and boost the proliferation and differentiation of osteoblast cells. Antibacterial properties are not only crucial to prevent dental caries for dental implants, but they also hinder initial post-surgery infections after the insertion of orthopaedic implants, which always leads to implant failure [5]. The combination of HAp or tricalcium phosphate with fluoride, is known as fluorapatite (FAp), and has been recently corroborated to bear the potential of reinforcing mineralization and crystallization of HAp on the coating surface. Therefore, FAp would extensively increase the performance and durability of dental implants and prostheses.

In order to incorporate these elements onto and into the coating layer, a surface treatment technique could be applied. Thus, surface treatment methods, such as conversion coating, electroplating, anodization, plasma electrolytic oxidation (PEO), organic coating, and vapor-phase processes have been exploited as means to accomplish this purpose. Among these methods, PEO, or micro-arc oxidation, has been employed on valve metals and its alloys, namely Mg, Ti, Al, and Zr, with alkaline or phosphate electrolyte solutions, which is then followed by micro-spark formation, resulting in a ceramic-like coating. PEO is one of the techniques that has gained attention in recent years [6]. The development of bio-functional PEO-treated coating with an electrolyte solution consisting of bio-functional elements such as Zn, Mg, Mn, Si and Sr recently seems to be necessary [7,8]. Besides, the incorporation of calcium and phosphate elements via the PEO of Ti alloys has been reported by several authors [3,9–12]. Despite the need for implants to have antibacterial properties, only several antibacterial coatings have been developed by using distinct antibacterial elements such as Cu, Ag, Zn, and Zr through the PEO technique, and their antibacterial properties have been examined [13–15]. PEO coating incorporated with fluoride element has been investigated until the present time for various purpose such as to enhance the corrosion resistance of Mg and Al [16–18]. More specifically, Santos-Coquillat et al. studied PEO coating enriched with fluoride to reinforce the biological performance of titanium implants [5,19]. PEO coatings are commonly amorphous or crystalline in phases, and the additional elements of the coating could be possibly tailored by adjusting the process parameters and electrolyte constituents. During PEO treatment, the TiO$_2$ layer is initially formed, then the cationic and anionic components are merged into the coating layer. As previously mentioned, by introducing calcium phosphate and fluoride to the substrate surface [9,20], it would promote a better bone ingrowth to the implant surface [21].

Santos-Coquillat et al. [5] developed a multifunctional PEO coating on a Ti6Al4V substrate for orthopaedic applications by incorporating Ca and P as bioactive elements and adding Si and F as antibacterial elements. Hydrogen fluoride, nitric acid, and calcium carbonate were used as the electrolyte solution in this study. The authors studied the effect of treatment time on the coating composition and its morphology, and they investigated the coating’s bioactivity and antibacterial effect with osteoblast cells and S. aureus, respectively. The result demonstrated that a longer treatment time altered the TiO$_2$ phase from anatase to rutile and promoted the generation of HAp and FAp. With respect to the antibacterial effect, the coating with the highest amount of fluoride exhibited the least number of bacteria on the coating surface, up to 60%, which is a significant result. Hence, this study exhibits that fluoride is an excellent candidate as an antibacterial element.

A prior study has documented the effectiveness of using a CaF$_2$ additive in a phosphate electrolyte solution to form an oxide layer on a magnesium alloy by PEO, which then successfully improved the corrosion resistance and bioactivity of the substrate [22]. In the present work, we extend the study on the growth of bio-functional coatings with a one-step PEO approach, using alkaline phosphate electrolyte solution containing trisodium orthophosphate, potassium hydroxide and calcium fluoride, on Ti6Al4V. The results discuss the electrical response, phase composition of the coating and the mechanism of coating growth, surface and cross-section morphologies, and the adhesion of the coating onto the substrate, which were determined by micro-scratch tests. Finally, the bioactivity of the
coating was analysed by evaluating the apatite formation ability with simulated body fluid (SBF) immersion test.

2. Materials and Methods

2.1. Substrate Preparation

Ti6Al4V substrates of 15 mm × 15 mm × 2 mm were abraded with SiC emery paper up to 2000 grade. Subsequently, they were polished with an Extec Chemic Cloth and diamond suspension. Then, the abraded substrates were rinsed with distilled water and cleaned ultrasonically with 70% ethanol and distilled water before PEO treatment. These cleaning procedures were conducted to ensure that the surfaces of the substrates were free from contaminants prior to the PEO experiments.

