

UTILISATION OF NATURAL HYDROXYAPATITE FROM WHITE BARRAMUNDI FISH (*LATES CALCARIFER*) SCALES FOR COATING TITANIUM ALLOY (Ti6Al4V ELI) USING DIP COATING METHOD TO IMPROVE BIOACTIVITY IN BIOMEDICAL APPLICATION

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Submitted October 1, 2022; accepted November 7, 2022

Keywords: Ti6Al4V ELI; Hydroxyapatite; White barramundi fish scales; Dip coating; Coating time

*Ti6Al4V ELI is a titanium alloy that is quite widely used in medical applications for prosthesis implants. Ti6Al4V ELI has high corrosion resistance and good biocompatibility, but is less bioactive for new bone growth sites. Improving the bioactive properties of titanium alloys can be undertaken by coating them with hydroxyapatite. In this study, the type of hydroxyapatite used is a natural hydroxyapatite which is processed from local Indonesian ingredients, namely white barramundi fish scales (*Lates calcarifer*). White barramundi scales are a type of waste that contains high calcium, so they have the potential to be a source of hydroxyapatite. The purpose is to determine the characteristics of the natural hydroxyapatite layer on the mechanical properties and surface properties of the Ti6Al4V ELI titanium alloy. The hydroxyapatite coating was conducted by the dip coating method with a coating time of 10, 14, 24, and 30 seconds. The characterisation was carried out using a Stereo Microscope and SEM. The results showed that the hydroxyapatite layer met the thickness parameters in biomedical applications in 14 minutes with a layer thickness of 199 μm . This result is better than previous studies using the electrophoretic deposition method at a voltage of 7 V for 10 seconds where the layer thickness was 72 μm . This indicates that such a simple method of dip coating with an optimum parameter is applicable for coating titanium alloys with a natural hydroxyapatite.*

INTRODUCTION

Fractures are one of the biggest causes of death in the world. According to the World Health Organization (WHO), the increase in the number of cases involving fractures can be caused by the increase in the number of vehicles. An increasing age can also be a factor in fractures due to the decreased bone mass [1, 2]. One of the treatments for patients with fractures is to carry out bone implants using biomaterials [3]. Some biomaterials that are commonly used for implants are: stainless steel [4, 5], cobalt alloys [6-8], and titanium alloys [9-11]. Ti6Al4V ELI is a titanium metal implant material that has good biocompatibility properties to prevent the corrosion rates [9] and the coating material falling into the body. However, it is less bioactive for the growth of new bone tissue and bone osseointegration in implants. To improve bioactive properties of titanium, it is necessary to coat the implant material with hydroxyapatite compounds [9].

Hydroxyapatite is a bioceramic material that has good biocompatibility [12], can increase the bioactive

properties [12, 13] on the surface of biomaterials, and is not toxic to the body [12, 14, 15]. Hydroxyapatite provides osseointegration (increases bone growth to form a bond with the implant surface) to implant material into surrounding bone tissue because its composition is similar to human bones [16]. Apart from commercial hydroxyapatite, natural hydroxyapatite can be obtained from bovine bones [17, 18], shellfish [19, 20], eggshells [14, 21], coral [22], and limestone [23] and white barramundi fish scales. A comparison of the energy-dispersive X-ray (EDX) analysis results of natural hydroxyapatite alternative materials can be seen in Table 1.

Table 1. EDX comparison of alternative natural hydroxyapatite materials.

Compound	Alternative Material			
	Bovine Bones	Shellfish	Snail Shell	Eggshells
C	5.22 %	18.83 %	6.22 %	17.17 %
O	40.60 %	30.98 %	38.43 %	40.79 %
Ca	33.96 %	49.67 %	55.35 %	23.79 %

Waste from fishery processing plants is known to reach 75 % of the total weight of fish. The waste is in the form of bones, skin and fish scales, which are considered to be of low value. The processing of fishery waste itself can minimise the volume of waste while increasing the added value of the resulting product [24, 25]. White barramundi fish scales are part of the fishery processing waste [24]. Fish scale waste contains high calcium so that it can be used to manufacture hydroxyapatite. White barramundi fish scales have also been used as a material for bone grafting [26]. In this study, white barramundi fish scale waste from local Indonesian fish was used as a titanium coating material to increase the bioactivity properties of the material.

