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Density of Hydroxyapatite-Gelatin Composite Scaffolds Derived from Blue Swimming Crab Shells

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Abstract
Hydroxyapatite-gelatin composite scaffolds have potential applications in bone tissue engineering as they combine the bioactive properties of hydroxyapatite with the capability of gelatin as a polymer matrix. This study aims to analyze the density of hydroxyapatite-gelatin composite scaffolds derived from blue swimming crab shells at varying gelatin concentrations of 2%, 3%, and 4%. Hydroxyapatite was synthesized from blue swimming crab shell powder through a microwave-assisted precipitation method, followed by characterization using X-ray diffraction (XRD). The scaffolds were fabricated via the slurry casting method using a total mass of 4 g of the hydroxyapatite-gelatin mixture. Density testing was conducted on three specimens for each variant using the hydrostatic weighing method based on Archimedes' principle, with 96% ethanol as the immersion fluid. The XRD characterization results demonstrated that the synthesized powder was dominated by a hydroxyapatite phase at 99.6%, with minor phases of calcite at 0.3% and portlandite at 0.1%. The average densities of the scaffolds at gelatin concentrations of 2%, 3%, and 4% were 2.161 g/cm^3, 2.259 g/cm^3, and 2.321 g/cm^3, respectively. Descriptively, an increase in gelatin concentration within the tested range was followed by an increase in the average scaffold density. Further testing on porosity, morphology, mechanical properties, degradation, and biocompatibility is required to determine the more optimal scaffold composition.

Keywords: *blue swimming crab shells, density, gelatin, hydroxyapatite, scaffold*

INTRODUCTION

Bone tissue engineering is a regenerative approach that utilizes biomaterials to facilitate the repair of damaged bone tissue. A crucial component in this approach is the scaffold. Scaffolds function as temporary three-dimensional structures that support cell attachment, proliferation, and differentiation during the formation of new bone tissue, which then gradually degrade as the regeneration process progresses (Farjaminejad et al., 2024; Qu et al., 2019). To perform these functions, scaffolds must possess biocompatibility, biodegradability, and a porous structure that supports cell migration, nutrient transport, and the formation of blood vessels within the tissue (Farjaminejad et al., 2024; Lestari et al., 2026).

Hydroxyapatite is widely used as a scaffold material because it is a bioactive and biocompatible calcium phosphate material naturally found in bones and teeth (Alkaron et al., 2024; Fiume et al., 2021). However, hydroxyapatite exhibits a high degree of brittleness and is difficult to mold into required sizes and shapes, which limits its application as a standalone material (Alkaron et al., 2024; Amiryaghoubi & Esfahlan, 2024; Fiume et al., 2021). To overcome these limitations, hydroxyapatite can be combined with a natural polymer, such as gelatin, to form a composite material (Nawafi et al., 2022). In hydroxyapatite-gelatin composites, gelatin acts as the polymer matrix, while hydroxyapatite serves as the inorganic phase or filler material that imparts osteoconductive properties. The combination of these two materials also has the potential to support cell adhesion and growth, as well as improve material stability (Nawafi et al., 2022; Wu et al., 2024).

In addition to the material composition, the raw material source of hydroxyapatite also needs to be considered. Natural hydroxyapatite can be obtained from various biological sources, including animal bones, fish bones, eggshells, and the shells of marine organisms (Osuchukwu et al., 2021). Blue swimming crab shells are a fishery waste containing calcium carbonate ($CaCO_3$), thus having the potential to be utilized as a calcium source in the synthesis of hydroxyapatite (Fitriyana et al., 2024). The utilization of these blue swimming crab shells can serve as an alternative for using fishery waste as a raw material for biomaterials. Hydroxyapatite derived from *Portunus pelagicus* shells has also been developed as a scaffold material and reported to increase the expression of osteonectin and VEGF-B in animal trials, demonstrating its potential as an alternative material for bone regeneration (Ashrin et al., 2025). In hydroxyapatite-gelatin composites, differences in the composition of the two materials can affect the morphology and pore homogeneity of the fabricated scaffolds (Nawafi et al., 2022). The addition of gelatin has also been reported to influence the pore size and porosity of scaffolds (Wahda et al., 2024). Density is related to porosity and can affect the mechanical strength, permeability, and the presence of structural defects in scaffolds. Therefore, density was used in this study as a parameter to evaluate the structural compactness level of the scaffolds at each gelatin concentration variant (Lestari et al., 2026).

