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# Hardness of Hydroxyapatite-Based Scaffolds Derived from Green Mussel Shells at Various Gelatin Concentrations

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**Abstract**
Large-sized bone defects require scaffolds capable of supporting tissue regeneration while possessing adequate mechanical characteristics. This study aims to analyze the effect of varying gelatin compositions on the hardness characteristics of hydroxyapatite-based scaffolds synthesized from green mussel shells (*Perna viridis*). Hydroxyapatite was synthesized using calcium precursors derived from calcined green mussel shells and subsequently characterized using X-Ray Diffraction (XRD). The scaffolds were fabricated using the slurry casting method with gelatin composition variations of 2%, 3%, and 4%, alongside hydroxyapatite compositions of 98%, 97%, and 96%, respectively. The mechanical characteristics of the scaffolds were tested using a Copley TBF 1000 Tablet Hardness Tester to obtain the breaking force values. The XRD results demonstrated that the synthesized material was dominated by a hydroxyapatite phase with a weight percentage of 98.7%, while the calcite and portlandite phases were 1.0% and 0.3%, respectively. The testing results indicated that the commercial scaffold exhibited the highest breaking force value of 3.51 kg. For the green mussel shell scaffolds, the highest value was obtained at the 2% gelatin variation at 1.37 kg, followed by 4% gelatin at 0.96 kg, and 3% gelatin at 0.69 kg. After conversion using the ASTM C496 equation, the mechanical value of the commercial scaffold was 0.609 MPa, whereas the 2%, 3%, and 4% gelatin variations were 0.238 MPa, 0.120 MPa, and 0.130 MPa, respectively. All of these values remained below the mechanical requirement of ≥1.5 MPa. Thus, the variation in gelatin composition influenced the mechanical characteristics of the scaffolds in a non-linear manner. The 2% gelatin composition produced the best mechanical characteristics compared to the other variations; however, it still requires further development to meet the mechanical requirements for bone graft materials.

**Keywords:** gelatin, green mussel shells, hardness, scaffold, slurry casting

## INTRODUCTION

Bone is a hard, mineralized connective tissue that plays a role in providing structural support for the body, protecting vital organs, and providing attachment sites for muscles (Cowan et al., 2024). Bone is also a metabolically active tissue with the ability to undergo continuous remodeling, repair, and regeneration throughout life (Ma et al., 2023). Despite its natural regenerative capacity, the bone healing process can be impaired in large-sized defects, particularly due to severe trauma, infection, tumor resection, vascularization disorders, or extensive bone tissue loss. Large bone defects are often incapable of undergoing optimal spontaneous healing, thus requiring reconstruction strategies or supplementary biomaterials to support the regeneration process (Migliorini et al., 2021). This issue has widespread clinical implications. The Global Burden of Disease Study 2019 reported approximately 178 million new fracture cases worldwide in 2019, an increase of 33.4% compared to 1990 (GBD 2019 Fracture Collaborators, 2021). This condition indicates that the development of materials to support bone tissue regeneration remains a crucial field of research. Despite possessing favorable biological characteristics, the use of hydroxyapatite as a standalone material still faces limitations, as HAp is relatively brittle and exhibits low mechanical toughness (Ratnayake et al., 2017).

This mechanical limitation becomes increasingly critical in porous scaffolds because an increase in porosity is generally followed by a decrease in mechanical strength, even though an interconnected pore structure is required to support cell infiltration and nutrient transport into the inner part of the scaffold (Deville et al., 2006). This condition prompts the development of HAp-based scaffolds frequently to be carried out through the formation of composite materials with natural or synthetic polymers to achieve a balance between biological and mechanical properties. Therefore, the selection of constituent materials and their composition becomes a vital aspect, as changes in the ratio between the ceramic and polymer phases can alter structural compactness, porosity, interphase interactions, degradation, and the scaffold's load-bearing capacity.