Methods that can be used in coating hydroxyapatite on the surface of implant materials are the plasma spraying method [27, 28], the pulsed laser ablation method [29, 30], the coating method [31], the sol-gel method [32, 33], and electrophoretic deposition methods [34, 35]. In this study, the coating method is the simplest, most inexpensive, providing a uniform deposition method that can be carried out at low temperatures and can coat irregular shapes and patterns [36]. The result of the coating using the dip coating method is influenced by the parameters of the coating time, the speed of the withdrawal, the sintering temperature, and the concentration of the solution [37]. The aim of the study was to determine the mechanical properties and surface properties of titanium alloy Ti6Al4V ELI after the hydroxyapatite layer coating was applied from the processed white barramundi fish scales.

EXPERIMENTAL

Sample Preparation

The Ti6Al4V ELI sample was cut in the form of a plate using a hand grinder to obtain a sample with a size of 20 mm × 20 mm × 4 mm as shown in Figure 1.

After the sample was cut, the solution treatment was carried out to increase the adhesiveness of the coating and remove the residual stress from the cutting. The solution treatment process consists of heating to a certain temperature, then a holding temperature, and then quenching using a GSL-1100 vacuum tube furnace. The process was carried out at a temperature of 950 °C for 1 hour with a heating and cooling rate of 5 °C·min⁻¹. Furthermore, a sanding process was carried out to smooth the surface of the sample so that the sample was perfectly coated. The sanding was carried out using sandpaper with a mesh of #500, 800, and 1500. After sanding, polishing was carried out to smooth the surface of the sample using alumina powder.

The samples were then washed using distilled water. Advanced cleaning was carried out using an ultrasonic cleaner to clean the surface from the solid particles that

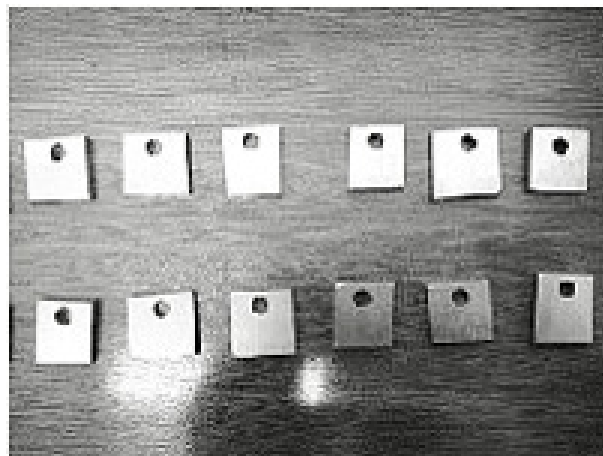


Figure 1. Ti6Al4V ELI titanium alloy samples.

were still attached. The cleaning process was carried out by immersing the sample in a 70 % ethanol solution for 30 minutes. After that, the sample was cleaned again with distilled water, soaked in acetone for 30 minutes. After that, the sample was separated from the acetone solution and then soaked again with a 0.1 M NaOH solution for 1 hour in a beaker. The samples were then cleaned and then dried using a hot plate stirring machine at a temperature of 50 °C for 5 minutes.

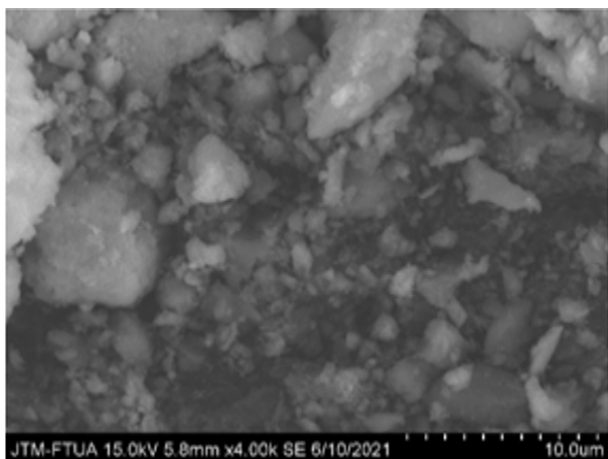
The preparation of the suspension solution was carried out by preparing polyvinyl alcohol (PVA), natural hydroxyapatite and distilled water. The suspension solution was made by dissolving 2 g of PVA into 50 g of distilled water at a temperature of 121 °C and homogenised it for 1 hour at a speed of 300 rpm using a magnetic stirrer. The PVA solution was used as a binder for coating the natural hydroxyapatite on the substrate. After homogenisation, 12 g of natural hydroxyapatite was added slowly. The used natural hydroxyapatite specifications can be seen in Table 2.

