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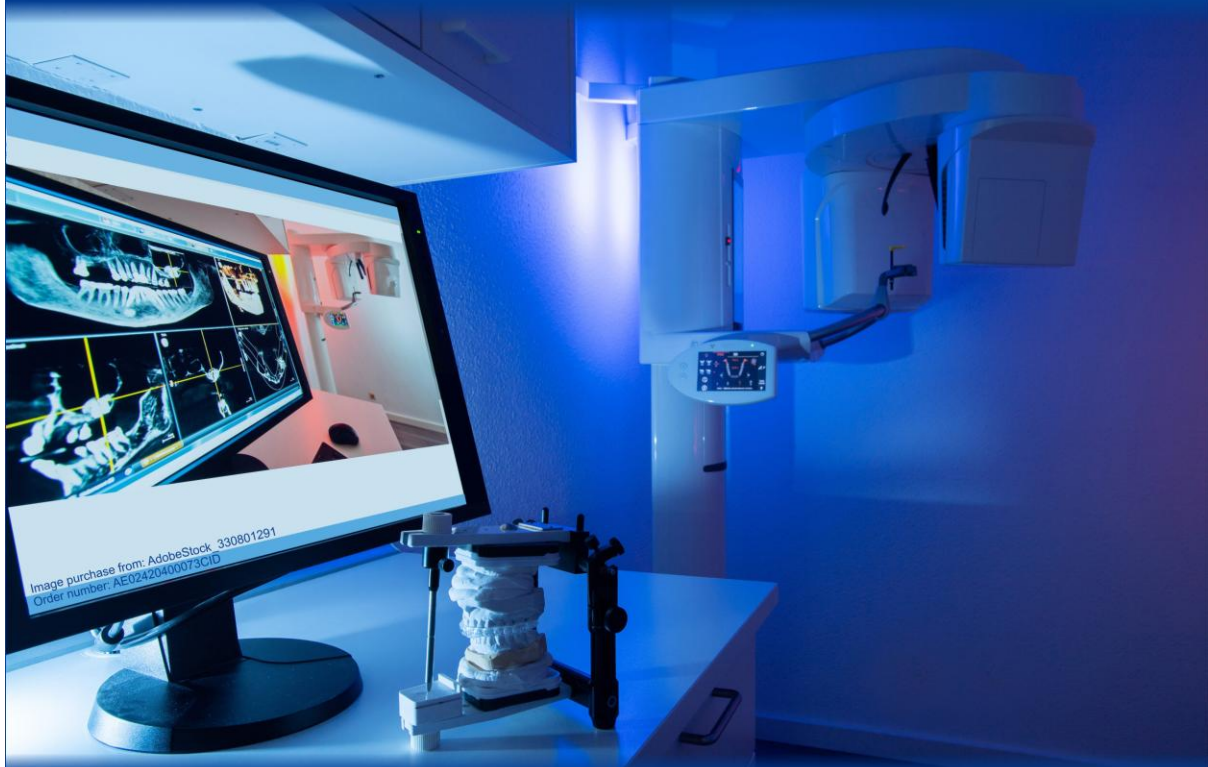
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Fabrication of composite block from beta-tricalcium phosphate and polyacrylic acid via freeze-drying method

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ABSTRACT

Background: Beta-tricalcium phosphate (β -TCP) is widely used in bone grafting due to its biocompatibility and bioresorbability. Recently, there has been growing interest in using sustainable materials, such as green mussel shells, as an alternative source for β -TCP. These shells, rich in calcium carbonate, provide a cost-effective and environmentally friendly alternative for β -TCP synthesis. **Purpose:** To fabricate composite blocks from β -TCP derived from green mussel shells, mixed with polyacrylic acid (PAA), using a setting reaction and freeze-drying method. **Methods:** Beta-tricalcium phosphate powder was obtained via wet precipitation, starting with calcium carbonate from green mussel shells, converting it to calcium oxide, and then to β -TCP. The resulting powder was mixed with PAA, set, and freeze-dried to form composite blocks. **Results:** Characterization of the composite blocks for porosity and diametral tensile strength (DTS) showed that blocks made with green mussel shell-derived β -TCP had rougher surfaces due to larger particles than control blocks made with commercial β -TCP. Composite blocks with 70% green mussel shell-derived β -TCP and 30% PAA exhibited significantly higher porosity ($26.97\% \pm 2.64\%$) and DTS (11.76 ± 1.59 MPa) than those made with commercial β -TCP (porosity: $13.40\% \pm 1.56\%$; DTS: 7.79 ± 1.29 MPa). Reducing β -TCP content to 60% resulted in increased porosity ($34.22\% \pm 1.84\%$) and lower DTS (6.41 ± 0.78 MPa). **Conclusion:** Composite blocks made from green mussel shell-derived β -TCP and PAA showed higher porosity and significantly higher DTS than blocks made from commercial β -TCP. Decreasing β -TCP content increased porosity but decreased DTS.