An alternative calcium source with the potential to be used in hydroxyapatite synthesis is green mussel shell (*Perna viridis*) waste. The utilization of mussel shells possesses added value because it can reduce seafood processing waste while simultaneously providing a calcium precursor that is relatively inexpensive and available in large quantities (Naubnome et al., 2025). Ismail et al. (2022) demonstrated that calcium carbonate obtained from green mussel shells possesses a chemical composition approaching that of commercial calcium carbonate and has the potential to be used as a raw biomaterial. Charlena et al. (2022) also successfully synthesized hydroxyapatite from green mussel shells via the sol-gel method, which exhibited the formation of a hydroxyapatite phase under alkaline conditions. A more recent study by Irfa'i et al. (2024) showed that calcium carbonate precursors from green mussel shell waste could be hydrothermally converted into nanocrystalline carbonated hydroxyapatite, with a hydroxyapatite content exceeding 90 wt.% under specific conditions. Ikaputri et al. (2024) also obtained HAp from green mussel shells using the sol-gel method with a Ca/P ratio of approximately 1.68, which is close to the stoichiometric ratio of hydroxyapatite at 1.67. These findings reinforce the potential of green mussel shells as a local calcium source for the development of hydroxyapatite-based biomaterials.

To improve the characteristics of HAp scaffolds, gelatin can be used as the polymer phase in composite formation. Gelatin is a product of collagen hydrolysis that possesses excellent biocompatibility and biodegradability, making it widely used as a biomaterial in tissue engineering (Mohanto et al., 2023). Gelatin also has functional groups, primarily carboxyl and amide groups, which enable interactions with the inorganic phase of hydroxyapatite; the interaction between $Ca^{2+}$ ions in HAp and the carboxyl groups of gelatin has been reported to play a role in the formation of gelatin-HAp composites (Bartmański et al., 2022). However, the proportion of gelatin used must be controlled because changes in the polymer composition can affect the arrangement of HAp particles, pore structure, crystallinity, water absorption, degradation rate, and mechanical characteristics of the scaffold (Diansari et al., 2026). A recent review by El-Bahrawy et al. (2025) indicated that gelatin-HAp composites hold promising prospects in bone tissue engineering due to their biocompatibility, biodegradability, and osteoconductivity, but the optimization of mechanical properties remains one of the primary aspects requiring development.

The relationship between gelatin-HAp composition and the mechanical characteristics of scaffolds has been reported in several studies. Nawafi et al. (2022), for instance, found that variations in gelatin and hydroxyapatite composition affected the pore morphology and compressive strength of the scaffolds. At a gelatin:HAp composition of 1:2, an average pore size of 225.12 ± 16.57 μm and a compressive strength of 18.1 ± 0.61 MPa were obtained. A study by Aminatun et al. (2024) on HAp/PCL/gelatin nanofiber scaffolds also demonstrated that changes in material composition resulted in differences in mechanical properties, porosity, and degradation characteristics. Further recent findings by Diansari et al. (2026) indicated that changing the ratio of HAp to gelatin could lead to differences in the crystallinity, porosity, density, degradation, and compressive strength of the scaffolds. These results demonstrate that gelatin composition is a key parameter that can determine the mechanical response of HAp composite materials.

Most studies on gelatin-HAp composites still focus on evaluating compressive strength, tensile strength, elastic modulus, porosity, and degradation, while studies regarding the effect of gelatin composition on the hardness of green mussel shell-based HAp scaffolds remain limited. Hardness is important to indicate a material's ability to withstand local deformation, whereas changes in the gelatin fraction can affect the bonding between HAp particles, structural compactness, and the balance of organic and inorganic phases. Therefore, this study aims to analyze the effect of gelatin concentration on the hardness of hydroxyapatite scaffolds synthesized from green mussel shells (*Perna viridis*) and to determine the composition that produces the most suitable hardness characteristics. The results of this study are expected to clarify the relationship between polymer

composition and the mechanical properties of scaffolds, while simultaneously supporting the utilization of green mussel shell waste as a value-added biomaterial.

## METHOD

The primary material used in this study was green mussel shells (*Perna viridis*) in their intact condition. The shells were first cleaned of soft tissues, mud, sand, and other impurities using distilled water until completely clean. Subsequently, the shells were dried until they contained no moisture. The dried shells were then crushed into small pieces and ground using a grinder for 5 minutes until they became a powder. The shell powder was subsequently sieved using a 100-mesh sieve to obtain a more uniform particle size. The sieved powder was stored in a sealed container before being used in the calcination stage. The preparation stages of the green mussel shells are shown in Figure 1.

