

UV-induced Precipitation of Bone-like Apatite on Anodised Titanium in Simulated Body Fluid

T.C. Lee, H.Z. Abdullah, and M.I. Idris

Abstract— Anodic oxidation is an electrochemical method to deposit ceramic coatings on the metals substrate. It has been widely used to modify the surface properties of titanium in order to improve the bioactivity. Recently, photocatalytic activity of biomaterials has attracted great deal of attention. This study aims to investigate the effect of UV irradiation on the precipitation of bone-like apatite in simulated body fluid (SBF). Specifically, the high purity titanium foils were anodised at various voltage (50V-350V) in mixture of β -glycerophosphate disodium salt pentahydrate (β -GP) and calcium acetate monohydrate (CA) for 10 minutes. Next, the anodised titanium foils were soaking in SBF for 7 days by illuminating with UVC light (peak wavelength of 254 nm). Field emission scanning electron microscopy (FESEM) and glancing angle X-ray diffractometer (GAXRD) were used to characterise the surface morphology and crystallinity of anodised titanium before and after soaking in SBF. After 7 days, bone-like apatite was covered on the surface of anodised titanium. Interestingly, smooth surface of anodised titanium also covered by bone-like apatite which contradict with the previous findings that concluded smooth surface lack of nucleation site for the growth of bone-like apatite. This finding indicated that \bullet OH groups produced during photocatalysis were the key factor to induce the precipitation of bone-like apatite. The result also showed that novel bioactive material is able to prepare by photocatalysis of anodised titanium in SBF.

Keywords— anodic oxidation, titanium, titanium dioxide, UV irradiation, simulated body fluid, bone-like apatite.

I. Introduction

Metals have been used as implant materials for more than 100 years when Lane used metal plate to fix the bone fracture [1]. Among all the metals, titanium is the most popular implant materials. Titanium is an element which is enclosed within the mineral ore in rutile form. It is well known that titanium and its alloys have become one of the popular biomaterial for implants application due to its properties such as biocompatibility, promote osseointegration, good mechanical properties, low modulus of elasticity and high corrosion resistance [2-4]. Nowadays, there is an increasing trend in titanium implants especially for dental implants, cochlear replacement, screw parts for orthodontic surgery, bone fixation, artificial heart valves, and surgical instrument. However, the titanium is bio-inert material and does not promote significantly better bone apposition after implantation [5].

The surface of implant plays a crucial role in the process of osseointegration. A number of efforts had been done by researchers on improving the surface roughness, crystallinity and wettability of implant material in order to accelerate

bone healing and enhance anchorage to the implant [5,6]. It is believed that rough topography may increase the bone in contact and improve the cell adhesion to the surface, thereby it can achieve better biomedical integrity. Rough surface displays a higher developed area than a smooth surface therefore it enhances the bone anchorage and reinforces the biomechanical interlocking of bone with implant material. Apart from that, high crystallinity surface provides better torque resistance value and better cellular responses if compared to the amorphous surfaces. Moreover, adhesion, proliferation, expression of osteoblastic markers and mineralised were increased due to the crystalline surfaces [7].

Surface modification methods such as anodic oxidation, gel oxidation and chemical treatment are applied to enhance the bioactivity of titanium. Anodic oxidation is an electrochemical method which combines electric field driven metal and oxygen ion diffusion for formation of oxide layer on anode surface. The advantages of anodic oxidation include simple, low cost, high adhesion bonding, easy to achieve the desired properties and increase of the corrosion resistance of titanium [8,9].

After implantation, anodised titanium will form a bone-like apatite layer on the surface in order to the bond with living bone. The composition and structure of bone-like apatite is very similar to the human bone [10]. Previously, anodic oxidation in mixture of calcium acetate (CA) and β -glycerophosphate (β -GP) provides calcium and phosphorous ions which will enhance anchorage of implant to the bone but it takes time to form bone-like apatite on the surface of anodised titanium [11]. Therefore, photocatalytic activity of biomaterials has become new research interests.

