FTIR, SEM and Micro-CT Scan results of biocomposite scaffold characteristics

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Abstract. CHA/PVA/Alginate based biocomposite scaffolds have been successfully synthesized by using freeze drying methods. Carbonated hydroxyapatite or CHA powder was firstly synthesized using calcium from chicken eggshells, phosphate acid, and sodium carbonates as starting materials. Polyvinyl alcohol (PVA) has been chosen in this experiment to reinforce the mechanical properties of the CHA/alginate scaffolds. The alginate concentrations of 3%, 4%, and 5% were used as porosifiers and CaCl₂ was added as a crosslink agent. Properties of the biocomposite were characterized by fourier transform infrared (FTIR) spectrophotometer, scanning electron microscope with energy dispersive X-ray (SEM-EDX), and micro computed tomography (Micro-CT) Scanner. FTIR results of the scaffolds show spectra corresponding to functional groups of CHA, PVA, and alginate which include O-H, C-O, C = O, C = C, O-Na, P-O and P = O. The morphology of the scaffolds was evaluated by SEM and clearly shows many interconnectivity pores with the values range from 4.92 to 28.48 µm which are sufficient for osteoblast cells to grow. The EDX results show that the scaffolds contain Ca, P, C, O, Na, Cl, and S with the Ca/P ratio ranges from 1.81 to 2.67. Porosity of the scaffolds was measured by micro-CT scanner presenting the range of 25.86% to 42.74%. These scaffolds reveal good prospects for bone substitute application.

1. Introduction
The need of biomaterial is increasing with the number of bone damage cases due to the increasing number of motor vehicle accidents. Every day thousands of surgical procedures are performed to replace or repair tissue that has been damaged. Bone is a highly dynamic connective tissue, which provides adequate mechanical strength and structural support to the body. At macroscopic level, it is classified as cortical or compact bone and cancellous or trabecular bone [1]. The inorganic substance of bone forms a structure that approximates the structure of calcium phosphate apatite carbonation. The main inorganic elements in bone are Ca²⁺ (24.5%) and P³⁻ (11.5%). In addition, bones also contain trace elements such as Na⁺ (0.7%), Mg²⁺ (0.55%), Cl⁻ (0.1%), K⁺ (0.03%), F⁻ (0.02%), and CO₃²⁻ (5.8%) [2]. The mineral composition in human bones differs slightly from hydroxyapatite (HA) stoichiometry due to the presence of impurities in human bones which include carbonate ions, chlorides, fluorides, magnesium, and sodium [3]. Apatite biology in bone has the characteristics of low crystallinity and non-stoichiometry [4]. Bone apatite contains 8% of carbonate ions in apatite crystal structure which HA does not have. The content of carbonate ions in the apatite structure plays an important role in the process of resorption by osteoclasts.

Apatite carbonate (CHA) can be synthesized by various synthesis methods such as precipitation, hydrothermal, ultra-sonication, sol-gel, mechanochemical techniques, and microwave. Microwave is an...
electromagnetic wave which has frequencies range from 300 MHz-300GHz and may provide a rapid and shorter synthesis time because the waves can penetrate and excite water and lipid molecules evenly. Microwave irradiation may produce nano size material, faster temperature rise, lower energy use, high yield and purity [5]. Synthesis of CHA will produce bone-like material that can stimulate bone growth. But CHA has a weakness that is fragile when it is 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. PVA is a nontoxic, water-soluble, biocompatible, and biodegradable synthetic polymer, which is widely used in biochemical and biomedical applications [6]. Alginate is derived from brown algae which has hydrophilic and biocompatible properties, and is relatively economic [7].

In the applications, biomaterials needed by orthopaedic doctors are in the form of scaffolds (porous scaffolds). For bone tissue engineering, therefore, it is necessary to control the porosity and pore characteristics, mechanical strength, biodegradation properties, and bioactivity of the scaffolds. The use of ceramics/polymer-based scaffolds will make the scaffolds osteoconductive and reinforce the scaffolds [8]. The scaffold pores can be produced using freeze drying method. Freeze drying is a simple method for producing scaffolds because it does not need high temperatures which may damage the polymer structures. The water contained in the scaffolds will leave the pores and make the scaffolds become soft and elastic. Based on these matters, in this study, CHA/PVA/alginate biocomposite scaffolds were developed through freeze-drying method. Calcium source used was chicken egg shell (CES) waste which compose of about 95% calcium carbonate, 3.5% glycoproteins, and proteoglycans [9]. Biocomposite scaffolds made from CHA, PVA, and alginate are expected to be one of biomaterial candidates that have potential to be developed for human bone application [10].

