Synthesis of 3D-porous scaffold from cockle shells waste-based hydroxyapatite with addition silica from tin tailings

F Afriani1,*, Evi.J1, R A Rafsanjani2, R Amelia3, M Hudatwi4 and Y Tiandho1

1Department of Physics, Universitas Bangka Belitung, Indonesia
2Department of Physics, Universitas Indonesia, Indonesia
3Department of Mathematics, Universitas Bangka Belitung, Indonesia
4Department of Marine Sciences, Universitas Bangka Belitung, Indonesia

*E-mail: fitri-afriani@ubb.ac.id

Abstract. This study aims to synthesize a porous scaffold based on hydroxyapatite and silica using the polymer sponge replication method. In bone tissue engineering technology, the development of porous scaffolds is a topic that is intensively studied because it is expected to be a solution to various problems of conventional bone therapy. In addition to proposing a porous scaffold synthesis method, we also utilize natural waste-based materials such as cockle shells and tin tailings as raw materials in this research. Investigation through x-ray diffraction (XRD) pattern with the goodness of fit coefficient, \( \chi^2 = 0.09 \) shows that the coprecipitation method is effective for the synthesis of hydroxyapatite. Analysis of XRD pattern of tin tailings sand with a value of \( \chi^2 = 0.008 \) showed that the diffraction pattern was related to silica with space group P 41 21 2. The polymer sponge replication method with polyurethane template succeeded in obtaining scaffolds with macropores above 300 \( \mu m \). Based on the diffraction pattern of the three porous scaffolds prepared with different percentages of HA, it is known that all porous scaffolds have peaks related to HA and silica. It indicates that the decomposition temperature of polymer does not provide sufficient energy for the HA and silica to transform or react chemically.

1. Introduction

The development of scaffolds in the bone therapy is expected to be a solution to the scarcity of donors in conventional bone therapy [1, 2]. The absolute requirement for the material to be applied as a scaffold is that it is biocompatible and does not have a toxic effect on humans [3, 4]. Candidate materials that are often developed because they meet these criteria usually come from the calcium phosphate family, such as hydroxyapatite (HA) with the chemical formula Ca\(_{10}\)(PO\(_4\))\(_6\)(OH)\(_2\). It is because calcium phosphate is the main constituent of human bones.

Hydroxyapatite can be synthesized from natural materials rich in calcium, such as eggshells, animal bones, and shells [3]. As an archipelago, the development of calcium phosphate-based materials such as hydroxyapatite derived from cockle shells is interesting. It is due to the high abundance of cockle shells in the Bangka Belitung Islands [5].

In addition to focusing on biocompatibility and biodegradability, an essential aspect in developing scaffolds is a good performance in the pores [6]. The pores will be the attachment space for cells and cavities for cells to migrate and obtain nutrients. However, the presence of pores often also causes problems in the mechanical strength of the scaffold.
Improvement solutions that can be made for HA-based porous scaffolds are through the development of composites. Materials that can improve these properties include silica [7, 8]. Silica is widely known as a material that can act as a reinforcement in scaffolds. Interestingly, silica is also known to be biocompatible. The presence of silica ions in the scaffold structure will also increase the bioactivity properties by forming the surface layer of apatite crystals on simulated body fluids (SBF) [9].

As one of the highest tin-producing areas globally, the Bangka Belitung Islands have various problems related to post-mining lands, such as tailings originating from the washing process of tin excavation. Physically, the composition of tin tailings waste is composed of sand, clay, and dust. While chemically, the main content of the tailings is dominated by silica. In several previous studies, we have developed several purification methods to increase the silica content in tin tailings, such as the acid leaching method [10] and the solid-state method [11]. Therefore, the utilization of silica from tin tailings into various functional products is an interesting topic to be studied.

In this article, we propose a method of synthesizing a porous scaffold consisting of a mixture of HA and silica via polymer sponge replication method. We utilize cockle shell waste as a source of calcium in the synthesis of HA and tin tailings sand as raw material for silica.

2. Method
To synthesize hydroxyapatite, we used cockle shell (Anadara granosa) powder calcined at a temperature of 1000°C. Afterward, the calcined powder was dissolved in distilled water and added (NH$_4$)$_2$HPO$_4$ as a source of phosphate until it reached a Ca/P ratio = 1.67. The precipitate was sintered at a temperature of 900°C for 5 hours. We used x-ray diffraction (XRD) to investigate the crystalline properties of HA.

To purify tin tailing, we applied the solids method as the previous study [11]. We combine the purified silica with HA to obtain a porous scaffold through the polymer sponge replication method. HA powder and tin tailings silica are formed into slurries with variations in the ratio of HA: silica is 80:20 (HA-80), 70:30 (HA-70), and 60:40 (HA-60), respectively. The slurry was prepared by PVA mediation. The slurry was impregnated into a polyurethane (PU) sponge and sintered at 900°C for 3 hours. The scaffolds were then analyzed for their crystalline properties using XRD.