2.2. PEO Treatment

Ti6Al4V (Tokyo Titanium Co., Ltd., Saitama Prefecture, Japan) substrates were immersed in the electrolyte. The electrolyte solution comprised 5 g of tri-sodium orthophosphate (Ajax Finechem, Australia), 3 g of calcium fluoride (Sigma-Aldrich, Saint Louis, MO, USA), and 2 g of potassium hydroxide (Sigma-Aldrich, USA) in 1000 mL distilled water with a pH of 12.55. Then, the solution was stirred using a magnetic stirrer for 2 h and subsequently sonicated using an ultrasonic bath for another 2 h. These two steps are crucial to ensure the chemical powders are well dissolved in distilled water and to prevent any settling and agglomeration of the powder particles. The substrates were set as the anode, and the stainless-steel beaker containing the electrolyte solution served as the cathode. Recommended material for the cathode in the PEO process is an inert material such as stainless steel with a purpose simply to complete the circuit. PEO treatment was performed for 10 min under an auto-ranging DC power supply unit (Keysight Technologies Deutschland GmbH, Model No: N8957A, Boeblingen, Germany) with a maximum voltage of 1500 V and a current capacity of 30 A. The setup was equipped with a stirrer to ensure the consistency of the electrolyte and a water-cooling system to maintain the electrolyte at a low temperature during the process (Figure 1). The temperature of the electrolyte was recorded before and upon completing the treatment. Before the process, the temperature was 23 °C, and it eventually increased to 53 °C upon turning off the power supply. Considering the safety of the PEO user, the temperature of the electrolyte was not measured during the process. The parameters that were used for the PEO treatment are shown in Table 1. The electrical response was recorded throughout the process.

Figure 1. Plasma electrolytic oxidation setup.
Table 1. Plasma electrolytic oxidation (PEO) parameters used during the process.

| PEO Parameters          | Quantity |
|-------------------------|----------|
| Voltage threshold (V)   | 400      |
| Maximum current applied (A) | 1        |
| Treatment time (min)    | 10       |

2.3. Coating Characterization

Surface morphology and elemental composition of the coated substrates were observed by FESEM and EDS (FESEM-EDS, JSM-7001FA, JEOL, Tokyo, Japan). Pore density population and sizes on the coating were estimated by using open source software ImageJ. The substrate was cross-sectioned and carbon-coated to study the thickness and the distribution of the elements across the coating. The coating layer formed on the Ti6Al4V substrate was analysed using a Rigaku Smartlab XRD (CuKα = 0.154056 Å) at a step size of 0.02° and a scanning speed of 2° per minute from a 5° to 90° scan range of 2θ. Then, the phase compositions of the PEO coating were analysed using PDXL Version 2.2 software (Rigaku Corporation, Tokyo, Japan), which is an integrated XRD analysis software for Rigaku Smartlab XRD.

2.4. Micro-Scratch Test

Micro-scratch tests (Micro Materials Ltd. Wrexham, UK) were performed using a conical Rockwell diamond tip of a radius of 25 µm. The scratch length was set to 1000 µm, and a linearly increasing load in the range of 0–2000 mN was fixed. The onset of delamination at the critical load Lci was taken as the adhesive strength of the coating substrate system. Finally, the scratch track was probed by optical microscopy to observe the failure modes of the coating.

2.5. In Vitro Study of Bioactivity

In vitro bioactivity of coated substrates was determined by evaluating the apatite-forming ability. Static simulated body fluid was used as a soaking solution with an ionic composition almost equal to human plasma. The solution was prepared according to the protocol by Kokubo et al. by dissolving reagents of NaCl, NaHCO3, KCl, K2HPO4, 3H2O, MgCl2·6H2O, CaCl2, Na2SO4 and (HOCH2)3CNH2 with 1000 mL. Tris-HCL (Tris-hydroxymethyl aminomethane—hydrochloric acid) served as buffer to maintain a constant pH value of 7.4 at 36.5 ± 0.5 °C. Uncoated substrates (control samples) and coated substrates were immersed in 30 mL of SBF for 14 and 28 days in an incubator at a constant temperature of 37 °C. After various immersion times, the substrates were washed thoroughly with distilled water and allowed to dry under room temperature. Dried immersed substrates were kept in a desiccator before surface characterization.