Photocatalysis can be defined as the process where a substance participates in modifying the rate of a chemical transformation of the reactant without being altered in the end of the process. Photocatalyst is widely used in common industrial applications such as photocatalytic water splitting, purification of pollutants, photocatalytic self-cleaning, photocatalytic antibacterial, photo-induced super hydrophilicity and photosynthesis [12]. It is seldom been used in biomedical application especially for implantation.

In order to induce the precipitation of bone-like apatite, UV irradiation has been employed to achieve a better bone-like apatite forming ability on the surface of anodised titanium. This is because UV irradiation is able to alter the chemical composition of surface by producing more hydroxyl groups (\bullet OH) which will improve the bioactivity of surface and promote the formation of bone-like apatite [13,14]. In the present work, the effect of UV irradiation on the precipitation of apatite in simulated body fluid was examined in order to understand the phenomenon basically.

Maizlinda Izwana Idris
Universiti Tun Hussein Onn Malaysia
Malaysia

II. Experimental Method

A. Sample Preparation

High-purity titanium foils with dimension of 25 mm x 10 mm x 0.05 mm were wet hand polished with 1200 grit abrasive paper (~1 μm) to remove native layer, and then immersed in an ultrasonic bath with acetone. Next, the titanium foils were rinsed with distilled water and dried using compressed air. Anodic oxidation was done by using programmable power supply (Genesys 600-1.3, Densel-Lambda, Japan) in 400 ml electrolytic solution of 0.04 M β -GP (Sigma, $\geq 98.0\%$) and 0.4 M CA (HmbG, $\geq 90.0\%$) with 50-350V and 70 mA.cm⁻² for 10 minutes at room temperature. The anodised titanium foils were cleaned by dipping in distilled water, followed by drying in air.

B. Simulated Body Fluid

Anodised titanium foils were immersed in 20 ml SBF (prepared by following Kokubo's recipe [15]), kept at 37 °C for 7 days and illuminated by UVC lamp (peak wavelength of 254 nm) to evaluate their bioactivity. The distance between UV lamp and samples was kept at 15 cm to avoid the heat transferred to the SBF in order to maintain the temperature. The SBF was refreshed every two days so that lack of ions would not inhibit the apatite formation. After removal from SBF, the anodised titanium foils were washed with distilled water and air dried.

C. Characterisation

The surface morphology was examined by using FESEM (JFM-7600F, Joel) at an accelerating voltage of 2.0 kV. The mineralogical composition of the foils was determined by using GAXRD (X'pert-Pan Analytical) at 40 kV and 40 mA, angle of incidence of 1° and scanning speed of 0.02° 2 θ .

III. Results and Discussion

A. Surface Morphology

Fig. 1 shows the surface morphology of anodised titanium at 50 V, 150 V, 250 V, and 350 V. It can be observed that the porosity of oxide layer increases with the applied voltage. The surface of anodised titanium exhibited relatively smooth surface after anodising at 50 V. However, at 150 V, some irregular craters with small pores were observed on the surface of anodised titanium. With continued anodising up to 250V, donut-shaped pores were formed due to the sparking during anodic oxidation. The pore size were approximately to 0.5-1.4 μm in diameter. Meanwhile, at 350 V, the size of donut-shape pores became larger (1-2.7 μm) and crack was observed on the surface. The summary on surface morphology of anodised titanium at different voltage is tabulated in Table I.

During the anodic oxidation, the newly-formed oxide layer on the anode is keeps growing until reaching the dielectric breakdown limit. The potential drop at the weak point exceeds the dielectric limit and cause the sparking occurs if the applied voltage increases. At this stage, the local temperature raise up to thousands Celcius and lead to the local melting on the surface of anode. Donut-shaped

pores are formed due to the local melting during the anodic oxidation. Generally, the anodised layer is not uniform due to the existence of flaws, defects, local stress, and non-uniform oxide thickness. The cracks can occur due to thermal gradients and shrinkage stress established during and after arcing. Combination of metal surface (titanium substrate), metal-ceramic surface (discontinuous layer between metal and ceramic surface) and ceramic surface (TiO₂ layer) lead to the thermal gradient and shrinkage stress during anodic oxidation [2-4,8,9].

