Coating of Nanotube Ti6Al4V Alloy with Hydroxyapatite-Chitosan-Polyvinyl Alcohol Composite

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Abstract

Hydroxyapatite (HAp) can be applied as a bone implant material. HAp was composited with chitosan and polyvinyl alcohol (PVA) and coated to Ti6Al4V to improve its biocompatibility. The composite HAp-chitosan-PVA coated to anodized and unanodized Ti6Al4V. Modification of the Ti6Al4V surface by anodizing was done using a 20 V voltage. The Ti6Al4V metal alloy was coated with a composite using the electrophoretic deposition (EPD) method with a voltage of 80 V. The coated composites in Ti6Al4V nanotubes and non-nanotubes were characterized by Scanning Electron Microscope (SEM), X-Ray Diffraction (XRD), Fourier Transform Infrared (FTIR), corrosion test with potentiostat, and in vitro bioactivity test with Atomic Absorption Spectroscopy (AAS). The XRD results showed a distinctive peak indicating that the composite comprises HAp and Ti. The SEM Ti6Al4V nanotube results show a pore size of 24 nm. The FTIR results show that there were functional groups of HAp, chitosan, and PVA. The corrosion rate on Ti6Al4V nanotubes without coating was greater than with coating. The In vitro bioactivity test showed that the decrease of Ca^{2+} Ti6Al4V nanotube concentration was faster compared to non-nanotube Ti6Al4V.

Keywords: chitosan, hydroxyapatite, nanotube, non-nanotube, Ti6Al4V

Abstrak (Indonesian)

Hidroksiapatit (HAp) dapat diaplikasikan sebagai bahan implan tulang. HAp dikomposit dengan kitosan dan polivinil alkohol (PVA) dan dilapisi dengan Ti6Al4V untuk meningkatkan biokompatibilitasnya. Komposit HAp-kitosan-PVA dilapisi Ti6Al4V anodized dan unodized. Modifikasi permukaan Ti6Al4V dengan anodizing dilakukan menggunakan tegangan 20 V. Paduan logam Ti6Al4V dilapisi dengan komposit menggunakan metode elektroforesis deposisi (EPD) dengan tegangan 80 V. Komposit yang dilapisi pada nanotube Ti6Al4V dan non-nanotube dikarakterisasi dengan Scanning Electron Microscope (SEM), X-Ray Diffraction (XRD), Fourier Transform Infrared (FTIR), uji korosi dengan potensiostat, dan uji bioaktivitas in vitro dengan Atomic Absorption Spectroscopy (AAS). Hasil XRD menunjukkan puncak yang khas yang menunjukkan bahwa komposit terdiri dari HAp dan Ti. Hasil nanotube SEM Ti6Al4V menunjukkan ukuran pori sebesar 24 nm. Hasil FTIR menunjukkan adanya gugus fungsi HAp, kitosan, dan PVA. Laju korosi pada nanotube Ti6Al4V tanpa coating lebih besar dibandingkan dengan coating. Uji bioaktivitas in vitro menunjukkan bahwa penurunan konsentrasi nanotube Ca2+ Ti6Al4V lebih cepat dibandingkan dengan non-nanotube Ti6Al4V.

Kata kunci: kitosan, hidroksiapatit, nanotube, non-nanotube, Ti6Al4V

INTRODUCTION

Bone damage such as bone cancer and bone loss require bone grafting to replace the damaged bone. The bone is a natural composite material composed of organic and inorganic components. One of the inorganic components of bone (45-65%) is hydroxyapatite (HAp). The $Ca_{10}(PO_4)_6(OH)_2$ compound is very stable under normal physiological conditions. Synthesized HAp is good for bone transplant because it can bind to bone and is biocompatible and osteoconductive. Synthesized Hydroxyapatite is the most widely used material for

bone regeneration such as coating for the implant and bone filler [1]. Synthesized HAp has a composition similar to that of human bones but has delicate properties (low mechanical strength) so there need to be additional polymers or the like which can sustain the usefulness of HAp itself [2]. Therefore, it is necessary to make a material containing HAp with mechanical strength equivalent to bone mechanical strength and is resistant to pressure. The composite used in this study is a material composed of a polymer and ceramic. The ceramics used are hydroxyapatite. Hydroxyapatite is mixed with natural and artificial polymers that will make the composite stronger when applied to the bone.

