Fabrication of porous hydroxyapatite/chitosan composite bodies through dip-coating method

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Abstract. Porous hydroxyapatite have been attracting considerable attention for bone surgery. Relatively low mechanical strength of porous hydroxyapatite hampers its application for permanent bone implant. The aim of this work was to study the effect of chitosan concentration and time of coating on the physical, chemical and mechanical properties of porous hydroxyapatite/chitosan composites prepared using dip coating technique. Firstly, chitosan powder was dissolved into acetic acid with a concentration of 0.50 %, 0.75 % and 1.00 % (w/v). Subsequently the porous hydroxyapatite bodies were coated with chitosan using dip-coating method at dip time during 30, 45, 60 min. The composites bodies were dried in room temperature for 24 h. The hydroxyapatite/chitosan bodies with compressive strength in the range of 1.52 - 4.05 MPa, porosity of 75 – 78 %, pore size of 22.8 - 26.61 μm, and density of 0.71 - 0.78 g/cm3 were obtained. The increase of chitosan concentration from 0.50 % to 0.75 % can decrease the porosity from 78 % to 76 % and pore size from 28.25 μm become 22.88 μm. The compressive strength of the composites increased with the coating time.

1. Introduction

Patients with bone damage such as osteoporosis increase annually. Women over the age of 50 years and one in between five men over 50 years suffering from osteoporosis [1]. Therefore, the handling of bone damage is very important considering that bone have a role as human body support. Implantation is the replacement of body tissues by using compatible materials [2]. Implant treatment has a high success rate that has been rated as high as 95 to 99% [3]. There are three material groups can be usually used as implant i.e. allograft, xenograft and autograft [4]. But, availability of natural materials is very limited. Therefore, it has been a lot of research to find a synthetic material that compatible of the human body. One of the applied implantation methods is a porous material [5]. The pore size of 100 μm creates the bone tissue grow and connect to the entire surface of the material, in this way the implant serves as a structural bridge or scaffold for bone formation. The advantage of porous implants is that mechanical stability increases with the growth of bone tissue in the material. Hydroxyapatite [Ca10(PO4)6(OH)2] a ceramic material that has the physical and chemical similarity to the minerals contained in the bones and teeth of humans so that biocompatible and can be used in orthopedic surgery [6]. Most of the resulting porous hydroxyapatite is still deficient in bone implant applications because the poor compressive strength [5]. To solve the problem, porous hydroxyapatite can be composited with polymer material for use as bone substitutes. Use of polymers as the composite porous hidrooksiapatiit aims to become harder and not easily brittle [7]. Polymer that can be used porous hydroxyapatite composite is chitosan. Chitosan contains glucosamine and N-
acetylglucosamine unit 1-4 glycosidic long chain [8]. Chitosan is a polymer that is considered suitable for composites with ceramics because of the nature of the chitosan that is biocompatible, biodegradable, osteoconductive and can reduce the brittle properties of calcium phosphate compounds-chitosan as bone substitution biomaterials. Chitosan can be produce from shrimp waste and the percentages of deacetylation obtained varied between 80.0–81.8 % near the degree of deacetylation of the commercial chitosan [9].

Composite alumina with potassium phosphate and poly vinyl alcohol as binder have been done using a coating method that produces alumina ceramics with a porosity level of 65 % and a compressive strength value of 3.0 MPa [5]. In addition, composite hydroxyapatite was produced a scaffold of bovine hydroxyapatite with poly vinyl alcohol coating where the optimum compressive strength of 3.2 MPa in 3 % poly vinyl alcohol [10]. Also, composite was produced hydroxyapatite/poly-l-lactid acid (PLLA) scaffold were prepared via Thermally Induced Phase Separation (TIPS) by using a ternary PLLA- dioxane-water solution (polymer-solvent-nonsolvent) [11]. The obtained results demonstrated the possibility to analyze the internal distribution of the particles via a non-invasive technique; furthermore a homogeneous hydroxyapatite content and a uniform size distribution in the whole sample was observed, which allows one to assess that the scaffold production technique does not create neither a filler gradient nor a particle sedimentation. In this paper reports the effect of chitosan concentration and coating time on compressive strength, pore size, porosity and density of hydroxyapatite/chitosan body as composite substitution of bone.

2. Materials and methods

2.1. Preparation of porous hydroxyapatite bodies
The foaming protein starch consolidation method was used to produce porous hydroxyapatite bodies [12]. Slurry was made by mixing 24 g of hydroxyapatite (Sigma Aldrich, German), 3 g of starch, 8 g Darvan (R.T Vanderbilt, USA), with 28 g of egg yolk in a glass beaker. The slurry was stirred at 200 rpm for 3 hours. Furthermore, the slurry was poured into a stainless steel mold and heated at 180 °C for 1 hour. The yolk were removed by burn-out in a furnace at 600 °C for 2 hours. Then heating was continued up to 1,200 °C ended by 2 hours holding time at the temperature.

