

Study of hydroxyapatite based on Aceh's bovine bone coating on 314L stainless steel as a candidate for coating dental implant materials

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ABSTRACT The use of hydroxyapatite (HAp) based on Aceh's bovine bone has been studied as a candidate for the coating dental implant materials. In this study, the effect of sintering temperature and viscosity on the thickness of the hydroxyapatite layer on the 316L stainless steel plaque was observed. The hydroxyapatite was synthesized from bovine bone and then coated on the plate using the dip-coating method. Then, the plate surface was dried at 110 °C for 30 minutes and sintered with various temperatures of 500, 600, 700, and 800 °C for 1 hour. The plate that has been coated with hydroxyapatite was characterized using a thickness meter to measure the thicknesses of the layer. The water composition of 10 grams/L had a better effect on the thickness of the implant material coated with HAp beef bone compared to the water composition of 4, 6, and 8 (gram/L) ($p < 0.05$). On the other hand, the temperature factor did not affect changes in the thickness of the implant material ($p > 0.05$). The higher water composition (10 grams/L) resulted in a decrease in the thickness of the implant material coated with beef bone HAp.

KEYWORDS: Hydroxyapatite, sintering temperature, viscosity, dip-coating, thickness, coating dental implant

INTRODUCTION

Hydroxyapatite with the formula $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ is chemically similar in mineral components in natural bone and hard tissue in mammals.¹ Hydroxyapatite (HAp) is a derivative of calcium phosphate, which is the main component of calcium from bone which is formed due to the crystallization process to become calcium orthophosphate hydroxide.² HAp can be synthesized from natural sources, for example, bovine bones. A previous study has been carried out and successfully synthesized HAp from Aceh's bovine bone by varying the sintering temperature.³

Hydroxyapatite is generally developed as an application for HAp coatings which are often used to coat metal implants to change the surface properties of the hydroxyapatite. One of the applications using HAp coatings is the osseointegration of dental implants. The implant surface that has been coated with hydroxyapatite shows a natural fit when there is contact between the bone and the artificial bone (e.g., dental

implants). The HAp coating technique was first used for alumina ceramics used for dental implants and metals (stainless steel, Co-Cr alloy, Ti alloy, and Ta) for bone replacement. Hydroxyapatite has been applied as an implant device, especially as a coating, considering its mechanical and chemical properties. The bond between hydroxyapatite and the substrate is the most significant concern because it is essential in the coating method.⁴

Implants using hydroxyapatite coating are more satisfying than using titanium implants.⁵ Hydroxyapatite coating can be done by the dip-coating method. The substrate is dipping in a liquid coating solution in the dip-coating process and then withdrawn from the solution at a controlled rate. The coating layer is deposited on the substrate surface without decomposition or reaction with the metal substrate.⁶ One of the factors that influence the adhesion strength of the hydroxyapatite coating is a binder, where the binder helps the deposition process on the substrate's surface.⁷ The HAp

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material which used as a coating material certainly has several characteristics that must be possessed. One of the characteristics that must be owned is the thickness of the layer. The American Dental Association (ADA) No. 8 classified the materials for zinc phosphate into two types: Type I: Small particle size with a maximum thickness of 25 μm . Type II: Medium particle size and maximum thickness is 40 μm .⁸

Previous studies have been carried out to determine and compare the coating thickness of new adhesive agents by conforming to ADA No. 8 for

zinc phosphate cement specification. Each of the 20 ingredients tested was handled as instructed by the manufacturer. Nine of the twenty materials conform to ADA type I specifications for film thicknesses less than 25 μm , and these include hydroxyapatite cement, glass ionomer cement, zinc phosphate cement, and polycarbonate cement.⁸⁻⁹ In this work, we reported the effect of sintering temperature and viscosity on the thickness of the hydroxyapatite layer using a 316L stainless steel plate and prepared by dip-coating method.