This composite material must remain stable in contact with body fluids and other aqueous solutions. The natural polymer that can be used is chitosan while the artificial polymer that can be used is polyvinyl alcohol (PVA). Chitosan is chosen because it is abundant in nature, has good biocompatibility, biodegradability, excellent physicochemical properties, and commercially available while being relatively cheap [3]. Polyvinyl alcohol is chosen because it has the advantage of having good chemical stability, is water soluble, biocompatible, biodegradable, non-toxic, can form pores, and can make hydrophilic interactions with the body tissues [4]. The hydroxyapatite used is synthesized using the wet method. According to Darwis and Warastuti [5], HAp synthesis by wet method produces HAp with excellent purity (Ca: 35% and P: 20%) and resembles the composition of calcium and phosphate content in bone, with a Ca/P ratio of 1.69. The HAp used in this study was synthesized from shells of snails found in rice fields (*Bellamya javanica*). The composite is coated on a metal surface to lower the corrosion rate and increase its mechanical strength when applied to the bone. The metal alloy used is Ti6Al4V. Aluminum-coated Ti6Al4V alloys are inert. Pure titanium and titanium alloys are commonly used for orthopedic implants because they have good mechanical strength, chemical stability, and biocompatibility [6]. Titanium alloys and pure titanium can be surface modified. Metal surface modification of alloys can be done by making nanotubes. The techniques of making nanotubes used in this study were through anodization [7]. Titanium coated composites are used because these metals have good capability to increase osteoblast bonds and can trigger cell development. Nano-sized titanium can be applied to biomaterial composites as they are biocompatible and bio-inert. In addition, the presence of nanotubes will trigger a significant acceleration of osteoblast bonds. The composites that have been made will be coated onto a Ti6Al4V alloy

of nanotubes and non-nanotubes. One method to be used for coating composites in Ti6Al4V metal alloys is electrophoretic deposition. Electrophoretic Deposition (EPD) has the advantage of being a simple, flexible tool in the use of materials and coatings, as well as rapid deposit rates [8]. The results of the composite HAp-chitosan-PVA were characterized by several characterization techniques.

MATERIALS AND METHODS
Materials
The materials used are Ti6Al4V metal alloys acetic acid, aquadest, ringer lactate infusion solution, $(NH_4)_2.HPO_4$, $Ca(OH)_2$, chitosan, NH_4OH, ethanol, acetone, polyvinyl alcohol, NH_4F, ethylene glycol, platinum electrodes (Pt), universal pH indicator, SiC paper or grit 320-1500 grit paper, and Whatman filter paper.

Surface Modification of Ti6Al4V Metal Alloys Being Nanotubes [9]
The Ti6Al4V metal alloy is cut with a diameter of 14 mm with a thickness of 5 mm. Pieces of the alloy are scrubbed using SiC paper or 320-1500 grit sandpaper. Subsequently, the metal is cleaned with ethanol, acetone, and distilled water with their own sonicator for 15 minutes. After that, the metal is dried with the room temperature. The formation of nanotubes on the surface of a metal is carried out by the method of anodization. The composition of the solution composition for anodizing is ethylene glycol: water of 9: 1. The NH_4F solids were weighed at 0.6 g and dissolved in the solution so the NH_4F concentration used was 0.6% (w/v). Pieces of Ti6Al4V are anodized in NH_4F 0.6% (w/v) with a voltage of 20 V from direct current, DC. Anodization is done within three hours. The anodizing process is carried out with two electrodes, the platinum electrode (Pt) as the cathode and the Ti6Al4V pieces as the anode. After the anodizing process, the Ti6Al4V pieces are removed, rinsed with distilled water, and dried at room temperature. The anodized metal is characterized by stereo and SEM microscopes.

Composite Coating on Metal Alloys Ti6Al4V Electrophoretic Deposition Method [10]
The Ti6Al4V alloy as a negatively charged electrode and platinum as positive electrode. The EPD process is performed at a constant cell voltage of 80 V for 1 h at room temperature. During the EPD process, the dispersed HAp-chitosan-PVA will move due to the electrical effect so that it will adhere to the surface of the Ti6Al4V metal alloy. During the deposition electrophoresis process, the dispersed HAp-chitosan-

PVA will move and attach to the surface of the Ti6Al4V metal alloy. The composite is attached to both anodizing (nanotube) and without anodizing metal alloys (non-nanotube). Next, the coated metal alloy will be dried.

Corrosion Test

The corrosion test uses potentiostat devices with potential values of -20 mV to 20 mV vs logarithmic current in corrosion media. Corrosion media, which is an infusion solution of 0.9% NaCl. Samples with a diameter of 1.4 cm are placed on the working electrode, then inserted in the potentiostat device. The comparative electrode, which is the calomel electrode while the carbon electrode as the auxiliary electrode. The corrosion rate can be determined due to the corrosion process caused by the movement of electrons in the electrochemical reaction.

In Vitro Bioactivity Test

The standard parent solution containing Ca^{2+} with a concentration of 1000 ppm was diluted to 5 ppm in 100 mL with distilled water. The standard curve of concentration and absorbance relationships with concentrations of 0, 0.5, 1, 1.5, 2.0, and 2.5 ppm was then made. The standard solution already prepared is then measured by AAS at a wavelength of 422.7 nm. Thereafter one Ti6Al4V metal which was coated with HAp-chitosan-PVA, was immersed in a ringer lactate infusion solution for 14 days. Immersion results were taken daily to 1, 3, 5, 7, 9, 11, and 14 by 10 mL. Then measured the calcium content using AAS at a wavelength of 422.7 nm.

