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Original Article

บทความวิชาการ

Calcium phosphate formation on anodized commercially pure titanium in simulated body fluid solution

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Abstract

Objective To investigate the surface characteristics of anodized commercially pure titanium grade 2 prepared in different electrolytes and the relationship between surface properties and calcium phosphate formation immersed in simulated body fluid solution.

Materials and methods Commercially pure titanium grade 2 plates sized 10 x 15 x 0.5 mm³ were divided into three groups, 10 specimens for each group, including control. Potentiostatic anodization was performed for 30 minutes at room temperature. Group I specimens were anodized in 0.5 M H₃PO₄ with potential of 200 V and Group II specimens were anodized in 1 M NaOH solution with potential of 20 V. All specimens were then immersed in simulated body fluid (SBF) solution for 7, 14 and 28 days at 36.5°C, pH 7.4. The x-ray diffraction, SEM and EDS analysis were used to investigate the surface characteristics of anodized titanium before and after immersion in SBF.

Results After anodic oxidation, the uniform structure consisting of numerous pores was observed on the surface of titanium specimens in H₃PO₄ solution, while irregular surface consisting of pores and elevated surface on titanium specimens in NaOH solution. Using XRD analysis, anatase crystalline structure was found on titanium anodized in H₃PO₄, but rutile crystalline structure was observed on titanium anodized in NaOH. After immersion in SBF, only titanium prepared in electrolyte of NaOH immersed in SBF for 28 days showed calcium phosphate formation. However, no calcium phosphate formation could be observed in any group of titanium specimens anodized in H₃PO₄ including control group.

Conclusion Crystalline structure of anodized titanium surface may influence partly on the ability of calcium phosphate formation after immersion in simulated body fluid solution.

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Key words: anodization; simulated body fluid; surface characteristics; titanium

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Introduction

Titanium is widely used in dental implants due to its unique properties among all metals for osseointegration, including excellent mechanical properties, high corrosion resistance, and biocompatibility.¹ Because of its reactivity in both air and aqueous electrolytes, a dense oxide film can spontaneously form on a titanium surface and this reaction product becomes a potent barrier against dissolution of the metal.² Clinical success of dental implants is related to their biocompatibility and early osseointegration. However, the rate and quality of implant osseointegration is related to its surface properties. As a result, multiple surface modification methods have been developed to improve the surface properties such as surface topography, composition, and wettability for implant–tissue interaction and osseointegration. Plasma spraying, blasting with ceramic, acid etching, and anodizing are methods currently used to treat the surface of titanium dental implants.^{3,4}

Anodization has become an attractive method for preparing an oxide film on the surface of titanium to induce calcium–phosphate formation in simulated body fluid (SBF) which has ion concentrations roughly equivalent to human blood plasma.^{5–9} SBF is a simplified model which can be used to understand the initial phase of bone formation around titanium implants. The apatite forming ability of materials in SBF can predict the degree of *in vivo* bioactivity of the material. If materials can form apatite in SBF in a short period, this implies that it can form apatite in the living body in a shorter period.⁷ Microarc oxidized (MAO) or anodization is an electrochemical method of surface modification which can alter its roughness, porosity, and chemical composition for improving biocompatibility by controlling factors such as electrolyte selection, and their concentration, applied voltage or current, temperature, and anodizing time. The difference in pore morphology generated on the metal

surface depends on the anodizing parameters. Different titanium oxide crystal structures can be found on titanium surfaces after anodization depending on the conditions used. At lower applied voltages the oxide film is amorphous, whereas at increasing voltages the oxide film structure can develop into either of three different crystal structures—anatase, rutile or brookite, each with different physical properties such as wettability.¹⁰ Both wettability and surface charge play an important role in protein absorption behavior and consequently bone apposition.^{11,12}

The purposes of this study were to investigate the crystal structure and surface morphology of anodized titanium metal in different electrolyte solutions under different anodizing conditions, and the relationship between anodized titanium and the ability of calcium phosphate formation in SBF solution.