Table 2. Specifications of the natural white barramundi fish scale hydroxyapatite.

No	Specification	Value
1	Name	Natural Bio Nano Hydroxyapatite (Bio-nHAp)
2	Particle Size	10 µm
3	Purity	Non-synthetic (99 %)
4	Raw material	White Barramundi Fish Scales
5	Making process	Mild Processing (Without Furnace)
6	Particle Shape	Spherical

In Table 2, it can be seen that the natural hydroxyapatite from the white barramundi fish scales has an average particle size of 10 µm with a non-synthetic purity of 99 %. The processing was carried out by using a mild processing process in the form of a spherical powder. White barramundi fish scales contain up to 66 % calcium and up to 33 % phosphorus.

The particle shape and chemical content of the natural hydroxyapatite processed from the white barramundi fish scales can be seen in Figure 2.



Element	Weight%	Atomic%
P K	33.62	39.59
Ca K	66.38	60.41
Totals	100.00	

Figure 2. SEM and EDX results from the natural hydroxyapatite processed from white barramundi fish scales.

After the natural hydroxyapatite was added to the PVA solution, homogenisation was carried out for 24 hours at a speed of 150 rpm using a magnetic stirrer. Furthermore, a solution of nitric acid (HNO₃) was added to obtain a solution with a pH of 4 for the coating process.

Coating with the dip coating method

The dip coating process was carried out using a self-manufactured dip coating machine at the Physical Metallurgy Laboratory, Mechanical Engineering, Andalas University. This tool is a relatively simple and cheap tool that can be easily reproduced as shown in Figure 3.

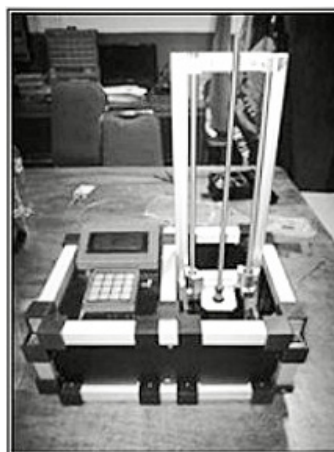


Figure 3. Dip coating machine.

The Ti6Al4V ELI samples were placed on a dip coating device with the appropriate withdrawal speed and coating time parameters. The sample was immersed with a constant withdrawal speed of 4 mm·s⁻¹ and a coating time of 10 seconds, 14 seconds, 24 seconds and 30 seconds. After coating, the sample was dried for 24 hours at room temperature. The variation of the coating time using the dip coating process can be seen in Table 3.

Sintering Process

The sintering process aims to make the coating to be not easily brittle and detachable. The sintering process was carried out using a Dentsply Ceramfire Vacuum Tube Furnace GSL-1100 with a CeramcoFire S model. This process consists of heating, holding, and cooling at the furnace temperature (annealing). This process was carried out at a temperature of 800 °C for 1 hour with a heating and cooling rate of 5 °C·min⁻¹.

Sample Characterisation

The surface morphology of the Ti6Al4V ELI titanium alloy samples was viewed using an Olympus SZX10 LG-PS2 stereo microscope and a Hitachi Horiba Scanning Electron Microscope (SEM) model S-3400. Observations were made to see the microstructure of the natural hydroxyapatite coating on the Ti6Al4V ELI titanium alloy. Determining the thickness of the natural hydroxyapatite layer was carried out using a Sanfix Thickness Gauge Series type GM-280.

