Coating of Hydroxyapatite-Chitosan Composite on TiAlV Alloy with Electrophoretic Deposition Method

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Abstract. Orthopedic implant nowadays often use TiAlV due to its high strength, lightweight and resistant to corrosion. However, TiAlV has poor biocompatibility and bioactivity. The objective of this research was to composite hydroxyapatite with chitosan and coat the surface with TiAlV under electrophoretic deposition method. Electrophoretic deposition process carried out by the potential difference of 80 V in an hour. The coated TiAlV was observed using scanning electron microscope (SEM). There were granules which were believed as hydroxyapatite. The density of those granules was quite high which was believed to be the existence of chitosan. X-ray diffraction and Fourier transformed infrared spectra showed that the alloy has been coated by the hydroxyapatite-chitosan composite. The coated TiAlV by the hydroxyapatite-chitosan composite lowered the corrosion rate and did not have toxic effect to endothelial cells based on the inhibition to the cells was only 35%.

Keywords. chitosan, electrophoretic deposition, hydroxyapatite, TiAlV

1. Introduction

Cases of bone damage such as broken bones had been increased from year to year. Bone damage can be overcome with the implant. Planting the implant will be able to replace the damaged bone or assist in the formation of new bone tissue. The implants used are usually made from metal, ceramic, polymer, or composites. The field of medicine has been frequently used as a metal implants because the metal has good mechanical properties, strong and malleable. Metals that commonly used as an implant material was stainless steel, titanium alloys, and cobalt alloys.

Titanium (Ti) and its alloys such as TiAlV is a metallic material that is now often used as orthopedic implants because it has high strength, easy on weight and excellent corrosion resistance compared to metal alloys based on cobalt and stainless steel. However, metal implants have a poor biocompatibility and bioactivity that can cause corrosion on the metal by body fluids. Metal corrosion can cause inflammatory reactions in surrounding tissue implantation. In addition, the product of this corrosion is toxic ions that harmful to the body [1].

One technique that can be used to solve this problem is to coat the metal with a material which has a high biocompatibility and bioactivity such as hydroxyapatite. Hydroxyapatite was used due to its structural and compositional similarity to that of the mineral phase of hard tissue. Hydroxyapatite also has an excellent binding bone ability [2]. Hydroxyapatite coating will enhance the biocompatibility to be an obstacle to the release of metal ions. The presence of hydroxyapatite implants will also enhance the bone bioactivity by virtue of its chemical components. However, hydroxyapatite has main
limitation of the use of difficult to kept it in place after being implanted in the bone because it is fragile. The weakness is overcome by combining hydroxyapatite with a polymer such as chitosan [3]. Synthesis of hydroxyapatite can be done in two ways, wet methods and dry methods. The usual method that used is a wet method. Wet method consists of precipitation, hydrothermal, and hydrolysis. This method is often to used because of high homogeneity and can be reached easily at low temperature [4]. In addition, with this method obtained a high yield and a pure product, as well as the cheap equipment. Hydroxyapatite can be synthesized using waste of rice fields snail shell (Bellamya javanica). Waste of this shell is an abundant waste but not yet used commercially significant. This waste contains calcium that can be used for hydroxyapatite synthetized [5].

After the synthesis process, hydroxyapatite will be composited within chitosan. Those composites will be coated on the TiAlV alloy. There are two kind of methods that use to make the composites, in-situ and ex-situ. Ex-situ method was selected because the composite will have higher degree of crystallinity. Coating process can be done by electrophoretic deposition (EPD), sol gel coating, and plasma spraying. Plasma spraying and EPD is a method that often to used for coating of hydroxyapatite on metal implants. However, plasma spraying technique can causes hydroxyapatite decomposition due to high temperatures [6]. On the other hand, the EPD method is a method that is easy to do, produce a uniform coating, and using simple and cheap equipment [7]. Based on that, this research will be synthesized hydroxyapatite-chitosan composite. Composites are then coated on TiAlV alloy which is base on the bone implant. The coating method to be used is Electrophoretic Deposition. This research aimed to synthesize and perform characterization of composite chitosan-hydroxyapatite (HAp-Ch) and coat the metal alloy TiAlV with hydroxyapatite-chitosan composite with electrophoretic deposition method. The characterization of TiAlV coated by hydroxyapatite-chitosan was performed.