3. Results and Discussion
Figure 1 shows the XRD pattern of the hydroxyapatite synthesized in this study. We used refinement calculations to analyze diffraction patterns. The peaks that appear in the diffraction pattern are related to the XRD pattern belonging to hydroxyapatite. The synthesized hydroxyapatite had a hexagonal crystal system with a space group P 63/ M. The three highest peaks produced were located at 20: 31.85°, 32.91°, and 32.44° and these peaks corresponded to orientations of 2 1 1, 0 3 0, and 1 1 2 respectively. With the goodness of fit coefficient value ($\chi^2$) = 0.09, we obtain the lattice parameters from HA that satisfy: $a = b = 9.4156$ Å and $c = 6.8830$ Å and have a cell volume of 528.3 Å$^3$.

![Figure 1](image_url)  
Figure 1. XRD pattern of HA synthesized from cockle shell waste. The + symbol indicates the experimental data (obs), and a solid line indicates the calculation (calc) of the refinement. The lower trace is the difference between experiment and calculation (diff). The vertical lines mark the position of the calculated Bragg peaks for HA.
Figure 2 presents a refinement of the silica x-ray diffraction pattern. The goodness of fit coefficient ($\chi^2 = 0.008$) indicates the refinement process has good quality. The synthesized silica has a group space of P 4 1 2 1 2 with lattice parameters $a = b = 4.9939 \text{ Å}$ and $c = 6.9612 \text{ Å}$ with a cell volume of 173.567 Å$^3$. The three highest peaks of the silica diffraction pattern are at positions 21.89°, 36.10°, and 31.34° and correspond to orientations 0 1 1, 1 1 2, and 0 1 2.

**Figure 2.** XRD pattern of silica synthesized from tin tailing waste. The + symbol indicates the experimental data (obs), and a solid line indicates the calculation (calc) of the refinement. The lower trace is the difference between experiment and calculation (diff). The vertical lines mark the position of the calculated Bragg peaks for HA.

Figure 3 presents the microscopy results of HA/silica scaffolds synthesized by the polymer sponge replication method. Through pore size analysis using the equivalent area method and the equivalent perimeter method, it was seen that all scaffolds had pores above 300 m. The relationship between the percentage of HA and the average pore size in Figure 4 shows that HA does not change the pore shape significantly.

**Figure 3.** HA/silica porous scaffold with polymer sponge replication method: (a) HA-80, (b) HA-70, and (c) HA-60.
Figure 4. Relationship between mean pore size and HA percentage in HA/silica scaffolds

Figure 5 presents an XRD analysis of the porous scaffold after sintering. After going through the calculation of the refinement of the XRD pattern, it is known that the sintering process at a temperature of 900°C does not change the resulting hydroxyapatite phase. The constituent phases of the scaffold material are HA and silica, which do not form a significant chemical reaction indicated by the XRD in Table 1. Therefore, this indicates that the synthesized mixture does not interfere with the biocompatibility properties of the two constituent materials. The absence of a chemical reaction between hydroxyapatite and silica in this study was related to the low thermal energy given to the sintering process. In addition, the hydroxyapatite and silica phases have good thermal stability.

Figure 5. XRD pattern of HA/silica scaffold. The + symbol indicates the experimental data (obs), and a solid line indicates the calculation (calc) of the refinement. The lower trace is the difference between experiment and calculation (diff). The vertical lines mark the position of the calculated Bragg peaks for HA and silica, respectively: (a) HA-80, (b) HA-70, and (c) HA-60.
Table 1. Lattice parameter of HA/silica scaffolds

| Sample | $\chi^2$ | HA (P 63/M) | Silica (P 41 21 2) | Volume Cell (Å$^3$) |
|--------|---------|-------------|-------------------|---------------------|
|        |         | $a$ (Å)    | $b$ (Å)           | $c$ (Å)            | $a$ (Å) | $b$ (Å) | $c$ (Å) | Volume Cell (Å$^3$) |
| HA-60  | 0.108   | 9.4128     | 9.4128            | 6.8809             | 527.975 | 4.9896 | 6.9696 | 173.516          |
| HA-70  | 0.098   | 9.4170     | 9.4170            | 6.8804             | 527.701 | 4.9861 | 6.9766 | 173.446          |
| HA-80  | 0.108   | 9.4081     | 9.4081            | 6.8776             | 527.195 | 4.9857 | 6.9818 | 173.548          |

4. Conclusion
After refining the x-ray diffraction pattern, it can be concluded that we have successfully synthesized HA from cockle shells through the coprecipitation method. Analysis of the silica XRD pattern also showed that the XRD peaks related to the silica peak with space group: P 41 21 2. Through the polymer sponge replication method with a polyurethane template, we have also succeeded in synthesizing a 3-dimensional porous scaffold based on HA and silica with the size of macroporous above 300 μm. Investigations on the XRD analysis pattern of the scaffolds showed that the method used did not provide sufficient energy for the HA and silica to react or undergo phase transformation chemically. Thus, the resulting scaffold can still maintain its biocompatibility properties because it is composed of biocompatible compounds.

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