TABLE I. THE SUMMARY ON SURFACE MORPHOLOGY OF ANODISED TITANIUM AT 50 V – 150 V.

| Voltage (V) | Surface Morphology | | | |
|-------------|--------------------|-----------------|-----------|----------|
| | Pore No. | Surface Profile | Pore Size | Cracking |
| 50 | No Appear | Even | No Appear | No |
| 150 | Few | Uneven | No Appear | No |
| 250 | Many | Uneven | Small | No |
| 350 | Many | Uneven | Large | Yes |

B. Surface Mineralogy

Fig. 2 shows GAXRD pattern of the anodised titanium samples obtained at various voltage. It is clear that the crystallinity of oxide layer was enhanced with the use of the higher voltage. At a low voltage (50 V and 150 V), the anodised titanium only consist of titanium. With increasing of applied voltage to 250 V, the intensity of titanium peaks were significantly decreased. Apart from that, anatase and rutile phase of TiO₂ began to appear on the oxide layer. However, at 350 V, more anatase and rutile phases of TiO₂ were detected due to the arcing process occurred during the anodisation.

The amorphous TiO₂ was transformed to anatase and rutile phases due to the high local temperature resulted by arcing process. This is because rutile and anatase TiO₂ becomes more stable than amorphous TiO₂ at higher temperature [11].

The following conclusion can be made from these patterns:

- The presences of the rutile peaks on the surface of titanium anodised at 250 V and 350 V showed that the local temperature during anodic oxidation was more than 600 °C (the transition temperature of anatase to rutile TiO₂).
- The decrease in the in intensity of Ti peaks as increasing of voltage indicated that the film thickness increased in increasing of voltage.

C. In Vitro Test

Fig. 3 shows the FESEM micrographs of the anodised titanium at various voltage after soaking in SBF for 7 days. The micrograph revealed that that surfaces of all samples undergo significant change after soaking in SBF. Precipitation of bone-like apatite were observed on the surface of anodised titanium and covered the whole surface. Some island-like agglomerated apatite was deposited on the surface. With increasing of applied voltage, the island-like agglomerated apatite became larger. The size of island-like agglomerated apatite for 50 V, 150 V, 250 V and 350 V

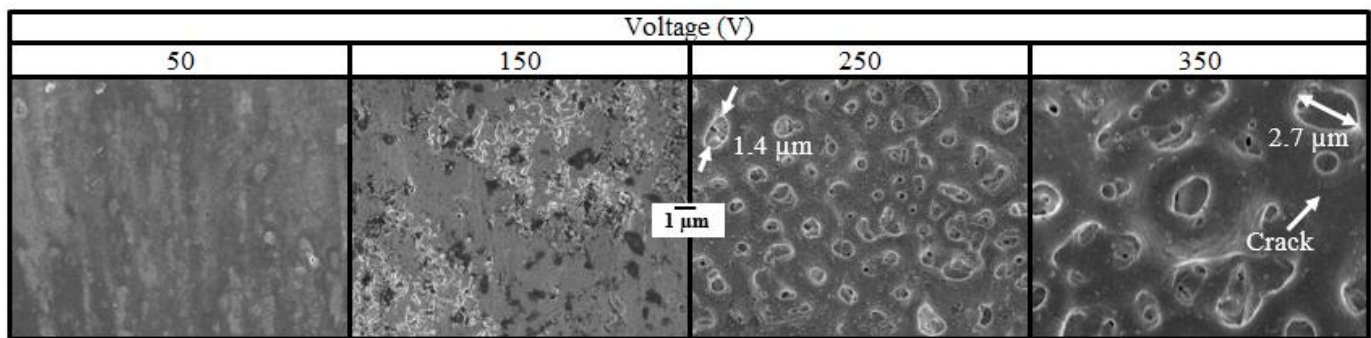


Fig. 1. FESEM micrographs of anodised titanium at 50 V-350 V.

