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Porosity of GEL-HA Composite Scaffolds Synthesized from Blue Swimming Crab Shells

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Abstract

Bone defects are a serious health problem requiring graft materials (scaffolds) with ideal porosity. This study aims to determine the effect of varying gelatin concentrations on the porosity of hydroxyapatite-gelatin composites based on blue swimming crab shells. Blue swimming crab shells were utilized as an alternative source of hydroxyapatite (HA) because they are rich in calcium content and possess biocompatible characteristics. However, pure HA exhibits brittle mechanical properties, necessitating the addition of gelatin to bind the structure and reduce brittleness. The HA was processed using the microwave method. Scaffolds with varying gelatin concentrations of 2%, 3%, and 4% were fabricated using slurry casting and oven drying methods. Characterization using X-Ray Diffraction (XRD) demonstrated that the HA synthesis successfully achieved a purity level of 99.6%. Porosity testing conducted using the liquid displacement method indicated that the pore percentage values ranged from 61.90% to 62.84%. The porosity trend exhibited a non-linear pattern, with the highest porosity value achieved by the HAG-3 specimen at 62.84%. Based on the conducted research, the obtained porosity values have met the minimum threshold for scaffolds, which is in the range of 50% to 90%.

Keywords: blue swimming crab shells, gelatin, hydroxyapatite, porosity, scaffold

INTRODUCTION

Bone defects are a serious public health problem with a significant impact on patients' quality of life. These bone defect cases are generally caused by several factors, such as trauma, infection, tumors, or metabolic disorders (Prasetya et al., 2025). Naturally, bone tissue possesses the intrinsic capacity to heal on its own; however, this capability is limited and insufficient to repair defects that have exceeded a critical size.

The magnitude of bone defect incidence and the high medical urgency are evident from global statistical data. Based on data from Zhao et al. (2021), it was recorded that globally, 2.2 million bone graft procedures were performed in 2021. This certainly involves the need for a massive supply of bone graft materials every year. To date, the high demand for these medical materials has not been matched by local production capacity and sustainability. Consequently, the supply of biomaterials in Indonesia is highly dependent on foreign sources, resulting in high-priced imported products continuing to dominate the Indonesian market (Susanto et al., 2020).

In an effort to overcome the limitations of conventional methods, bone tissue engineering has been widely developed as a reliable alternative approach. Although minor bone defects can regenerate on their own, the recovery of large-sized bone defects is often hindered by a lack of blood supply or the presence of systemic diseases (Abbasi et al., 2020). Therefore, bone tissue engineering is carried out by restoring and maintaining the natural function of bone tissue through the implantation of a scaffold in the defective bone area (Islamillennio, 2023). This scaffold functions as a temporary template for cells to attach, migrate, and proliferate until new tissue is formed (Butarbutar, 2026;

Hutmacher, 2000). One of the most crucial scaffold characteristics in supporting successful bone regeneration is porosity. According to Karageorgiou & Kaplan (2005), porosity is a highly important physical parameter in bone scaffold design, as high porosity accompanied by large pores has been proven to enhance bone ingrowth post-operation (Hutmacher, 2000).

Hydroxyapatite is a highly beneficial bioceramic that has been widely utilized in both medical and non-medical fields. Hydroxyapatite possesses a chemical composition resembling organic compounds and can be obtained using synthetic or natural calcium sources. Common natural sources used include crab shells, bovine bone, and fish bone (Islamillennio, 2023). Pure hydroxyapatite has drawbacks in the form of its brittle nature, low mechanical resistance, and slow resorption rate, necessitating a combination with other materials to eliminate these deficiencies (Rasmiyanti et al., 2022). Gelatin, a natural polymer extracted from collagen, can provide structural strength (Maulina et al., 2024). This gelatin can improve or eliminate the drawback of hydroxyapatite, namely its brittleness.

