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In Vitro Degradation Rate of Gelatin/Hydroxyapatite Composite Scaffolds Derived from Blue Swimming Crab Shells

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Abstract
Bone tissue damage is a serious health problem that requires bone graft treatments. Blue swimming crab shell waste has proven effective for utilization as a hydroxyapatite (HA) source due to its high calcium content. However, HA possesses a drawback in the form of brittle mechanical properties, making the addition of an organic polymer matrix such as gelatin necessary. This study aims to analyze the effect of varying gelatin and HA compositions (6:94, 7:93, and 8:92 wt%) on the in vitro degradation rate of HA-Gel scaffolds. HA was calcined and synthesized using a microwave-assisted precipitation method, and subsequently characterized using X-Ray Diffraction (XRD). The scaffolds were fabricated using the slurry casting method and then dried using the oven drying method. The in vitro degradation rate testing was conducted in a Phosphate Buffered Saline (PBS) solution at a temperature of 37°C for 1, 2, 3, 6, 12, 18, and 24 hours. The XRD results validated that blue swimming crab shells are effective in producing HA with a phase purity level reaching 99.1%. The fabrication of HA-Gel scaffolds using the slurry casting method successfully formed uniformly shaped scaffolds, although surface defects in the form of voids were present. The in vitro degradation rate exhibited a residual mass of 100–101.8% across all variants, indicating that degradation had not yet occurred; instead, swelling by the gelatin matrix took place. The variant with the highest HA content (94%) demonstrated the largest and most fluctuating mass increase, while the variant with the highest gelatin content (8%) was the most stable, indicating good mass resistance of the HA-Gel scaffolds during the initial immersion phase.

Keywords: blue swimming crab shells, gelatin, hydroxyapatite, in vitro degradation, scaffold

INTRODUCTION

Bone tissue damage is one of the most common and serious problems in human health, where bone transplantation remains the most effective method for repairing damaged bone tissue (Wei et al., 2023). Globally, it is estimated that more than two million bone transplantation procedures are performed annually (Wei et al., 2023). In Indonesia, the incidence of bone fractures reaches 1.3 million annually, making it the country with the highest bone fracture incidence rate in Southeast Asia (Kemenkes RI, 2025). In the treatment of bone damage, there are various types of commonly used bone graft substitute materials, such as autografts, allografts, xenografts, and synthetic or alloplastic materials (Fatima et al., 2023). Synthetic bone grafts have become a popular choice due to their availability, minimal risk of disease transmission, and lower morbidity rates compared to autograft or allograft procedures (Archunan & Petronis, 2021). Scaffolds play a crucial role in bone healing and regeneration by providing structural integrity, mechanical support, and a conducive environment (Todd et al., 2024). Factors influencing the characteristics of an ideal scaffold include porous morphology, biocompatibility, controlled biodegradability, and mechanical strength (Zhou et al., 2024). During the grafting process, the scaffold functions as a microenvironment for cells to facilitate new bone formation (Mahanani, 2022).

Blue swimming crabs are an export commodity in the fishery sector whose byproducts contribute to a massive amount of shell waste (Husni et al., 2020). These blue swimming crab shells are known to contain high minerals, primarily calcium (Yanuar, 2013). Blue swimming crab shell waste possesses an extremely high calcium carbonate content, reaching 93.78%, thus demonstrating great potential to be extracted as a local raw material for hydroxyapatite fabrication (Zuliana et al., 2025). The chemical characteristics contained in blue swimming crab shell waste are highly ideal for conversion into bone implant bioceramics (Romadhona et al., 2023). In its processing, the chemical synthesis of hydroxyapatite is considered more effective as it offers much better control over the structure and final properties of the material, with one efficient technique being the microwave-assisted precipitation method (DileepKumar et al., 2022). Furthermore, the fabrication process of biocomposite scaffolds can be conducted using the solvent casting technique, which involves dissolving gelatin in distilled water as a solvent and mixing it with hydroxyapatite powder before pouring it into a mold (Saputra et al., 2026).