RESULTS AND DISCUSSION

Nanotube and Non-Nanotube Alloy Metal Ti6Al4V

The Anodizing technique is a modification of metal surface using an electrochemical process. The anodizing technique is the process of forming oxide layer by reacting metal with the electrolyte solution. According to the research of Kaczmarek-Pawelska and Krasicka-Cydzik [7], the oxide layers that can form on titanium metal are TiO_2, Al_2O_3, VO_2, V_2O_3, and V_2O_5. The Ti6Al4V metal alloys used in this study were given 2 treatments, without anodization and anodization. The anodizing process will produce nanotubes on the metal surface so that it will affect whether or not the composite is coated onto the metal surface. Nanotubes are a form of a metal or compound that has a tube morphology at nanometer size. The process is described in **Fig. 1**, the Pt electrode is used for the anodizing process and is immersed in the electrolyte solution so that nanotubes will form on the

metal surface. The Pt electrode was used as the cathode while the metal Ti6Al4V was used as the anode.
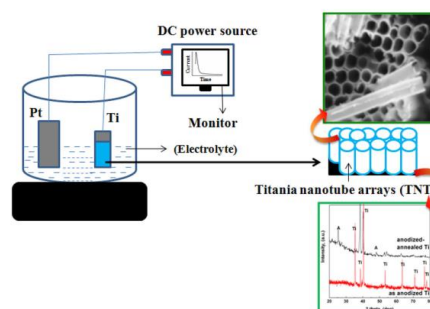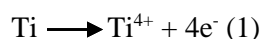

Figure 1. Illustration of the manufacture of nanotubes in Ti [9]

The things that will affect the anodizing process are electrolyte pH, electrolyte concentration, temperature, time, current, and voltage. There are generally two types of electrolyte solutions that can be used in the anodizing process, organic and aqueous electrolytes. The aqueous electrolyte has an acidic pH and high-water content whereas the organic electrolyte has a small amount of oxygen. Electrolyte solutions can affect the diameter of nanotubes in metals. Electrolyte solutions that can be used to make nanotubes in titanium are those of F⁻ ions such as NH_4F/CH_3COOH, H_2SO_4/HF, and Na_2HPO_4/NaF [9].

In the first-generation anodizing process HF was used as an electrolyte. However, HF was replaced with NH_4F for the second generation. The third and fourth generation electrolytes for the manufacture of nanotubes added organic and water electrolytes (0-5% w/v). The aqueous electrolyte selected in this study was NH_4F 0.6% (w/v) in a mixture of ethylene glycol and water with a ratio of 9:1. According to the research of Kaczmarek-Pawelska and Krasicka-Cydzik [7], the concentration of 0.6% (w/v) NH_4F in ethylene glycol and water mixtures can form nanotubes in Ti6Al4V metal alloys with a diameter of 50±5 nm. Ethylene glycol is chosen because of its properties as one of the high viscosities (viscous) organic electrolytes. Organic electrolytes are selected to produce fine, long nanotubes in nanotubes. Meanwhile, the addition of water to ethylene glycol is intended to make the tube geometry in pore nanotubes longer and smoother [10]. The titanium metal alloy is immersed in an electrolyte solution containing F⁻ ions, titanium metal is placed on the positive pole (anode) while the Pt electrode is placed at the negative (cathode) pole with a voltage of 20 V. Time used for this process was 3 hours at room temperature. The optimal temperature for the manufacture of nanotubes with organic electrolytes is between 0-40 °C [13]. If the temperature used exceeds

40 °C, the oxide layer on the surface of Ti formed is unstable and not uniformly shaped [11]. The current used is a direct current (DC) with a potential value of 20 V. The DC current is used because this current is a direct current so that the F$^-$ ion of NH$_4$F will move in the direction of the alloy surface and will form small pores of nanometer size (nanotube). The potential value used will affect the diameter, thickness, and distance between the pores of the nanotubes. A potential value of 20 V is chosen for the anodizing process because at a voltage above 40 V, nanotubes cannot be formed whereas if the voltage is below 20 V of thin pore nanotubes. According to research Ghicov et al. [14] reported that the optimum potential value for pore nanotube formation is 20 V.