Commercial synthetic hydroxyapatite is an effective material for bone substitutes, but it is highly expensive. Therefore, the selection of blue swimming crab shells as the base material for synthesizing hydroxyapatite will reduce the costs incurred to obtain it. Crab shells are an industrial waste from seafood processing that is abundant in Indonesia. According to Rafianto et al. (2021), crab shell waste contains a calcium level of 22.93%. This calcium content allows crab shells to be processed into the base material for hydroxyapatite. The utilization of this crab shell waste is not only to suppress production costs and reduce dependence on imported products, but it can also provide a solution to the suboptimal management of fishery industry waste.

Based on the above discussion, further research is needed to determine the effect of gelatin and HA concentrations on the porosity of GEL-HA composite scaffolds derived from blue swimming crab shells. This study focuses on the experimental investigation of the effect of varying gelatin and HA concentrations on the porosity characterization of HA-Gel composite scaffolds made from crab shells using the oven drying method to determine the optimal composition.

METHOD

The primary material of this study was hydroxyapatite synthesized from blue swimming crab shells using the microwave method and calcined at 900°C. Other materials included blue swimming crab shell powder as the base material and calcium source, distilled water as the solvent, diammonium hydrogen phosphate ((NH₄)₂HPO₄) from Merck as the phosphate source, and type B gelatin as the polymer matrix. The total number of specimens in this study was 9, divided into 3 variants.

The preparation process began with sieving the blue swimming crab shell powder to 100 mesh to achieve a uniform particle size. The sieved crab shell powder was subsequently placed into a furnace using a porcelain crucible for the calcination process. The calcination process was conducted by holding the furnace temperature at 600°C for a specific period, which was then raised to 900°C and held for 5 hours. This was intended to convert the carbonate elements in CaCO₃ into calcium oxide (CaO) (Hadiwinata et al., 2021). After the calcination process was completed, the samples were naturally cooled until they reached room temperature.

The calcined powder was then synthesized using the microwave method. The composition used in this study followed Fitriyana et al. (2025), comprising 3.1568 g of calcined crab shell powder and 2.942 g of diammonium hydrogen phosphate (Merck Analytical Reagent/AR). The first step of the synthesis involved dissolving the calcined CaO in distilled water to form a calcium hydroxide suspension. This suspension was reacted drop-by-drop with the diammonium hydrogen phosphate ((NH₄)₂HPO₄) solution while being stirred using a sonicator until a hydroxyapatite precipitate was formed. The diammonium hydrogen phosphate reagent functioned as an analytical reagent and as a precursor for the HA synthesis (Fitriyana et al., 2024). The hydroxyapatite precipitate subsequently underwent an aging process by being placed in a microwave for 3 minutes. The microwave method was selected because it is a cost-effective and environmentally sustainable heating method, capable of rapid heating, enhancing reaction kinetics, and facilitating the production of HA with high purity and uniformity (Fitriyana et al., 2025). Subsequently, the hydroxyapatite underwent a washing process by being soaked in distilled water at several time intervals. The washed hydroxyapatite was then filtered using filter paper, leaving only the precipitate, which was subsequently dried in an oven. The synthesized hydroxyapatite was further characterized using X-Ray Diffraction (XRD) testing to identify the crystalline phases formed within the hydroxyapatite. The produced hydroxyapatite was

then fabricated into scaffolds by combining it with gelatin as the polymer matrix. The total mass of the HA-GEL composite was 4 grams. The gelatin and HA were weighed using a Fujitsu FSR-A analytical balance with the variations presented in Table 1.

Table 1. HA-GEL Composition

Specimen	HA:GEL Ratio	HA (g)	Gelatin (g)
HAG-2	98:2	3.92	0.08
HAG-3	97:3	3.88	0.12
HAG-4	96:4	3.84	0.16

The required amount of weighed hydroxyapatite was then poured into the heated gelatin solution and stirred using a magnetic stirrer until the mixture became homogeneous. The mixture was molded using the slurry casting method. The molds used were cylindrical silicone molds with a diameter of 6 mm and a height of 6 mm, as shown in Figure 1. The molded scaffolds were then dried in an oven at a temperature of 40°C for 24 hours. After being molded and dried for 24 hours, the scaffolds were removed from the molds and stored in a dry place prior to testing.