Previous studies have developed various types of hydroxyapatite-based scaffolds using different fabrication methods for defect repair and bone regeneration applications (Fendi et al., 2024). Nawafi et al. (2022) evaluated gelatin-hydroxyapatite composites through the characterization of the crystalline phase, morphology, pore size, and compressive strength. The results of the study indicated that differences in the composition of hydroxyapatite and gelatin affected the crystallinity, pore homogeneity, and compressive strength of the scaffolds. Aminatun et al. (2024) developed HA/PCL/gelatin nanofiber scaffolds and demonstrated that changes in gelatin composition influenced porosity as well as cell interactions with the scaffolds. Meanwhile, Wahda et al. (2024) developed hydroxyapatite/SiO_2/gelatin composites and evaluated their pore size, porosity, and compressive strength. In addition, Kadi et al. (2024) developed hydroxyapatite/PCL/gelatin scaffolds and evaluated their mechanical properties, bioactivity, and biological responses.

Nevertheless, studies regarding the density of gelatin-hydroxyapatite composite scaffolds utilizing hydroxyapatite synthesized from blue swimming crab shells remain limited in the reviewed literature. Therefore, this study was conducted to analyze the density of hydroxyapatite-gelatin composite scaffolds at gelatin concentrations of 2%, 3%, and 4%. The novelty of this research lies in the utilization of hydroxyapatite synthesized from blue swimming crab shells, the specific variations in gelatin concentrations used, and the measurement of density as a characterization parameter.

METHOD

The materials used included 100-mesh blue swimming crab shell powder as the calcium source, diammonium hydrogen phosphate ($(NH_4)_2HPO_4$) grade EMSURE® ACS, Reag. Ph Eur, for analysis as the phosphate source, gelatin as the polymer matrix, distilled water as the solvent and washing medium, and 96% ethanol as the immersion fluid for density testing.

The equipment used included a furnace for the calcination process of the crab shells, a sonicator to homogenize the mixture during hydroxyapatite synthesis, a microwave as a heating device to assist the synthesis process, a magnetic stirrer to dissolve the gelatin, an oven to dry the hydroxyapatite powder and scaffolds, silicone molds to form the specimens, and an analytical balance with a readability of 0.001 g to measure the mass of the materials and specimens. A porcelain crucible was used as a container during the calcination process, while ziplock plastics, airtight plastic containers (thinwall), and silica gel were used to keep the powder and scaffolds dry during storage.

The 100-mesh sieved blue swimming crab shell powder was placed in a porcelain crucible and then inserted into a furnace. The temperature was raised to 600°C and maintained for 1 hour, then continued until it reached 900°C and held for 5 hours. After the calcination process was completed, the powder was left inside the furnace until it reached room temperature. The calcined powder was then stored in a ziplock plastic placed inside an airtight plastic container along with silica gel before being used in the hydroxyapatite synthesis stage.

Hydroxyapatite was synthesized using a microwave-assisted precipitation method. The precipitation method has been used in the synthesis of hydroxyapatite from blue swimming crab shells because it involves a relatively simple process, has low synthesis costs, produces water as a byproduct, and carries a low risk of contamination (Fitriyana et al., 2024). The use of a microwave assisted by

ultrasonic mixing has also been applied in the synthesis of hydroxyapatite from blue swimming crab shells (Zuliana et al., 2025).

Diammonium hydrogen phosphate was used as the phosphate source, while the calcined powder was used as the calcium source. Both materials were homogenized in distilled water, and the mixture was then heated using a microwave. The synthesized product was subsequently washed, precipitated, filtered, and dried until a hydroxyapatite powder coded as HA-CR was obtained. The synthesized hydroxyapatite powder coded as HA-CR was characterized using X-ray diffraction (XRD) at the Physics Laboratory, Universitas Negeri Semarang. This test was conducted to identify the crystalline phases formed and to confirm the presence of hydroxyapatite in the synthesized powder. The obtained diffraction pattern was then compared with hydroxyapatite reference data to determine peak conformity and the possible presence of other phases.

