Synthesis of Bio-composite CHA/PVA/Alginate as Bone Substitute Material

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Abstract. The main inorganic constituent of human bone is carbonated-hydroxyapatite (CHA). The CHA powder had been successfully synthesized by using chicken eggshell through microwave assisted precipitation methods. In the application, the biomaterials needed by orthopaedic doctors were in the form of scaffold. The addition of PVA and alginate to CHA can improve mechanical properties and affect the pores. The freeze-drying method was used to synthesize bio-composite scaffold. The FTIR result showed that spectrums correspond to functional group of PO\textsubscript{4}\textsuperscript{3-}, OH\textsuperscript{-}, CO\textsubscript{3}\textsuperscript{2-} which were the characteristic of CHA. The elastic scaffold was produced in this research. The scaffold mechanical test results showed that the higher PVA content showed the higher mechanical properties. The phase composition was evaluated by XRD and indicated the presence of CHA. The morphology of the sample was evaluated by SEM which formed pores. These scaffolds showed good prospects for bone substitute material.

1. Introduction

The development of synthetic bones as alternative graft materials become a major advance in biomaterial technology. Natural bone is containing 50-70% of apatite minerals, 20-40% organic material, 5-10% water, and 3% of proteglycan, peptides, and lipid [1]. Synthetic bone grafts commonly are made by hydroxyapatite (HA) Ca\textsubscript{10}(PO\textsubscript{4})\textsubscript{6}(OH)\textsubscript{2} which has a similar composition to mineral apatite. Although HA has the ability to bond to bone, but the rate of osseointegrations are relatively slow. The way to enhance the osseointegration process is add an incorporate ion that are present in bone mineral such as carbonate ion. Apatite carbonate or carbonated hydroxyapatite (CHA) is a promising material because it has more bio-resorbable and bioactive on in-vivo test than stoichiometric HA [2,3].

Apatite carbonate (CHA) can be synthesized using precipitation method assisted by microwave irradiation. Microwave is an electromagnetic wave which has frequencies range from 300 MHz-300GHz and is located between radio and infrared waves in electromagnetic spectrum. It provides a rapid and shorter synthesis time because the electromagnetic waves can penetrate and excite water and lipid molecules evenly. The advantages of using microwave irradiation are can produce nanometer size of the material, more precision, shorter synthesis time, faster temperature rise, lower energy use, high yield and purity [4]. Synthesis of CHA can produce bone-like material that can stimulate bone growth. But CHA has a weakness that is fragile when used as a single element. For this reason, there is a need to mix CHA with elastic compounds such as polyvinyl alcohol (PVA) and alginate. The function of polymer as a matrix can help the mineralization process while reducing the fragile nature.
of CHA. PVA and alginate had stable properties in in-vivo tests and good mechanical properties that are suitable to be applied as bone implants in human body [5].

In the application, the biomaterials needed by orthopaedic doctors are in the form of scaffold (porous scaffold). Scaffold is very necessary as a medium for infiltration of osteoblast cells which can accelerate bone mineralization processes [6]. The scaffold pores can be produced using the freeze drying method. Freeze drying is a simple method for producing scaffold because it does not use high temperatures which can damage the polymer structure. The water contained on the scaffold will come out and leave the pores which make the scaffold become soft and elastic. Based on these matters, in this study bio-composite scaffold CHA/PVA/Alginate was developed through the freeze-drying method. Calcium sourced that used is s from chicken egg shell (CES) waste which is composed of 95% of calcium carbonate, 3.5% of glycoproteins, and proteoglycans [7]. Bio-composite scaffolds which made from CHA, PVA, and alginate are expected to be biomaterials candidate that have potential to develop in human bone engineering.