Keywords: beta-tricalcium phosphate; diametral tensile strength; freeze drying; medicine; polyacrylic acid; porosity

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INTRODUCTION

In addition to its osteoconductivity, the higher bioresorbability of beta-tricalcium phosphate (β -TCP) compared with hydroxyapatite makes it a popular choice in orthopedic and oral surgery applications. In this field, β -TCP is widely used as a scaffold material due to its ability to support cell attachment, proliferation, and differentiation, as well as its suitable resorption rate, which matches new bone formation. Clinically, β -TCP is commonly used for bone graft substitutes in dental implants, fracture repair, and spinal fusion, where it provides temporary structural support while promoting new bone growth. These properties make β -TCP an ideal candidate

for use in bone tissue engineering applications, where the balance between mechanical support and biological remodeling is crucial. This characteristic is expected to promote improved bone formation while replacing the resorbed materials.^{1–7} The combination of β -TCP and polymers presents an attractive material of choice for application in bone substitution.⁸ Polyacrylic acid (PAA) is a synthetic polymer that exhibits notable properties, such as biocompatibility, strong adhesion, and biodegradability. In dentistry, particularly in glass ionomer cement (GIC) applications, PAA contributes to dental cement, enhancing mechanical properties and setting reactions of calcium phosphate materials through crosslinking. The chelation reaction between carboxyl groups ($-\text{COOH}$) from PAA and

calcium ions from calcium phosphate contributes to scaffold stability.^{9–11}

An investigation by Moradi et al.⁹ discovered that a composite scaffold consisting of TCP and PAA can be fabricated through a crosslinking reaction and freeze-drying process involving various reagents as precursors. However, although successful polymerization and the desired mechanical properties were achieved, the research involved a complex mixing procedure with several chemicals. Putri et al.¹¹ suggested a simpler method involving the chelation reaction between calcium ions from β -TCP and $-\text{COOH}$ groups from PAA, similar to the setting mechanism in GIC. The current study adopts a similar approach by mixing β -TCP and PAA.

Despite the advantages of β -TCP, its widespread clinical application is limited by factors such as high production costs, limited availability in some regions, and reliance on synthetic or mined sources. These drawbacks are prompting the exploration of alternative, cost-effective, and sustainable sources for producing β -TCP. Natural calcium-rich waste materials, such as mammalian bones, coral, and shellfish, have been reported as potential candidates for synthesizing calcium phosphate materials.¹² Among them are green mussel shells (*Perna viridis*), which are abundantly consumed in Southeast Asia and generate large quantities of shell waste. These shells are primarily composed of calcium carbonate and thus present an excellent renewable resource for calcium-based biomaterials, including β -TCP.^{12,13}

The novelty of the present study lies in the development of a β -TCP–PAA composite block using β -TCP fabricated from green mussel shells through a simple setting and freeze-drying process. This research introduces a new, low-cost source for biomaterials and demonstrates that waste from green mussels can produce materials with similar or better physical and mechanical properties than commercial β -TCP. Compared with other synthetic biomaterials such as hydroxyapatite, β -TCP made from green mussel shells offers improved biodegradability and resorption rates that better match natural bone remodeling. Unlike autologous bone grafts, which have excellent biological compatibility but are limited by donor availability and morbidity, this form of β -TCP provides a cost-effective and abundant alternative without donor-site complications. Additionally, it avoids risks associated with allografts, such as immune rejection and disease transmission, while still supporting osteoconductivity and new bone growth.^{12,13}

When combined with PAA through a setting and freeze-drying process, β -TCP synthesized from green mussel shells produces composite blocks with comparable or improved physical and mechanical properties, such as porosity and diametral tensile strength (DTS), compared with commercial β -TCP-based composites, making it a viable, cost-effective alternative biomaterial for bone tissue engineering. Therefore, this study evaluates the feasibility of combining β -TCP fabricated from green mussel shells with PAA through a setting reaction and freeze-drying method.

MATERIALS AND METHODS

The green mussel shells used in this study were obtained from Muara Angke Port, Jakarta, Indonesia. Only fresh shells were selected, and any shells showing signs of decay, unpleasant odor, visible dirt, or other contamination were excluded to ensure the quality of the raw material. The selected shells were thoroughly cleaned and heated to remove any remaining organic components. Following this, the shells were ground into particles smaller than 350 μm and calcined in a furnace at 1,000°C for 5 hours to obtain calcium oxide (CaO). The resulting CaO powder was reacted with a phosphoric acid solution (calcium/phosphate ratio: 1.5, typical for β -TCP) and calcined at 1,000°C to obtain β -TCP.