MATERIALS AND METHODS

The bovine bones as a Hydroxyapatite (HAp) source were taken from a slaughterhouse in Banda Aceh City, Aceh, Indonesia. HAp generated from bone was characterized by XRD. Furthermore, the coating on the dental implant material is Stainless Steel 316L to check the composition of the water and its thickness. Hydroxyapatite (HAp) was firstly synthesized from Aceh's bovine bones which the content of calcium (CaO) in bovine bones becomes the raw material in HAp synthesis. Based on the XRD test, the HAp has been successfully formed.³ The preparation of the HAp suspension and the coating method is based on previous research.¹⁰

Boiled beef bones are cleaned of fat that is still attached using distilled water until clean. Then cut into smaller sizes and soaked in 0.1 M NaOH solution for eighth to release the still attached fat. Then washed with distilled water and then dried in the sun to dry to obtain a constant mass. Furthermore, it is calcined at a temperature of 1000 °C for six h using a furnace. Then it was made into powder and sifted (140 mesh), and then milled at 250 rpm for five h, with a ball to powder ratio (ball to powder ratio, 10:1). The bovine bone powder was then characterized using an XRD tool to see the content of the CaO phase.

The synthesis of hydroxyapatite started by reacting CaO powder with a solution of phosphoric acid (%wt), beef bone, and phosphoric acid at 50:50 using the solid-state reaction method. The mixture of the two was then milled with a rotation speed of

350 rpm for 10 hours with a ball to powder ratio (ball to powder ratio, 10:1). The milled CaO powder and phosphoric acid were then sintered using a furnace at a temperature of 900 °C for 2 h. After that, the powder is sintered until cold. Furthermore, XRD testing was carried out to see the hydroxyapatite phase formed

The coating process with HAp suspensions is prepared with various viscosity by varying the composition of water. A dip-coating device was ready for the suspension coating treatment. SS 316L metal plaque measuring 25x10x1 mm was cleaned using grit-500 sandpaper and continued using grit-1000 until the surface looked clean. The SS 316L metal plaque has been sanded then sterilized using ethanol solution for 15 minutes, then drying it in an oven with a temperature of 110 °C for 10 min. Coating by immersing is done in a suspension for 10 sec for 1-time immersion. The same procedure is carried out for suspensions of varying viscosity. After the HAp coating process is complete, the sample is dried using an oven at 110°C for 30 minutes and then sintered with temperature variations of 500, 600, 700, and 800°C for one h.¹⁰ After the cooling process, the HAp layer is formed on the metal plaque SS 316L that tested the thickness of the coating as many as five points on the diagonal plane using a thickness meter. Based on the data obtained from the thickness meter, it can be observed the thickness of the coating from the coating result.

RESULTS

Fig 1 A. shows that the results of the data adjustment indicate that the CaO phase has been formed in the pure beef bone powder sample, which has been calcined at a temperature of 1000 °C. The formation of the CaO phase can occur because at temperatures above 900°C there has been a

transformation of the calcium phase, which was previously in the form of calcium carbonate (CaCO_3) into calcium oxide (CaO) by releasing carbon dioxide (CO_2). Figure (B) shows the hydroxyapatite phase profile formed based on XRD testing with the JCPDS database, PDF#740566.

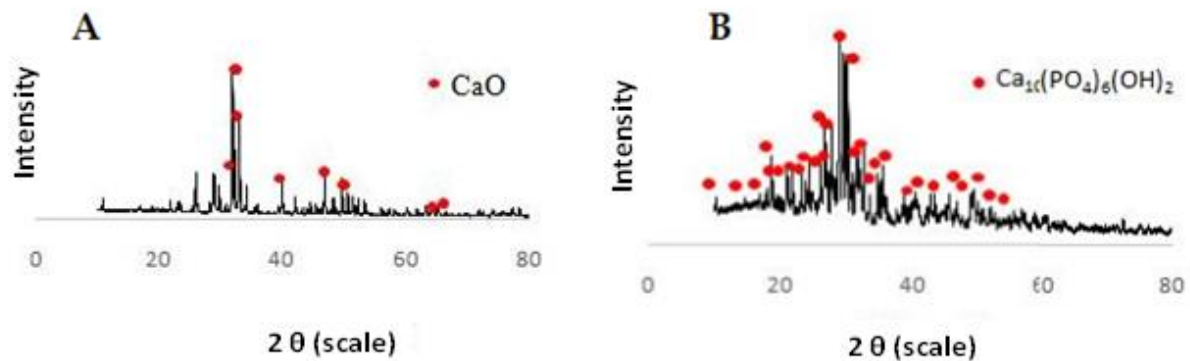


Figure 1. XRD profile of bovine bone powder. (A) phase formation of CaO at an angle of 2θ by adjusting the JCPDS database for CaO with PDF#821691 number. (B) formation of hydroxyapatite

