Synthesis Hydroxyapatite/Collagen/Chitosan Composite as Bone Graft for Bone Fracture Repair

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ABSTRACT
Composite hydroxyapatite-collagen-chitosan as bone graft materials for bone fracture has been synthesized using ex-situ method. Combining the advantages of hydroxyapatite with chitosan and collagen as composites bone material that it show good biocompatibility and can bond with surrounding host tissues inherent from hydroxyapatite. In this research hydroxyapatite was synthesized from cow bone with calcination method. The main of this research is to determine the chemical and physics characteristics from the bone graft. The analysis data from Spectra IR indicated vibration of $\text{OH}^-$, $\text{PO}_4^{3-}$ and $\text{CO}_3^{2-}$ from hydroxyapatite, also $\text{N}-\text{H}$, $\text{C}=\text{O}$ and $\text{C}-\text{H}$ from collagen and chitosan along with the peak wave number shifting. The result from SEM characterization was indicated surface morphology formed mineral apatite layer with rough crystal.

Keywords: Hydroxyapatite/chitosan/collagen, Bonegraft, Bone fracture.

1. INTRODUCTION
Bone consists of organic and inorganic materials composed in a very complex structure. Once it gets damaged, the whole human body will not function and it consequently bothers the human’s activities. Many causes inflict the bone damage such as a working accident, handicap born, and age. One surgical procedure to address such damage is by performing bone graft, an implant transplanted from one to another place. Bone graft is classified into three types namely autograft, allograft, and xenograft. First, autograft is the most used healing method for bone damage. However, auto graft method lacks of available bone mass and handicap structure in which it makes auto graft less effective [1]. Second, allograft might be the alternative of auto grafting which the implant isobtained from other persons. Unfortunately, allograft seems to be less interested as it easily confronts with some disease and immunology cases like hepatitis [2]. At last, xenograft is conducted by implanting animal tissue to human being body. This typical bone grafting is seldom to perform since the risk of rabies and bone cancer is too high [3].

Bone graft materials should be biocompatible, osteoconductive, and non-toxic [1,3]. The materials could consist of collagen, hydroxyapatite, and chitosan. Hydroxyapatite is considered as one of the materials since it is non-toxic and biocompatible as well as having equal mineral phase with inorganic composition of dental bone [4]. One of the materials that is able to be used in hydroxyapatite synthesis is from ox bone because its inorganic composition of hydroxyapatite is about 93% [5]. Obtaining hydroxyapatite can be conducted through wet precipitation and sol-gel methods. Unfortunately, both methods are less effective when using chemicals. In this research, hydroxyapatite is obtained by calcination of ox bone. Cahyaningrum (2018)[4] reveals that hydroxyapatite becomes less osteoconductive during the process of calcinations, in which this phenomenon conveys its weakness [2].

Human bone consists of inorganic (70%), organic (20%), and cell including osteoblast, osteocyte, and water [6,7]. It needs to be enhanced by polymer to improve the nature of osteoconductive of hydroxyapatite. Moreover, its organic compounds comprise collagen and chitosan. Collagen represents protein which is mostly contained in bone, in which 90% organic mass of human bone contains it [8]. It is easily permeated by human body, as well as forming and arresting, detaining bone strain, improving the
nature of osteoblast, and measuring the biocompatibility [9].

Even if human body can produce collagen, that amount is about to decrease eventually along the accretion of age. Henceforth, it can be used for handling related patients who need bone implants. Additionally, chitosan is polysaccharide which characters are non toxic, biodegradable, and measuring up to biocompatible [10]. It can be produced with deacetylation of chitin. It has similar structure to glycosaminoglycan which are also existed in human body [11]. Glucosamine can improve the process of bone repair by stimulating cartilage cell to form proteoglycan [12,13]. Chitosan can enhance the nature of osteoconductive, biocompability, and biodegrability, yield composite to hold up pressure, and quicken forming of bone mineral by creating pore the composite produced [13,14]. Therefore, this research aims to produce bone graft with hydroxyapatite-collagen-chitosan as the materials for substituting bone. The synthesis of hydroxyapatite-collagen-chitosan is carried out through ex situ method.

2. METHOD

Materials which are used in this research are crab shell, some material pro analysis quality there are H₃PO₄, NaOH, HNO₃, Aquademineral, hydroxyapatite standart come from Bank Jaringan of RSUD Dr. Soetomo Surabaya. The collagen was from PT. Nitta Gellatin Japan and the chitosan was from PT. Indo Chittin, Indonesia

Some instruments are used in this research are: FTIR from Perkin Elmer Spectrum RXI was used to perform functional group analysis. The surface morphology was analyzed using SEM (Zeiss. EVO MA 10, Germany). Universal Testing Machine (UTM) was carried out to strengthen hydroxyapatite and bone graft. Some chemical materials with excellent quality were from Merck.

2.1. Synthesis of Bone Graft from Hydroxyapatite-Collagen-Chitosan

Amount 1.75 g of hydroxyapatite synthesized from ox bone was dissolved in 10 mL H₃PO₄ 2% (v/v). Solution condensation was mixed by using stirrer magnetic until being homogeneous. 0.50 g of collagen was dissolved in 10 mL CH₃COOH 2% (v/v). After that, 0.25 g of chitosan was dissolved in 10 mL CH₃COOH 2% (v/v) by using stirrer magnetic until being homogeneous. Hydroxyapatite was dissolved later then was added into chitosan by putting it into the solution. Then, the solution was added with collagen dripped and at the same time swirled using stirrer magnetic. After that, the bone graft was neutralized with NaOH 1M for producing solution with pH7. Afterwards, the produced bone graft was dried using Freeze-Dry. The bone graft that produced was characterized with some instrument.