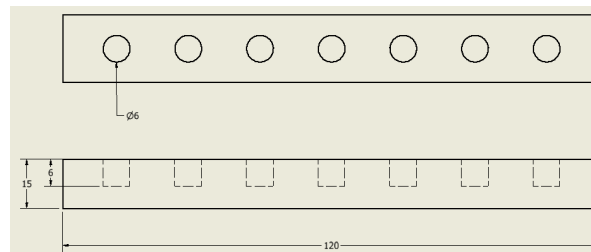


Figure 1. Slurry Casting Molding

The porosity of the scaffolds can be measured using Archimedes' principle. The method employed was the liquid displacement method. The procedure was as follows: W_1 represents the dry weight of the scaffold. Next, the scaffold was soaked in ethanol for 12 minutes to ensure the pores were filled with ethanol. Following the soaking, the scaffold was weighed again (W_2), and the final step was measuring the mass of the scaffold while submerged in the ethanol liquid (W_3). The porosity testing procedure can be seen in Figure 3. W_2 is the saturated mass of the scaffold measured in the air, W_1 is the dry mass of the scaffold, and W_3 is the saturated mass of the scaffold measured in the liquid (Maji et al., 2015). The porosity was calculated using the equation:

$$Porosity = \frac{W_2 - W_1}{W_2 - W_3} \times 100\%$$

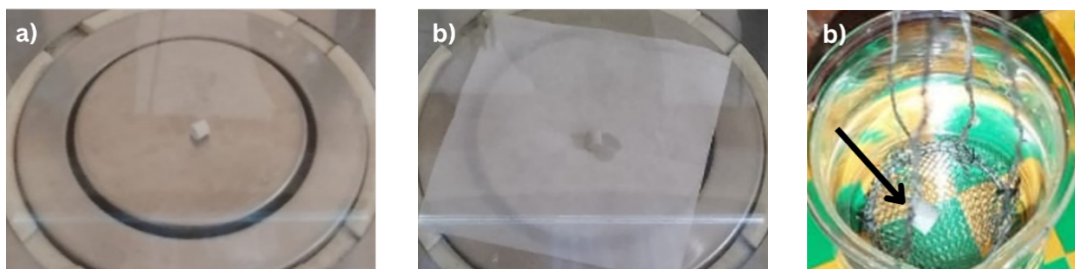


Figure 2. Porosity Testing Procedure. a) dry mass, b) saturated mass, c) submerged mass

RESULTS AND DISCUSSION

Scaffold fabrication in this study was conducted using the slurry casting method. The scaffolds were prepared based on gelatin and hydroxyapatite variants according to Table 1. The synthesized hydroxyapatite subsequently underwent an X-Ray Diffraction (XRD) process. This aimed to identify the crystalline phases formed in the hydroxyapatite prior to undergoing the slurry casting process.