Materials and methods

Preparation of titanium specimens

Thirty commercially pure titanium grade 2 plates sized 10 x 15 x 0.5 mm³ were ground with series of silicon carbide abrasive paper from grid no. 400 up to no. 1,200 under water coolant, followed by ultrasonic cleaning in acetone for 5 mins, and subsequently pickled in 1% HF solution for 1 min to remove the native oxide. All specimens were then rinsed with distilled water and air-dried.

Anodization process

The specimens were divided into 3 groups, 10 specimens for each group, including control. Potentiostatic anodization was performed using titanium plate 50 x 150 x 0.5 mm³ as cathode. Two groups of titanium specimens were anodized in two different electrolyte solutions, 0.5 M H₃PO₄ and 1.0 M NaOH for 30 mins at room temperature. The H₃PO₄ treatment was carried out at a direct constant potential

of 200 V whereas the NaOH treatment was performed at a direct constant potential of 20 V. The applied potential voltages and electrolyte concentration for each condition are received from our pilot study. During anodization, the temperature was maintained at 25°C in a water-cooled bath with stirring to maintain a homogenous electrolyte solution and reduce gas evaporation due to the electrochemical reaction at the titanium substrate surface. After anodization, the specimens were gently rinsed with de-ionized water and air-dried. The control samples were simply rinsed in de-ionized water and air-dried without anodization.

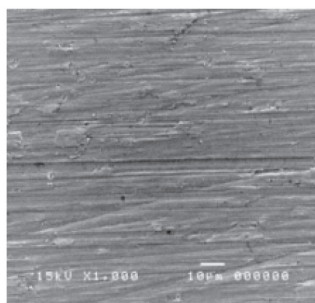
Calcium phosphate formation after immersion in SBF

The representative images of titanium surface and percentage of ion elements after immersion in SBF for each period of condition are shown in Fig. 3 and

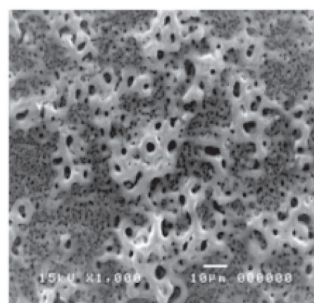
Table 3. This study showed that after the titanium was anodized in 0.5 M H_3PO_4 solution at 200 V for 30 mins and then immersed in SBF for 7, 14, and 28 days, no calcium phosphate formed on the titanium surface. Only phosphate was detected with no significant change on the titanium surface. On the other hand, the titanium anodized in 1 M NaOH solution at 20 V for 30 mins, only calcium could be detected without phosphate after immersed in SBF for 7 and 14 days. Moreover, the surface texture appeared smoother than without immersion. However, after immersion for 28 days, the elevated smoother surface was increased while the pores remained present. There was a significant change on the surface of the titanium anodized in NaOH solution showing the white layer covered on the surface observed with SEM. By EDS analysis, the amount of calcium ion increased with increased time of immersion. The ratio of calcium and phosphate (Ca/P) formed on the titanium surface of NaOH group was 1.70.

Table 1 Norminal ion concentrations of SBF in comparison with those in human blood plasma

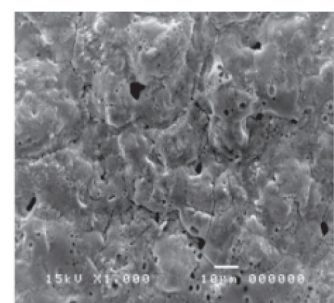
Ion concentrations (mM)	Na^+	K^+	Mg^{2+}	Ca^{2+}	Cl^-	HCO_3^+	HPO_4^{2-}	SO_4^{2-}	pH
Blood plasma	142.0	5.0	1.5	2.5	103.0	27.0	1.0	0.5	7.2-7.4
SBF	142.0	5.0	1.5	2.5	147.8	4.2	1.0	0.5	7.4