3. RESULTS AND DISCUSSION

Hydroxyapatite-collagen-chitosan composite was synthesized by using ex-situ method through dissolving hydroxyapatite, collagen, and chitosan. In this research, hydroxyapatite dissolved in phosphoric acid 2% (v/v), collagen, and chitosan was dissolved in acetate acid 2% (v/v). The acetate acid solution should be appropriate for dissolving collagen and chitosan [15][16]. The phosphoric acid was added in hydroxyapatite because hydroxyapatite got dissolved in the condensation of phosphate ion, representing strong acid with pH under 4 [17].

![Figure 1](A IRSpectra of HAp,(B) Chitosan, (C) Collagen, and (D) Bone graft)

Figure 1 shows the analysis of functional group for hydroxyapatite (HA), collagen, and bone graft. The characteristic of hydroxyapatite was having functional group of PO₄³⁻ and –OH, while the characteristic of collagen and chitosan is having functional group of C=O, C=O - NH₂ and - OH. Figure 1A shows that hydroxyapatite Bank Jaringan (BJ) had functional group of -OH at 3435. 83 cm⁻¹ and PO₄³⁻ at 571.18 cm⁻¹ and 1050.33 cm⁻¹. Meanwhile, Figure 1B depicts that hydroxyapatite ox bone had functional group of OH at 3570.76 cm⁻¹ and PO₄³⁻ at 571.18 cm⁻¹ and 1050.33 cm⁻¹. As comparison, the carbonate
functional group of CO$_2$ at the calcination process could be performed. Figure 1D shows that the FTIR spectra from bone graft had functional group of -OH, -NH, PO$_4^{3-}$, CO$_3^{2-}$, and C=O. Vibration at 3291 cm$^{-1}$ represented functional group of -OH from chitosan and that of at 3391.39 cm$^{-1}$ represented functional group of -OH from collagen. The interaction between hydroxyapatite with amida I of collagen was at 1665.22 cm$^{-1}$.

**Table 1. The strength of bone graft**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Strength (MPa)</th>
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<tbody>
<tr>
<td>Bone graft 7:2:1</td>
<td>2.31</td>
</tr>
<tr>
<td>Bone graft 7:1.5:1.5</td>
<td>1.07</td>
</tr>
<tr>
<td>Bone graft 7:1:2</td>
<td>1.29</td>
</tr>
</tbody>
</table>

Table 1 shows the result of strength test with autograph instrument, of which the result yielded was irrelevant with the standard of human bone. Bone human had strong standard value depress equal to 2-12 Mpa [15]. In accordance with the standard of prop bone, the organic composition was obtained from collagen and chitosan that influenced the strong depress from bone graft [2]. The composite still could be used by at part of other bones like nose, shoulder, and tooth bones.

**Figure 2** Diffractogram of bone graft 7:1:2 (A); bone graft 7:1.5:1.5 (B); bone graft 7:2:1 (C); hydroxyapatite Ox bone (D), and hydroxyapatite BJ (E)

Hydroxyapatite BJ (Figure 2E) dominated with HA phase with 20 was 31.80°. The HA from ox bone (Figure 2D) dominated by HA phase with 20 was 31.8 and the existence of carbonate with 20 was 3.27°. HA phase and theapatite carbonate type A (AKA) were also existing at hydroxyapatite (HA)-collagen-chitosan composite. In composite 7:2:1 (Figure 2C), AKA phase at20 was 31.75° and the HA phase with 20 was32.89°. In composite 7:1.5:1.5 (Figure 2B), HA phase at 20 was 31.86° and the AKA phase with 20 was32.32°. In the composite 7:1:2 (Figure 2A), HA phase with 20 was 31.8° and the AKA phase at 20 was 32.2°, of which hydroxyapatite was more stable in this condition. At pH base, condensation of hydroxyapatite was progressively lower that caused saturated hydroxyapatite and formed amorphous calcium phosphate (ACP). The value of amorf was very low so that the characteristic of bone graft was brittle and incompatible, which means that it can be used for human bone implant. Figure 2 shows the result of XRD from bone graft using Na$_2$HPO$_4$ in hydroxyapatite solution, which yielded crystallinity amounted of 66%. Moreover, the hydroxyapatite had changed phase into ACP. The interaction between Ca$^{2+}$ ion from hydroxyapatite and -NH$_2$ from chitosan formed coordinative bond. In addition, when collagen composite was added, the interaction happened to be a coordinative bond between functional group of Ca$^{2+}$ with -NH from the collagen. The comparison of composition at bone graft might influence functional group, crystallinity, and surface morphology of the produced bone graft.

**Figure 3** The analysis of surface morphology from bone graft 7:1.5:1.5 (A) and bone graft 7:1:2 (B), 10,000x

Figure 3 represents the analysis of surface morphology with magnification 10,000x from the hydroxyapatite and bone graft. In details, Figure 3A shows that hydroxyapatite had a certain structure in form granule with different size among others and also had a short distance. Figure 3A and 3B portray the result of composite with magnification 10,000x. Figure 3A and 3B depict the surface morphology in a form of apatite mineral coat with crystal which was harsh enough at pore wall. Moreover, Figure 3B conveys that there were less disseminating fibers in form of granule because the apatite coat was not formed perfectly. The produced composite in form of granule could transform into the form of smooth item that has small pore size measure thus, it could tie the bone graft with human being bone.
4. CONCLUSION

Bone graft synthesized from collagen-hydroxyapatite-chitosan composite has functional group of OH−, PO43−, and CO32− from hydroxyapatite and the functional group of -NH2, C=O from chitosan and collagen. The functional group emerges to tie between hydroxyapatite, collagen, and chitosan composite. The organic composition was obtained from collagen and chitosan that influenced the strong depress from bone graft.

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