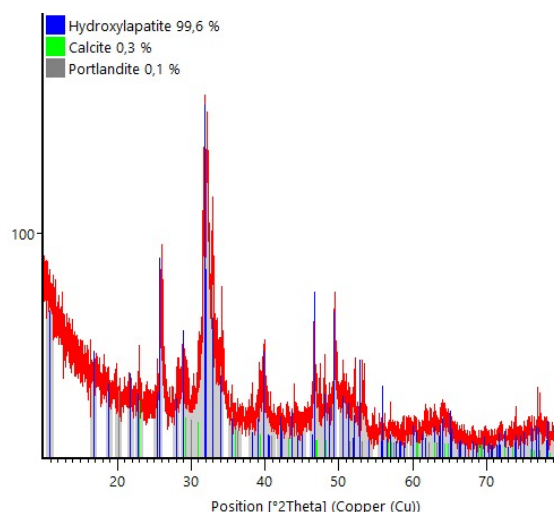


Figure 3. XRD of Blue Swimming Crab Shell HA

The hydroxyapatite characterization results using XRD in Figure 4 demonstrated that the synthesized product successfully achieved a hydroxyapatite phase content of 99.6%. This dominant phase could be observed at the 2-theta angle range between 31° and 33° . In addition, minor impurity phases were detected in very small amounts, namely calcite (CaCO_3) at 0.3% and portlandite (Ca(OH)_2) at 0.1%. The calcite phase most likely originated from the residual calcium carbonate (CaCO_3) inherent in the blue swimming crab shells that was not entirely eliminated during the calcination process (Pu'ad et al., 2019). The dominance of this hydroxyapatite phase was supported by the high natural calcium content in the crab shell waste. According to Raya et al. (2015), the calcium content in crab shells after calcination is 66.62%; thus, when subjected to an appropriate calcination process and precise synthesis parameters, this material is capable of producing a dominant hydroxyapatite phase with minimal impurity phases.

The scaffold fabrication process was influenced by various technical variables, where one of the primary determining factors was the physical characteristics of the generated slurry prior to the molding stage. The slurry characteristics, particularly those related to the viscosity level and mixture homogeneity, played a crucial role in determining the final quality of the scaffolds, especially in terms of shape. Based on the conducted research and experiments, a slurry condition with a viscosity level that was too low (too liquid) caused the scaffolds to tend to not form according to the target. The formed scaffolds were typically smaller than the predetermined dimensions or exhibited chipping at the corners. Conversely, if the slurry condition had an adequate viscosity level, the scaffolds could form according to the desired dimensions and shape.

In several instances in this study, the scaffolds experienced physical defects in the form of significant size variations or chipping at the corners. Based on the conducted research, a higher HA content (and consequently a lower gelatin content) caused the slurry mixture to dry very easily, and vice versa. This phenomenon occurred due to shrinkage during the drying process using the oven drying method. Structural shrinkage during the process (if the solvent was excessive) was suspected to occur due to the high solvent content in the mixture, which subsequently evaporated when heated. This excessive evaporation caused the total dimensional reduction of the scaffold itself.

Besides the slurry factor, the gelatin composition factor also influenced the scaffold structure. The function of gelatin is as a binder (Pratiwi et al., 2017). A study by Pratiwi et al. (2017) explained that gelatin strengthened the hardness (kg) of the specimens used (chewable tablets). Nevertheless, in this study, the composite scaffold specimens were ultimately successfully molded and formed with a stable appearance using the composition variations summarized in Table 1. Furthermore, the scaffolds were also safe and did not crumble when subjected to physical handling by hand.

In determining the appropriate slurry mixture formula, it was highly necessary to consider several technical aspects, such as the addition of distilled water solution and the appropriate fabrication process stages. Based on the experimental results, the specified amount of distilled water used as a solvent in each slurry mixture was 5 mL. The determination of this volume dosage was not done randomly but was determined by referring to the evaluation results of a series of previous trial-and-error experiments. In the initial trials, a solvent volume less than this amount actually produced

a slurry that was too dense, hardened quickly, and was extremely difficult to distribute evenly into all corners of the silicone mold. Figure 5 shows visual proof that the scaffold fabrication stages proceeded successfully. The resulting specimens were able to maintain their appropriate cylindrical shape, follow the mold pattern, and successfully achieve the final dimensional precision according to the target predetermined from the beginning of the design.



Figure 4. Scaffolds

The scaffold composition in terms of gelatin and HA concentration variants significantly influenced the scaffold structure. An increase in the gelatin composition certainly resulted in a reduction in the HA composition. According to Landi et al. (2008), an increase in the HA composition is inversely proportional to an increase in porosity. This is because the water content in the slurry also had an impact. A larger HA composition resulted in a smaller amount of water in the slurry mixture and made the mixture highly dense and cohesive; consequently, when the water evaporated, the empty spaces left behind became smaller. Thus, the porosity in the scaffolds was formed by several important factors that could not be ignored.

