Effect of Stirring Duration on Hardness and Antibacterial Characteristics of Polyethylene Glycol-Hydroxyapatite Nanocomposites

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Abstract. We study the effect of stirring duration on the synthesis of polyethylene glycol-hydroxyapatite (PEG/HA) nanocomposites and their hardness and antibacterial characteristics. The HA was used from natural deposit calcite rock from Druju-Malang. In this study, PEG/HA composite with a composition ratio of 20:80 in %Wt was obtained through co-precipitation method with various stirring duration of 1, 2, 4, and 6 hrs. The results indicate that the variation of stirring duration of the PEG/HA composite promotes a change of functional bonds, grain size, and hardness value. The optimum hardness was achieved by stirring duration of 2 hrs. Furthermore, the PEG/HA composite exhibited a relatively consistent value of antibacterial zone in a variation of stirring duration, but it still showed an antibacterial performance. Our study would give a good understanding of the technique to improve mechanical and antibacterial properties in HA-based biomedical applications for bone regeneration.

Keywords: Polyethylene glycol, hydroxyapatite, nanocomposite, hardness, antibacterial

1. Introduction
The research on biomaterials received great attention due to their attractive properties for human rehabilitation. The increasing problems regarding bone fracture lead to the high use of biomaterials in bone rehabilitation. To date, several studies have been performed to find and develop alternative materials for bone tissue repairing with high biocompatibility. Hydroxyapatite [Ca$_{10}$(PO$_4$)$_6$(OH)$_2$] (HA) is one of the biomaterials that is widely used in bone tissue rehabilitation due to its resemblance to human bone tissue minerals and another potential characteristic [1].

Hydroxyapatite (HA) shows the high bioactivity, biocompatibility for bone regeneration, but it still lacks mechanical properties. As a bone graft material, a material that has good mechanical properties is needed. To improve the mechanical properties of HA, it is necessary to modify the HA preparation technique. In this study, a composite, containing HA and polymers, which have mechanical properties, was proposed to solve the problem. Polyethylene Glycol (PEG) is a potential polymer for a composite system, which has high biocompatibility, non-toxic, and high antibacterial properties [2,3]. Therefore, the introducing PEG in HA is expected to have good antibacterial properties in biomedical applications. Furthermore, because the mechanical properties become one of the essential parameters of the HA, in
this work, we also investigate such properties. The samples were synthesized using co-precipitation method because this method becomes an effective and simple process that allows the production of a nanoscale particle with high homogeneity distribution [4]. In this study, the variation of stirring duration is performed to modify morphology, mechanical, and antibacterial properties of PEG/HA. Here, the morphology, mechanical, and antibacterial properties were investigated by comprehensive analysis.

2. Methods

2.1. Preparation
Calcite rock with calcium content of 98.68%, collected from natural deposit materials, Druju-Malang, were grounded and sieved with 200 mesh, continued with calcination at a temperature of 1000 °C for 5 hrs to remove CO₂ content, obtaining Ca(OH)₂ as a starting material of calcium in the synthesis of HA.

2.2. Synthesis

2.2.1. Synthesis of HA
Diammonium hydrogen phosphate [(NH₄)₂HPO₄] (DHP) and NH₄OH was used as a source of phosphate and pH controller, respectively. The Ca(OH)₂ powder was reacted with HNO₃ 2M to produce Ca(NO₃)₂. The Ca(NO₃)₂ was reacted with DHP and NH₂OH for 2 hrs. Furthermore, the solution was precipitated for 24 hrs with the pH of ~ 9-10. The precipitate was filtered and dried at 100 °C, obtaining the HA powder.

2.2.2. Synthesis of PEG/HA
A composite was produced from HA powder and PEG with the PEG/HA ratio of 20:80% using a variation of stirring duration of 1, 2, 4, and 6 hrs. The solution was precipitated for 24 hrs with a neutral pH. The precipitate was further filtered and dried at 100°C for 8 hrs.