Control (without anodization)



H_3PO_4



NaOH

Fig. 1 SEM photographs of titanium from control group, after anodization in H_3PO_4 and NaOH (x1,000)

Table 2 Summary of surface morphology of titanium specimens investigated by SEM and XRD before and after anodization

Group	Before anodization		After anodization	
	SEM	XRD	SEM	XRD
Control	Machined surface	Non crystalline structure	Machined surface	Non crystalline structure
H ₃ PO ₄	Machined surface	Non crystalline structure	Elevated area with pores	Anatase
NaOH	Machined surface	Non crystalline structure	Irregularly elevated surface	Rutile

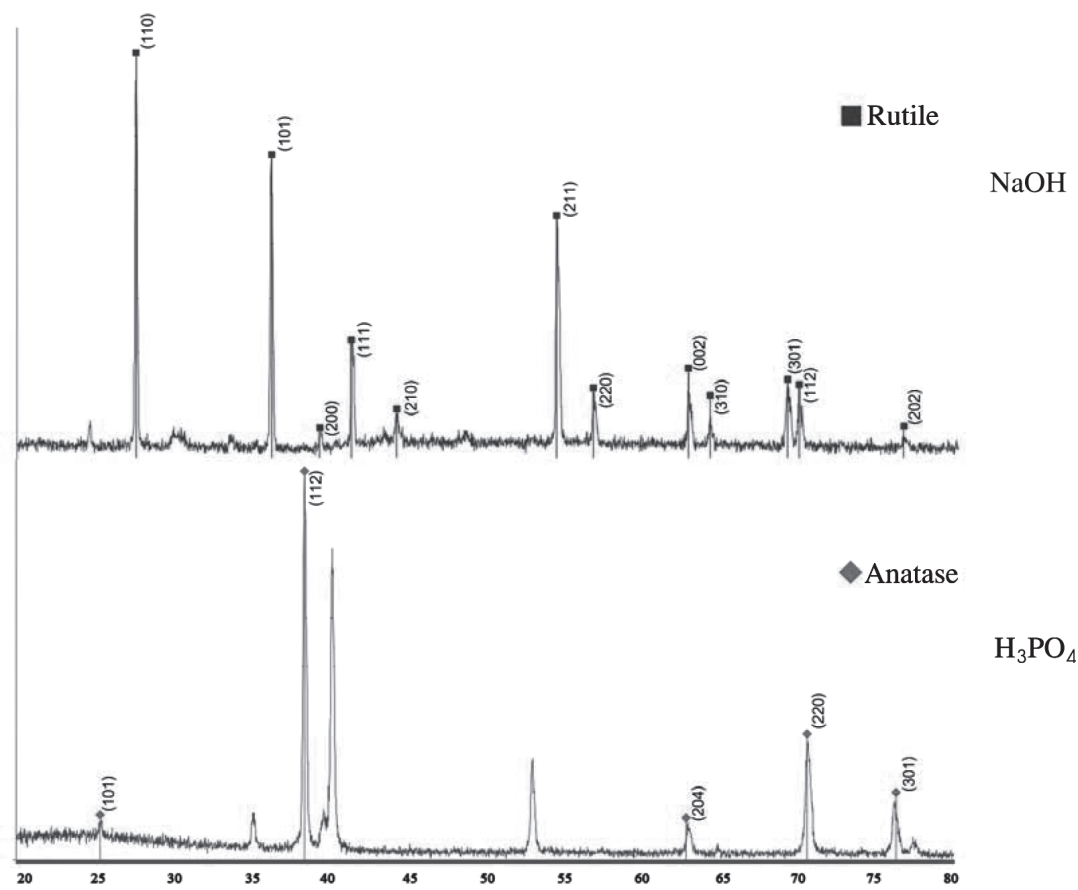
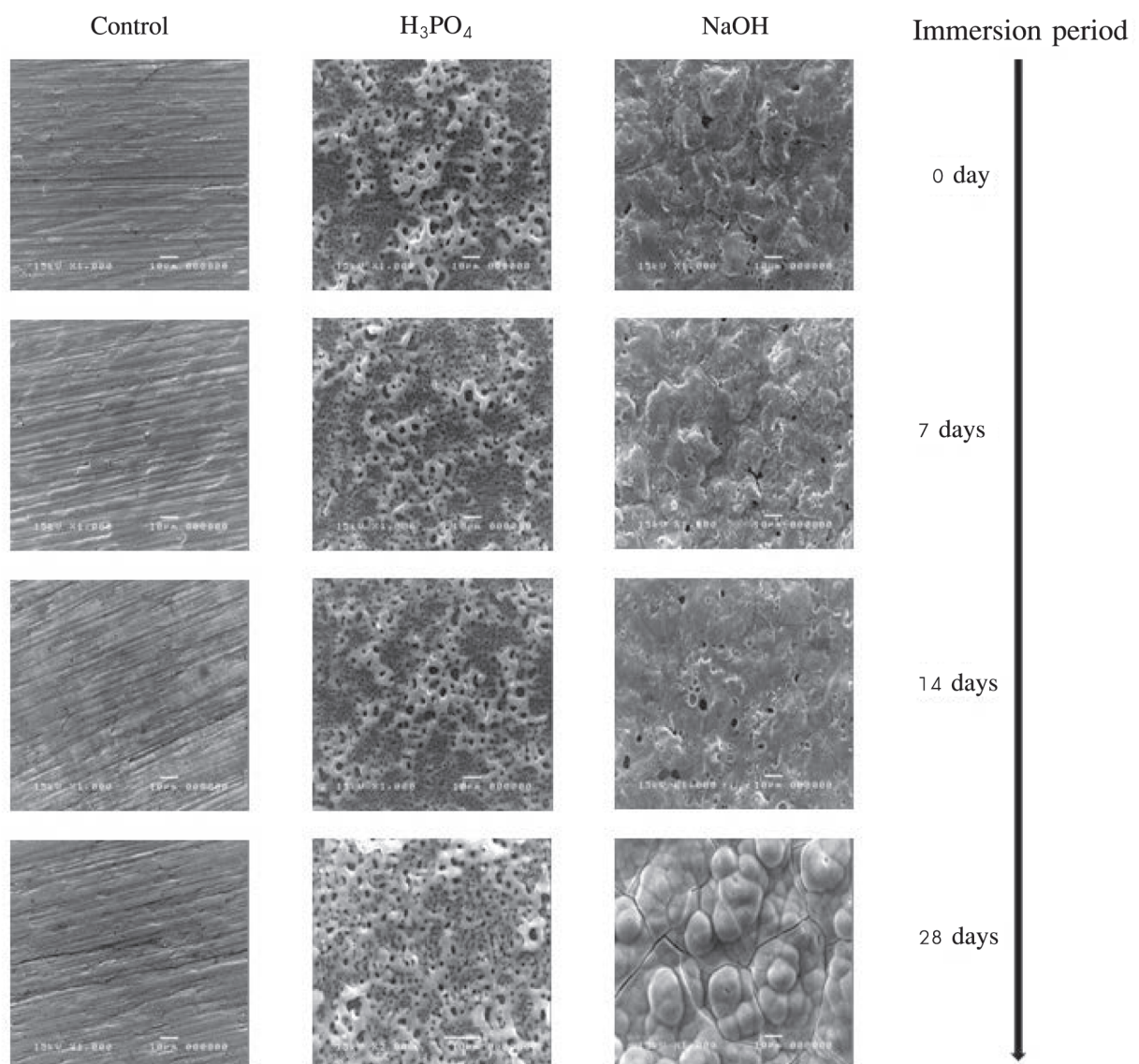


