Synthesis hydroxyapatite/collagen/chitosan composite for tissue engineering

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Abstract. Hydroxyapatite/ Collagen/Chitosan composite has been synthesized by ex-situ methods. The purpose of this research is to study the composition’s effect of the composite HA/Coll/ Chi to some characters such as a functional group and phase as well as the physical character such as crystallinity, the compressive strength and surface morphology. HA synthesized from the precursor calcium derived from duck eggshell reacted with H_3PO_4. HA sintered at 900 °C. HA was added with collagen and chitosan solution with a mass ratio of the composition HA/Coll/Chi are 7: 2: 1; 7: 1.5: 1.5; 7: 1: 2. FTIR analysis showed a band PO_4^3- and OH^- adsorption of HA, as well as a shift vibration of spectra in the group C=O and NH_2 from chitosan and collagen that shows there has been a bond between collagen, chitosan and HA. Data of XRD showed that the phase of HA and chitosan in the composite of HA/Coll/Chi. Composite with collagen component height has low crystallinity.

1. Introduction

Bone as part of human body has very important function, so that in the event of fracture bone will the serious of health and needed technique healing for correct bone. One of the techniques for healing of bone is use synthetic bone graft biomaterial. The good synthetic of bone graft have to biocompatible, bioactive, nontoxic, supporting the nature of and osteoconductive of osteoinductive [1-2], beside that the bone graft have to composition and structure which is equal with bone, that is containing > 69% calcium phosphate, especially hydroxsiapatite; 21% collagen; 9% water and 1% other component [3]. Hydroxyapatite as especial component have the power of unfavorable mechanic, brittle and breakable, so that in synthesis of synthetic bone graft, the hydroxyapatite has to be composite with other material. Composite of polymer matrix have some advantage among others avoid the problem of shielding stress and eliminate second surgery procedure to eliminate implant. Mineral phase especially function of hidroxyapatite give inertia and delaying, while organic matrix give interest strength and bone flexibility [8]. Collagen measure up to good biocompatibility to degradation and permeated by body. In composite material, collagen and hydroxyapatite play role which is same as at natural bone [4]. The collagen can isolation from various sources of that are ox bone, goat, scrawl and fishbone. At this research the collagen got from scrawl. Characteristics osteoconductive and osteointegrated of graft bone relate to porosity storey level and porosity size. To form pore at graft bone need materials that added which is function as porogen. Porogen that use must have characteristic biocompatible, biodegradable and nontoxic, one of them is chitosan.
Chitosan is deacetylation chitin processes. This material can use as filler in composite produse. Chitosan have some characteristic there are biocompatible, biodegradable, non-toxic, non-antigenic and osteoconductive [5-7]. The osteoconductive character of chitosan is it can quicken growth of osteoblast at is composite of HA-chitosan so that can quicken forming of bone. In this research the bone graft made by composite three materials that is hidroxyapatite/collagen of Chitosan with mass composition variation of which look like with composition a period of bone. The syntheses there of bone graft are two methods able to be used are ex-situ and in-situ. Method of ex-situ represent method addition of polymer when collagen, chitosan after especial materials in the form of hidroxyapatite have been formed, while at method of in-situ addition collagen and chitosan done at the time of process of synthesis hidroxyapatite take place. Some research indicates that method of ex situ yield product having compared to higher purity than other method.

2. Material and Method

The calcium oxide was isolated from eggshell (*Gallus gallus*), the collagen was synthesis from scrawl (*Gallus gallus*) obtained from Mojosari, Indonesia. Chitosan (85% DD), Acetic acid, nitrate acid, natrium hydroxide p.a quality purchase from Merck.

2.1. *Synthesis of hydroxyapatite*

Synthesis hydroxyapatite has been done by reacting precursor of calcium and phosphate precursors with comparison of concentration of Ca/P 1.67. CaO powder was added by aquademineral to yield hydroxide calcium. After that it was added with phosphate and stirrer until homogeneous. And then the solution added with natrium hydroxide until pH 10. Mixture was aging at room temperature during 24 hours. The solution yielded to filter and its sediment is washed with aquademineral, dried at oven in temperature 110 °C during 2 hours. After that, it was added with nitrate acid and sintering in 900 °C during 2 hours. The crystal that produces was cooled in furnace to produce of hydroxyapatite.

2.2. *Synthesis of hydroxyapatite-Chitosan- collagen composite*

Amount of chitosan was dissolved in acetate acid solution. It solution was stirred until homogeneous. Hydroxyapatite powder have been dissolved with water was added in chitosan solution with wish drop method. After that it solution was added with collagen solution. The composite that produce was treatment with freeze dry and then condensed on natrium hydroxide solution. After that the composite was dried in freeze dry again. The processed repeat again for composite with variation Massa composition HA/Coll/Chi 7:1.5:1.5 and 7:1:2. Composite HA/Coll/Chi was characterized functional group and crystalinity.