Apatite formation on the coating surfaces of SBF-immersed samples was characterized by FESEM, EDS and XRD. The morphologies were observed by FESEM and the chemical compositions and crystallization of dispersed apatite on the coating surfaces was examined by EDS and XRD with similar setting parameters in 2.3. The FESEM images of apatite deposited on the coating surfaces was examined by ImageJ software.

3. Results and Discussion

3.1. Electrical Response of PEO Coatings

Figure 2 displays a typical output voltage shift and current behaviour which were acquired during the PEO process of the Ti6Al4V substrate. According to few studies [23–25], the plot could be segregated into four different stages, indicating the changes in coating resistance, viz., Stage I, Stage II, Stage III, and Stage IV.
Figure 2. A real-time plot of the voltage and current acquired for 10 minutes of treatment time.

Stage I is the onset of the process. The voltage and current escalate rapidly for 10 s only, and this pattern coincides with the passive oxide film growth, known as anodic oxidation in the conventional electrochemical method [26]. The passive oxide film functions as a barrier layer between the metal substrate and electrolyte during the early stage of the process, as illustrated in Figure 3a.

Stage II is when the voltage transient begins to decline, but the current keeps linearly increasing until the end of this stage. The micro-arc sparks could be noticed to emerge across the substrate surface, during the process which suggests the breakdown of the passive oxide layer (Figure 3b) [27]. Furthermore, a higher voltage causes local heating at the substrate/electrolyte interface with high temperature and pressure, and it eventually induces the melting of the substrate where the Ti, Al and V ions and electrons are ejected, as shown in Equations (1)–(3).

Stage III shows the current becoming constant, and the voltage change is much slower compared to Stage II. The coating grows thicker and augments the resistance of the PEO coating; thereby, it modifies the feature of the plasma and larger sparks could be observed during the process.

Stage IV is when the voltage reaches its threshold of 400 V, and the current begins to oscillate to find the stability of the circuit. The sparks become more powerful, which may cause thermal cracking. The instability at points 1 and 2 may be inferred as the crack formation and lead to disturbances in current flow through the coating. In addition, emergence of the oscillating current may represent the stage of oxide breakdown and regrowth, which maintains the thickness of the layer since the voltage is limited to 400 V only.

3.2. The Phase Composition of the Coating and the Mechanism of Growth Coating

The element composition was probed on the coating surface and the Ca/P ratio was determined. The coating had a Ca/P ratio of 1.303 ± 0.01, which is lower than the ratio of stochiometric hydroxyapatite (1.67) [28]. Figure 4 illustrates the XRD pattern of Ti6Al4V and the PEO coating, which exhibited seven distinct phase compositions, namely FAp, HAp, tricalcium phosphate, anatase, rutile, aluminium oxide and vanadium (V) oxide which were obtained during the PEO process. XRD process was carried out to corroborate and to analyse the phases that were incorporated in the coating layer. As reported in a previous study by Laveissière et al. [29], all the compounds used in the electrolyte solution preparation would be involved in the process. However, the elements that do not play any key role to fulfil the purpose of the coating development were not analysed. Anatase and rutile are two polymorphs from TiO2 that have a tetragonal crystal structure and are favourable for bone implants, owing to their
bioactivity and biocompatibility [9,12]. According to Hussein et al. [27], the coating layer growth mechanisms could always be classified into two different layers, inner layer (inward growth) and outer layer (outward growth). The dissolution of the Ti, Al and V from the substrate and oxygen diffusion produce TiO₂, Al₂O₃ and V₂O₅ in the inner layer.

![Figure 3](image_url). Schematic of the PEO process during the four different stages (adapted from [27]): (a) passivation of oxide film in Stage I; (b) breakdown of oxide layer in Stage II; (c) incorporation of chemical species from electrolyte into the coating layer in Stage III; and (d) the repetition of the process with three distinct types of plasma discharge in Stage IV.