Fig. 2 XRD patterns of titanium after anodization in NaOH and H₃PO₄

Table 3 EDS analysis (% element) of titanium surfaces immersed in SBF for 7, 14, and 28 days after anodization in H_3PO_4 and NaOH, respectively including control group

Group	7 Days			14 Days			28 Days		
	Ca	P	Ca:P	Ca	P	Ca:P	Ca	P	Ca:P
Control	–	–	–	–	–	–	–	–	–
H_3PO_4	–	6.42	–	–	6.72	–	–	6.95	–
NaOH	3.61	–	–	10.27	–	–	21.36	12.6	1.70

**Fig. 3** SEM images of titanium surfaces immersed in SBF for 7, 14, and 28 days after anodization in H_3PO_4 and NaOH including control group (x1,000)

Results

Structure and composition of titanium surfaces

The titanium plate anodized in 0.5 M H_3PO_4 solution was randomly chosen for SEM observation. The surface showed a three-dimensional structure consisting of numerous elevated areas with pores of different sizes generated by the spark-discharge when the voltage reached 200 V. Titanium anodized in 1 M NaOH had a spark-discharge at potential of 20 V, and SEM image exhibited a three dimensional irregularly elevated surface with various sized holes over the surface. Moreover, the presence of cracks was observed. The representative SEM images of surface morphology of these two anodization groups compared to the control are shown in Fig. 1. By x-ray diffraction, anatase phase of TiO_2 was found in the titanium oxide surface anodized in 0.5 M H_3PO_4 solution while the oxide surface of the anodized titanium in NaOH solution displayed the rutile phase of titanium dioxide (as shown in Fig. 2). The summary of surface morphology investigated by SEM and XRD is also shown in Table 2 while the mean element percentage of phosphate and sodium in the oxide film after the anodization process before immersion in SBF is shown in Table 3.

Calcium phosphate formation after immersion in SBF

The representative images of titanium surface and percentage of ion elements after immersion in SBF for each period of condition are shown in Fig. 3 and Table 3. This study showed that after the titanium was anodized in 0.5 M H_3PO_4 solution at 200 V for 30 mins and then immersed in SBF for 7, 14, and 28 days, no calcium phosphate formed on the titanium surface. Only phosphate was detected with no significant change on the titanium surface. On the other hand, the titanium anodized in 1 M NaOH solution at 20 V for 30 mins, only calcium could be detected without phosphate after immersed in SBF for 7 and 14 days. Moreover, the surface texture appeared smoother than

without immersion. However, after immersion for 28 days, the elevated smoother surface was increased while the pores remained present. There was a significant change on the surface of the titanium anodized in NaOH solution showing the white layer covered on the surface observed with SEM. By EDS analysis, the amount of calcium ion increased with increased time of immersion. The ratio of calcium and phosphate (Ca/P) formed on the titanium surface of NaOH group was 1.70.

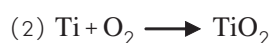
Discussion

The titanium surface characteristics of dental implants plays an important role in their osseointegration. The topography of the the titanium surface relates to the rate and quality of bone apposition to the implant.¹³ Proper implant surface chemistry and topography can improve the interaction between bone and implant surfaces in a short time.³ This study was aimed to investigate the potential bioactive property of anodized titanium plates by observing their ability to form calcium phosphate on their surfaces during immersion in simulated body fluid. It has been known that the ability of a material to form apatite on their surface in SBF indicating the degree of *in vivo* bone bioactivity has been based on the rate of apatite forming on the surface. X-ray diffraction, fourier transform infrared spectroscopy (TF-XRD), scanning electron microscopy, and transmission electron microscopy showed surface apatite formed in SBF had a similar composition and structure to bone mineral.^{14,15}