2. Methods

2.1 HAp Synthesis
Ca(OH)$_2$ powder prepared from the ashes of calcination of rice field snail shell that have been hydrated. Hydroxyapatite synthesized by react the suspension of Ca(OH)$_2$ and solution of (NH$_4$)$_2$HPO$_4$. The solution (NH$_4$)$_2$HPO$_4$ 0.3 M dropped wisely on the suspension of Ca(OH)$_2$ 0.5 M using a biuret at a temperature of 40 ± 2°C and stirred using a magnetic stirrer. pH is monitored and corrected to obtain a 10 of pH. The solution was then sonicated for 12 hours and then aged over the next 24 hours. After that the result was sintering at 900°C for 2 hours. The precipitate is finely ground using a mortar. The HAp was characterized using XRD, FTIR, and SEM.

2.2 Hydroxyapatite-chitosan composite coating
Before coating process, pre-treatment on TiAlV must be done. TiAlV was formed with a diameter of 14 mm and a thickness of 2.5 mm. it must be mashed with 800-grit silicon carbide paper, then washed with water and degreased with ethanol, and allowed to dry on air [8]. After that the alkali treatment was given by soakaed it in NaOH 10% for 24 hours. 3% chitosan solution made by dissolving 3 g of chitosan in a solution of acetic acid 3% to 100 mL. Chitosan solution is then stirred with a magnetic stirrer for 5 hours [9]. Chitosan solution that has been made is taken about 1 mL and then dissolved in 50 mL ethanol, then added about 0.2 g of hydroxyapatite gradually while stirring using a magnetic stirrer. Hydroxyapatite-chitosan colloid solutions that have been formed are connected to two electrodes, as the negatively charged electrode used TiAlV which is the target of coating and platinum as the electrode with positively charged. During the process of electrophoretic deposition, hydroxyapatite-chitosan that were dispersed will move under the influence of an electric current so that it will stick on TiAlV surfaces. The instrument was conducted at a constant cell voltage of 80V for 60 minutes.
2.3 *In vitro test in SBF solution*
TiAlV that has been coated by hydroxyapatite-chitosan, soaked in SBF solution for 10 days. Results immersion taken daily to 1, 3, 5, 7, and 10 day of immersion for 10 ml. After that calcium levels were measured using AAS on 422,7 nm of wavelength.

2.4. Cytotoxicity on Endhotel cells CPAE
Cells were grown using a culture plate 6 wells with a cell number of $2 \times 10^4$ cells / well and the cells were incubated for 24 h at 37°C with 5% CO$_2$. TiAlV metal alloys that was coated by hydroxyapatite-chitosan composite sterilized by 15 kGy gamma-ray irradiation. Furthermore, the sterile sample put in CPAE cells culture that has been flocculated (24 hours old cell culture). Endothelial cell cultures without treatment is used as a negative control. The sample in culture medium were incubated for 72 hours at 37°C with 5% CO$_2$. The number of cells is determined using a hemocytometer. Cell viability checked by Trypan blue dye and percent of inhibition was determined.

3. Results and Discussion

3.1 Hydroxyapatite synthesis
Hydroxyapatite synthesized by snail shell powder that has been converted into Ca (OH)$_2$ as a source of calcium and (NH$_4$)$_2$HPO$_4$ as a source of phosphate. The process of synthesis used wet precipitation method for high yield, low cost, and pure products [10]. Synthesis of hydroxyapatite is running through the reaction

$$10\text{Ca(OH)}_2 + 6(\text{NH}_4)_2\text{HPO}_4 \rightarrow \text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2 + 6\text{H}_2\text{O} + 12\text{NH}_4\text{OH}$$

Based on the reaction above, the ratio of calcium and phosphate is 1.67, so that the concentration of each precursor is determined by the stoichiometry. (NH$_4$)$_2$HPO$_4$ solution flowed Ca(OH)$_2$ solution with 1 mL / min of flow rate. This is done with the aim to get uniformity size of hydroxyapatite partici. Hydroxyapatite can be formed at room temperature, but the precursors are reacted with keeping the temperature at 40 ± 2°C and pH 10. The temperature used in the reaction process will increase the rate of reaction of formation of hydroxyapatite [11]. According to Dahlan *et. al* [12], pH 10 was the optimal condition of hydroxyapatite formation. The pH setting is done by adding dropwise NH$_4$OH when the mixing reach pH below 10. If the reaction run at pH <7, it will be the formation of calcium monophosphate dehydrated that easily soluble in water, whereas at pH 8 will be formed Ca$_2$P$_2$O$_7$ (β-TCP), at pH 9 will be formed mixture (β-TCP) and hydroxyapatite, and at pH 10 will form hydroxyapatite. The use of NH$_4$OH in order to restrain the other chemical compounds because NH$_4$OH was the by product of reaction above. Hydroxyapatite were then characterized using SEM and XRD.