3. Results and discussion

At hidroxyapatate synthesis, CaO powder was soluted in aquademineral form Ca(OH)\(_2\) solution. It solution was added with phosphoric acid solution drop to drop so that pH not go down drastically. The rate of phosphoric acid addition very related to obtained pH in the end of synthesis. Degradation at pH under 7 causing imperfect dissociation of phosphoric acid so that yield β-Ca\(_3\)(PO\(_4\))\(_2\) and CaO. Phosphoric acid solution that was added with slowly can increase the homogeneity of solution [8,9]. At the research, the synthesis of hydroxyapatite is undergone at temperature 60 °C, this function is to crystal maximalized produce and reduces produce monoclinic crystal structure. The monoclinic crystal structure can produce in temperature under at 60 °C [10]. Hydroxyapatite that synthesis is expected has structure same with bone structure, there are hexagonal structure. When phosphoric acid was added in calcium hydroxide solution the solution slowly condensation become to have the character of acid, while process of crystallization take place effective at base condition [11] that the solution must be added with natrium hydroxide until pH 10, hydroxyapatite crystal stable at pH 10 [12]

The hydroxyapatite from synthesis with variation sintering temperature compared with hydroxyapatite standard from Bank Jaringan. Fig 2 showed the spectra of CaO, hydroxyapatite that
sintering at 800 (HAp-800); 900 (HAp-900); 1000 °C (HAp 1000); hydroxyapatite without sintering (HAp-TS) and hydroxyapatite from Bank Jaringan (HAp-BJ).

Sample HAp-TS has characteristic spectra at wave number 411.07; 1095; 576.97; 1400.36; 659.96; and 1648.76 cm\(^{-1}\). At HAp-TS spectra don’t band at 630 cm\(^{-1}\) because these phase HAp has more impurities than HAp that was undergo sintering processes. The characteristic of HAp-800 are have peak at 963.26; 495.2; 1070.5; 570; 602.5; 1420.21; 631.27; 1637.49; and 3401.04 cm\(^{-1}\). Sample HAp-900 has band at around 454.76; 1072.5; 528.5; and 163801 cm\(^{-1}\). The characteristic spectra for HAp-1000 there are have spectra at around 403.26; 1074.5; 575.5; 1638.01 and 3435,21 cm\(^{-1}\). The wave number for spectra samples A-F are similar there is characteristic for CO\(_3\)^2-, PO\(_4\)^3-, and OH\(^-\) functional groups.

Figure 1. FTIR spectra of (A) CaO, (B) HAp-TS, (C) HAp-800, (D) HAp-900, (E) HAp-1000, (F) and HAp-BJ.

Figure 2. Bone graft 7:2:1 (A); 7:1.5:1.5 (B) and 7:1:2
At Figure 2 seen that all bone graft have physical character of solid with white in color. All bone graft has hollow fiber which result bone graft do not too hard when depressed. Pursuant to perception of physical seen bone graft have been mingled is homogeneously. Bone graft of Ha/Coll/Chi 7:1:2 with content of chitosan at most brass white in color, more and more content of chitosan hence its color tend to turn white brass. Composite of Ha/Coll/Chi 7:1:2 also more solid and resilient when compared with bone graft Ha/Coll/Chi 7:1.5:1.5 and bone graft Ha/Coll/Chi 7:2:1. Progressively the increasing of chitosan the bone graft seen it more resilient.

| Table 1. Crystalinity of Bone graft |
|-------------------------------------|
| Sample  | 2θ 1 (°) | 2θ 2 (°) | 2θ 0 (rad) | 2θ 02 (rad) | Intensity | Fraction of crystal Amorph (count) | Fraction of crystal Crystalline (count) | Crystallinity (%) |
| Bon graft 7:2:1  | 3  | 33  | 0.5  | 0.58  | 0  | 44.9  | 146  | 0  | 2.9  | 77.02 |
| Bon graft 7:1,5:1.5  | 3  | 33  | 0.5  | 0.58  | 0  | 76.5  | 283  | 1  | 4.9  | 78.98 |
| Bon graft 7:1:2  | 1.88  | 0.77  | 6  | 0.58  | 0  | 84.6  | 211  | 1  | 3.9  | 71.65 |

At table 1 can be seen that the crystalinity of bone graft HA/Coll/Chi is experiencing of degradation. Degree of crytalinity express of the composition of crystal content in a material (Samsiah, 2009). It’s crystalinity degree more high the bone graft hence progressively material crystal. Bone graft Ha/Coll/Chi have crystalinity degree more lower than hydroxyapatite caused by addition of organic material at bone graft which result bone graft is amorf. This matter is because collagen and chitosan are disseminated and has been bonding with apatite compound. At is bone graft of Ha/Coll/Chi 7:2:1 owning lowest crytalinity among other bone graft, this is indicate that materil organic have effect in crystalinity of bone graft.

![SEM analysis of Bonegraft](image)

**Figure 3.** SEM analysis of Bonegraft (A) 7:2:1; (B) 7:1.5:1.5; (C) 7:1:2 magnification 500x

The Surface morphology with SEM analysis for three bone graft saw at Figure 3. At Figure 3 can be seen that the result of analysis of SEM with magnification 500x which is yield for graft bone
Ha/Coll/Chi. At Ha/Coll/Chi graft bone have surface of third surface surface of refinement. Morphology of graft bone Ha/Coll/Chi consist of granula regular small, existence of formed pore.

4. Conclusion
Hydroxyapatite that produce from eggshell with precipitation wet method have functional group similar with hydxyapatite from Bank Jaringan (HAp-BJ), there are characteristic for $\text{CO}_3^{2-}$, $\text{PO}_4^{3-}$, and $\text{OH}^{-}$ functional groups. Bone graft Ha/Coll/Chi with composition 7:2:1 has lowest crytalinity among other bone graft, this matter indicate that collagen has effect in bone graft characteristic.

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