The thickness of the coating is usually determined by the grain size and viscosity of the suspension. However, in this study, various sintering viscosities and temperatures were carried out to assess their effect on coating thickness. The results of the resulting average thickness

measurement Table 1. It can be obtained a graph of the relationship between sintering temperature variations and viscosity variations (based on variations in the composition of water in the suspension) to the layer thickness (Fig 2)

Table 1. Results of thickness calculation

Sintering Temperature (°C)	Composition of water (gram/L)	N	SDV	Thickness (μm)	Frequency
500	4	3	0.01	780	9%
	6	3	0.01	700	8%
	8	3	0.11	580	6%
	10	3	0.11	410	5%
600	4	3	0.00	750	8%
	6	3	0.02	670	7%
	8	3	0.1	550	6%
	10	3	0.11	340	4%
700	4	3	0.12	700	8%
	6	3	0.02	650	7%
	8	3	0.21	530	6%
	10	3	0.001	360	4%
800	4	3	0.31	670	7%
	6	3	0.22	600	7%
	8	3	0.22	480	5%
	10	3	0.11	260	3%

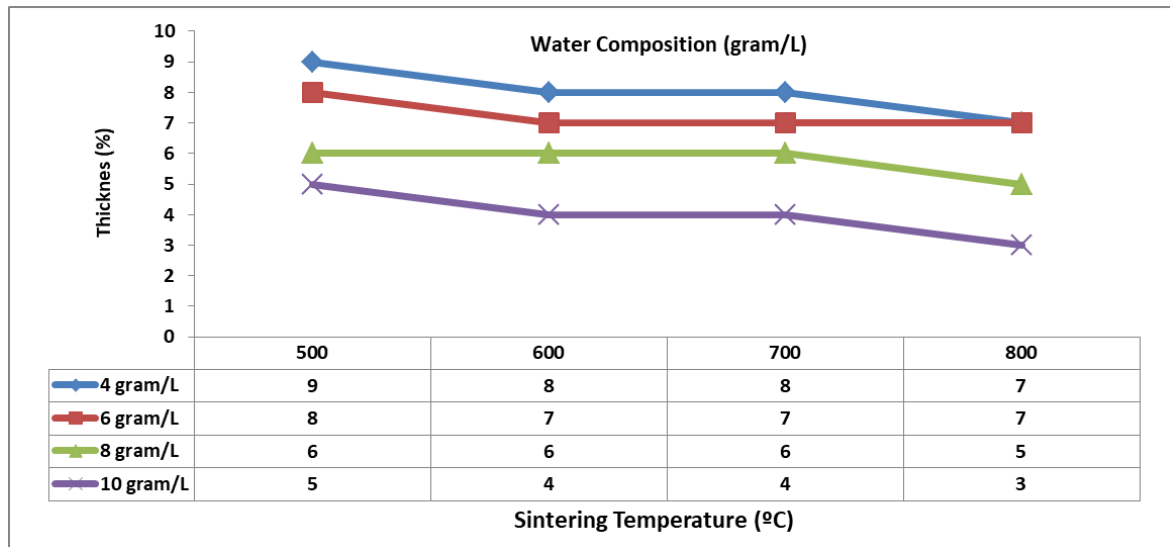


Figure 2. The effect of sintering temperature and water composition variations on the thickness value of the hydroxyapatite lay

The One Way ANOVA analysis showed no significant difference between the temperature groups and each other ($p > 0.05$; 0.809). In addition, the correlation analysis showed that there was no relationship between the effect of temperature on changes in the thickness of the implant material after being coated with HAp from bovine bone ($r = -$

0.268). On the other hand, the degree of water composition used in this study was significantly different between and among groups ($p < 0.05$; 0.000), but there was no relationship between the water composition and the thickness of the implant material ($r = -0.937$).