**Figure 1.** Preparation of green mussel shells

The green mussel shell (*Perna viridis*) powder that passed the 100-mesh sieve was placed into a porcelain container. Next, as shown in Figure 2, a calcination process was conducted using a furnace at 900°C with a holding time of 5 hours. This calcination process was performed to remove organic compounds and impurities, as well as to decompose calcium carbonate ($CaCO_3$) into calcium oxide (CaO) through a thermal decomposition process (Ismail et al., 2022). After the calcination process lasted for 5 hours, the furnace was turned off without opening the heating chamber, and the samples were allowed to cool inside the furnace until they reached room temperature. Ismail et al. (2022) explained that during the cooling process, the formed CaO could react with water vapor in the air, thus undergoing hydration and forming calcium hydroxide or portlandite ($Ca(OH)_2$). The calcined powder was subsequently removed once the furnace reached room temperature. Next, the powder was sieved using a 100-mesh sieve to ensure a more uniform particle size. The powder that passed the sieve was then stored in a sealed container prior to being used in the hydroxyapatite synthesis stage.

**Figure 2.** Calcination process of green mussel shell powder

Hydroxyapatite synthesis was conducted using calcium hydroxide [$Ca(OH)_2$] derived from the calcined green mussel shells as the calcium source and diammonium hydrogen phosphate [$(NH_4)_2HPO_4$] as the phosphate source. A total of 3.16 grams of $(NH_4)_2HPO_4$ was dissolved in 20 mL

of distilled water, then subjected to ultrasonic treatment at a power of 600 Watts for 10 minutes. Separately, 2.94 grams of $Ca(OH)_2$ was mixed with 20 mL of distilled water and subjected to ultrasonic treatment at the same power for 20 minutes. During the mixing process, the $(NH_4)_2HPO_4$ solution was gradually added to the $Ca(OH)_2$ suspension until the mixture became homogeneous. This composition was used to obtain a Ca/P ratio approaching 1.67, corresponding to the stoichiometric ratio of hydroxyapatite (Priyani et al., 2025). The homogeneous mixture was then processed using a microwave for 3 minutes. The use of a microwave for this duration was also applied by Zuliana et al. (2025) in synthesizing hydroxyapatite from blue swimming crab shells, demonstrating that microwave treatment can be applied in the synthesis of hydroxyapatite derived from shell-based natural calcium sources (Zuliana et al., 2025). After the microwave process was completed, the sample was washed using distilled water in six stages. The first washing was conducted for 30 minutes, while the subsequent five stages lasted 15 minutes each. The formed precipitate was then separated through filtration and dried using an oven at 120°C for 60 minutes until dry hydroxyapatite powder was obtained (Priyani et al., 2025).

The synthesized and dried hydroxyapatite powder was used as the sample for X-Ray Diffraction (XRD) testing. Before the testing was conducted, the powder was ensured to be in a dry and non-agglomerated condition so that the sample possessed a relatively uniform distribution. The sample was then prepared on a sample holder, and its surface was leveled before being inserted into the XRD instrument. The testing was conducted to identify the crystalline phases formed, as well as to determine the crystallite size and the percentage of the hydroxyapatite phase in the synthesized powder (Priyani et al., 2025).

The scaffolds were fabricated using hydroxyapatite (HAp) synthesized from green mussel shells and gelatin as a binder. The fabrication procedure was modified from Saputra et al. (2026), who used a gelatin/HAp composite with a total solid mass of 4 grams, 5 mL of distilled water, a 6 mm × 6 mm silicone mold, and drying at 40°C for 24 hours. In this study, the scaffolds were fabricated using the slurry casting method with a distilled water volume of 5 mL and varying gelatin concentrations of 2%, 3%, and 4%, while the HAp composition was adjusted so that the total solid mass remained 4 grams. These variations were used to evaluate the changes in the scaffold hardness values due to differences in gelatin composition. The composition of each sample was prepared with a total solid mass of 4 grams, as presented in Table 1:

