The Influence of Chitosan Concentration on Synthesis of Hydroxyapatite Scaffold on Crystallinity and Surface Morphology

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Abstract. Hydroxyapatite (HA) is a bioceramic material having a chemical formula (Ca₁₀(P0₄)₆(OH)₂) similar to the chemical structure of bone and hard tissue in humans. Recently, HA-scaffold has been intensively developed by many researchers due to its potential application in dealing with tissue injured. The implantation of HA-scaffold into bone defect aims to help and stimulate the growth of new bone tissue. Crystallinity and microstructure are properties that need to be considered to synthesis HA scaffold. Although, HA is biocompatible and osteoconductive but low biodegradable, to solve these problems added chitosan. Chitosan is a biopolymeric material with specific properties, in terms of biocompatible, non-toxic, osteoconductive, biodegradable, and not carcinogenic. In addition, a natural polymeric chitosan has similarities with the extracellular matrix, in terms of good biological performance and inherent cellular interactions. In this study, HA-scaffold was synthesized using Ca(OH)₂ as the raw material of natural calcite mineral, H₃PO₄, and a solid polyurethane polymer (sponge) as forming of pores by sol-gel method. The addition of chitosan concentration of 0 %, 2 % and 3 % in hydroxyapatite scaffold evaluated its influences on crystallinity and surface morphology. The phase, crystallinity and crystal size are evaluated by XRD. Functional groups in HA scaffold and HA compounds were evaluated with FTIR. The results showed that HA has Ca/P ratio of 1.64. The addition of chitosan concentration caused the decrease of crystallinity and increased the pore size. Meanwhile the crystal sizes were obtained: 49.99 nm (HA), 54.37 nm (HA-scaffold without chitosan), 54.98 nm (HA-scaffold with 2% chitosan), and 40.44 nm (HA-scaffold with 3% chitosan).

Keywords. Chitosan, crystallinity, HA scaffold, and surface morphology.

1. Introduction

Hydroxyapatite (HA; Ca₁₀(P0₄)₆(OH)₂) is an attracting biomaterial due to its excellent biocompatibility, bioactivity, and osteoconductivity [1]. HA materials are also potential candidates for use in fluorescence labeling, cell targeting, imaging and diagnosis materials [2, 3]. The chemical composition, size, crystallinity, and morphology of the HA crystals and their aggregates play critical roles in determining their properties and potential applications [4–8]. Furthermore, HA nano-bioceramics exhibit better higher resorbability and bioactivity than in micro-scale sizes [9]. In addition, the crystal grain size can also regulate the degradation rate. Comparing with micro-size, HA
nano-sized possesses higher dissolution rate because possess much more boundaries and the dissolution always occurs most easily on grain boundaries [10]. In the natural apatite, the low crystallinity, ion substitution, and small crystal size are important factors that are related to their good solubility [11]. The surface morphology design is considered as potent approaches to increase the bioactivity and biological response [12, 13]. In fact, the human bone mineral is composed of co-substituted essential trace elements such Na, Mg, Zn, Sr, F, Cl, Si, and CO$_3^{2-}$ in crystal lattices that was done by Hartatiek et al [14, 15]. HA is measured as a potential candidate as scaffold for bone tissue engineering application, which acts as an excellent temporary substrate to allow cell in-growth, proliferation, and differentiation.

Natural biopolymers have received much more attention in the field of orthopedic and other biomedical applications due to their excellent biocompatibility and biodegradability. In recent years, chitosan and its applications in the field of tissue engineering have attracted considerable attention [16, 17]. Chitosan is a unique polysaccharide that shares a number of structural and chemical similarities with collagen and has been used as DNA and drug delivery vehicle, as skin grafting template, and as wound healing material [18, 19]. Also, chitosan is a natural polymer obtained by deacetylation of chitin, with properties that are biocompatible, biodegradable, cationic and relatively non-toxic in natural [20]. Hence, chitosan is appropriate when it is compositied with HA to improve its bioactivity and biological response that is related with surface morphology while crystallinity that is related with to good solubility (biodegradable). In this study, the effect of chitosan content on HA was carefully examined the effect of crystallinity and surface morphology.

2. Materials and methods

2.1. Preparation of hydroxyapatite/chitosan nanocomposite

Four synthesizing stages were conducted in order to obtain the HA-chitosan nanocomposite material as briefly listed as follows: (1) the preparation of chitosan solution, (2) synthesis of hydroxyapatite by means of co-precipitation method, (3) synthesis of hydroxyapatite-scaffold sponge, and (4) preparation of HA-chitosan composites via sol-gel route.

Chitosan powder from shrimp shells (brand Sigma-Aldrich) was dissolved in 2\% (v/v) acetic acid solution to obtain a polymer solution. Different composition of chitosan aqueous solution was prepared by dissolving the different amount chitosan powder into 100 cm$^3$ distilled water containing acetic acid and stirred for 3 h at 300 rpm until a good suspension was achieved and then the solution is allowed to stand for 24 h. Samples of this experiment consisted of 0 g, 0.5 g, 0.75 g of chitosan. In this experiment, HA powder was prepared by natural calcite from Druju Malang Jawa Timur. CaO powder was dissolved in 50 mL ethanol 96\% and blend with H$_3$PO$_4$ solution and stirred for 30 min at 37 °C and 300 rpm. The solution was precipitated for 24 h and then stirred at 60 °C until change be gel and calcined at 1000 °C for 3 h. HA powder was dissolved into 10 mL aquaest and stirred at 300 rpm, 37 °C for 30 min. Solid polyurethane polymer (sponge) with dimension (2 cm × 2 cm × 0.5 cm) was soaked into the gel for 5 h and then dried at 110 °C for 24 h. Finally, one gram of HA powder was dissolved into 10 mL aquaest, and then solution of chitosan was dropped and stirred with 300 rpm at 37 °C for 4 h. The solution was precipitated at room temperature for 24 h. The gel sponge was soaked into the solution and dried at 110 °C for 24 h. The sample was calcined at 1000 °C for 4 h.