When the electrode is has flowed a potential value, there will be a migration event on the load. The hydrogen ion will migrate to the cathode (Pt) and undergo reduction to form the hydrogen gas at the cathode [13]. The negative ion from F$^-$ will move toward the anode (Ti6Al4V). At the anode, Ti6Al4V metal alloys will oxidize so that the charge value increases to Ti^{4+}. When Ti^{4+} ions have formed at the anode, the ions from F$^-$ moving toward the anode will react with Ti^{4+} ions to form an oxide layer. The mechanism of the formation of nanotubes occurs in three stages, namely (1) oxidation of Ti, (2) the formation of the oxide layer, and (3) the dissolution of the oxide layer. The reaction to the anodizing process occurring in the Ti6Al4V metal alloy follows the following reactions:

$$Ti \longrightarrow Ti^{4+} + 4e^- \quad (1)$$

$$Ti^{4+} + 2H_2O \longrightarrow TiO_2 + 4H^+ \quad (2)$$

$$TiO_2 + 6F^- + 4H^+ \longrightarrow [TiF_6]^{2-} + 2H_2O \quad (3)$$

The result of the observation of Ti6Al4V metal alloy surface with stereo microscope can be seen in **Figure 2**. From the figure it can be seen that there are uniform scratches so that it is suspected that the nanotube has formed on the surface of Ti6Al4V metal alloy. The observation of nanotubes with stereo microscopy was poor compared to observations with SEM because it did not show any significant results.
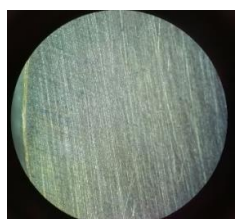

Figure 2. Observation of stereo microscope on Ti6Al4V surface with anodized treatment

Aluminum metal alloys Ti6Al4V without anodization (**Figure 3a**) shows no pores on the metal surface while on anodized Ti6Al4V metal alloy (**Figure 3b**) there are pores of nanometer size. The mean diameter of the pores of the metal alloy surface is 28.04 nm. The morphology of the nanotube form is uniformly produced, but there are still parts of the surface of the metal alloy that do not form nanotubes. This is because of the α and β phases in Ti6Al4V. The α phase is enriched with Al and the β phase is enriched in the presence of V. Nanotubes will grow uniformly in the α phase but the solubility of vanadium in the electrolyte leads to the more dominant phase of β compared with the α phase. The use of ethylene glycol (pH = 6.5) also prevents attacks from the β phase but vanadium has a much faster solubility than Al [15]. In addition, the longer the anodizing process the larger the tube diameter and tube length but not exceeding 6 hours [15]. The morphology of the nanotubes being less uniform may also be due to the sanding not being even and smooth enough. In addition, could also be due to oxide layers of vanadium such as VO_2, V_2O_3, and V_2O_5 being dominant.
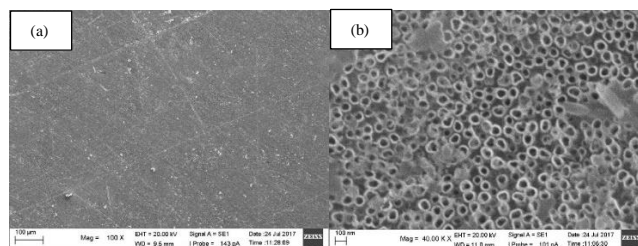

Figure 3. Morphology of SEM test of Ti6Al4V metal alloys without anodizing (non-nanotube) (a) and anodizing (nanotube) (b)

Coating of Ti6Al4V with Composite

Before the coating of Ti6Al4V, HAp was composited with chitosan and polyvinyl alcohol (PVA). This treatment is intended to correct the deficiencies of HAp. According to Firnanelty [16], based on the viscosity and the cytotoxicity of the composite HAp-chitosan-PVA can be applied as a bone implant material due to its fragile hydroxyapatite properties so it is necessary to add polymers such as chitosan and PVA. The suspected interaction between the HAp-chitosan-PVA composite is shown in **Figure 4**. The NH2 clusters in chitosan have an interaction with Ca^{2+} ions in HAp thus forming a coordination complex [17]. In addition, chitosan has an intramolecular hydrogen bond. Meanwhile, OH groups of PVA have intermolecular hydrogen interactions with OH groups of chitosan.
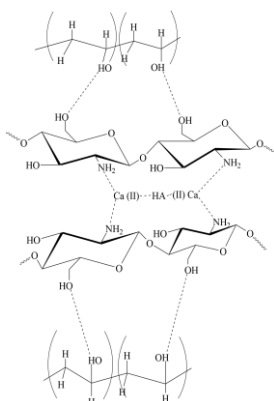
Figure 4. Suspected interaction of the composite HAp-chitosan-PVA

Chitosan is dissolved with acetic acid, it is intended for chitosan to be protonated so that chitosan content becomes positive and coating process can be done. The formed composite has an agglomerated physical appearance. Furthermore, the composites added ethanol. The function of ethanol to form a suspension on composites. In addition, an aqueous medium added to the composite in the EPD must have an inert nature. In this study, the Electrophoretic Deposition (EPD) method is used to attach the composite to the metal surface. The EPD is coating method that uses component separation techniques or charged molecules based on their different migration rates in an electric field. According to Abdeltawab et al. [18], the voltage and timing values applied to the metal will be directly proportional to the weight and thickness of the coated composite. The potential value used is 80 V. The time used in this study was 1 hour. The greater the value of voltage and time given, the greater the weight and thickness of the composite. The EPD process was carried out at ±29 °C. The use of room temperature was chosen because of the higher temperatures causing the formation of hydrogen gas at increased cathode, the hydrogen gas that formed diffuses through the sediment layer resulting in a more porous layer [19].
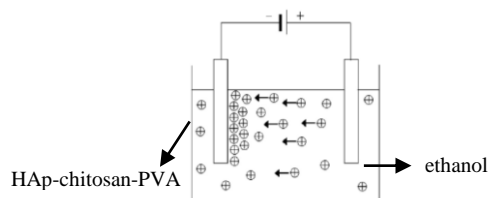