**Table 1.** Scaffold Composition

| Test Group | HAp (%) | Gelatin Mass (g) | HAp Mass (g) |
|---|---|---|---|
| Gel 2 % | 98% | 0.08 | 3.92 |
| Gel 3 % | 97% | 0.12 | 3.88 |
| Gel 4 % | 96% | 0,16 | 3.84 |

The gelatin, according to each variation, was dissolved in 5 mL of distilled water at 60°C until completely dissolved. The HAp powder was then gradually added into the gelatin solution while being stirred using a magnetic stirrer. The stirring was performed to achieve an even distribution of HAp particles within the gelatin matrix and to produce a slurry consistency suitable for the molding process. The use of gelatin as a binder in HAp composites is based on its biocompatible, biodegradable, and flexible properties, making it suitable for use in materials for bone tissue engineering applications (Saputra et al., 2026). Subsequently, the slurry was transferred to a mortar to be ground until the hydroxyapatite and gelatin were perfectly mixed.

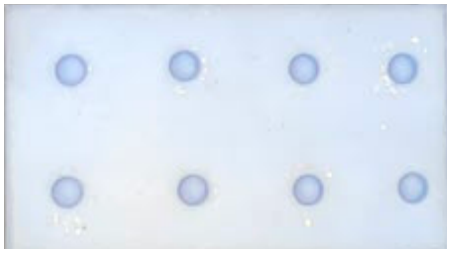
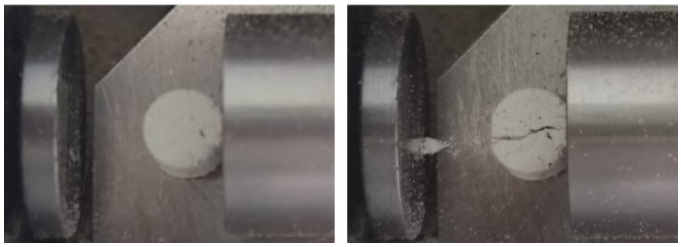


**Figure 3.** Silicone scaffold molds

The homogeneous slurry was subsequently poured slowly into cylindrical silicone molds with a diameter of 6 mm and a height of 6 mm, as shown in Figure 3. The pouring process was carried out using a spatula until all parts of the molds were filled. The samples were then left at room temperature

for 15 minutes to allow the slurry to undergo initial compaction. Once compacted, the scaffolds were removed from the molds and subsequently dried in an oven at 40°C for 24 hours until hardened. These drying conditions referred to Saputra et al. (2026), who utilized 40°C for 24 hours in fabricating green mussel shell-based gelatin/HAp composites. The scaffolds that met the criteria were then stored in a sealed container prior to hardness testing.

The scaffold hardness was tested using a Copley TBF 1000 Tablet Hardness Tester. In this study, hardness was operationally defined as breaking force or crushing strength, which is the maximum force required to cause a solid sample to undergo failure or fracture on a specific plane (Alhusban et al., 2024). The Copley TBF 1000 operates by placing the sample between a fixed jaw and a motorized jaw. The jaw moves, gradually applying force to the sample until a fracture occurs, as shown in Figure 4. The change in force detected by the load cell was then recorded as the breaking force in kg. Each gelatin composition group was fabricated in triplicates. With three composition groups, the total number of scaffolds tested was 9. Each scaffold was tested only once because the testing is destructive.



(a) (b)

**Figure 4.** Scaffold (a) before testing and (b) after testing

## RESULTS AND DISCUSSION

Based on the XRD diffraction pattern in Figure 5, the synthesized sample was dominated by a hydroxyapatite phase with a weight percentage of 98.7%, while the calcite and portlandite phases were only about 1.0% and 0.3%, respectively. The dominance of the HAp phase indicated that the synthesis process from green mussel shells proceeded well and produced hydroxyapatite with a high purity level. The presence of calcite and portlandite in small amounts indicated the presence of residual precursors that had not reacted completely. These results are consistent with Priyani et al. (2025), who obtained green mussel shell hydroxyapatite with a purity of 98.5%.