The primary approach in fabricating synthetic scaffolds relies on hydroxyapatite due to its biocompatibility, osteoconductive properties, and its chemical composition ($Ca_{10}(PO_4)_6(OH)_2$), which is highly similar to natural bone minerals (Wen-Wen & Jiang-Ying, 2018). This material is highly effective in overcoming bone damage and enhancing regeneration through its three-dimensional structure (Fendi et al., 2024; Obada et al., 2020). However, HA possesses drawbacks in its mechanical strength, being brittle and prone to fracture due to its hygroscopic nature (Noviyanti et al., 2023). Therefore, HA needs to be combined with a polymer matrix, such as gelatin, which is capable of increasing cell attachment, improving mechanical strength, and facilitating the controlled release of bioactive molecules (Boran, 2013; Tomić et al., 2021).

A highly crucial parameter in scaffold evaluation is the *in vitro* degradation rate, as this parameter directly influences cellular interactions and the body's tissue regeneration response, where an appropriate degradation rate must align with the timing of new bone formation (Ghosh Dastidar et al., 2023). Therefore, this study was conducted to validate the phase purity of blue swimming crab shell-based hydroxyapatite through XRD analysis, observe the physical shape of the HA-Gel composite scaffolds, determine the *in vitro* degradation rate values, and analyze the effect of varying gelatin and blue swimming crab shell-based hydroxyapatite compositions on the *in vitro* degradation rate of the HA-Gel scaffolds.

METHOD

The primary materials used in this study included blue swimming crab shells obtained from Cirebon Regency, diammonium hydrogen phosphate ($(NH_4)_2HPO_4$) (Merck), gelatin, distilled water, and PBS (Phosphate Buffered Saline) solution. The blue swimming crab shells were ground using a disc mill and sieved using an 80-mesh sieve to reduce the particle size of the crab shell powder. The uniformly sized blue swimming crab shell powder was then calcined using a muffle furnace at a temperature of 900°C for 5 hours (Chandra Muhamadin et al., 2023) to obtain calcium elements from the decomposition of calcium carbonate ($CaCO_3$) into calcium oxide (CaO) (Wati et al., 2025).

The synthesis process of hydroxyapatite (HA) was carried out using the microwave-assisted precipitation method. A total of 52 wt% $(NH_4)_2HPO_4$ was dissolved in 20 mL of distilled water using a sonicator at a power of 600 W for 10 minutes, while 48 wt% of the calcined blue swimming crab shell powder was dissolved in 20 mL of distilled water for 20 minutes. The phosphate solution was then gradually mixed into the calcined crab shell solution. The homogeneous solution was subsequently processed in a microwave at a power of 450 Watts for 3 minutes (Chandra Muhamadin et al., 2023). The precipitate was washed, soaked, filtered, and then dried in an oven at a temperature of 110°C for 2 hours (Chandra Muhamadin et al., 2023). The generated HA powder was then characterized using X-Ray Diffraction (XRD) to confirm the formation of the HA phase.

The fabrication of the HA-Gel scaffolds was conducted using the slurry casting method by varying the weight ratio (wt%) of gelatin to the blue swimming crab shell HA. The mixed mass formulation was set at 4 grams, as presented in detail in Table 1. Gelatin was dissolved with 5 mL of distilled water in a beaker, then placed on a magnetic stirrer at 60°C for 10 minutes until it formed a solution. After the gelatin was completely dissolved, the gelatin solution was poured into a mortar previously filled with blue swimming crab shell HA and subsequently ground until homogeneous. Cylindrical molds with a diameter of 6 mm and a height of 6 mm were used to mold the mixture of gelatin and blue swimming crab shell HA, which was then left at room temperature for approximately

30 minutes. The scaffolds were removed from the molds and then dried in an oven at 40°C for 24 hours (Saputra et al., 2026). The fabrication results of the HA-Gel scaffolds are shown in Figure 1.

Table 1. Gelatin:HA composition ratio

Sample Code	Gelatin:HA Composition Ratio (wt%)	Gelatin (g)	HA (g)
G6-HA94	6:94	0.24	3.76
G7-HA93	7:93	0.28	3.72
G8-HA92	8:92	0.32	3.68