Figure 5. Schematic of electrophoretic deposition process

The coating process occurs due to the transfer of the composite HAp-chitosan-PVA to the negatively charged electrode (cathode), the Ti6Al4V (**Figure 5**). The positively charged electrode (anode) used is Pt. The Pt electrode was chosen because of its inert nature. The composite coating process with the EPD method consists of 2 stages. The first stage is the stage of migration of charged particles that reside in the liquid solvent due to the electrical voltage (electrophoresis). The second stage is the coagulation stage of the particles to form a salt (deposition) [20].
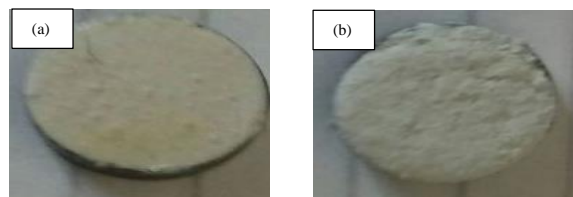

Figure 6. Ti6Al4V coated composite HApchitosan-PVA (a) non-nanotubes and (b) nanotubes

According to **Fig. 6**, the HAp-chitosanPVA composite is coated onto a Ti6Al4V metal alloy. In **Figure 6a** it can be seen that the composite HAp-chitosan-PVA coated on non-nanotube Ti6Al4V metal looks smoother compared to the more rough and thicker Ti6Al4V nanotube metal (**Figure 6b**). This is possible because of the effect of the nanosized pores on the metal so that more and more composites are attached to the metal surface [20].

Characterized Composite with SEM

In **Figure 7** it can be seen that the composite attached to the metal surface is thicker on the metal Ti6Al4V nanotube (**Figure 7b**) than the non-nanotube metal (**Figure 7a**).
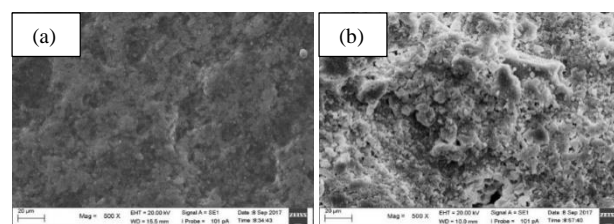

Figure 7. Appearance of the surface of the composite HAp-chitosan-PVA morphology on non-nanotube (a) and nanotube (b) metal alloys

The average particle size on Ti6Al4V surface nanotubes and non-nanotubes were 47.01 and 69.78 nm. The mean particle size at cross section of nanotube and non-nanotube Ti6Al4V were 116.31 and 171.98 nm. In Ti6Al4V nanotube metal alloys (**Figure 7b**), the composites attached to the metal alloy surface appear less evenly or the presence of uncoated metal parts. The particle size of the composite on non-nanotubes is

greater than that of composites in Ti6Al4V metal alloy nanotubes. The unevenness of composites attached to metal alloys may be due to the potential used in the EPD process were not optimum. The use of potential values in the EPD process varies depending on the type of metal. **Figure 8** shows the agglomerated granules which are believed to be HAp. Santos et al. [21] suggested that the form of HAp is a granule or needles that are not sharp in the meantime, the clumps on the SEM test result show that the lump is a combination of HAp, chitosan, and PVA.
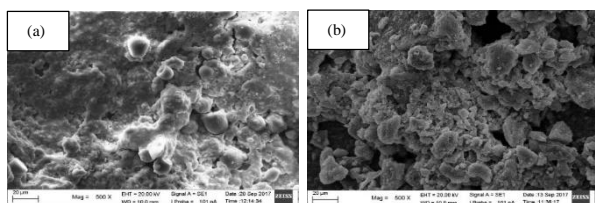

Figure 8. Appearance of cross-sectional morphology of composite HAp-chitosan PVA in non-nanotube (a) and nanotube (b) metal alloys