2.2. Process of chitosan coating
Chitosan solution was prepared by dissolving chitosan powder (Sigma Aldrich, German) with concentration 0.50 %, 0.75 %, 1.00 % w/v into 2 % v/v acetic acid solution (Merck, Germany). Then the mixture was stirred at room temperature until a homogeneous solution. Coating process of porous hydroxyapatite with chitosan was conducted using dip-coating technique during 30, 45 and 60 min dip time. Furthermore, porous hydroxyapatite/chitosan composite bodies were dried at room temperature for 24 hours.

2.3. Analysis method
Analysis of morphological of the composite bodies were conducted using SEM (JSM-6390A, Japan). The XRD (PanAnalytical, German) analysis was used to determine the composite crystalline phases whereas the compressive strength was test using universal testing machine. Composite density was measured by comparing the weight with the composite volume and porosity was calculated using the following equation 1.

\[
Porosity (P_p) = 1 - \frac{\rho_s}{\rho_t} \times 100\%
\]

Where \(\rho_s\) is sample density and \(\rho_t\) the theoretical density 3.14 g/cm³.

Pore size distribution of porous bodies were analysed using ImageJ 1.5 j8 application from Wayne Rasband National Institute of HealtUSA [13].
3. Result and discussion

3.1. Characteristics of porous hydroxyapatite bodies

Porous hydroxyapatite/chitosan composite bodies obtained after drying process were performed resulted in samples without significant deformation which indicated good homogeneity of the materials. Figure 1 shows three porous hydroxyapatite/chitosan composite samples with cylindrical shape.

Figure 1. Hydroxyapatite/chitosan composite bodies with cylindrical shape

Coating time and chitosan concentration influence the density, porosity and pore size of the composite bodies. Figure 2 shows the effect of chitosan solution concentration on the density of hydroxyapatite bodies. Density plays an important role in determining the microstructure of the dried composite bodies. Figure 2 shows the density of porous hydroxyapatite before and after coated with chitosan.

![Figure 2](image)

Figure 2. Density of composite bodies versus (a) chitosan concentration and (b) coating time

The density of composite body was found to increase from 0.73 g/cm³ at 0.50 % chitosan concentration to 0.75 g/cm³ at 0.75 % concentration. However, the density decrease again when the concentration increased to 1.00 %. It is believed that high viscosity chitosan solution resulted in thick chitosan layer at surface body of the composite, so that the density also increase. But, when the concentration increase to 1.00 % resulted in higher viscosity too. It will make the chitosan solution can not coat the surface of hydroxyapatite bodies in the pores, moreover addition of chitosan concentration produce high density of bodies. Similarly, longer coating time leads to higher density of the hydroxyapatite bodies and corresponding thicker chitosan layer at hydroxyaptite bodies [5].
Porosity is considered to have a significant impact on compressive strength of hydroxyapatite bodies. Figure 3 shows the effect of chitosan concentration and coating time of hydroxyapatite bodies porosity. The control of density and porosity not only by the chitosan concentration but also by managing coating time. Porosity of hydroxyapatite before chitosan coated on the body wall surface is 78%. Increasing coating time can decline the porosity of the composite body. While varied chitosan concentration will make the fluctuation of hydroxyapatite porosity [5].

**Figure 3.** Porosity of hydroxyapatite bodies at different (a) chitosan concentration and (b) coating time

Physical Porperties of Porous Hydroxyapatite Bodies.

Many studies have been made to establish a relationship between strength and microstructural properties such as pore structure and pore size distribution. The strength of a porous body is strongly affected by the strength of the body wall and the surface flaws on the wall. Figure 4 shows the effect of chitosan concentration and coating time on compressive strength of the hydroxyapatite/chitosan bodies. The compressive strength value of porous hydroxyapatite before coated is 1.43 MPa and it increase when chitosan coated on the body. The highest compressive strength of the obtained composite is 4.05 MPa at 0.75 % chitosan concentration and 60 min coating time. Compressive strength of composite body at 1.00 % chitosan concentration decreased compared to the 0.75 % concentration. It can be explained that pore size and and pore distribution are the dominant factors in controlling the strength of material mechanisms, so that the chitosan layer distribution can be evenly distributed throughout the pore and surface of hydroxyapatite bodies [5].

**Figure 4:** Compressive strength of hydroxyapatite bodies
3.2 Micro and macrostructure of hydroxyapatite body

Figure 5 shows the morphology of the hydroxyapatite bodies coated with chitosan 0.50 % and 0.75 %. The figure depict a layer of chitosan seen starting to form but still imperfect in which there is a layer of chitosan ruptures. In contrast to Figure 5b chitosan layer is formed not rupture but distribution still uneven.