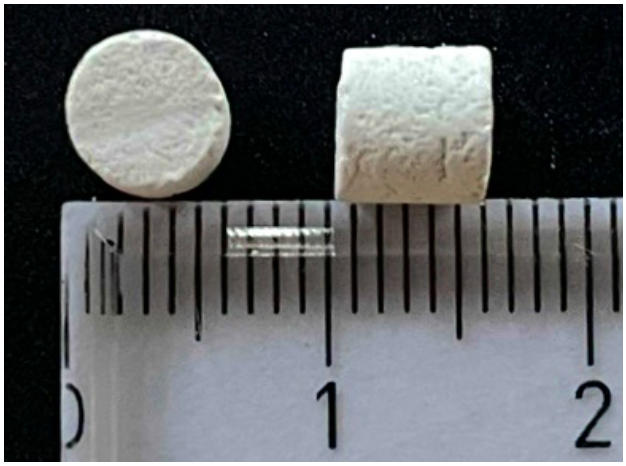

Figure 1. Dimensions of the HA-Gel scaffold samples

The *in vitro* degradation rate testing was conducted to evaluate physical stability by immersing each scaffold sample, with a known initial mass (W_0), into a container filled with 0.2 mL of Phosphate Buffered Saline (PBS) solution at a constant physiological pH of 7.4, as shown in Figure 2. All samples were incubated in an oven at a constant temperature of 37 ± 2°C, in order to simulate the biological environment of the human body. The degradation rate evaluation was performed by sampling periodically at time intervals of 1, 2, 3, 6, 12, 18, and 24 hours, with three replications conducted for each variant. The samples were rinsed with distilled water to stop the degradation process and dried in an oven at 37°C for 24 hours prior to the final weighing (W_t). The degradation rate can be determined using the residual mass percentage, calculated using the following equation:

$$ResidualMass(\%) = \frac{W_t}{W_0} \times 100$$

Where:
W_0 = Initial dry weight of the scaffold sample before immersion (g)
W_t = Final weight of the scaffold sample after drying (g)

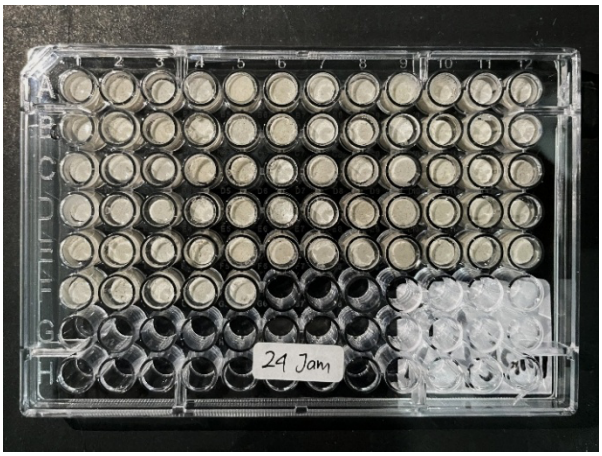

Figure 2. Immersion of HA-Gel scaffold samples

RESULTS AND DISCUSSION

X-Ray Diffraction (XRD) analysis was conducted to validate the success rate of hydroxyapatite synthesis from blue swimming crab shell waste raw materials (Romadhona et al., 2023). The XRD results were analyzed using HighScore Plus software, which demonstrated a formed hydroxyapatite crystallinity percentage of 99.1%. The portlandite crystalline phase formed at 0.3% and the calcite crystalline phase formed at 0.6%, as shown in Figure 3. The microwave-assisted precipitation method proved effective in producing a pure hydroxyapatite crystalline phase with a high percentage. These results indicate that the thermal decomposition and subsequent reactions successfully removed carbonate impurities optimally (Romadhona et al., 2023). This testing confirms that the HA powder synthesized from blue swimming crab shells possesses high phase viability and meets the standards as a base material for biomedical applications.