2. Research Methods
CES, H₃PO₄, and NaHCO₃ were used as starting materials for CHA synthesis. CES were collected and washed using distilled water and their membranes were peeled off. This step aims to remove macro dirt from CES that we used. The cleaned and dried CES were then calcined using furnace at 900°C for 8 hours to remove the organic components of the sample. CES which is containing CaCO₃ will release CO₂ and another organics compound and produce CaO. CaO then ground to fine powder using mortar and pestle. A calcium solution 1M Ca(OH)₂ 50 ml was mixed with an aqueous solution of 50 ml 0.6M H₃PO₄ and 50 ml 0.3M NaHCO₃ at molar ratio Ca²⁺:PO₄³⁻:CO₃²⁻ = 1.67:1:0.5 using a magnetic stirrer and the aqueous solution was adjusted to pH 13. The solution was immediately transferred into microwave oven with power 400W for 30 minutes.

The initial stage of making scaffold was dissolving PVA powder (5 gr, 7.5 gr, 10 gr) into a 100 mL distilled water solvent at temperature 60°C until homogenous. CHA powder was added to the PVA solution and alginate was added slowly to the solution until gel was formed. After the solution is stirred, 2 ml of 0.03 M CaCl₂ was added as crosslink agent. The mixtures of CHA/PVA/Alginate were then put in a multi-plate well. The quenching gel process was carried out in the freezer for 24 hours, and then followed by the freeze drying process using a freeze dryer until the sample was dried at a temperature of -50°C. Scaffolds were characterized by FTIR, XRD, SEM EDX and mechanical tests. FTIR measurements were carried out to determine the functional groups of samples. The compressive strength test is tested by universal testing machine with speed 0.5 mm/minute and pressed 80% of the original length. Crystallinity and material structure were analysed using XRD with sources Cu-Kα (λ = 1.5405 Å). Morphology analysis, pore size, and element content were observed using SEM EDX with magnification 50x-1000x.

3. Results and Discussion

3.1 CHA Powder Characterization

![Figure 1. FTIR spectra of CHA powder](image-url)
The results of the FTIR spectrum in the figure above showed the presence of the functional groups PO$_4^{3-}$, CO$_3^{2-}$, and OH which were CHA groups. The PO$_4^{3-}$ group was detected at wavenumber 470 cm$^{-1}$ and 568 cm$^{-1}$ for the vibration group P-O, while the vibration P=O was detected at wavenumber 1049 cm$^{-1}$. The transmittance band of the carbonate apatite group was identified at wave number 870 cm$^{-1}$ and 1420 cm$^{-1}$. The OH group was detected at wavenumber 3452 cm$^{-1}$. The results of the identification of functional groups on CHA powder indicate the compatibility with the absorption area for each functional group.

Furthermore CHA powder was characterized using XRD to identify the phase and structure of the material. In Figure 2, CHA peaks were detected at angles 2θ 25.91°, 29.14°, 32.04°, 33.07°, 34.05°, 40.02°, 46.81°, 49.86°, and 53.19°. The presence of carbonate ions in apatite causes decreased crystallinity [8]. With Cohen method, the values of the lattice parameters a and c are obtained 9.419 Å and 7.742 Å. The EDX (energy dispersive x-ray) test results showed that the sample contained elements of Ca (29.63%), P (14.02%), C (15.13%), O (39.25%), and Na (1.97%). The results of the quantitative analysis showed that the Ca/P ratio of the CHA sample was 1.63. These results showed a smaller Ca/P ratio than stoichiometric HA which has a Ca/P ratio of 1.67. This is due to the presence of carbonate ions which substitute the phosphate group to produce non-stoichiometric apatite. However, the value obtained is in accordance with the range of the Ca/P CHA ratio in the literature, which is 1.6-2.0.

![Figure 2](image1.png)  
**Figure 2.** (a) XRD CHA powder (b) EDX CHA

3.2 Compressive Strength of Scaffold CHA/PVA/Alginate

In this study CHA was mixed with PVA and alginate to produce an amorphous biodegradable scaffold. The results of mixing the three ingredients produce elastic scaffold like a sponge. The factors that influence scaffold that will be obtained include the material used, the composition of the material, the size of plate, density, temperature, and the freeze drying time used.