An hydroxyapatite surface can improve the capacity for bone apposition on the implant surface but there are some concerns about the technique of HA deposition on titanium surfaces. HA deposition by a plasma-spray technique is not effective for coating a complex shaped implant. Delamination and loosening of the HA coating has been reported especially when inserting implants into dense bone.³

Anodization is an implant surface treatment method to produce apatite on the surface using a

2-step process. Firstly, titanium is anodized to produce titanium oxide on the surface and to change the surface topography and then the anodized titanium is immersed in simulated body fluid to induce apatite to form on the surface.¹⁶ The anodic oxidation process can be used to design the roughness, porosity, and chemical composition of the oxide. Voltage and current of DC power supply, type and concentration of electrolyte, and electrolyte temperature are the controlling factors to produce an anodized surface. Anodic oxidation processing under lower applied voltages results in an amorphous oxide film. On the other hand, anodization at voltages above the breakdown limit or spark anodizing, the oxide film will change from amorphous to crystalline oxide. Increased voltages not only changes the crystallinity, but also increases the pore size and roughness. The surface roughness, thickness of the oxide layer, crystallinity of TiO_2 , Ca and P content of oxide layer were increased after being anodized in calcium acetate and calcium glycerophosphate at increased voltages.¹⁷ Increasing the temperature resulted in an acceleration in electrochemical reaction.¹⁰ The electrolytes used in anodic oxidation can be either acid or non-acid electrolytes. Anodization in acid electrolyte results in a porous microstructure with craters and pores on the surface. The study of Afshar *et al.*¹⁸ showed the breakdown voltage of 0.5 M H_3PO_4 is 178.1 V while Chen *et al.*²⁷ indicated for a 1 M H_3PO_4 anodization the sparking occurs when the voltage increased to 200 V. In the present study, the H_3PO_4 group sparked at 200 V and the NaOH group began to spark from 13 V to 20V around the surface to the forming of pores on.



The above equations show the reactions occurring during the anodized titanium process. Reactions (1) and (2) occurred at the Ti/oxide interface and the oxide layer on the titanium surface respectively,¹⁰ resulting in an oxide layer forming on the surface. Titanium oxide ions can dissolve into the electrolyte as

cations and TiO_2 can precipitate and form a layer on the porous structure. The dissimilar morphological appearances of the anodized titanium surfaces as observed by SEM, resulted from anodization in different electrolyte solutions. Titanium anodized in H_3PO_4 showed a porous surface originating from the spark-discharge on the surface between oxide and electrolyte. The NaOH electrolyte treatment resulted in a different anodized titanium surface morphology of an elevated surface with some pits. The oxide morphology and oxide constituents can be designed by the current density, electrolyte concentration, and bath temperature.¹⁰ Moreover, impurities of electrolyte can increase processing time of crystal structure formation. However, crystal structure of oxide layer is complicated. Further investigation of crystal structure formation is necessary.¹⁹⁻²⁰ The composition of the oxide on the surface after anodization in H_3PO_4 and NaOH contained titanium and oxygen which were the dominant elements of titanium oxide, while phosphate and sodium originated from electrolyte solutions.

The crystalline structures of generated oxide films is related to the type of electrolyte used, applied voltage, and dielectric breakdown of the layer.²¹ There are three crystalline forms of titanium oxide known as anatase, rutile and brookite, each with different properties. In the present study, XRD peaks of anatase and rutile phases of titanium oxide were observed on the surfaces of the H_3PO_4 and NaOH groups, respectively. This indicates the process parameters, electrolyte composition and voltage had affected the structure and morphology of the anodic oxide film of titanium.