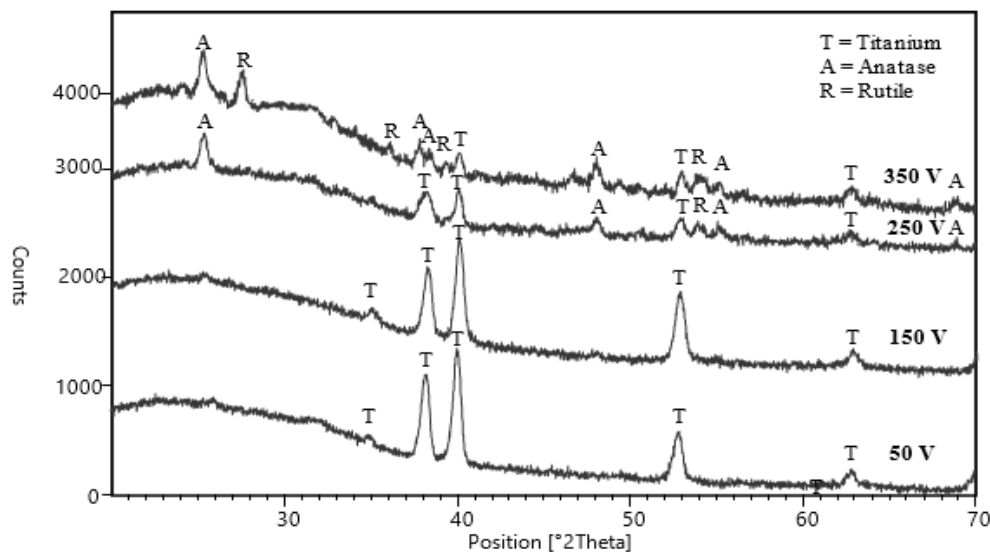


Fig. 2. GAXRD patterns of anodised titanium 50 V- 350 V.

were 1.4 μm , 5.1 μm , 6.4 μm and 9.3 μm , respectively. Interestingly, the smooth/partially porous surface which anodised at 50 V and 150 V also covered by bone-like apatite. The result was contradict with the previous findings [16,17] that concluded smooth surface lack of nucleation site for the growth of bone-like apatite. These results indicated nucleation site of bone-like on smooth surface was activated by UV irradiation.

The GAXRD results (Fig. 4) indicated the presence of bone-like apatite and matched with the apatite standard (JCPDS 9-432). Broad peaks of hydroxyapatite at 30° - 33° attribute to bone-like apatite with low degree of crystallinity. Intensities of apatite peaks increased with the increasing of applied voltage. This result indicated that more crystalline bone-like apatite were deposited on the surface of anodised titanium with higher applied voltage.

Generally, photocatalysis occurred during UV light irradiation. The $\bullet\text{OH}$ groups will be produced after UV reacts with TiO_2 . Next, the $\bullet\text{OH}$ groups will combine with Ti to form Ti-OH groups. As a result, the surface of anodised titanium will change to negatively charged. Negatively charged surface will attract the Ca^{2+} ions in SBF and combine with Ti-OH groups to form amorphous calcium titanate [18-20]. According to Takadama *et al.* (2001), after a long soaking time, amorphous calcium titanate will react with phosphate ions (PO_4^{3-}) and form bone-like apatite [21].

Moreover, more porous and crystalline surface were produced by anodisation with higher voltage. Anil *et al.* (2011) claimed porous induce the growth of bone-like apatite due to the increasing of surface area and nucleation site [7]. Moreover, the crystallinity of anodised titanium also play a crucial role for the growth of bone-like apatite. Anatase and rutile TiO_2 are good photocatalyst and active during photocatalysis. More $\bullet\text{OH}$ groups were produced from the anodised titanium with anatase and rutile TiO_2 due to the increasing of photocatalytic activity. Thus, larger and high crystalline bone-like apatite was formed on the surface of anodised titanium with higher voltage.

iv. Conclusions

In conclusion, porous and high crystallinity TiO_2 layer was produced by using anodic oxidation in mixture of $\beta\text{-GP}$ + CA. UVC irradiation induced the precipitation of bone-like apatite in simulated body fluid. Formation of $\bullet\text{OH}$ groups on the surface induced the precipitation of bone-like apatite. The precipitation of bone-like apatite depended strongly on the surface properties of anodised titanium. Porous and high crystallinity provided more nucleation site for the growth of bone-like apatite. The bone-like apatite was deposited on the smooth surface of anodised titanium after UV irradiation after soaking in SBF for 7 days. UV illumination during SBF is an efficient method and acts as catalyst to enhance the bioactivity of anodised titanium.