Characterized Composite with XRD

The XRD test is performed to determine the 2θ phase of the coated composite on Ti6Al4V by matching the 2θ degree of JCPDS. According to **Fig. 9** the highest peak phase 2θ HAp of a composite HAp-chitosan-PVA with Ti6Al4V non-nanotubes appears at an angle of 26.23°, 32.13°, 33.26°, 47.04°, and 49.58°. The highest intensity is at an angle of 32.13°. The peak is then matched with JCPDS data number 09-432 and shows matches with JCPDS HAp data. Typical X-ray diffraction patterns in Ti emerging from non-nanotube Ti6Al4V are 35.67°, 38.52°, and 40.70°. The highest peaks of the typical X-ray diffraction pattern of Ti occur at an angle of 40.70°. Meanwhile, the highest 2θ peak HAp peak of the composite with Ti6Al4V nanotubes appears at an angle of 29.33°, 31.80°, 32.91°, 35.30°, and 38.50°. The 2θ Ti phase peaks of the highest intensity Ti6Al4V nanotubes are present at an angle of 35.30°, 38.50°, and 40.35°. X-ray diffraction pattern with the highest intensity at an angle of 40.35°. The peak comes from Ti. Peaks with the highest intensity Ti are matched with JCPDS data number 44-1294. Measured crystal size of HAp on non-nanotube Ti6Al4V and composite coated nanotubes, 105.17 and 65.65 nm. The crystallinity of HAp in composites is good because the 2θ phase peaks are sharp or not widened. The peaks of chitosan, PVA, Al, and V do not appear due to small concentrations, below 10 %. Meanwhile, the concentration of HAp used in this study was 10% (w/v). In addition, due to the amorphous nature of chitosan and PVA, X-ray

diffraction peaks in the diffractogram do not appear. There is a slight shift in the pure X-ray diffraction HAp with HAp composed of chitosan and PVA. Examples of peak shifts occur at an angle from 31.96° to 32.13° and 31.80°. The highest intensity of non-nanotube Ti6Al4V in the HAp phase while Ti6Al4V nanotubes has the highest intensity in the Ti phase (**Figure 9**). It is possible that on the Ti6Al4V nanotube coating with EPD there is still a surface of the metal alloy not covered by the composite so that the peak Ti has the highest intensity and the phase peak HAp ranks second. Meanwhile, on the peak non-nanotube Ti6Al4V HAp is the 2θ phase peak with the highest intensity. In addition, Ti intensity on Ti6Al4V nanotubes can be increased after being anodized due to the effect of TiO_2 coating. This is in line with Wang et al.[22] who states that the diffraction peak at Ti is increased because anatase and rutile of TiO_2 can be detected with the appearance of a typical Ti peak.
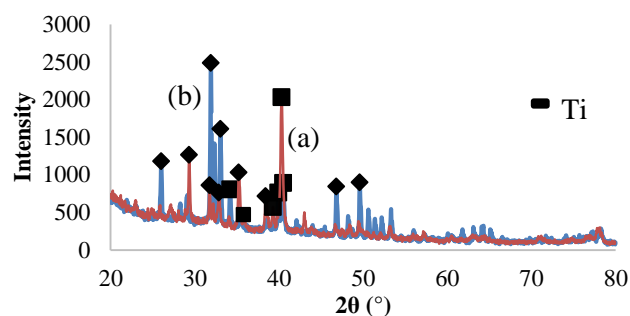

Figure 9. Differentiated Ti6Al4V non nanotube (a) and nanotube (b) coated composite HAp-chitosan-PVA

Characterized Composite with FTIR

The FTIR analysis was also applied to the composite HAp-chitosan-PVA to detect the functional groups of the composites that appear on the FTIR spectrum (**Figure 10**). In the wavelength 3500-3000 cm-1 there is a widening of the peak, at wave number 3331.07 cm^{-1} due to the combined vibrations of OH chitosan,PVA,HAp,and NH of chitosan. In the wave number 2912.51 cm^{-1} is the stretching vibration of CH/CH_2 in the composite. This peak is formed because inside the composite there are chitosan and PVA which have a CH/CH_2 group. Stretching vibration C=O of amide I appears at wave number 1610.56 cm^{-1}. In wave numbers 1571.99 and 1101.35 cm^{-1} is the bending vibration of NH amide II and C-O-C from chitosan. The HAp characteristics of the Hap-chitosan-PVA composite uptake band also appear on the spectrum with the 1433.11 cm^{-1} wave number is the C-O stretch vibration of CO_3^{2-}. The appearance of the carbonate absorption band (CO_3^{2-}) indicates that there is