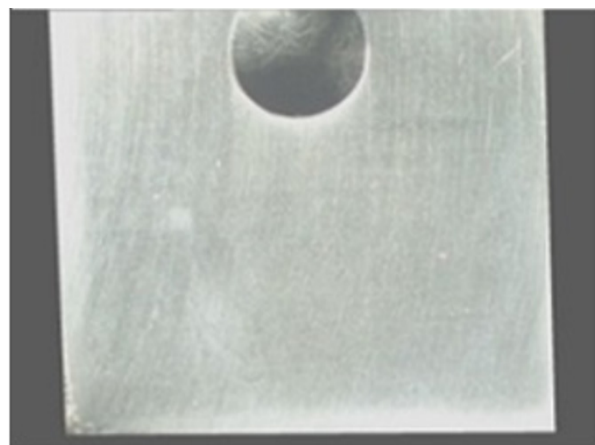
Table 3. Variation of the coating time by the dip coating method.

Sample	Variation	
	Withdrawal Speed (mm/sec)	Coating Time (seconds)
1	4	10
2		14
3		24
4		30

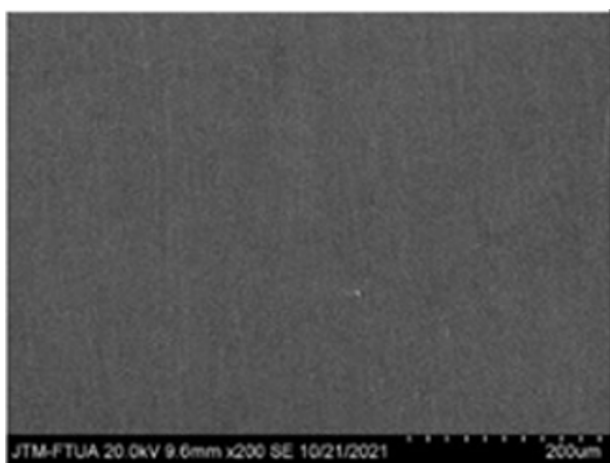
RESULTS AND DISCUSSION

Morphology of the natural hydroxyapatite coating on the Ti6Al4V ELI titanium alloy

Based on the results of the research that has been carried out, it shows differences in the surface characteristics of Ti6Al4V ELI before and after being coated with natural hydroxyapatite.



a)

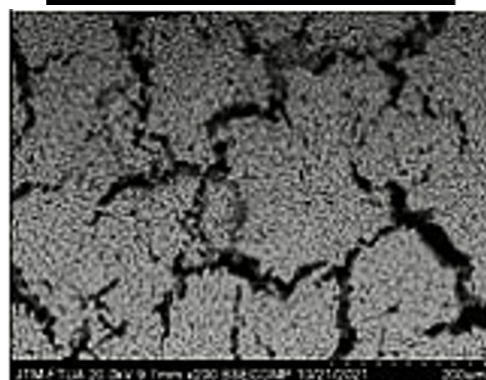


b)

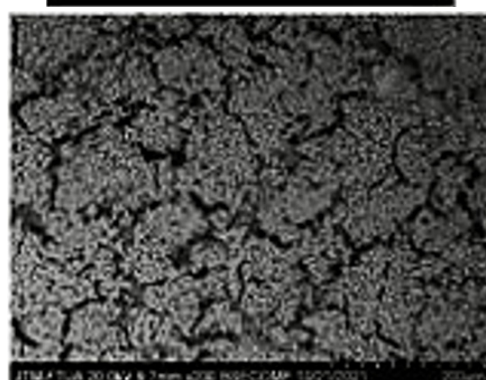
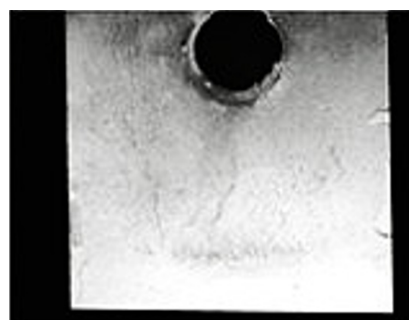
Figure 4. Surface morphology of Ti6Al4V ELI before coating (a) Stereo microscope observations and (b) SEM observations with 200x magnification.