The hydroxyapatite-gelatin scaffolds were fabricated using the slurry casting method. Each variant was prepared with a total mass of 4 g for the hydroxyapatite and gelatin mixture. The gelatin concentrations used were 2%, 3%, and 4%, while the hydroxyapatite mass was adjusted so that the total mixture mass for each variant remained constant. The gelatin was dissolved in 5 mL of distilled water at 60°C using a magnetic stirrer for 10 minutes. The HA-CR coded hydroxyapatite powder was then added to the gelatin solution and stirred for 3–4 minutes until a homogeneous slurry was formed. All variants used the same volume of distilled water. The mass of the gelatin and hydroxyapatite powder for each variant was determined based on the total mixture mass of 4 g, as shown in Table 1.

Table 1. Composition for hydroxyapatite-gelatin scaffold fabrication

Gelatin (%)	Number of test specimens	Gelatin (g)	HAp (g)
2	3	0,08	3,92
3	3	0,12	3,88
4	3	0,16	3,84

The slurry was subsequently poured into silicone molds, each with a diameter and height of 6 mm. The silicone molds used in the scaffold fabrication process are shown in Figure 1.

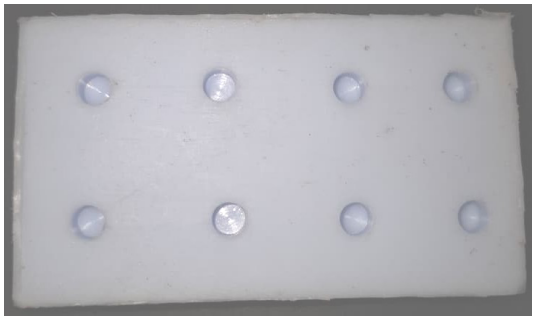

Figure 1. Scaffold molds

A single batch of the mixture produced approximately 15–20 scaffold pieces. The specimens were then dried in an oven at 40°C for 1 hour to ensure the initial shape of the scaffolds was sufficiently stable. Afterward, the specimens were removed from the molds and dried again in an oven at 40°C for 24 hours. The dried scaffolds were subsequently stored in ziplock plastics placed inside an airtight plastic container along with silica gel until the density testing stage.

Scaffold density testing was conducted using the hydrostatic weighing method based on Archimedes' principle, with 96% ethanol as the immersion fluid. This method determines density based on the difference in the specimen's mass when weighed in air and in the fluid (Alanazi et al., 2024). A total of three specimens from each gelatin concentration variant were used in the testing. Each specimen was first weighed in air using a balance with a readability of 0.001 g to obtain the mass in air (W_0). The specimens were then immersed in 96% ethanol in a separate container for 12 minutes. After the immersion process, the specimens were weighed while submerged in the ethanol to obtain the apparent mass of the specimen (W_3). The scaffold density value was calculated using an equation adapted from Diansari et al. (2026):

$$D_s = \frac{W_0}{W_0 - W_3} \times (\rho - d) + d$$

Where:
D_s : scaffold density (g/cm³)
W_0 : specimen mass in air (g)
W_3 : specimen mass in ethanol (g)
ρ : density of 96% ethanol
d : density of air

The density data from the three specimens for each gelatin concentration variant were processed using Microsoft Excel to obtain the average and standard deviation. The data processing results are presented in the form of tables and bar charts, with error bars indicating the standard deviation. The analysis was conducted descriptively by comparing the average values and the data spread across each variant.