2. Experimental
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 to remove macro dirt. The cleaned and dried CES were then calcined at 900°C for 8 hours to remove the organic components. CaCO₃ will release CO₂ and other organic compounds to produce CaO. CaO was then ground to fine powder using mortar and pestle. 50 ml of 1M Ca(OH)₂ was mixed with 50 ml of 0.6M H₃PO₄ and 50 ml of 0.3M NaHCO₃ at molar ratio of Ca²⁺:PO₄³⁻:CO₃²⁻ = 1.67:1:0.5. The mixing processes were stirred using a magnetic stirrer and the pH was maintained at 13. The resulted solution was then immediately placed into a microwave oven 400 W for 30 minutes.

The initial stage of making scaffold was dissolving 7.5 g PVA powder into a 100 mL distilled water at a temperature of 60°C until homogenous. CHA powder was added to the PVA solution and alginate (3%, 4%, and 5%) 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 a crosslink agent. The mixtures of CHA/PVA/alginate were then put in a multi-plate well. The quenching gel process was carried out in a freezer for 24 hours, and then followed by freeze drying processes using a freeze dryer until the sample was dried at a temperature of -50°C. The scaffolds obtained were then characterized by using FTIR, SEM-EDX, and micro-CT scanner. FTIR measurements were carried out to determine the functional groups of samples. Morphology analysis, pore size, and element content were observed by using SEM-EDX with magnification 50x-1000x. Porosity analysis was obtained from micro-CT scan results.

3. Results and Discussion
3.1. Biocomposite Scaffolds of CHA/PVA/Alginate
CHA powder has hard and brittle properties. Meanwhile, scaffolds needed in medical applications are biodegradable amorphous scaffolds. Therefore CHA needs to be composites with polymers, such as PVA and alginate, to improve the mechanical properties and form pores. The Micro CT scan images of the CHA/PVA/alginate biocomposite scaffolds is shown in Figure 1. The results of mixing the three ingredients (CHA-PVA-alginate) form a gel mixture and after freeze drying processes produce a scaffold that is elastic like a sponge. Pores in the scaffold appear as a side effect of the disappearance of water (ice crystals) through the sublimation processes at low temperature of about -50°C for 48 hours.
Variation in temperature and time during freeze drying processes greatly affects the performance of the scaffolds. Some experiments suggested that, before freeze drying, it is necessary to consider some factors describing the characteristics of the scaffold such as material composition, mold size, and density.

Figure 1. Micro-CT scan images of the CHA/PVA/alginate biocomposite scaffolds

3.2. FTIR Spectroscopy Analysis
Na-alginate compound is one of the natural polymers with R-O-R groups containing carboxyl groups (C-O), alkana (C-H), carbonyl (C=O), sodium isomers (O-Na), and hydroxyl groups (O-H) [11]. The FTIR spectra identified by the functional groups of alginate compounds are shown in Figure 2a. The absorption peak of the C-O group was detected at wave number 1127 cm\(^{-1}\), the C-H group was identified at wave number 2925 cm\(^{-1}\), the C=O group at wavenumber 1645 cm\(^{-1}\), the O-Na group at wave number 1423 cm\(^{-1}\), and the group O-H at wave numbers 615 cm\(^{-1}\) and 3447 cm\(^{-1}\). Meanwhile FTIR spectra for polyvinyl alcohol are shown in Figure 2b which shows the functional groups of O-H, C-H, C = C, and C-O, respectively, at wave numbers 3447 cm\(^{-1}\), 2924 cm\(^{-1}\), 1632 cm\(^{-1}\), and 1035 cm\(^{-1}\).