Figure 4 shows the surface of the Ti6Al4V ELI sample which had been sanded and polished so that the surface was even and smooth. Figure 4a shows the result of the stereo microscope where the surface is flat, but there are a few scratches from the sanding. Figure 4b shows the result of the SEM observations of the surface of the Ti6Al4V ELI sample after sanding and polishing.

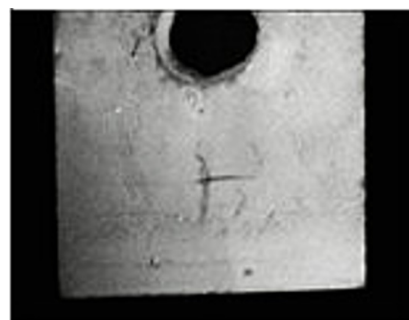
Figure 5 shows the result of the surface observations of the Ti6Al4V ELI sample using a stereo microscope and SEM with a magnification of 200 \times after the dip coating method was applied. Figure 5a shows the results of the coating sample for 10 s. The results show that the sample was completely coated, but there was still natural hydroxyapatite that was not fully bonded so that there were cavities. This is because the coating time was too short and the natural hydroxyapatite had not been deposited properly and did not form a full bond. Figure 5b shows the result of the coating for 14 s. The results of the observations show that the sample was evenly coated, but there was agglomeration on the surface. Agglomeration causes the natural hydroxyapatite particles to not optimally disperse

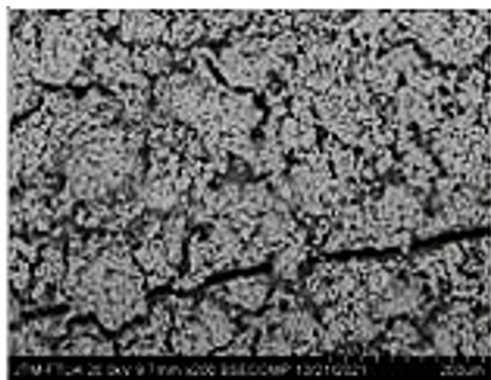


a)

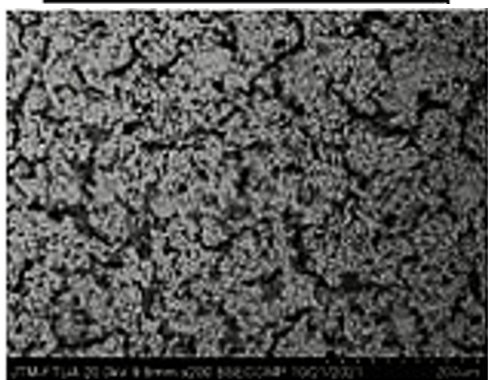


b)





b)



b)

Figure 5. Surface morphology of the natural hydroxyapatite coating using an optical microscope and SEM (a) 10 seconds, (b) 14 seconds, (c) 24 seconds and (d) 30 seconds.

and produces a thin layer. Figure 5c shows the result of the coating for 24 seconds. The results showed that the surface was evenly coated, but there were cracks on the surface. Cracks occurred because a layer that was too thin formed in the centre of the surface. The natural hydroxyapatite layer that is too thin has a higher crack rate than the thicker layer. Figure 5d shows the result of the coating for 30 seconds. The results of the observations show that a layer was thoroughly and evenly formed on the surface of the Ti6Al4V ELI sample. The results of the observations show a good bond between the natural hydroxyapatite particles on the surface of the sample. This is because the coating time of the Ti6Al4V ELI substrate in the solution suspension was longer, thus the deposition time was longer.

Based on the results of the morphology observations of the natural hydroxyapatite layers on the surface of the Ti6Al4V ELI titanium alloy with the dip coating method, it was found that the optimum layer morphology occurred at a variation of the coating time of 30 s. The results show that the surface of the sample had been evenly and thoroughly coated with natural hydroxyapatite. The cracks can be reduced by adding Zirconia as recently reported by Sanny et al. [47-48].

Effect of the variation in the coating time on the surface coverage measurement results on coating surfaces

The surface coverage measurement results were obtained based on the results of the analysis using the imageJ software. The effect of the variations on the surface area of the natural hydroxyapatite layer on Ti6Al4V ELI can be seen in Figure 6.