absorption of air from CO_2. In addition to the presence of absorbent bands of carbonates, the notion that in the composite there is a HAp is the presence of a phosphate group (PO_4^{3-}). Vibration stretch from PO_4^{3-} appears at wave number 1049.28 cm-1. In addition, there are also bending vibrations of PO_4^{3-} which appear at wave numbers 630.72 and 570.93 cm^{-1}. From the composite FTIR spectrum it appears that there are still compounds of HAp incorporated in the composites as evidenced by the absence of alteration of the changed chemical components in the composites as indicated by the presence of CO_3^{2-} and PO_4^{3-} uptake bands. The main functional groups that make up HAp are OH^- and PO_4^{3-} groups, but sometimes CO_3^{2-} groups appear as impurities. Based on research by Rodríguez-Lugo et al. [23], CO_3^{2-} absorption appears at wave numbers 1321 and 1417 cm^{-1}, and phosphate ion absorption appears at wave numbers 469.567 and 603 cm^{-1}.
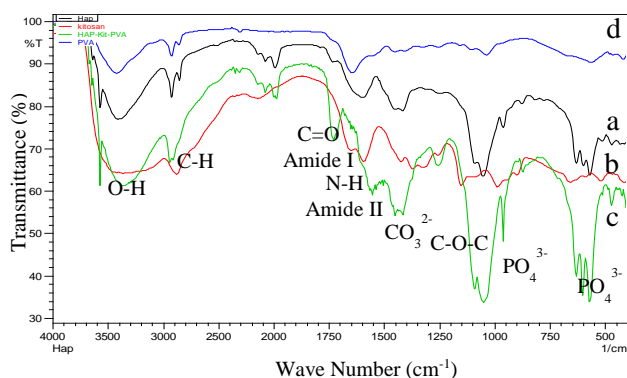

Figure 10.   FTIR Spectrum of HAp (a), chitosan (b), HAp-chitosan-PVA (c), and PVA (d)

Corrosion Rate of Ti6Al4V

Corrosion events are a material degradation process that progresses little by little due to electrochemical attacks that occur when the metal is placed in physiological solutions such as the solution in the human body. The corrosion rate test was performed to compare the corrosion rate between non-nanotube Ti6Al4V and nanotubes before and after coating (**Figure 11**). The unit value of the corrosion rate is mpy (mils per year). The corrosion rate value obtained in this study exceeds the established European standard for implantable materials, which is 0.486 mpy. But the corrosion rate of titanium for biomaterial applications usually has a corrosion rate value between 0.8-5.0 mpy. The corrosion current (Icorr) shows the amount of metal ions that enter the physiological solution. Icorr values of non-nanotube and nanotube Ti6Al4V without measurable coatings were 1.68 and 1.89 ($\mu A/cm^2$). Meanwhile, Icorr values of non-nanotube and nanotube Ti6Al4V with measured

coatings were 0.75 and 1.86 ($\mu A/cm^2$). If the Icorr is highly measured, many metal ions will dissolve into the physiological solution. The smaller the Icorr value the better the corrosion resistance of a metal. The value of Icorr obtained in this study is in accordance with the research undertaken by Dihn et al. [24] which explains that the value of Icorr Ti6Al4V without coating will be greater than the coating. Based on Fig.12, it is seen that the corrosion rate of the coated composite product is smaller than without coating. The corrosion rate of non-nanotube Ti6Al4V and nanotube blank or without coating with Hap-chitosan-PVA composite is 1.15 and 1.29 mpy. Meanwhile, the non-nanotube Ti6Al4V corrosion rate and nanotubes coated by Hap-chitosan-PVA composites were 0.51 and 1.27 mpy. This event occurs because the composite will block the surface of Ti6Al4V so that the composite will be lost first. Therefore, the corrosion rate with coating is less than without a composite coating because the composite layer blocks the release of metal ions from Ti6Al4V. In contrast to Ti6Al4V without composite coatings that will directly interact with a 0.9% NaCl physiological solution. In addition to the composite on Ti6Al4V, the metal oxide coating also affects the rate of corrosion. As described in the previous section that the oxide layers formed in the anodizing process are TiO_2, Al_2O_3, and V oxide. The oxide layer can block the release of metal ions in Ti6Al4V so that the corrosion rate will be low.
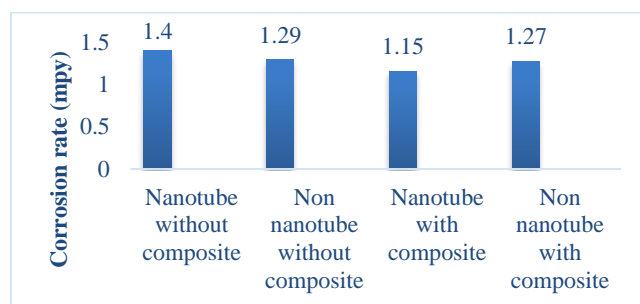

Figure 11. Corrosion Rate of Ti6Al4V

The corrosion rate on composite coated Ti6Al4V nanotubes has a higher corrosion rate value than non-nanotube Ti6Al4V corrosion rate. This event is possible because the composite coating layer Ti6Al4V has an uneven thickness so that Ti6Al4V corrosion rate will increase. The thickness of the barrier layer on the metal becomes an important factor in corrosion resistance. The thicker the composite layer that covers the metal gives more protection against the coated metal. The thickness of the composite layer of HAp-chitosan-PVA attached to Ti6Al4V depends on the potential value and time used in the coating process by

the EPD method. In addition, the presence of metal ions of Ti6Al4V such as Ti^{4+} that have not formed TiO_2 oxide layer (Fig. 1). This factor is confirmed by the presence of Ti6Al4V surface parts that have not yet formed nanotubes. The concentration of NaCl physiological solution affect on the corrosion process. The concentration of NaCl used is directly proportional to the rate of corrosion of the metal. As Shahba et al. [25], the higher the NaCl concentration used, the higher the corrosion rate.