Porosity testing was conducted using the liquid displacement method on 9 GEL-HA composite scaffold specimens. This aimed to observe the extent to which variations in the gelatin composition affected the percentage of empty space within the scaffold structure. Based on the data presented in Table 2 and Figure 5, the scaffold porosity percentages ranged from 61.90% to 62.84%. The three tested variants did not show significant differences. This was indicated by HAG-2 and HAG-4, which had almost identical values of 61.90% and 61.95%. The highest porosity value was achieved by the HAG-3 specimen with a value of 62.84% and a standard deviation of approximately 1.944. Conversely, the lowest porosity percentage was recorded in HAG-2, which decreased to 61.90% with a relatively large deviation value of approximately 2.261. Figure 5 shows that the HAG-2 specimen had a porosity value of 61.90%. This percentage value then experienced an increase until it reached its peak in the HAG-3 specimen, which had a value of 62.84%. However, this upward trend was not linear; in HAG-4, a decrease in the porosity value was recorded, dropping to 61.95%. The presence of a standard deviation indicates a range of variation in each repetition of the test specimen.

The increase in porosity from the HAG-2 to the HAG-3 specimen was suspected to be because, at a 3% concentration, gelatin functioned proportionally as a binder. This concentration was sufficient to build the structure without excessively increasing the slurry's viscosity, allowing the distilled water to be distributed evenly and leave maximum pore cavities upon evaporation in the oven. Conversely, the decrease in porosity that occurred in HAG-4 was suspected to be caused by the phenomenon of ethanol evaporating during the testing process. According to Mahanani et al. (2024), a good porosity percentage for bone-like scaffolds is in the range of 50 to 90%. Based on the research findings, these results have exceeded the minimum threshold for a good scaffold; thus, the generated porosity was classified as sufficient.

Table 2. Scaffold Porosity	
Specimen	Porosity (%)
HAG-2	61.90 ± 2.261
HAG-3	62.84 ± 1.944
HAG-4	61.95 ± 1.454

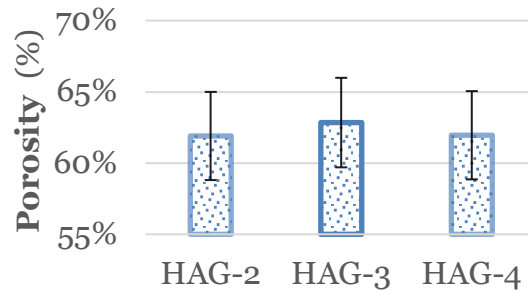


Figure 5. Porosity Chart of HAG-2 to HAG-4

CONCLUSION

Based on the research and testing results that have been conducted, it can be concluded that the utilization of blue swimming crab shell waste possesses immense potential and can serve as a superior natural calcium source in the synthesis of hydroxyapatite (HA) bioceramics. The use of this marine waste not only provides an environmentally friendly and economical solution but also proves to be highly effective as a scaffold material. Through the optimization of a microwave-based synthesis method combined with a calcination process at a temperature of 900°C, this study successfully produced a blue swimming crab shell-based hydroxyapatite phase with a purity level reaching 99.6%. In addition to this purity level, the success of the synthesis was also marked by a minimal amount of residual impurity phases, namely a calcite phase of 0.3% and a portlandite phase of 0.1%, where these impurity phases will not significantly interfere with the material's biocompatibility. Furthermore, alongside the synthesis purity results, this study also fabricated a composite of hydroxyapatite and gelatin into a three-dimensional structure (scaffold) through a slurry casting method, followed by solvent evaporation using an oven drying method. The analysis results of the porosity testing using the liquid displacement method indicated that the porosity of the 9 specimens across 3 variants exhibited a value range of 61.90% to 62.84%. In the porosity testing analysis results, no linear trend was observed, likely due to the phenomenon of ethanol evaporation while conducting the liquid displacement method. When compared to the journal by Mahanani, these porosity results are already classified as good for bone applications. As a recommendation for future research, it is suggested to refine the liquid displacement method so that the measurement results are more precise. Additionally, future studies could also explore other characterization tests.

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