2.3. Characterization
X-ray diffractometer (XRD) was used to characterize the crystallinity of the sample. Besides, the grain size of the sample was determined by using the Scherrer following equation

\[ D = \frac{k\lambda}{B\cos\theta} \]

with \( D \) is the grain size (Å), \( k \) is the constant (~0.9), \( \lambda \) is the wavelength of Cu-Kα X-ray source, \( B \) is the full width at half maximum (FWHM) of diffraction peak (rad), and \( \theta \) is the Bragg angle (rad) [5]. Scanning electron microscopy and energy dispersive X-ray spectroscopy (SEM-EDX) were used to investigate the morphology and determine the Ca/P ratio, respectively. Fourier transforms infrared (FTIR) spectroscopy was used to investigate the functional bonds of the sample. Furthermore, the hardness of the sample was determined by using a microhardness Vickers tester. Antibacterial characteristic of the sample was characterized by Mueller Hinton Agar diffusion method using Staphylococcus Aureus [6].

3. Results and Discussion

3.1. Hydroxypatite (HA)
Figure 1 presents the diffraction pattern of HA fitted using Match software. The result shows that HA exhibits the same phase with HA database (COD code: 96-721-7895). This result reveals that the synthesized HA has 100% HA phase. The lattice parameter of HA is determined by a fitting process using AMCSV-0004924 model, with obtained lattice parameters of \( a = b = 9.4439\text{Å}, c = 6.8569\text{Å} \) and \( \gamma = 120° \). This result shows a good agreement with a previous report [7], which obtained the lattice parameter of \( a = b = 9.407\text{Å} \) and \( c = 6.872\text{Å} \). Furthermore, the HA grain size calculations using the
Scherrer equation is presented in Table 1. The obtained grain size is in the range of 25-32 nm. This size is a good agreement with HA in human bone (20-80 nm) [8]. A little larger grain size of 35-50 nm for PEG/HA composites was reported by previous work [9].

In order to investigate the morphology and Ca/P ratio of the HA, SEM-EDX characterization is used. Figure 2 shows the morphology of HA tends to be agglomerated. The obtained Ca/P ratio of synthesized HA is 1.45, smaller than standard Ca/P value of 1.67 [8]. The difference in the value of Ca/P ratio is presumably due to the presence of impurities. The synthesis is conducted in the non-isolated environment, which can promote the presence of other materials on the HA surface. The small ratio of Ca/P is supported by the presence of other phases (carbonate and TCP) in HA.

![Figure 1. The diffraction pattern of the HA sample fitted by HA database (COD code: 96-721-7895) using Match software.](image)

Table 1. The grain size of the samples by the Scherrer equation.

| Stirring duration | Grain size (nm) |
|-------------------|-----------------|
| HA                | 31.19           |
| 1 hr              | 27.91           |
| 2 hrs             | 25.69           |
| 4 hrs             | 27.75           |
| 6 hrs             | 29.12           |
Figure 2. SEM image of the HA sample.

3.2. Polyethylene glycol-hydroxyapatite (PEG/HA) nanocomposite
Figure 3 shows the functional bonds group of HA, PEG, and PEG/HA composites. The presence of P-O and O-H vibration mode confirm the presence of HA. The presence of carbonate compounds in 1384 cm\(^{-1}\) confirms the presence of the contact between carbon dioxide (CO\(_2\)) and solvents (aquades) on HA surface.

![FTIR spectra of HA, PEG, and PEG/HA samples.](image)

Figure 3. FTIR spectra of HA, PEG, and PEG/HA samples.
The presence of carbonate in HA will reduce thermal stability so that the presence of carbonate needs to be removed. By introducing the longer stirring duration and addition of PEG, the presence of carbonate was successfully reduced. The result confirms that C-O vibration mode decrease at 1386 cm$^{-1}$, which is associated with the addition of PEG in HA. In addition, the stirring duration also promotes the weakening of the C-O vibration mode. The longer stirring duration promotes more homogeneous PEG/HA and reduces the presence of a carbonate bond in PEG/HA due to the inert character of PEG-coated the surface of HA. A new peak indicates the presence of PEG/HA at 3566 cm$^{-1}$ which indicated by a solid arrow. On the other hand, we observed a loss of weak peaks indicated by an empty arrow at around 2470 cm$^{-1}$. This peaks confirmed that PEG and HA also share an interaction. The increase of stirring duration affects to the more prominent of the interaction. Dhanalaksmi et al. reported that a new peak was observed at 2944 cm$^{-1}$, which was ascribed to PEG/HA [9].