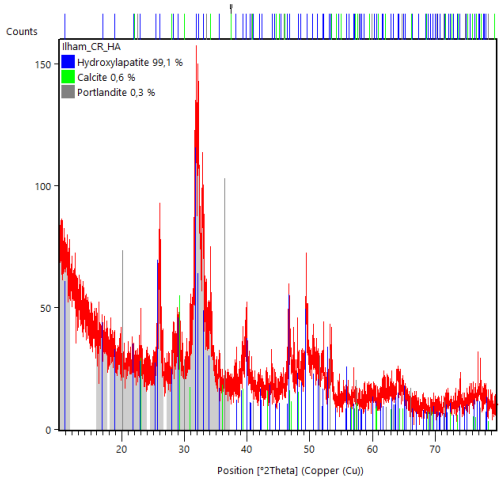

Figure 3. Diffraction pattern of blue swimming crab shell HA

Based on the fabrication process using the slurry casting method, the HA-Gel composite scaffolds were successfully formed according to the dimensions of the silicone molds. Physically, the generated HA-Gel scaffolds exhibited a cylindrical physical shape that was generally appropriate and uniform. Nevertheless, closer observation indicated the presence of some physical defects, such as small cavities or voids, in certain parts of the HA-Gel scaffolds, as shown in Figure 4. The successfully formed HA-Gel scaffolds demonstrated the success of the slurry casting method in the fabrication process of the HA-Gel composite scaffolds. This is because the homogeneous mixture of the gelatin and blue swimming crab shell HA solution was formed following the dimensions of the silicone mold. The formation of physical defects in the form of cracks in some parts of the specimens was generally influenced by an imperfect hand-mixing process and the evaporation of the water solvent trapped within the gelatin matrix during oven drying. The presence of these defects in the form of voids is a crucial factor affecting the internal pore distribution and the mechanical characteristics of the scaffolds (K. R. Razali, 2014).

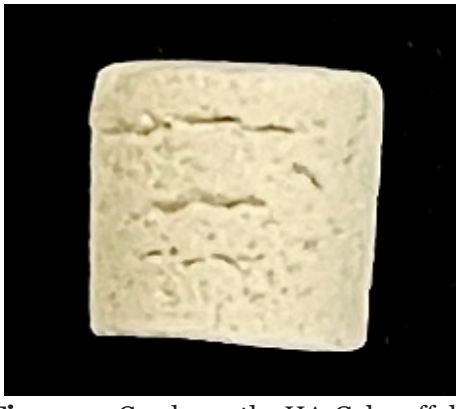

Figure 4. Cracks on the HA-Gel scaffold

The *in vitro* degradation rate was observed to evaluate the mass stability of the composite scaffolds when interacting with a physiological PBS solution (Ghosh Dastidar et al., 2023). Based on the test data presented in Table 2 and Figure 5, the degradation rate for the compositional variations of 6% gelatin (G6-HA94), 7% gelatin (G7-HA93), and 8% gelatin (G8-HA92) yielded a residual mass profile ranging from 100% to 101.8% over a 24-hour immersion period. The increase in residual mass (100–101.8%) across all HA-Gel scaffold variants during the first 24 hours of immersion in PBS indicates that the dominant process at this stage was water absorption (swelling) by the gelatin matrix, rather than degradation. Gelatin is hydrophilic—its amine, carboxyl, and hydroxyl groups form hydrogen bonds with water, thereby drawing water into the scaffold structure (Petros et al., 2020).

When compared across variants, a fairly clear pattern emerged: G6-HA94, which possessed the lowest gelatin ratio, exhibited the largest and most fluctuating mass increase, whereas G8-HA92, with the highest gelatin ratio, showed the smallest and most stable mass increase, with G7-HA93 falling in between. The stratified graph among the variants (G6-HA94 being the highest and most fluctuating, G8-HA92 being the lowest and flattest) is related to the ratio of HA to gelatin. In HA-gelatin systems, a higher HA ratio has been proven to decrease the swelling ratio and biodegradation rate, as well as reduce the role of gelatin (Ghorbani et al., 2016), while a higher gelatin concentration drastically affects the pore size, porosity, and swelling of the scaffold (Jalili AhmadAbad et al., 2018). In the slurry casting method utilizing gelatin as a gelling agent, good dispersion of ceramic particles in the slurry prior to the addition of gelatin is a crucial prerequisite for producing a dense and homogeneous microstructure of the ceramic walls and struts around the pores (Lombardi et al., 2009). The voids formed created a non-uniform pore structure, as seen in Figure 4, which is suspected to have caused inconsistent water inflow and outflow rates during the measurement period for G6-HA94, resulting in a sharply fluctuating residual mass curve. Conversely, the higher gelatin ratio in G8-HA92 allowed for a more even gelatin dispersion and a more homogeneous scaffold structure, resulting in a stable and flat water absorption pattern. Another suspected reason why a mass increase occurred after testing is that, prior to drying, the samples were rinsed twice with distilled water for 1 minute to remove the residual PBS solution, following a common practice in other scaffold degradation studies that rinse samples specifically to remove deposited minerals from PBS before mass measurement (Bazgir et al., 2021). Thus, the contribution of salt residues to the mass increase has likely been minimized, although in a less homogeneous porous structure like G6-HA94, it cannot be completely ruled out as a minor additional factor. Swelling by the gelatin matrix remains the primary and more dominant explanation for the observed mass increase.