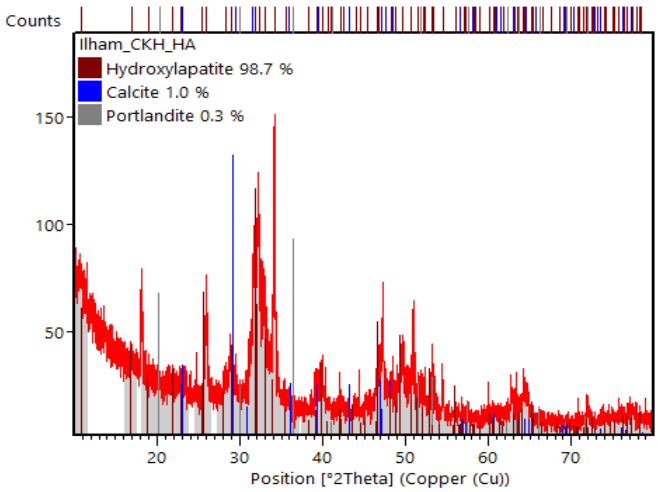



**Figure 5.** XRD pattern of green mussel shell hydroxyapatite

Green mussel shell (*Perna viridis*) hydroxyapatite-based scaffolds were successfully fabricated using the slurry casting method with gelatin composition variations of 2%, 3%, and 4%, alongside hydroxyapatite compositions of 98%, 97%, and 96%, respectively. Visually, the generated scaffolds were cylindrical, white in color, and possessed a fairly compact structure, as shown in Figure 6. The cylindrical shape was obtained from the molding process of the hydroxyapatite-gelatin slurry, followed

by a drying process until a solid structure was formed. Gelatin in the HAp composite acted as both the polymer phase and the interparticle binder. The presence of gelatin can improve the cohesion and structural stability of the scaffold by filling the spaces between the hydroxyapatite particles (Dressler et al., 2011). However, increasing the gelatin content also decreased the proportion of HAp as the inorganic phase, which could affect the structural compactness, porosity, and mechanical properties of the scaffold (Diansari et al., 2026). Therefore, the gelatin composition needs to be optimized to achieve a balance between binding capacity and the mechanical strength of the scaffold.

**Figure 6.** Green mussel shell scaffolds

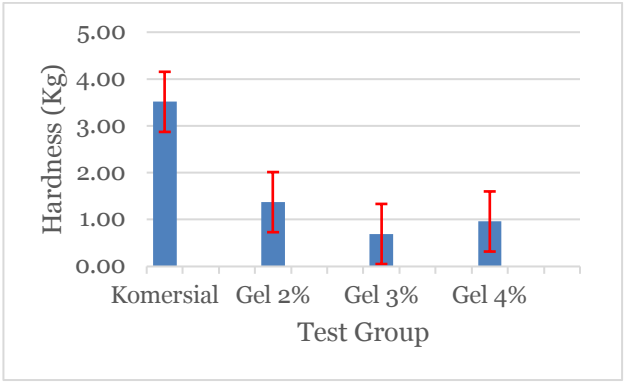



**Figure 7.** Graph of average scaffold hardness values

Based on Figure 7, the average hardness test results demonstrated that the commercial scaffold had the highest hardness value of 3.51 kg, higher than all green mussel shell scaffold specimens fabricated using the slurry casting method. For the green mussel shell scaffolds, the hardness values ranged from 0.69 to 1.37 kg, with the highest value obtained at the 2% gelatin variation at 1.37 kg, followed by 4% gelatin at 0.96 kg, while the lowest value was found at the 3% gelatin variation at 0.69 kg. The increase in gelatin concentration from 2% to 3% showed a considerable decrease in the hardness value. This condition is suspected to be related to the change in composition between the polymer and hydroxyapatite phases as well as the pore structure formed during the fabrication process. Gelatin as the polymer phase plays a role in binding the hydroxyapatite particles, but changes in its concentration can affect the structural compactness, pore distribution, and interfacial interactions between gelatin and hydroxyapatite.