Diffractogram hydroxyapatite synthesis (Figure 1) shows that the powder obtained is hydroxyapatite. This is demonstrated by the emergence of a distinctive diffraction pattern of hydroxyapatite at 20 31.84°, 32.17° and 32.86°. It is quite appropriate when referring to JCPDS to hydroxyapatite, which shows that the typical peak of hydroxyapatite is at 20 31.77°, 32.19°, and 32.90°.
3.2. Hydroxyapatite-chitosan composite coating on TiAlV metal

Metal implants when used in the long term will lead to the release of metal ions that can cause side effects. This can be prevented by coating the metal with some biocompatible materials. As a biocompatible material, hydroxyapatite is used to coat TiAlV, in addition, this material has high bioactivity. However, hydroxyapatite has a low mechanical resistance. Hydroxyapatite is fragile, so its requires some materials to improve its mechanical properties such as chitosan [3]. Enhancement of this mechanical properties had cause by hydrogen bonds (Figure 2) between the OH, NH$_2$ groups of chitosan and OH groups of hydroxyapatite.

The formation of hydroxyapatite-chitosan composite made with ex-situ method, a method that is done by adding chitosan after the synthesis of hydroxyapatite. It its made by in-situ method it can cause chitosan disappear and leave pores on hydroxyapatite when the composite was sintered [13]. In-situ method made by dissolving chitosan with hydroxyapatite powder in ethanol to form a colloidal mixture. Colloid solutions are then used to coat the TiAlV with the EPD.

Metal TiAlV given some treatment before it is coated with a colloidal solution. The metal surface sanded with sandpaper 1200 grid so that the dirt on the surface of the metal disappear and it make smoothest surface of this metal. Rough metal surface can disrupt the coating results. After that the metal is washed with water and soaked with ethanol in order to degrease the metal surface [8]. Furthermore, 10% NaOH solution was used to soak the metal for 24 hours to allow the metal surface more hydrophilic, so that the composite easier to stick to metal surfaces. In addition, the hydrophilic surface on the metal also able to improve implant osseointegration [14].

TiAlV metal that pre-treated with platinum then used as the electrode on the circuit EPD tool. The coating process in this method occurs in two stages, migration stage of charged particles in solution by the electric field (phase electrophoresis) and the coagulation stage of particles that formed a layer on the electrode (phase deposition) [13]. Chitosan particles have a positively charged NH$_2$ group so that the negative electrode (cathode) used TiAlV and the positive electrode (anode) used platinum. Illustration assembly EPD tools used are shown in Figure 3.

![Diffraction pattern of synthesize HAp](image)

**Figure 1.** Diffraction pattern of synthesize HAp

![Hydrogen bonds on hydroxyapatite-chitosan composite](image)

**Figure 2.** Hydrogen bonds on hydroxyapatite-chitosan composite
Figure 3. EPD illustration

Potential that used in the coating process was 80V and the process is conducted for one hour. When the EPD is run, then the suspension of hydroxyapatite-chitosan will migrate towards.

Figure 4. Composite hydroxyapatite-chitosan on TiAlV alloy

TiAlV and deposited on its surfaces. After the coating process is completed, TiAlV was dried on air so the composite will hard to release from the surface. Figure 4 shows the results of coating processes by EPD method

3.3. SEM characterization

Hydroxyapatite and TiAlV coated by hydroxyapatite-chitosan composite then characterized by electron microscopy to determine the morphology Microscopy. The difference of hydroxyapatite and hydroxyapatite-chitosan composite will show the existence of chitosan in composite. These morphologys shown at Figure 5.

Figure 5. Surface morphology (a) hydroxyapatite (b) TiAlVcoated by hydroxyapatite-chitosan composite

Hydroxyapatite morphology (Figure 5a) taken at 2000x magnification. The morphology shows agglomerated granules that believed as hydroxyapatite. This is supported by Santos et. al [11] which
states that the morphology of hydroxyapatite that was agglomerated granules and needle form. These results are then compared to the morphology of the hydroxyapatite-chitosan composite for the presence of chitosan in the composite.