![Figure 3](image2.png)  
**Figure 3.** Biocomposite scaffold CHA/PVA/Alginate

Scaffolds were then tested for compressive strength in samples with variations in PVA 5%, 7.5% and 10% and fixed alginate (5%). This test aims to find out the optimum percentage of PVA which produce good mechanical strength for scaffold. Based on these results, it can be seen that the higher PVA concentration, will increase the mechanical properties. According to the requirements, the
material can be used as bone substitute if it has a compressive strength about 2-12 MPa for cancellous or sponge bone [9]. The compressive strength of scaffold CHA/ PVA/ Alginate with variation of 10% PVA is suitable for scaffold application in tissue engineering.

![Figure 4. Compressive strength of scaffold](image)

3.3 Structural Analysis of CHA/ PVA/Alginate

Figure 5 showed the diffraction pattern of composite samples, namely the composition of 10% PVA with variations in alginate (3%, 4%, and 5%). The peaks that appear characterize CHA's phase. The peaks of each variation are at relatively equal angles with not much different intensities. The results of this analysis indicated that there is no indication for new crystalline phase formed due to the addition of alginate.

![Figure 5. X-ray diffraction pattern of scaffold](image)

The calculation results of the lattice parameters explained that there is no effect of using alginate on the lattice parameter values. This is due to the use of alginate, PVA, and the freeze drying technique did not damage the crystals. The results of the calculation of crystallite size and crystallinity showed that the addition of alginate reduced the crystallite size and the degree of crystallinity. This is due to the influence of the use of alginate as a porosifier which has an amorphous diffraction pattern which can reduce the crystallinity. This is in accordance with the application of scaffold to be developed because material degradation is easier to occur in materials with low degrees of crystallinity and small crystallite size [10]. Based on these results, the degree of crystallinity and the smallest size of crystallite were obtained in sample Al5.

| Sample ID | Lattice parameter (Å) | Crystallite size (nm) | Crystallinity (%) |
|----------|----------------------|---------------------|------------------|
| Al3      | 9.755 8.132          | 27.40               | 47.75            |
| Al4      | 9.178 7.593          | 26.49               | 46.06            |
| Al5      | 9.164 7.611          | 26.18               | 39.75            |
3.4 Pore Analysis on Scaffold
The results of pore size measurements using ImageJ software obtained the average pore size of scaffold as in Table 2. The pore size found in this scaffold tends to be small. This is because the mass ratio of CHA used was large. However, the pore size in some of the samples produced is sufficient for the growth of osteoblasts, namely 20-30 µm.

Table 2. Pore size of scaffold bio-composite

| Sample ID | Pore size (µm) |
|-----------|----------------|
| Al3       | 12.25          |
| Al4       | 17.65          |
| Al5       | 21.02          |

Figure 6. Morphology and pore size of scaffold

3.5 Analysis of Elemental Composition on Scaffold
The EDX results showed that scaffold is composed of carbon, oxygen, phosphorus, sodium, calcium, and other minor elements. The distribution of the elements contained in the composite is not evenly distributed in all composite parts. Based on the percentage of elements contained in the composite, the Ca/P molarity ratio was calculated and the results can be seen in Table 3. These results indicate that the Ca / P composite molarity ratio is greater than the stoichiometry HA molarity ratio 1.67. The increasing the Ca / P molarity ratio because of using CaCl2 as a crosslink agent, so that the amount of Ca contained in the composite increases. From these results it can also be concluded that the greater the alginate content in the sample, the smaller the Ca/ P ratio produced.

Table 3. Ca/P Ratio of scaffold

| Sample | Ca/P Ratio |
|--------|------------|
| Al3    | 2.69       |
| Al4    | 2.67       |
| Al5    | 2.00       |

4. Conclusion
Bio-composite scaffold CHA/PVA/Alginate had been successfully synthesized by freeze drying methods. The FTIR result showed that spectrums correspond to functional group of PO$_4^{3-}$, OH$^-$, CO$_3^{2-}$. The scaffold mechanical test showed that the higher PVA content, the higher mechanical properties. The XRD analysis results showed that the addition of PVA and alginate reduced both the crystallite size and the crystallinity. The morphology of the sample showed pores in the surface. The EDX results
showed that Ca/P ratio increased. From the results, it was found that the Al5 sample was the most optimal sample to be used as bone replacement material because it has good compressive strength, low crystallite size and crystallinity, and the largest pore size.

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