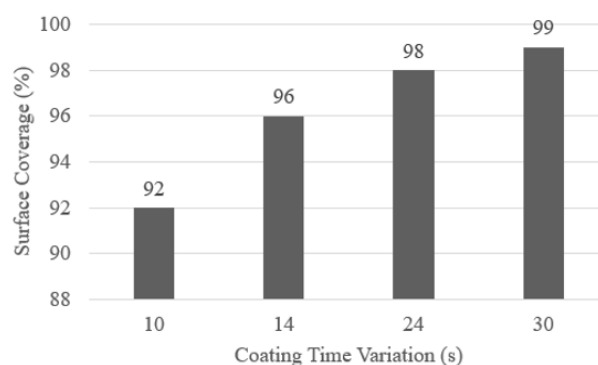


Figure 6. Effect of the variation in the coating time on the surface coverage.

Based on the measurement results in Figure 6, the highest surface coverage value of 99 % was obtained in the sample with a coating time of 30 seconds. The lowest surface coverage value of 92 % was obtained at a coating time of 10 seconds. From the graph, it can be seen that the relationship between the coating and surface coverage is directly proportional, the longer the coating time in the dip coating, the higher the surface coverage value.

Effect of the variation in the coating time on the thickness of the natural hydroxyapatite layer on Ti6Al4V ELI

The coating thickness measurement of the natural hydroxyapatite layer on Ti6Al4V ELI against the coating time can be seen in Figure 7.

Based on the graph in Figure 7, it is known that the average layer thickness ranges from 194 – 213 μm . The highest average layer thickness value was 213 μm at a coating time of 30 s. At 10 seconds, 14 seconds and 30 s, the coating thickness was 194 μm , 199 μm , and 208 μm , respectively.

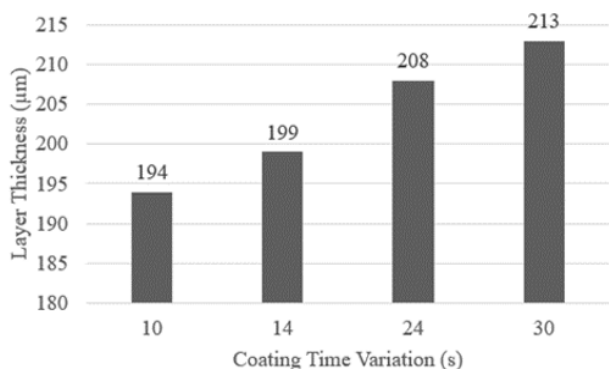


Figure 7. Effect of the variation in the coating time on the layer thickness.

Based on the results of the measurement of the thickness of the obtained layer, the results are in accordance with the parameters required for biomedical applications, namely those coating with the time of 10 seconds and 14 seconds. The thickness parameters required for the natural hydroxyapatite coating for biomedical applications based on the research that has been performed is 50 – 200 µm.

CONCLUSIONS

The surface of the Ti6Al4V ELI sample from the dip coating method showed that the entire surface was coated with natural hydroxyapatite. The most optimal morphology of the natural hydroxyapatite layer on the surface of the titanium alloy Ti6Al4V ELI occurred at a variation in the coating time of 30 seconds, where the entire surface was thoroughly and evenly coated. The highest surface coverage value of the natural hydroxyapatite layer of 99 % on the Ti6Al4V ELI surface was obtained at 30 seconds. The increase in the coating caused the mass and thickness growth of the titanium alloy Ti6Al4V ELI samples. The natural hydroxyapatite layer that meets the parameters of the thickness of biomedical applications is 14 seconds with a thickness of 119 µm and a surface coverage of 96 %. This result is better than previous studies using the EPD (electrophoretic deposition) method at 7 V for 10 seconds, where the layer thickness is 72 µm with a surface coverage of 86 %. This indicates that the simple method of dip coating is applicable for coating titanium alloys with a natural hydroxyapatite.

Acknowledgments

The authors would like to thank the Ministry of Education and Culture for providing the PMDSU Scholarships and research budgets from DRTPM Dikti in 2022.

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