Figure 2. FTIR spectra of Na-alginate and polyvinyl alcohol

Figure 3. FTIR Spectra of Al3, Al4, and Al5 samples
Figure 3 shows the FTIR spectra of the CHA/PVA/alginate biocomposite with the different concentrations of alginate (3%, 4%, and 5%). The spectra show that the functional groups identified increase in number due to the combination of the functional groups of CHA, PVA and Na-alginate. Based on the FTIR test results, it is obtained that the spectrum with the same patterns and wavenumbers with absorption rates are not too much different. The groups identified by this FTIR spectrometer include carboxyl (C=O), carbonyl (C = O), isomer sodium (O-Na), hydroxyl, and phosphate. The O-H group arises because it is the main group of CHA, alginate and PVA. The C=O, C=C, and C-O bonds confirm the presence of alginate and PVA in the sample, and the P-O and P = O groups are characteristic groups of calcium phosphate. Sodium O-Na isomer shows the presence of sodium alginate in the sample. The results of this characterization do not show the emergence of new functional groups in the scaffold due to the addition of PVA and alginate. In other words the addition of PVA and alginate does not change the composition of compounds chemically, but only changes the properties of the material physically.

3.3. Scanning Electron Microscopy (SEM) Analysis
The results of pore size measurements using ImageJ software reveal the average pore size of the scaffolds as presented in Table 1. The pore size found in these scaffolds tends to be small. This is because the mass ratio of CHA used was large. However, the pore size in some samples is sufficient for the growth of osteoblast cells. Interconnected pore structure is important to bone ingrowth and interface support. The micro-porous structure is beneficial to capillary in-growth, nutrient transport and biological properties. Figure 4 shows the SEM images of the morphology of the scaffold surface.

| Table 1. Pore size of scaffold biocomposites |
|---------------------------------------------|
| Alginate Content (%) | Pore Size (µm) |          |
|                 | Min (µm) | Max(µm) |
| 3                | 6.69     | 11.92   |
| 4                | 4.92     | 18.35   |
| 5                | 8.78     | 28.48   |

Figure 4. Morphology and pore shapes of the scaffolds

3.4. Analysis of Elemental Composition of the Scaffolds
The EDX results show that the scaffolds compose 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 result is shown in Table 2. This result indicates that the Ca/P composite molarity ratio is greater than the stoichiometry of HA molarity ratio 1.67. The increase of Ca/P molarity ratio may be due to the presence of CaCl₂ used as a crosslink agent, so that the amount of Ca contained in the composite slightly increases. However, this result clearly shows the relation between alginate content and Ca/P ratio which drives a conclusion that the greater the alginate content in the sample, the smaller the Ca/P ratio produced.
### Table 2. Ca/P ratio of the scaffolds

| Alginate Content (%) | Ca/P Ratio |
|----------------------|------------|
| 3                    | 2.67       |
| 4                    | 2.65       |
| 5                    | 1.81       |

#### 3.5. Porosity Measurements

Porosity of the samples for different alginate concentrations was measured by using a Micro-CT scan. Figure 5 shows the relation between the porosity and the alginate concentration. Porosity obtained at each scaffold of 3%, 4%, and 5% alginate, respectively, are 37.17%, 25.86%, and 42.74%. The values indicate that the porosity is low. Macropores hydroxyapatite for bone filler in spongious femur bone requires porosity of about 70% [12]. However, scaffold porosity greater than 80% can reduce the mechanical strength of the scaffolds. Meanwhile, commercial scaffolds made by Sigma Aldrich have an average porosity value of about 55%.

![Porosity](image)

**Figure 5.** Scaffold porosity of Al3, Al4, and Al5 samples

#### 4. Conclusion

CHA/PVA/Alginate biocomposite scaffolds, with Na-alginate concentration variation of 3%, 4%, and 5%, have been successfully synthesized. FTIR results confirm the presence of O-H, C-O, C = O, C = C, O-Na, P-O and P = O spectra which correspond to the functional groups of CHA, PVA, and alginate. SEM images show the surface morphology of the scaffolds with the interconnected pore structure. The size of the pores range from 4.92 to 28.48 µm. EDX results reveal the Ca/P ratios range of 1.81 to 2.67. The value of the pore sizes found is directly proportional to the concentration of the alginate used in this research. Micro-CT analysis presents that the porosity of the scaffolds obtained is less than 50%. From this experiment, it is found that the sample with alginate content of 5% is the most optimal scaffolds to be used as a bone replacement material since it has large pore size, high porosity and Ca/P ratio close to pure HA.

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