Figure 4 presents the diffraction pattern of the HA and PEG/HA nanocomposites. Although not a linear effect of the stirring duration, it is confirmed that the stirring duration shows a slight change diffraction patterns. Fitting results with the AMCSD-0004924 model confirmed the lattice parameters of the sample of $a = b = 9.4212$ Å and $c = 6.8927$ Å. This result has a consistent value with Swain's report, which obtained a lattice parameter value of $a = b = 9.42$ Å and $c = 6.87$ Å [10]. Further analysis on a single first peak around 26.5° shows that the induced of PEG and employing stirring reduce the grain size. The shift of peak to a lower position represent the small enlarging the lattice parameters.

Figure 5 presents SEM images of PEG/HA samples. All of the samples exhibit agglomerated grain. The PEG/HA with stirring duration of 1 hr shows inhomogeneous grain size distribution and morphology. The PEG/HA with stirring duration of 6 hrs has a finer morphology than other samples, but there are many pores that affect the value of the hardness produced. Here, the percentage of porosity and hardness value of the samples are presented in Table 2.
Figure 5. SEM images of PEG/HA samples with a variation of stirring duration of (a) 1 hr, (b) 2 hrs, 6 hrs, and (d) 6 hrs.

Table 2. Percentage of porosity and hardness value of the samples.

| Sample     | Porosity (%) | Hardness (HVN) |
|------------|--------------|----------------|
| HA         | 10           | 10             |
| PEG/HA 1 hr| 6.45         | 16.9           |
| PEG/HA 2 hrs| 3.7         | 23.93          |
| PEG/HA 4 hrs| 4           | 21.2           |
| PEG/HA 6 hrs| 7.69        | 17.73          |

The stirring duration affects the homogeneity particles distribution, resulting in a dense structure and subsequently promotes the higher hardness value. Table 2 shows that the HA show the porosity percentage of 10%, while PEG/HA nanocomposites with stirring duration of 1, 2, 4, and 6 hrs have the porosity percentage in the range of 3-10%. The PEG/HA nanocomposites with stirring duration of 2 hrs have a small porosity percentage of 3.7%. The result shows that the highest hardness value is obtained at a stirring duration of 2 hrs, with a hardness value of 23.93 HVN.

The antibacterial test is performed by using Mueller Hinton Agar diffusion method. List of clear zone of the samples is presented in Table 3. The result confirms that the highest clear zone of 0.785 mm is obtained at the stirring duration of 2 hrs. These results indicate that the sample has antibacterial properties, but only inhibits bacterial growth (bacteriostatic). The stirring duration does not affect the inhibition zone because the sample is not dissolved so that it settles in the media. The homogeneity of the sample and low solution diffusion of the Agar are due to insolubility of sample also promote non-optimal clear zone. Dhanalaksmi et al. reported that PEG-20/HA nanocomposites showed the highest antimicrobial activity compared to other PEG/HAp nanocomposites [9].
Table 3. Clear zone value of the samples.

| Sample         | Average zona (mm) |
|----------------|-------------------|
| HA             | -                 |
| PEG/HA 1 hr    | 0.76              |
| PEG/HA 2 hrs   | 0.785             |
| PEG/HA 4 hrs   | 0.68875           |
| PEG/HA 6 hrs   | 0.73              |

4. Conclusion
The morphology, hardness and antibacterial characteristics of PEG/HA nanocomposites have been investigated by varying the stirring duration. The longer the stirring duration produced, the smaller grain size. The highest hardness value of 23.93 HVN was found in the PEG/HA with stirring duration of 2 hrs, but the stirring did not affect the antibacterial characteristic. In another word, the resistance to bacteria was relatively the same in all of variation stirring duration.

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