![Figure 4](image_url). XRD pattern of the different phase compositions on the coating surface layer.
A high voltage is one of the features of PEO process, and in most cases, the dissolution of the substrate occurs when the voltage passes the breakdown voltage, where it then instantaneously generates a micro-arc discharge and eventually induces the dissolution of the Ti, Al and V of the substrate (Equations (1)–(3)). Potassium hydroxide (KOH) is ionized to form hydroxyl ions (Equation (4)), which then evolve into gaseous O\(_2\) (Equation (5)). The O\(_2\) appears in either two forms; a gas or O\(^{2-}\) ions, which leads to the formation of TiO\(_2\). Ti\(^{4+}\) would subsequently be attracted to O\(^{2-}\) (Equation (7)) and slightly react with OH\(^-\) (Equation (6)) resulting from the electrostatic attraction to form TiO\(_2\). Likewise, Al\(_2\)O\(_3\) and V\(_2\)O\(_5\) were formed through the direct oxidation and dissolution of Al and V of the substrate. However, the substrate has a higher constituent of Ti compared to Al and V, thus, the coating layer is predominantly composed of TiO\(_2\).

\[
\begin{align*}
\text{Ti} & \rightarrow \text{Ti}^{4+} + 4e^- \quad (1) \\
\text{Al} & \rightarrow \text{Al}^{3+} + 3e^- \quad (2) \\
\text{V} & \rightarrow \text{V}^{5+} + 5e^- \quad (3) \\
\text{KOH} & \rightarrow \text{K}^+ + \text{OH}^- \quad (4) \\
4\text{OH}^- & \rightarrow \text{O}_2 + 2\text{H}_2\text{O} + 4e^- \quad (5) \\
\text{Ti} + 4\text{OH}^- & \rightarrow \text{TiO}_2 + 2\text{H}_2\text{O} + 4e^- \quad (6) \\
\text{Ti}^{4+} + 2\text{O}^{2-} & \rightarrow \text{TiO}_2 \quad (7) \\
\text{Al}^{3+} + \text{O}^{2-} & \rightarrow \text{Al}_2\text{O}_3 \quad (8) \\
2\text{V}^{5+} + 5\text{O}^{2-} & \rightarrow \text{V}_2\text{O}_5 \quad (9)
\end{align*}
\]

In addition, the thickening of the coating was due to the ejection of the oxidized molten Ti through the discharge channels to the surface. The erupted molten TiO\(_2\), as interpreted in Figure 3c, rapidly cooled down and solidified at the coating/electrolyte interface [30,31]. As described by Hussein et al. [27], discharge cavities repeatedly occur throughout the process, allowing the molten oxidized Ti to pass through the channels and solidify on the surface. As a matter of fact, this phenomenon is committed to the growth of the outer layer.

Ionization of the electrolyte causes the compounds calcium fluoride and tricalcium phosphate to ionize into ions such as Ca\(^{2+}\), HPO\(_4^{2-}\) or PO\(_4^{3-}\), OH\(^-\), and F\(^-\), which form a sea of ions in the electrolyte solution. These ions then incorporate into the TiO\(_2\) layer and TiO\(_2\), assisting the nucleation and crystallization of HAp, tricalcium phosphate, and FAp by micro-discharges on the coating surface, as depicted in Figure 3d. Moreover, electrical attraction during the process draws fluorine and phosphorus anions to the substrate and contributes to the formation of HAp and FAp [22]. The equations of the reactions are as below:

\[
\begin{align*}
3\text{Ca}^{2+} + 2\text{PO}_4^{3-} & \rightarrow \text{Ca}_3(\text{PO}_4)_2 \quad (10) \\
5\text{Ca}^{2+} + 3\text{PO}_4^{3-} + \text{OH}^- & \rightarrow \text{Ca}_5(\text{PO}_4)_3(\text{OH}) \quad (11) \\
5\text{Ca}^{2+} + 3\text{PO}_4^{3-} + \text{F}^- & \rightarrow \text{Ca}_5(\text{PO}_4)_3\text{F} \quad (12)
\end{align*}
\]

Hence, an outer layer at the coating surface/electrolyte interface was formed by micro-discharge and the emission of molten Ti to the surface, which eventually oxidized and solidified.