Furthermore, from Figure 5c and 5e show the morphology of bodies hydroxyapatite coated with chitosan 0.75 % for 30 min. Analysis result showed that the chitosan layer is formed evenly. Layers are not rupture and can coat all of bodies but the grain size on the bodies still visible. This indicates that the layer of chitosan coat the bodies is not perfect (Figure 5e). Similarly, Figure 5d and 5f, show chitosan layer are evenly and thick to the hydroxyapatite bodies. In addition, the grain size on the bodies begin invisible (Figure 5f). This indicates the layer of chitosan coated throughout the bodies so, compressive strength of the bodies can achieve great point. The unevenness of chitosan layers can be due to uneven distribution of pore, porosity and density of hydroxyapatite bodies. Other causes are the density and viscosity chitosan solution. The lower density of solution formed the layer of chitosan becomes thinner and if the density solution is high then chitosan layer formed thicker, highy density makes difficult solution to coat all the pores. And this phenomena causes the chitosan layer is formed unevenly.

![Figure 5](image)

**Figure 5.** Macro and microstructures of hydroxyapatite/chitosan composite for (a) 0.50 %, 30 min; (b) 0.50 %, 60 min; (c, e) 0.75 %, 30 min; (d, f) 0.75 %, 60 min.

Pore size of hydroxyapatite before composited with chitosan was 28.25 µm. Pore size distribution of hydroxyapatite bodies before the coating was 1 - 20 µm with a pore number of 111 pores. The pore size distribution of obtained composite body was 25.56 - 26.62 µm and 22.88 – 23.14 µm at chitosan concentration of 0.5 % and 0.75 %, respectively.
Table 1. Pore size distribution of composite bodies.

| Pore Size (μm) | Number of Pore | Before Coating | 0.50 % chitosan | 0.75 % chitosan |
|----------------|----------------|----------------|-----------------|----------------|
|                |                | 30 min | 60 min | 30 min | 60 min |
| 1 – 20         | 111            | 106    | 114    | 129    | 242    |
| 21 – 100       | 91             | 46     | 69     | 102    | 76     |
| > 100          | 6              | 6      | 6      | 2      | 9      |
| Total          | 208            | 158    | 189    | 233    | 327    |

3.3. XRD analysis

The chemical composition and crystallinity of hydroxyapatite bodies were investigated using XRD analysis. XRD diffractogram of the composite bodies at various concentration of chitosan and coating time shown in Figure 6. The presence of hydroxyapatite and chitosan is noticeable. The hydroxyapatite peaks is obtained at an angle of 2θ i.e. 25.8287°; 31.7259°; 32.1370° and 32.8529°, while at 2θ i.e. 29.3467° and 34.0105° are the chitosan peaks.

Figure 6. XRD analysis of hydroxyapatite body with chitosan concentration and coating time of (a) 0.50 %, 30 min, (b) 0.50 %, 60 min, (c) 0.75 %, 30 min, (d) 0.75%, 60 min.
Diffractogram at various concentrations of chitosan and coating seen in Figure 6. The results of data processing shows the position of the hydroxyapatite and chitosan peaks. The hydroxyapatite peaks is obtained from figure. 6 a with an angle of 2ϴ i.e. 25.8287°; 31.7259°; 32.1370° and 32.8529° with intensity peak 2929.68 cts. figure 6 b shown peaks of hydroxyapatite 25.8420°; 31.7277°; 32.1478° dan 32.8647° with the intensity peak 3743.55 cts. While from the figure 6 c hydroxyapatite peaks formed i.e. 25.8300°; 31.7079°; 32.1288° and 32.8480° with the intensity peak 3478.81 cts. Furthermore, from Figure 6 d peak s of hydroxyapatite formed 25.8398°; 31.7360°; 32.1533° and 32.8719° with the intensity peak 3311.75 cts.

The chitosan peaks from figure 6 a with angle 2ϴ i.e. 29.3467° and 34.0105°. Figure 6 b shows that chitosan peaks 29.3623° and 34.0162°. While from figure 6 c chitosan peaks formed i.e. 28.0684° and 33.9957°. Furthermore figure 6 d chitosan peaks formed that is 28.1043° dan 34.0230°.

However, the addition of chitosan concentration from 0.50 % to 0.75 % (Figure 6) and coating time from 30 min to 60 min (Figure 6) are not significantly influence crystallinities of because low amount of chitosan compared to hydroxyapatite content.

4. Conclusion
The porous hydroxyapatite/chitosan composite bodies were successfully prepared via dip coating technique. The control of properties of the composites bodies not only by varying chitosan concentration but also by managing the coating time. As the coating time increased, the compressive strength of the composites increased as well and was found in range of 1.43 MPa to 4.03 MPa. If coating time was increased that made chitosan particle fill in pores but coating time overlong made pore enclosed and uneven. While chitosan concentration affect the porosity and pore size. The porosity of composite decreased with addition of chitosan concentration and coating time with pore size in the range of 22.88 – 28.25 µm.

5. Acknowledgments
Authors are thankful to the Ministry of Research, Technology and Higher Education of the Republic of Indonesia (Kemenristekdikti) for providing financial support to undertake this research work.

6. References
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