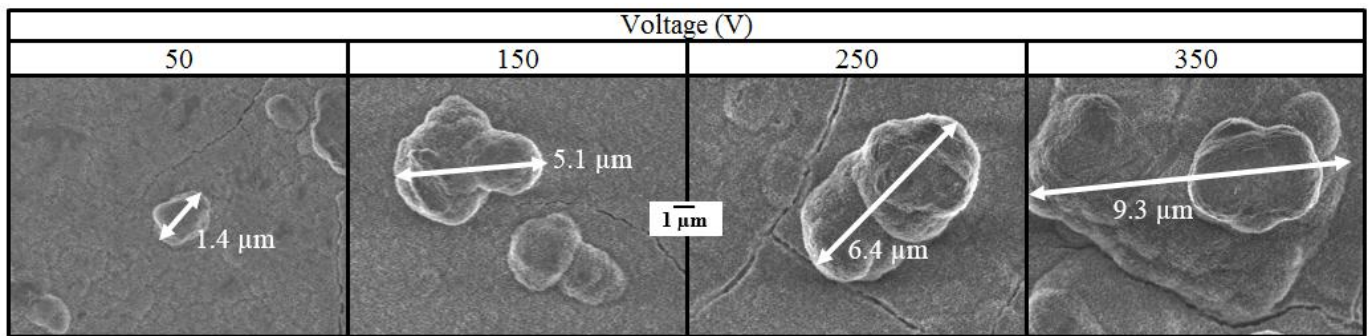


Fig. 3. FESEM images of anodised titanium at 50 V- 350 V after soaking in SBF for 7 days under UV irradiation.

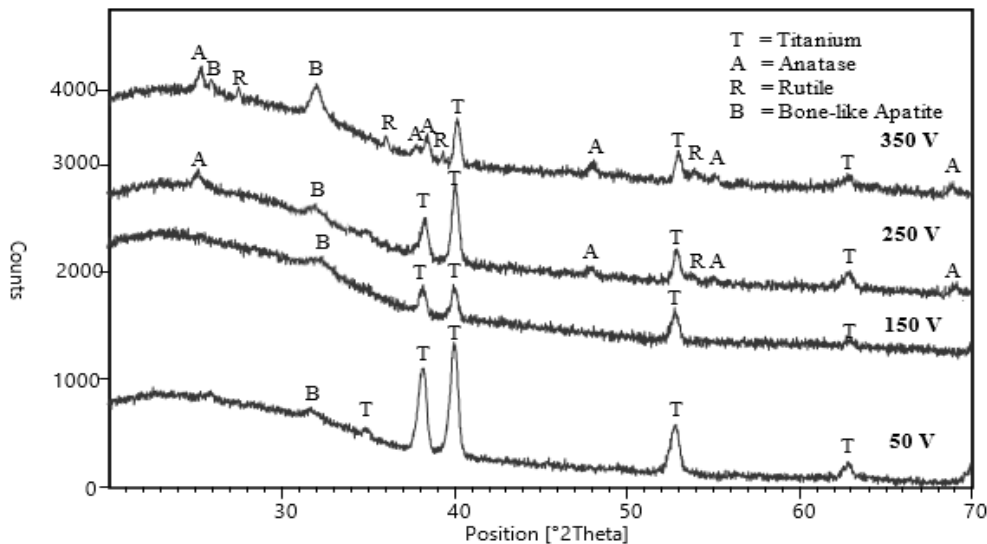


Fig. 4. GAXRD patterns of anodised titanium at 50 V- 350 V after soaking in SBF for 7 days under UV irradiation.

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