DISCUSSION

The XRD assay was performed to determine the phase of the powder being tested. The phase is then analyzed using the graph results by adjusting each peak with the Joint Committee on Powder Diffraction Standards (JCPDS) database with the desired stage. The XRD test results are matched with this database by detecting angle two and the lattice gap (d). Calcium in the bones of local Aceh cattle was revealed by adjusting the characterization data with the JCPDS database for CaO. (Fig 1).

Fig 2 shows that the variation of sintering temperature affects the thickness of the coating on the surface of the 316L stainless steel plaque. Where the sintering temperature increases, the thickness value will decrease. It is related to the value of the porosity of a material. A sample has pores as well as void space. Many pore holes and empty spaces will be closed during the sintering process, but these pore holes will not disappear completely, but the porosity value will decrease. In the sintering process, a heated sample will make several microstructure changes. At the same time, heating will occur grain movement resulting in forming a

grain boundary due to action between the grains due to the heat given. Then there is a shrinkage of the pores between the grain boundaries so that the porosity will decrease, and the space that appears due to the shrinkage of the pores will slowly experience grain growth. This pore shrinkage generally has smaller pores than before.¹¹ By decreasing the porosity value and the increase in the given sintering temperature, the resulting thickness value will also decrease. The grain size is getting closer together, which causes the space that was previously still large. It will slowly shrink due to heat treatment. This sintering process will cause the heated samples to close each other so that the empty cavities will gradually disappear.¹²

In Fig 2, it can also be seen that the effect of viscosity variations on the thickness of the hydroxyapatite layer is also inversely proportional to the increase in sintering temperature. The thickness measurement results obtained show that the more the addition of the water composition in the suspension, the smaller the thickness of the hydroxyapatite layer. It is related to the viscosity of

the rest. The viscosity level of each HAp suspension is various based on the composition of the water in the suspension used. Viscosity is the stability of a solution or a fluid to maintain its state due to internal friction in the liquid.¹³ So, the molecules that make up a fluid rub against each other when the fluid flows. Fluid is also greatly influenced by adhesion and cohesion forces. Cohesion is the attractive force between similar molecules, whereas adhesion is the attractive force between unlike molecules.

The adhesion force acts between the walls and the fluid layer (fluid molecules and wall molecules attract each other), while the cohesion force acts between the liquid membranes (fluid molecules attract each other). The thicker a suspension, the greater the energy required to make it flow at a certain speed. The adhesion cohesion force causes the interaction between the HAp suspension and the stainless steel plaque in the coating process. Another reference states that the coating agent's factors affecting the coating's thickness consist of the coating agent's viscosity value and the coating agent's molecular composition.

If the viscosity value is high, it will make it more difficult for the layer to move so that the layer formed is getting thicker. The increase in the thickness of the coating is caused by the viscosity of

the suspension, where if the concentration of the solute in the suspension increases, there will be an increase in the viscosity value.¹⁴ This is following previous research. The addition of water to the hydroxyapatite layer mixture affects the thickness of the hydroxyapatite coating deposited on 316L stainless steel. The more water composition in the suspension, the thickness of the coating will decrease.¹⁵

From the results of the measurement of the thickness values obtained, it can be seen that the thickness values produced in this study have not to match with the characteristics that required to be applied as a coating dental implant material, where the expected thickness is according to the specifications of the American Dental Association (ADA) No. 8 is $\leq 25 \mu\text{m}$.

The use of water composition in the coating process with dental implant material needs to be increased for further research because the findings of this study are not close to the minimum thickness of dental implant material standards. The use of a water composition above 20 grams/L with a temperature of 500-800 °C is highly recommended to obtain a material thickness according to the principles of dental implant materials.

CONCLUSION

The higher water composition (10 grams/L) resulted in a decrease in the thickness of the implant material coated with beef bone HAp. The resulting coating thickness value is found at 260 μm -780 μm

(>25 μm). However, this value does not match with the characteristics of the coating dental implant mater

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