To investigate ability of calcium phosphate formation, after immersion in SBF for 7, 14, and 28 days, no apparent change was observed on the surface of control and H_3PO_4 groups from SEM images and EDS analysis. Initially, the composition of the anodized surfaces consisted of phosphate but no significant change was seen after soaking in SBF for 7, 14 and 28 days, with approximately the same amount of

phosphate present on the surface. A certain thickness of titanium oxide is necessary to generate the apatite forming ability or bioactivity of titanium surface. Although titanium has the anatase phase of TiO_2 on its surface, no apatite formed on the titanium surface under the condition without spark-discharge.^{5,22} This is also found in the present study that no apatite was formed on the surface even though anatase crystal structure was produced on its surface at dielectric breakdown. The relationship between an amount of the titanium oxide on the titanium surface and ability of calcium phosphate formation cannot be clarified as differences in the thickness of oxide layers between H_3PO_4 group and NaOH group were not assayed in this study. Although anodized for the same amount of time, the oxide layer thickness may not have been the same. Further investigation of the relationship between oxide thickness and calcium phosphate formation ability would be beneficial. Furthermore, our result is in consistent with previous studies^{20,23–25} that calcium phosphate could form on titanium surfaces anodized in NaOH and immersed in SBF for 28 days. After anodizing and soaking in SBF for 7 days, the results of EDS showed calcium existed on the surface and calcium amount increased when immersed for 14 days. Finally, after 28 days of soaking in SBF, calcium and phosphate existed on the surface with Ca:P ratio 1.70 similar to hydroxyapatite. The calcium phosphate coating mechanism on alkali treated titanium in SBF solution as proposed by Kokubo *et al.*²⁵ who stated that Ti–OH groups on the surface are essential for the formation of calcium phosphate. The more Ti–OH groups present, the greater the ability to induce apatite nucleation. The Ti–OH groups on the titanium surface are negatively charge and react with positively charged Ca^{2+} ions to become calcium titanate. As the Ca^{2+} ions accumulate, the calcium titanate becomes positively charged and combines with negatively charged of phosphate to form calcium phosphates and transforms to apatite.²⁶ In our study, after anodizing in NaOH and subsequently soaking in SBF, Na^+ ions from the surface reacted with H_3O^+ ions forming TiOH groups on the surface

which led to the formation of calcium phosphates. This could explain the finding in this present study that calcium phosphate could form on the titanium surface which was anodized in NaOH and not in H_3PO_4 . Notably different crystalline structures of anatase and rutile are found on anodized titanium, and they may have an influence on surface chemistry leading to an effect osseointegration. However, it might not be directly related to calcium phosphate formation in SBF and should be further investigated.

Conclusions

In summary, calcium phosphate was formed on the titanium surface anodized in NaOH after 28 days in simulated body fluid solution. Different parameters of the anodizing process such as type of electrolyte, DC power voltages, and periods of anodization process can alter the oxide morphology and structure. This study suggests that titanium anodized in NaOH solution could improve surface characteristics of titanium leading to accelerate bone formation, however, an influence of crystalline structure of titanium oxide on calcium phosphate formation cannot be concluded.

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การเกิดแคลเซียมฟอสเฟตบนพื้นผิว คอมเมอร์เชียลลีย์เพียวไทเทเนียมที่ผ่านการ อะโนไดซ์ในสารละลายจำลองของน้ำในร่างกาย

บุศรินทร์ ล้อกตระกูล ท.บ.¹

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บทคัดย่อ

วัตถุประสงค์ เพื่อศึกษาลักษณะและโครงสร้างผลึกของชั้นออกไซด์บนผิวคอมเมอร์เชียลลีย์เพียวไททาเนียมเกรด 2 ทั้งก่อนและหลังการปรับสภาพผิวด้วยวิธีอะโนไดซ์ในสารละลายที่ต่างกัน และความสัมพันธ์ระหว่างคุณสมบัติของพื้นผิวกับการเกิดแคลเซียมฟอสเฟตที่ชั้นในสารละลายจำลองของน้ำในร่างกาย