### 3.3. Surface and Cross-Section Morphologies

SEM images of the surface morphologies of the PEO coating produced from the phosphate–alkaline electrolyte are presented in Figure 5a. The coating surface was overlaid with irregular micropores with different sizes and dominated by crimped shape pores. As shown in Figure 5d, the micropores size was
in the range of 0.158 µm² to 34.013 µm² (surface characteristics of the coating can be found in Table 2), and these pores may promote cell adhesion to the coating surface [32]. Sowa et al. [33] investigated the bioactivity of a coating containing Ca and P on a Ti alloy substrate, where the mesenchymal stem cells proliferated into the osteoblast cell with regards to the nature of Ca and P as natural bone elements. On top of that, the cells were well spread on the coating surface as they responded to the pores that formed on the PEO coating, which looked like the structure of porous bone. In fact, the biological response of the cell depends on surface features such as the chemical composition and surface topography [34–39]. Therefore, the micropore features developed by the PEO technique would enhance the proliferation and differentiation of cells [39]. The micropores could be interpreted from the release of gaseous oxygen and tracks of the micro-arc discharge channels. During the process, Ti ions from the substrate were dissolved and ousted through the discharge channels to the coating/electrolyte interface; meanwhile, oxygen ions diffused into the coating layer, which contributed to the formation of TiO₂. In short, the rapid solidification of the Ti oxide occurred, thereby sintering and accumulating on the coating surface, which eventually contributed to the growth of the coating.

Owing to the rising local temperature on the substrate and the electrolyte temperature that was kept as low as possible during the process, the molten Ti oxide promptly solidified and induced thermal stress and residual stress. The evidence points to the likelihood that, in most cases, these may contribute to the formation of cracks on the coating surface, as illustrated in Figure 6.

Table 2. Surface characteristics of the coatings.

| Properties of the Coatings                  | Quantity         |
|--------------------------------------------|------------------|
| Population density of pores (pores/mm²)    | 760,423.599      |
| Pore size (µm²)                            |                  |
| Min.                                       | 0.158            |
| Max.                                       | 34.013           |
| Average size (µm²)                         | 1.315            |
| Standard deviation                         | 2.952            |

![Figure 5](image-url)  
**Figure 5.** Image analysis to measure the population density of pores: (a) original image; (b) thresholded image; (c) segmentation after using the built-in Analyze Particles Image J plugin; and (d) histogram of pores distribution.
The presence of crooked lines at the coating/substrate interface resulted from the dissolution of the substrate in the early stage of the PEO process [40]. A typical PEO coating always comprises two particular layers (as mentioned before), namely the inner layer and outer layer where inhomogeneous pores and defects are spread on both layers. The inner layer is well adhered, less perforated, and has better mechanical properties compared to the outer layer. Then, the extent of connected porosity can be observed at the cross-section, as shown in Figure 7a,b. The total thickness of the coating surface varied in the range of 5–9 µm. The thickness of the coating was measured at ten distinct points, and the average coating thickness was calculated from those points; thereby, the average coating thickness was 7.89 µm with a standard deviation of 0.46. It appears that the cracks on the outer layer may be associated with the grinding process of the cross-section sample for SEM analysis and proving that the outer layer is flimsy and not dense. According to the Pilling–Bedworth ratio (P–B ratio), titanium’s P–B ratio is approximately 1.73, where the underlying metal is protected against further oxidation or corrosion by the passivated oxide coating; Figure 7b shows the contrary—there were open micropores on the coating layer. Repeated melting, melt-flow, and the re-solidification of the oxide coating from the plasma discharges and large sparks probably caused the formation of these open micropores. Despite these microstructures, corrosion might not happen due to titanium’s outstanding resistance to corrosion properties.

It was found from cross-sectional SEM images and accompanying EDS maps (Figure 8) that Ti, Ca, P, and F spread over the surface layer of the sample, while Al and V elements came from the dissolution of the substrate. V can scarcely be found compared to Ti and Al due to lower composition in the substrate. The distribution of chemical elements confirmed that the compounds dissolved in the electrolyte play an important role during the PEO process, according to the ratio of compounds used during the preparation of the electrolyte solution. Oxygen typically can be identified covering up the coating layer, ascribed to the oxidation during the process, which eventually nurtured the nucleation and adsorption of HAp and FAp on the outer layer of the coating. Because calcium was entangled along the coating surface, tricalcium phosphate, HAp, and FAp could be found on the outer layer
only. The power supply for PEO treatment plays a vital role in the resulting coating characteristics. DC power supply introduced positive bias where it would diminish the ability to attract positively charged ions to the surface by electrostatic forces. Therefore, the Ca incorporation in Figure 8 appears deficient, thus the condition was unconducive for the nucleation of HAp and FAp onto the surface coating during the treatment.

![Figure 7](image)

**Figure 7.** The cross-section morphology with: (a) two typical layers: inner layer and outer layer; (b) the open micro-discharge channels developed in the coating layer.