The surface morphology of TiAlV coated by hydroxyapatite-chitosan composite (Figure 5b) taken at 2500x magnification. Hydroxyapatite-chitosan composite surface is clearly different from pure hydroxyapatite. This shows hydroxyapatite incorporated in the matrix of chitosan. The matrix of chitosan causes the hydroxyapatite particles closer. Penetration hydroxyapatite particle into the matrix of chitosan led to the clots formation on the surface of the composite [15].

3.4. XRD characterization
To confirm that hydroxyapatite and chitosan not made new compound, the XRD characterization has been done. The diffraction pattern obtained is shown in Figure 6b.

These results indicate that the chitosan-hydroxyapatite coating successfully formed with the advent of the typical peaks of hydroxyapatite at 31.74°, 31.95°, and 32.05°. In addition, there are also the typical peaks of Titanium at 40.13 ° and 77.84° Ti. These results were then correlated with the data JCPDS to hydroxyapatite and titanium, which shows three typical peaks of hydroxyptite appear at 31.77° 32.19°, 32.90° and one of the typical peak of titanium is at 40.16 ° 77.80°. These results do not show any diffraction peaks for chitosan due to its amorf properties.

The existence of chitosan can be evidenced by the shifting peaks compared hydroxyapatite diffraction pattern before it mixed with chitosan (Fig 6a). The pattern of diffraction peaks shifted from 31.84°→31.77°, 32.17°→31.95°, and 32.86°→32.05°. This shift may be caused by the compression of a chitosan matrix through chitosan -hydroxyapatite bonds [16]. In addition of these shifting, the peak intensity of the diffraction pattern of hydroxyapatiten was decrease. Nikpour et. al [15] explains that the decline of intensity is due to the interaction between the hydroxyapatite with chitosan.

3.5. FTIR characterization
Analysis of the functional groups were identified using FTIR spectroscopy instruments. This analysis aimed to prove that the coat on TiAlV is hydroxyapatyte-chitosan composite. The result of this characterization shown in Figure 7.
Absorption bands typical of hydroxyapatite appears at wave number 3450 cm\(^{-1}\) (OH stretch vibration); wave number 1036 cm\(^{-1}\) and 865 cm\(^{-1}\) (asymmetry bending vibration of PO\(_4^{3-}\)); and 602 cm\(^{-1}\) and 566 cm\(^{-1}\) (symmetry bending vibration of PO\(_4^{3-}\)) [17]. The presence of chitosan according to Raut and Khairkar [18] indicated by the appearance of an absorption band at wave number 3439cm\(^{-1}\) (C-H and O-H stretch vibration); wavenumber 2925cm\(^{-1}\) (strain symmetry CH3); wavenumber 1666cm\(^{-1}\) (C=O stretch vibration); wavenumber 1438cm\(^{-1}\) (C-N stretch vibration); wavenumber 1363cm\(^{-1}\) (bending vibration CH3); wavenumber 1155cm\(^{-1}\) (C-O-C bending vibration); and wavenumber 1073cm\(^{-1}\) (C-OH stretch vibration). Based on the spectrum in Figure 7, it is typical for chitosan absorption band hve been seen. The results of the analysis of functional groups using FTIR show that composite of hydroxyapatite-chitosan has been formed.

3.6 Corrosion behavior

Metal implants in the body will undergo a process of corrosion by body fluids will cause inflammation and produce ions harmful toxic from the body. Hydroxyapatite-chitosan composite coating on a metal alloy TiAlV done one of them in order to inhibit the corrosion of the metal. Therefore, the rate of corrosion of the metal TiAlV need to be measured to prove that implants made of metal TiAlV has a corrosion rate that meets the standards for medical applications. After being coated by a hydroxyapatite-chitosan composite metal corrosion rate is again measured to see a decrease in the rate of corrosion of the metal.

Corrosion rate measurements performed in the form of NaCl 0.9%. This is done because the fluid body similar to the solution. The test results obtained corrosion rate shows that the rate of corrosion of metals TiAlV before and after coating, respectively for mpy 0.4152 and 0.3980 mpy. TiAlV metal corrosion rate after coating decreased by 4.14%. The decline shows that the corrosion rate of hydroxyapatite-chitosan composite coating can improve corrosion resistance of TiAlV. Drevet et al. [19] states that the electrodeposition coating serves as a protector for the substrate of titanium and improve corrosion resistance. TiAlV metal corrosion rate can also be seen from the its \(I_{corr}\) value. \(I_{corr}\) value will be directly proportional to the rate of corrosion. \(I_{corr}\) value obtained in accordance with those reported by Kwok et al. [8] which states that the value \(I_{corr}\) TiAlV that has been coated with hydroxyapatite ranging between 0.40-0.75 uA cm\(^{-2}\).