When compared to a commercial bone graft product that experienced 34.67% degradation on day 7 (Saputra et al., 2026), the blue swimming crab shell HA-Gel scaffolds demonstrated better structural and mass endurance during the initial phase of *in vitro* degradation. A comparison can also be made with a similar HAp/Gel scaffold, which actually exhibited rapid wet mass degradation (25.1% residual mass after 24 hours) (Tsai et al., 2025). This difference is suspected to be caused by the more dominant role of gelatin as the main structural matrix in that study, which differs from this study that utilized gelatin only as a minor binder (6–8%) within the dominant inert HA matrix (92–94%), as well as a wet weighing protocol without re-drying that also influenced the results. Among the three variations, the G8-HA92 sample exhibited the flattest stability profile, approaching the 100% line, while the G6-HA94 and G7-HA93 variations displayed slightly more dynamic water absorption fluctuations. This confirms that within the 6–8% gelatin composition range, the dominating process during the first 24 hours is water absorption, not structural degradation. HA-Gel scaffolds tend to maintain mass stability in the initial phase before new bone tissue begins to regenerate. Thus, these results represent the initial phase of water absorption equilibrium, not an indication of degradation failure, and further testing with a longer duration is recommended to compare with commercial scaffold products and to observe the reduction phase in the *in vitro* degradation rate test.

Table 2. *In vitro* degradation rate of the scaffolds

Time	G6-HA94	G7-HA93	G8-HA92
1 hour	101.62%	101.24%	100.29%
2 hours	101.25%	100%	100.75%
3 hours	100.76%	100.27%	100.79%
6 hours	101.02%	100.77%	100.28%
12 hours	100.47%	100.52%	100%
18 hours	100.75%	100.57%	100%
24 hours	101.69%	101.24%	100.50%

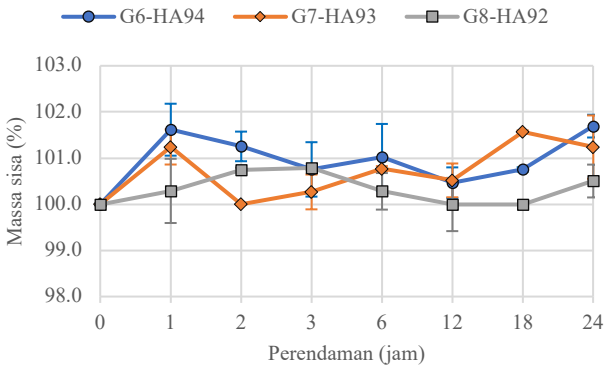

Figure 5. *In vitro* degradation rate

CONCLUSION

Blue swimming crab shell waste proved to be an effective and potential precursor for hydroxyapatite fabrication, where the XRD test validated a high HA phase purity level reaching 99.1%. The HA-Gel scaffold fabrication process using the slurry casting method successfully formed scaffolds with a uniform physical shape at 6%, 7%, and 8% gelatin variations, although defects in the form of cracks were still present in the HA-Gel scaffolds. The *in vitro* degradation rate testing results demonstrated that the three HA-Gel scaffold variants (G6-HA94, G7-HA93, G8-HA92) had not yet undergone degradation (mass reduction) during the 1–24 hour immersion period in PBS; instead, they exhibited an increase in residual mass in the range of 100–101.8% due to the water absorption (swelling) process by the hydrophilic gelatin matrix. The ratio of HA to gelatin was proven to influence the magnitude and consistency of this mass increase: G6-HA94, with the highest HA content and lowest gelatin, showed the largest mass increase and the most fluctuating pattern, suspected to be due to less homogeneous HA dispersion in the slurry, resulting in a non-uniform pore structure. Conversely, G8-HA92, with the highest gelatin proportion, exhibited the most stable and flat mass increase pattern, indicating a more homogeneous matrix structure. Overall, these results indicate that the three HA-Gel scaffold variants possess good mass endurance during the initial immersion phase; however, the 24-hour testing time window was insufficient to observe the final mass reduction phase, which is the true indicator of degradation. Further testing with a longer duration, alongside the characterization of porosity and pore morphology for each variant, is recommended to confirm the long-term degradation pattern and the underlying mechanisms of the different swelling behaviors during the initial phase of the *in vitro* degradation rate among the variants.

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