![Figure 8](image)

**Figure 8.** Cross-section EDS maps of the coating with different chemical elements, namely Ca, P, F, Ti, Al and V.

3.4. **Micro-Scratch Test**

The micro-scratch test was carried out to assess the adhesive strength of the coating layer on the Ti6Al4V substrate. This test was extensively used in the coating industry and coating research laboratories to assess the tribological properties of coatings [41]. HAp and FAp were ceramic coatings that were considered brittle, especially in bulk form, and yet it is somehow proven they were indemnified of this limitation when they were deposited as a coating. Thus, it is substantial to understand its mechanical behaviours and its failure modes with crack patterns when stresses are applied to the coating.

During the micro-scratch test, a progressive normal load was applied through a diamond indenter on the coating surface from left to right, as shown in Figure 9. The key transition points of the coating failure mechanisms are determined by a critical load, and these were confirmed by microscopy.
The coating started to indicate traces of failure when the diamond indenter was pulled over the surface of the coating. Coating failure can be determined with critical loads, and in this study, $Lc_1$ and $Lc_2$ were the two values generally depicting the different degrees of failure. The failure commenced with the plastic deformation where no spallation of the coating with minor cracks could be found. In this study, $Lc_1$ was designated as the onset of delamination or major cracks, where transverse, semi-circular chips on the trackside were observed after this point. The indenter penetrated more into the coating, which generated mild tensile cracking, and the indenter tip applied stress on the coating, where stress on the coating was relieved by delamination on the trackside. The $Lc_2$ was recorded when the coating broke through the scratch path and uncovered the substrate as a result of the induction of an adequate normal load and penetration depth of the indenter into the coating layer. The critical loads were quantified in accordance with the scratch profile of the load-displacement plot, and the scratch image was obtained from an optical microscope, as shown in Figures 9 and 10. The $Lc_2$ happened approximately at the scratch length of 717.13 µm with a critical load of 1553.38 mN, a depth of 522.56 nm, and a frictional load of 577.91 mN. This indicated that the diamond touched the substrate surface. This result is comparable with previous work by Adeleke et al. [10].

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3.5. In Vitro Bioactivity: Simulated Body Fluid (SBF) Immersion Test

In general, the in vivo bioactivity of biomaterial could be explained by bone bonding mechanisms. It involves five stages where the first two stages could be mimicked by an in vitro solution with an ion concentration almost equal to human blood plasma to predict the ability of bone tissue to interlock onto the surfaces of biomaterial. The five stages of the bioactivity mechanisms of biomaterials related to bone application are: the reduction of homeostatic pH at the implant/bone interface environment, the dissolution of calcium and phosphate ions from the biomaterial and concurrent apatite formation as a foothold of bone bonding on the material surface, the generation of extracellular matrix, the mineralization of collagen fibrils, apatite and the reinforcement of bone fixation at the bone/implant interface by mineralized collagen [42–44].
To evaluate the apatite formation ability of the coating, static SBF immersion test was performed for two distinct immersion periods (14 days and 28 days). After the immersion test was completed, the morphologies of the apatite formed on the coating were analysed by FESEM as shown in Figure 11. The size range of apatite formed on the coating surface was analysed by ImageJ as shown in Table 3. We observed that the white flocculent products were present for both coating surfaces with different immersion periods. After 14 days of immersion in SBF solution, the flocculent apatite was randomly dispersed, especially around the edges of micro-pores on the surface coating with a size range of 0.098–0.237 μm² (Figure 11b). Larger size of flocculent conglomerates could be seen for the SBF-immersed substrate of 28 days with a size range of 0.258–1.093 μm² generated on the coating surface as shown in Figure 11d. Measurement of the Ca/P ratio of these two SBF-immersed substrates is revealed by EDS analysis. SBF-immersed coated substrates exemplify a slightly lower Ca/P ratio compared to the coated substrate before the SBF immersion test as discussed earlier. These differences resulted from the partial dissolution process of tricalcium phosphate, HAp and FAp from the coating layer to upsurge the concentration of Ca²⁺ and PO₄³⁻ in the solution environment which eventually provoked the nucleation and precipitation of apatitic crystallization while merging with carbonate, hydroxyl and various cations from the SBF solution [44,45].