RESULTS AND DISCUSSION

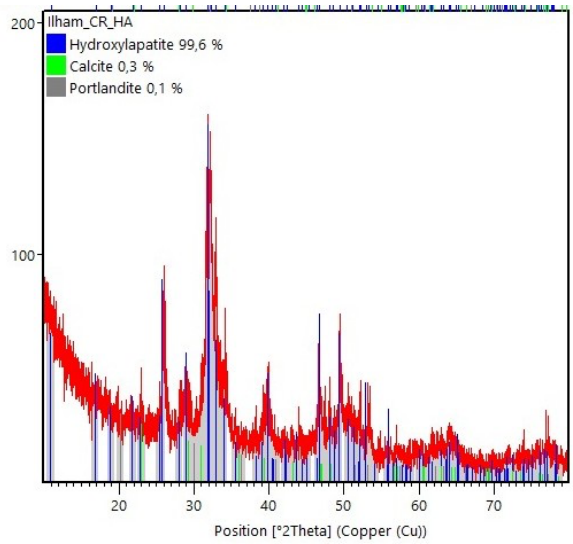

Figure 2. XRD of HA-CR powder

Figure 2 shows the XRD characterization results of the HA-CR powder synthesized from blue swimming crab shells. Based on the phase identification results, the HA-CR powder was dominated by a hydroxyapatite phase at 99.6%. Besides hydroxyapatite, a calcite phase of 0.3% and a portlandite phase of 0.1% were still detected. The diffractogram displayed several distinct diffraction peaks, indicating the formation of a material with a crystalline structure. The significantly higher percentage of hydroxyapatite compared to the other two phases indicates that hydroxyapatite was successfully formed as the primary phase. Meanwhile, the presence of calcite and portlandite in minute amounts indicates that the synthesized powder did not entirely consist of a single phase, but rather these two phases were merely minor phases.

Numerically, the percentage of the hydroxyapatite phase obtained in this study was higher compared to the findings of Zuliana et al. (2025). That study reported that synthesizing hydroxyapatite from blue swimming crab shells using a microwave method with ultrasonic mixing for 15 minutes produced a hydroxyapatite percentage of 74.5% and a crystallite size of 20.25 nm. This difference in results is suspected to be related to differences in synthesis conditions, the mixing process, microwave heating power and duration, washing, drying, and the characteristics of the raw materials used. However, this comparison cannot be directly used to state that one synthesis method is superior, as the process conditions and analytical methods in the two studies were not entirely identical. After hydroxyapatite was identified as the primary phase in the HA-CR powder, the powder was utilized as the constituent material for the gelatin-hydroxyapatite composite scaffolds with gelatin concentrations of 2%, 3%, and 4%. An example of the generated scaffolds is shown in Figure 3.

Figure 3. Scaffolds

The generated hydroxyapatite-gelatin composite scaffolds were successfully formed into solid cylindrical specimens following the silicone molds used. After being removed from the molds and undergoing the drying process, the specimens were able to maintain their basic shape and did not collapse. Thus, the fabrication process can be declared successful at the physical scaffold formation stage. Nevertheless, some specimens exhibited surfaces that were not completely even. After the fabrication and drying processes were completed, the scaffolds were subsequently tested to determine the density value for each gelatin concentration variation of 2%, 3%, and 4%. The testing was conducted on three specimens for each variation. The density values of these three specimens were then averaged. The average density testing results of the hydroxyapatite-gelatin scaffolds are presented in Table 2.

Table 2. Scaffold Density and Standard Deviation

Gelatin Concentration (%)	Average *Scaffold* Density (g/cm³)	Standard Deviation (g/cm³)
2	2,161	0,104
3	2,259	0,115
4	2,321	0,114

Note: Values were obtained from three specimens for each variation (n = 3).