วัสดุและวิธีการ แบ่งชิ้นงานแผ่นโลหะคอมเมอร์เชียลลีย์เพียวไททาเนียมเกรด 2 ขนาด 10 x 15 x 0.5 มม.³ จำนวน 10 ชิ้นต่อกลุ่ม ทั้งหมด 3 กลุ่ม รวมทั้งกลุ่มควบคุม โดยกลุ่มทดลองจะผ่านกระบวนการอะโนไดซ์ในสารละลายด้วยความต่างศักย์คงที่เป็นเวลา 30 นาทีที่อุณหภูมิห้อง ชิ้นงานทดลองกลุ่มที่ 1 จะถูกอะโนไดซ์ในสารละลายกรดฟอสฟอริกความเข้มข้น 0.5 โมลาร์ที่มีความต่างศักย์ 200 โวลต์ และชิ้นงานทดลองกลุ่มที่ 2 จะถูกอะโนไดซ์ในสารละลายโซเดียมไฮดรอกไซด์ความเข้มข้น 1 โมลาร์ที่มีความต่างศักย์ 20 โวลต์ จากนั้นนำชิ้นงานทั้งหมดไปแช่ในสารละลายจำลองของน้ำในร่างกาย (เอสบีเอฟ) เป็นเวลา 7 14 และ 28 วัน ตามลำดับ ที่อุณหภูมิ 36.5 องศาเซลเซียส และค่าความเป็นกรด-ด่าง 7.4 งานวิจัยนี้ศึกษาลักษณะและโครงสร้างผลึกของชั้นออกไซด์โดยใช้เอกซเรย์ดิฟแฟรกชัน กล้องจุลทรรศน์อิเล็กตรอนแบบส่องกราด และเทคนิคอีดีเอสวิเคราะห์ความแตกต่างของพื้นผิวของโลหะไทเทเนียมที่ผ่านการอะโนไดซ์ในสารละลายทั้งก่อนและหลังการแช่ในสารละลายเอสบีเอฟ

ผลการศึกษา หลังจากผ่านการอะโนไดซ์ในสารละลายกรดฟอสฟอริก พื้นผิวของชิ้นงานไททาเนียมที่เกิดขึ้นมีลักษณะเป็นรูพรุนสม่ำเสมอ ในขณะที่พื้นผิวที่ผ่านการอะโนไดซ์ในสารละลายโซเดียมไฮดรอกไซด์มีลักษณะขรุขระ เมื่อศึกษาด้วยเทคนิคเอกซเรย์ดิฟแฟรกชันก็พบโครงสร้างผลึกเป็นชนิดอะนาเทสในพื้นผิวโลหะไททาเนียมที่ผ่านการอะโนไดซ์ในสารละลายกรดฟอสฟอริก ขณะที่พื้นผิวโลหะไททาเนียมที่อะโนไดซ์ในสารละลายโซเดียมไฮดรอกไซด์มีโครงสร้างผลึกเป็นชนิดรูไทล์ หลังแช่ชิ้นงานในสารละลายเอสบีเอฟพบว่าเมื่อไทเทเนียมที่ผ่านการอะโนไดซ์ในสารละลายโซเดียมไฮดรอกไซด์แล้วแช่ในสารละลายเอสบีเอฟเป็นเวลา 28 วันเท่านั้นที่พบการสร้างแคลเซียมฟอสเฟตขึ้นบนพื้นผิวโครงสร้างผลึกบนพื้นผิวไทเทเนียม อย่างไรก็ตามไม่พบการสร้างแคลเซียมฟอสเฟตในกลุ่มที่ผ่านการอะโนไดซ์ในสารละลายกรดฟอสฟอริกจนถึงชิ้นงานไททาเนียมในกลุ่มควบคุม

สรุป โครงสร้างของพื้นผิวไทเทเนียมอาจมีผลกับความสามารถของการเกิดแคลเซียมฟอสเฟตภายหลังการแช่ในสารละลายจำลองของน้ำในร่างกาย

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คำสำคัญ: ไทเทเนียม; ลักษณะพื้นผิว; สารละลายจำลองของน้ำในร่างกาย; อะโนไดซ์

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