Therefore, the biomaterial for orthopaedic application is necessary to have the ability to marginally dissolve to induce the apatite formation process of osseointegration between adjacent bone tissue and implants. The prolonged immersion time in the SBF could also enhance the formation of apatite as evidenced in Figure 12a. The increase in the Ca/P ratio in SBF-immersed substrate of 28 days (control sample); and (c) coated Ti6Al4V immersed for 28 days. These differences resulted from the partial dissolution process of tricalcium phosphate, HAp and FAp from the coating layer to upsurge the concentration of Ca²⁺ and PO₄³⁻ in the solution environment which eventually provoked the nucleation and precipitation of apatitic crystallization while merging with carbonate, hydroxyl and various cations from the SBF solution [44,45].

Therefore, the biomaterial for orthopaedic application is necessary to have the ability to marginally dissolve to induce the apatite formation which will promote the chemical bond with the host bonny tissue. Uncoated Ti6Al4V substrates were immersed for 14 days and 28 days as well and they act as control samples to ascertain the Ti alloys could not activate any apatite nucleation on the surface. FESEM-EDS analysis upheld that uncoated Ti alloy could not provoke any apatite formation where Ca and P elements could not be detected, hence it confirmed that biofunctional coating would strengthen the bone bonding mechanism between the implant and the adjacent bone tissue.

**Figure 11.** Morphologies of apatite formed after the SBF immersion test: (a) uncoated Ti6Al4V immersed for 14 days (control sample); (b) coated Ti6Al4V immersed for 14 days; (c) uncoated Ti6Al4V immersed for 28 days (control sample); and (d) coated Ti6Al4V immersed for 28 days.
Table 3. Apatite sizes and Ca/P ratio at post-immersion in simulated body fluid (SBF).

| Properties of Apatite | 14 Days    | 28 Days    |
|-----------------------|------------|------------|
| Size range of apatite (µm²) | 0.098–0.237 | 0.258–1.093 |
| Ca/P ratio            | 1.296      | 1.294      |

The phase compositions of the SBF post-immersion PEO coating and titanium substrates were investigated by XRD, as shown in Figure 12a,b. The XRD pattern of SBF post-immersion of titanium substrate shows that the crystallinity of apatite could not be found. The phases of anatase, rutile, tricalcium phosphate, HAp and FAp already existed in the coating, as shown in Figure 4, and the phases were enriched under the SBF condition after 14 days and 28 days of immersion time. The XRD pattern for titanium substrate indicated that the Ti6Al4V substrate could not induce apatite formation on the surface while PEO-coated titanium with bio-functional elements spontaneously allow the growth of apatite on the surface. This suggests that PEO coating with HAp and FAp has good bioactivity and Ti6Al4V surface could not prompt the nucleation of HAp, thus it would delay the process of osseointegration between adjacent bone tissue and implants. The prolonged immersion time in the SBF could also enhance the formation of apatite as evidenced in Figure 12a. The increase in tricalcium phosphate, HAp and FAp compounds on the coating is the result of the reaction between the Ca²⁺ and PO₄³⁻ in SBF solution with TiO₂ and F⁻ in the coating during the immersion test. According to [46], the presence of FAp in the coating assists in improving the bioactivity of the coating, therefore the incorporation of fluoride elements into the coating becomes an excellent alternative for more robust orthopaedic implants application.

Figure 12. XRD analysis results after the SBF immersion test with two immersion times (14 days and 28 days): (a) coated Ti6Al4V substrates; and (b) uncoated Ti6Al4V substrates.
4. Conclusions

The chemical element constituents from the species contained in the electrolyte solution were successfully incorporated into the coating layer. The TiO$_2$ coating layer promotes the nucleation and formation of tricalcium phosphate, HAp, and FAp, and this was assisted by plasma discharges that happened during the process. The presence of HAp and FAp in the coating layer could contribute to the bioactivity and antibacterial properties of the TiO$_2$–PEO coating layer. The irregular micropores, with sizes approximately up to 34.013 $\mu$m$^2$ on the coating surface, are the traces of discharges that may offer a better proliferation of osteoblast cells on the coating. However, the bioactivity test of the actual osteoblast cells response to these PEO-treated coatings will require specific investigations in a further step of the work. Overall, the PEO hydroxyapatite fluorapatite coating exhibited the potential to overcome the deficits of titanium alloys and supported the application of the coating for orthopaedic implants. Future work shall investigate the optimal amount of fluoride in the coating layer that obtains excellent results without causing adverse effects in adjacent tissue.

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