The corrosion rate before and after coated TiAlV better than the corrosion rate of 316L stainless steel metal on research Sari et al. [20] who obtain corrosion rate by 0.4872 mpy. TiAlV meets the standards to be applied as an implant if it refers to the corrosion rate of the European standards for medical applications by 0.457 mpy. The presence of hydroxyapatite-chitosan composite layer on the metal surface TiAlV be advantages in the use of these metals into the implant. In addition to helping the growth of bone tissue, the coating also improves corrosion resistance on metal TiAlV. TiAlV metal coated hydroxyapatite-chitosan composite would be better used than TiAlV without coating.
3.7. Ca release in SBF solution

TiAlV that has been coated with hydroxyapatite-chitosan soaked in SBF (simulated body fluid) for 10 days to see the release or binding of calcium ions. SBF solution was used because it contains ions that are approximately equal to the human body fluids. The concentration of calcium during the immersion was observed on days 1, 3, 5, 7, and 10 using AAS. The results of the measurement of the concentration of calcium in SBF solution is shown in Figure 8.

![Figure 8. Ca enhancement rate in SBF solution](image)

Figure 8 shows that Ca release rate decrease. This indicates that on hydroxyapatite will occur the deposition process of calcium ions to the apatite crystals. The results are quite consistent with Sharma et al. [21] that said the first step in the apatite crystal growth seen after immersion for seven days, because at that period occurred Ca\(^{2+}\) deposition process. According Mohandes and Niasari [22] a decrease in the concentration of Ca\(^{2+}\) ions occurs on the 3rd day of immersion, while in this study, up to the 10th day of immersion has not decreased the concentration of Ca\(^{2+}\) ions. This is caused by the presence of chitosan is able to withstand the release of Ca\(^{2+}\) ions into the SBF solution.

3.8 Cytotoxicity CPAE

TiAlV metal implants should be non-toxic when implanted into the body. Thus, it is necessary to test the citotoxicity TiAlV implants that have been coated with hydroxyapatite-chitosan. Citotoxicity test was done with the help of endothelial cell culture media. Endothelial cell culture selected as the media for an important role in the process of angiogenesis. Angiogenesis is a physiological process of the body that assist in the formation of bone tissue [23]. Cytotoxic properties of the sample can be seen through the light microscope magnification of 80x. this can be done by comparing the morphology of endothelial cells in the control (Figure 9a) with cell morphology on coated TiAlV samples after 72 hours’ post-incubation (Figure 9b).
The morphology showed that on sample coated TiAlV has a few endothelial cell alive. This indicates that the coated TiAlV inhibit the life expectancy that can show by the value of percent inhibition. Percent inhibition showed inhibition of endothelial cell growth. The higher the percent inhibition of the implant more toxic the implant is. Coated TiAlV have been tested showed that the percent inhibition of endothelial cell amounted to 35.90% (Table 1). Value percent inhibition obtained is good. It is powered by a ISO 10993-5: 2009 that stated that the percent inhibition of few than 70% is considered to haven’t a cytotoxic effect. However, in a study of Zhang et al. [14], metal alloys Mg-Nd-Zn-Zr have a better inhibition percent is below 20%.

| Table 1. percent inhibition endothel cells after 72 h incubated |
|---------------------------------------------------------------|
|                  | Cell amount/mL | % inhibition |
| Coated TiAlV     | 125,000        | 35.90        |
| Control          | 195,000        | 0.00         |

4. Conclusions
TiAlV metal alloy has been successfully coated with hydroxyapatite-chitosan composite using electrophoretic deposition method. Particles of hydroxyapatite looks still not uniform. Coated TiAlV released calcium ions slowly into the SBF solution. Corrosion test results have shown that the coating process can reduce the rate of corrosion of metal TiAlV. Based on the rate of corrosion, coated TiAlV will be worthy for being implant material according to European standards for biomedical applications. Coated TiAlV implants haven’t cytotoxic effect to endhotelial cell according to ISO 10993-5: 2009.

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