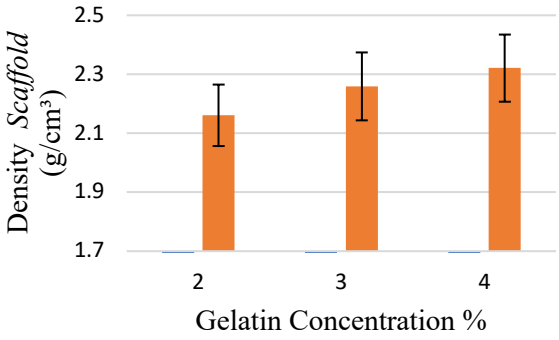

Figure 4. Scaffold density

Based on Table 2 and Figure 4, the average density of the scaffolds at gelatin concentrations of 2%, 3%, and 4% ranged from 2.161 to 2.321 g/cm³. The lowest density value was obtained at a 2% gelatin concentration, which was 2.161 g/cm³. The density increased to 2.259 g/cm³ at a 3% gelatin concentration and reached the highest value of 2.321 g/cm³ at a 4% gelatin concentration. Thus, within the tested concentration range, an increase in gelatin concentration was followed by an increase in the average scaffold density. The standard deviations at gelatin concentrations of 2%, 3%, and 4% were 0.104 g/cm³, 0.115 g/cm³, and 0.114 g/cm³, respectively. The 2% gelatin concentration had the smallest standard deviation, indicating a relatively lower spread of density values among the specimens. Meanwhile, the 3% gelatin concentration had the largest standard deviation, although its value did not differ significantly from that of the 4% gelatin concentration.

For comparison, Saputra et al. (2026) reported that a commercial bone graft product had a density of 1.58 g/cm³, while a gelatin-hydroxyapatite composite with a composition of 5% gelatin and 95% hydroxyapatite fabricated using the solvent casting method had a density of 1.44 g/cm³. The

scaffold density values in this study, which were 2.161 g/cm³ at 2% gelatin, 2.259 g/cm³ at 3% gelatin, and 2.321 g/cm³ at 4% gelatin, were all higher compared to the density value of the commercial product. The difference in density values is likely related to variations in the hydroxyapatite source, material composition, fabrication method, pore structure, and the density measurement method employed. Therefore, the higher density values in this study do not directly indicate that the generated scaffolds possess better quality than the commercial product.

The change in density values indicates an increase in the material's compactness level within the tested gelatin concentration range. In porous materials, the presence of open pores can decrease the apparent density value of the material (Mohammadi et al., 2020). Changes in material composition and the addition of hydroxyapatite into the polymer network can also affect scaffold porosity (Tomic et al., 2021). Therefore, the change in the ratio of hydroxyapatite to gelatin in this study is suspected to have also influenced the compactness of the formed scaffolds. However, this assumption cannot be directly confirmed as porosity testing and morphological observation using SEM were not conducted.

A different density range was reported by Diansari et al. (2026) on bovine hydroxyapatite-gelatin scaffolds, reporting a density value of 0.30–0.36 g/cm³. These values are lower compared to the density range in this study, which is 2.161–2.321 g/cm³. These value differences are suspected to be related to differences in the hydroxyapatite source, material composition, fabrication method, and internal scaffold structure. The completely uneven surfaces of some specimens are suspected to be associated with variations during the fabrication process. In scaffold fabrication, controlling the shape, porosity, density, pore size, pore distribution, and pore interconnectivity is a crucial aspect, while some conventional methods have limitations in producing structures that can be reproduced consistently (Mirmusavi et al., 2026). In this study, the manual mixing of the slurry and pouring into the molds, the presence of entrapped air, and shrinkage during the drying process are suspected to have contributed to the differences in surface evenness and compactness among the specimens. The 4% gelatin concentration produced the highest density value, but it cannot yet be declared as the most optimal scaffold composition. Determining the optimal composition cannot be sufficiently based solely on density values but also requires testing for porosity, morphology, mechanical strength, degradation, and biocompatibility. Therefore, the findings of this study are limited to the characterization of scaffold density at gelatin concentrations of 2%, 3%, and 4%.

CONCLUSION

The XRD characterization results demonstrated that the HA-CR powder synthesized from blue swimming crab shells was dominated by a hydroxyapatite phase at 99.6%, with minor phases of calcite at 0.3% and portlandite at 0.1%. The average scaffold densities at gelatin concentrations of 2%, 3%, and 4% were 2.161, 2.259, and 2.321 g/cm³, respectively. Descriptively, within the tested concentration range, an increase in gelatin concentration was followed by an increase in the average scaffold density. All of these density values were higher than the commercial bone graft density of 1.58 g/cm³ reported by Saputra et al. (2026). The 4% gelatin concentration produced the highest density value, but it cannot yet be declared as the most optimal composition because this study only evaluated the density parameter. Future studies need to combine density testing with analyses of porosity, morphology, mechanical strength, degradation, and biocompatibility to obtain a more comprehensive scaffold characterization.

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