3. Results and discussion

The compound groups of the resulting product was determined by an FTIR spectrometer (Prestige-21 Shimadzu). HA had OH-, HPO$_4^{2-}$, CO$_3^{2-}$ and PO$_4^{3-}$ groups. The FTIR spectroscopy has provided valuable information regarding the formation of HA powder, HA scaffold, and HA/chitosan composite. Figure 1 shows the results of FTIR spectroscopy for HA powder. Based on Figure 1, it can be inferred that all the HA groups exist in the FTIR graph indicating the formation of HA in the sample. Other clusters, such as C=C and C-H, indicate the presence of calcite compounds.
Figure 1. FTIR spectra of HA powder, HA scaffold, and HA scaffold-chitosan

The phase identification and crystal structure formation were evaluated by XRD. The average crystallite size \( D \) was determined according to the Scherrer equation, \( D = \frac{0.9\lambda}{\beta \cos \theta} \), where \( D \) is the average crystallite size (Å), \( \beta \) is the peak broadening of the diffraction line measured at half of its maximum intensity in radian, \( \lambda \) is the wavelength of X-ray (nm), and \( \theta \) is the Bragg’s diffraction angle [21]. Figure 2 shows the results of HA, HA scaffold, and HA scaffold/chitosan.

For the HA powder (blue) the presence of 2\( \theta \) peaks at about 26\( ^\circ \), 28\( ^\circ \), 31.07\( ^\circ \), and 32\( ^\circ \) corresponding to the diffraction planes (002), (102), (210), (221), and (300) indicates the formation of HA in the product by the co-precipitation method. There is still a CaCO\(_3\) phase at 2\( \theta \) in the Bragg plane of (210). The highest peaks for all the diffraction patterns, in Figure 2, were around 31\( ^\circ \) with small peaks shifts. This peak angle shift of HA indicates the addition of sponge and chitosan. This condition indicates that the synthesis of HA, HA scaffold and HA scaffold/chitosan are successfully synthesized by sol-gel method.

The crystallinity of HA was determined by the fraction of crystalline area over (crystalline crystalline area and amorphous area) \( \times 100\% \). Table 1 shows the degree of crystallinity of HA scaffold and HA scaffold/chitosan.

| Variation             | Net area | Amorphous area | Background area | Crystalline area | Crystallinity (%) |
|------------------------|----------|----------------|-----------------|------------------|------------------|
| HA scaffold/Chitosan   | 2710.21  | 647.14         | 240.18          | 1822.89          | 73.08%           |
| 0%                     |          |                |                 |                  |                  |
| HA scaffold/Chitosan   | 2602.36  | 644.89         | 244.12          | 1713.34          | 72.65%           |
| 2%                     |          |                |                 |                  |                  |
| HA scaffold/Chitosan   | 2635.18  | 826.24         | 221.75          | 1587.19          | 65.77%           |
| 3%                     |          |                |                 |                  |                  |
Based on Table 1 it can be stated that the addition of chitosan to HA scaffold decreases the degree of crystallinity of HA-chitosan. This result corresponds to the XRD pattern in Figure 2. The shift and fall of the degree of crystallinity in the diffraction pattern indicates the composite of the HA scaffold/chitosan composite which means the presence of a bond between the HA scaffold and the polymer matrix. The 2θ peak at 31.07° (221) on the HA powder weakens after the composite was formed, also indicating the participation of HA in bonding with the polymer. This can be due to the molecular interaction between HA scaffold and chitosan very well. The change in the width of the peak on the XRD pattern relates to the size of the crystallite. Table 2 below shows the crystallite size of the HA scaffold and HA scaffold/chitosan.

| Properties       | HA scaffold/ Chitosan 0% | HA scaffold/ Chitosan 2 % | HA scaffold/ Chitosan 3% |
|------------------|-------------------------|---------------------------|--------------------------|
| Crystallite size (nm) | 54.37                   | 54.98                     | 52.32                    |

Table 2 shows that the addition of chitosan can decrease the size of insignificant HA scaffold crystals. The size of the HA scaffold crystallite is still within the approximate size range of 50 nm, according to Kokubo [1, 22] that the crystallite size in bone ranges from 20 nm to 80 nm.

Figures 3.a to Figure 3.d indicate the SEM micrographs of surface morphology of the pure HA and composite samples with different contents of chitosan.

Based on Figure 3.a, it appears that pure HA represents homogeneously distributed morphology. The addition of chitosan causes more rough morphology and pores appear much more than pure HA. Hence composite HA scaffold/chitosan is best suited for tissue engineering. The pores provide a place for the growth of new bone tissue that can blend into the host tissue. Pores also provide a place for cell proliferation.
4. Conclusions
Natural calcite from Druju Malang, Jawa Timur, has been effectively used the raw material for HA production. In this present report, chitosan was well introduced into the as prepared HA forming HA/chitosan composites. The addition of chitosan decreased the crystallinity of HA/chitosan composites. In addition, the presence of chitosan in HA caused the pores to increase so that HA/chitosan Composite is potential to be applied for regeneration of tissue.

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Figure 3. SEM micrographs of (a) HA (b) HA scaffold/chitosan 0 % (c) HA scaffold/chitosan 2 % (d) HA scaffold/chitosan 3 %
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