In Vitro Bioactivity Test Result

In vitro bioactivity test was performed for 14 days. The Ti6Al4V coated metal alloy with HAp-chitosan-PVA composite is immersed in intravenous fluids. In vitro bioactivity test was performed to determine the concentration of Ca^{2+} ion release in lactated ringer infusion fluid using AAS. The lactated ringer infusion fluid is chosen because the ion content is similar to the fluid in the body such as Ca^{2+}, Cl^-, K^+, and Na^+ ions. Ca^{2+} ion content in lactated ringer infusion fluid is in $CaCl_2$ form. In **Fig. 12** we can see the yield curve of Ca^{2+} ions in lactate ringer with non-nanotube and nanotube Ti6Al4V. Ca^{2+} concentrations were observed by immersion of 1, 3, 5, 7, 9, 11, and 14 days. The relationship generated in the in vitro bioactivity test was the relationship between soaking days and Ca^{2+} concentration (mg/L).
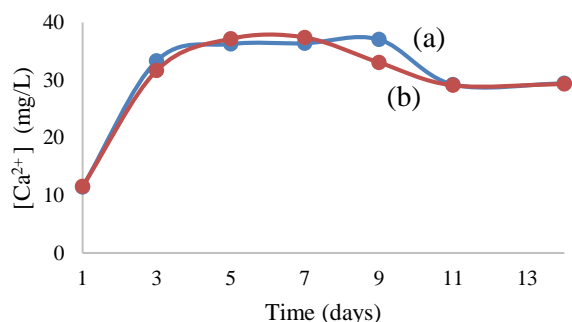

Figure 12. In vitro curves of Ti6Al4V composite coated with non-nanotube (a) and nanotube (b) infusions

Figure 12 shows that the concentration of non-nanotube Ca^{2+} Ti6Al4V ions increased during the 1st day to the 9th day. But on the 11th day there was a decrease of Ca^{2+} concentration, which was 29.25 ppm. Meanwhile, in Ti6Al4V nanotubes, Ca^{2+} ion concentrations increased from day 1 to day 7 and decreased on day 9, to 33.08 ppm. When compared to the release of pure Ca^{2+} HAp ions, the release of Ca^{2+} ions on compounded polymer HAp will be less than pure HAp due to the presence of chitosan and PVA

incorporated in HAp. Supposedly, the presence of chitosan and PVA as a glue between HAp and Ti6Al4V will make the release of Ca^{2+} ions to be slow or gradually increase. The immersion effect causes cracks in the composite layer. The gap is generated from the diffusion and reaction between the surface layer and the SBF solution. Ca^{2+} ions from HAp will be released to the SBF solution so that the calcium ion in the SBF solution will increase. The exchange of ions between HAp and SBF solution can occur due to differences in chemical potential. In addition, after immersion also occurs chemical dissolution between HAp with Ti from Ti6Al4V. The soaking of metal alloys coated by composites should be done for more than 14 days to obtain saturation point on the release of Ca^{2+} ions from HAp to SBF solution. Gu et al. [26] observed the effect of release of Ca^{2+} ions during 56 days of immersion and resulted after immersion of 14 days there was a new granule attached to the metal surface layer. Moreover, Ti6Al4V nanotubes have a slow release rate of Ca^{2+} ions due to an oxide layer on the metal surface. On Ti6Al4V nanotubes Ca^{2+} concentrations decreased faster than on non-nanotube Ti6Al4V. This is because the concentration of Ca^{2+} in the composite attached to Ti6Al4V has nothing to do with the physiological solution. Ca^{2+} ions in the physiological solution precipitate and form a new apatite with PO_4^{3-} ions from a physiological solution that can be attached to a metal alloy.

CONCLUSION

The Ti6Al4V metal alloy was successfully coated with a composite Hap-chitosan-PVA. Ti6Al4V nanotubes were also successfully formed at NH4F concentrations of 0.6% (w/v) with an ethylene glycol-water electrolyte solution. The pore diameter of nanotubes obtained was 24.04 nm. Characterization of Ti6Al4V metal alloys with nanotube and non-nanotube morphology showed that the crystalline size and particle size of Ti6Al4V composite coated nanotubes were smaller than non-nanotube Ti6Al4V. Composite Ti6Al4V coated metal alloys, both nanotubes and non-nanotubes, have a lower corrosion rate than those not composite coated. The faster Ti6Al4V nanotube coatings decreased Ca^{2+} concentrations in physiological fluid than non-nanotube Ti6Al4V.

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