At a 4% gelatin concentration, the hardness value experienced a slight increase to 0.96 kg compared to 3% gelatin, although it remained lower than that of 2% gelatin. These results indicate that the relationship between increasing gelatin concentration and scaffold hardness is not linear because the mechanical properties of the scaffold are also influenced by porosity, pore size and distribution, homogeneity of hydroxyapatite particles, and the quality of the bond between the polymer and ceramic phases (Wu et al., 2024). According to ISO 13779-1:2015, bone grafts have a mechanical property requirement of ≥ 1.5 MPa. The hardness value in kg was converted to MPa using the ASTM C496 equation for cylindrical specimens subjected to a lateral load as follows:

$$\sigma = \frac{2P}{\pi LD}$$

In this equation, $P$ is the load (N), $L$ is the specimen height, and $D$ is the specimen diameter. In this study, specimens with a height of 6 mm and a diameter of 6 mm were used. The conversion results are presented in Table 2 as follows:

**Table 2.** Compressive strength values

| Scaffold | Compressive Strength Value (MPa) |
|---|---|
| ISO 13779-1: 2015 | ≥ 1,5 |
| Commercial | 0,609 |
| GEL 2 % | 0,238 |
| GEL 3 % | 0,120 |
| GEL 4 % | 0,130 |

Based on Table 2, the conversion results of the breaking force indicated that the commercial scaffold possessed the highest mechanical value of 0.609 MPa. In the green mussel shell hydroxyapatite scaffolds, the highest value was obtained at the 2% gelatin variation at 0.238 MPa, followed by 4% gelatin at 0.130 MPa and 3% gelatin at 0.120 MPa. These results indicate that variations in gelatin concentration are related to the mechanical characteristics of the scaffolds, but changes in the compressive strength values do not exhibit a linear pattern. This condition is in line with the study by Diansari et al. (2026), which reported that variations in the hydroxyapatite and gelatin ratio produced compressive strengths of 0.22–0.44 MPa, with the highest value at a BHA:GEL ratio of 6:4, although the differences between formulations were not statistically significant. These findings are also supported by Nawafi et al. (2022), demonstrating that the gelatin/hydroxyapatite composition possessed different compressive strength values for each formulation. The highest compressive strength value was obtained at the GHA 1:2 composition at 18.1 ± 0.6 MPa, while formulations with excessively high proportions of either hydroxyapatite or gelatin produced lower compressive strength values. This indicates that the mechanical properties of the scaffolds are not solely determined by an increase in gelatin concentration, but rather by the compositional balance between the gelatin matrix and hydroxyapatite particles. An excessively high hydroxyapatite composition can lead to particle deposition and matrix discontinuity, whereas a remarkably low hydroxyapatite content causes the gelatin to not bond optimally with the hydroxyapatite particles.

## CONCLUSION

This study successfully synthesized hydroxyapatite from green mussel shells (*Perna viridis*) with a high purity level of 98.7%, while the calcite and portlandite phases were only 1.0% and 0.3%, respectively. The synthesized hydroxyapatite was subsequently successfully utilized as the base material for fabricating scaffolds using the slurry casting method with gelatin concentration variations of 2%, 3%, and 4%. The mechanical testing results demonstrated that the scaffold with the 2% gelatin variant exhibited the highest hardness value of 1.37 kg, followed by 4% gelatin at 0.96 kg and 3% gelatin at 0.69 kg, whereas the commercial scaffold showed a value of 3.51 kg. After converting based on the specimen dimensions, mechanical values of 0.238 MPa, 0.130 MPa, and 0.120 MPa were obtained for 2% gelatin, 4% gelatin, and 3% gelatin, respectively, while the commercial scaffold possessed a value of 0.609 MPa. Based on these results, the 2% gelatin variation provided the best mechanical characteristics among the tested green mussel shell hydroxyapatite scaffolds. The changes in mechanical values with increasing gelatin concentration did not exhibit a linear pattern, indicating that the mechanical characteristics of the scaffolds are determined not only by the gelatin concentration but also influenced by the hydroxyapatite-gelatin compositional balance, structural compactness, porosity, particle homogeneity, and the interfacial interactions of both phases. All converted values remained below the mechanical reference value of 1.5 MPa used in this study. Therefore, optimization of the composition and fabrication process is still required to enhance the mechanical strength of the scaffolds.

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