The β -TCP powder was subsequently mixed with a PAA solution (Mw: approx. 250,000, 35 wt% in H_2O , Sigma-Aldrich, St. Louis, Missouri, USA) at three varying percentage ratios of β -TCP to PAA: 60:40, 65:35, and 70:30. Each ratio was prepared with a total mixture weight of 2 g. For the 60:40 ratio, 1.2 g of β -TCP was mixed with 0.8 g of PAA solution; for the 65:35 ratio, 1.3 g of β -TCP was mixed with 0.7 g of PAA solution; and for the 70:30 ratio, 1.4 g of β -TCP was mixed with 0.6 g of PAA solution. The resulting mixture was placed into a mold with a diameter of 6 mm and a thickness of 3 mm. The mixture was subjected to deep-freezing at -80°C to facilitate the formation of ice crystals and then underwent the lyophilization process for 48 hours. As a control, we used commercial β -TCP (Sigma-Aldrich, St. Louis, Missouri, USA) for mixing with PAA.

The porosities of the blocks were measured using the equations below. Here, the relative density was calculated by dividing the bulk density by the theoretical density of both β -TCP (3.07 g/cm^3) and PAA (1.15 g/cm^3).^{14,15} The DTS of the samples was measured utilizing a universal testing machine (UTM: AGS-X, Shimadzu, Kyoto, Kyoto Prefecture, Japan) at a crosshead speed of 1 mm/min:

$$\text{Relative density (\%)} = \frac{\text{Bulk density}}{\text{Theoretical density}} \times 100 (\%)$$

$$\text{Total porosity (\%)} = 100 - \text{relative density (\%)}$$

One-way analysis of variance was employed to investigate significant differences among all groups. Following this, post hoc analysis was conducted using Tukey's honestly significant difference test to compare significance between each group. The statistical analysis was performed using KaleidaGraph 4.01 software (Synergy Software, Reading, Pennsylvania, USA), with the significance level set at $p < 0.05$.

RESULTS

The β -TCP and PAA mixtures were successfully set into composite blocks, as illustrated in Figure 1. At the macroscopic level, the sample containing the control

β -TCP displayed a smoother surface than the other samples (Figure 1A). Moreover, as the quantity of β -TCP powder derived from green mussel shells was increased, the surface appeared to become rougher (Figure 1B–D).

As Figure 2 shows, at the same ratio, composite blocks incorporating β -TCP derived from green mussel shells exhibited higher porosity ($26.97\% \pm 2.64\%$) than those containing commercial β -TCP ($13.40\% \pm 1.56\%$). An increase in the powder amount from 70% to 65% resulted in an increase in porosity ($35.00\% \pm 2.05\%$). However, there was no significant difference in porosity observed

between blocks containing 60% ($34.22\% \pm 1.84\%$) and 65% β -TCP.

The DTS values of the samples are shown in Figure 3. At a ratio of 70% β -TCP and 30% PAA, the samples containing β -TCP fabricated from green mussel shells (11.76 ± 1.59 MPa) displayed a significant increase in DTS value compared with those containing commercial β -TCP (7.79 ± 1.29 MPa). However, reducing the amount of powder to 65% resulted in a significant decrease in DTS value (7.35 ± 0.61 MPa). There was no significant difference between blocks containing 60% (6.41 ± 0.78 MPa) and 65% β -TCP.

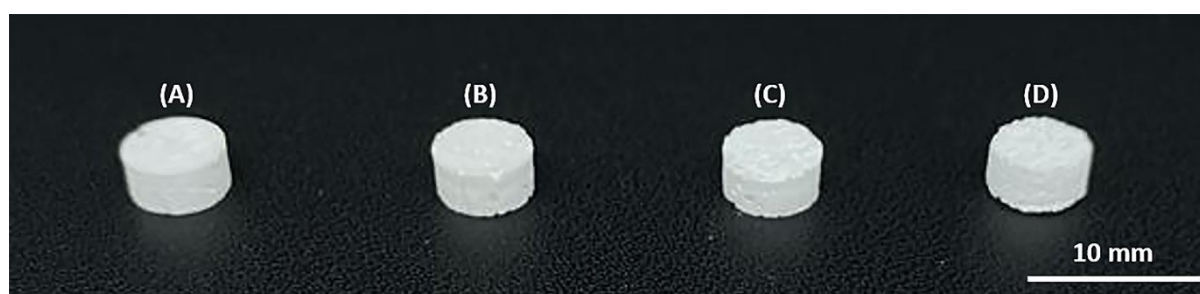


Figure 1. Composite blocks containing (A) 70% Sigma-Aldrich beta-tricalcium phosphate (β -TCP) control with 30% polyacrylic acid (PAA) (SA-P), (B) 60% green mussel shell-derived β -TCP with 40% PAA (B60-P40), (C) 65% green mussel shell-derived β -TCP with 35% PAA (B65-P35), and (D) 70% green mussel shell-derived β -TCP with 30% PAA (B70-P30).

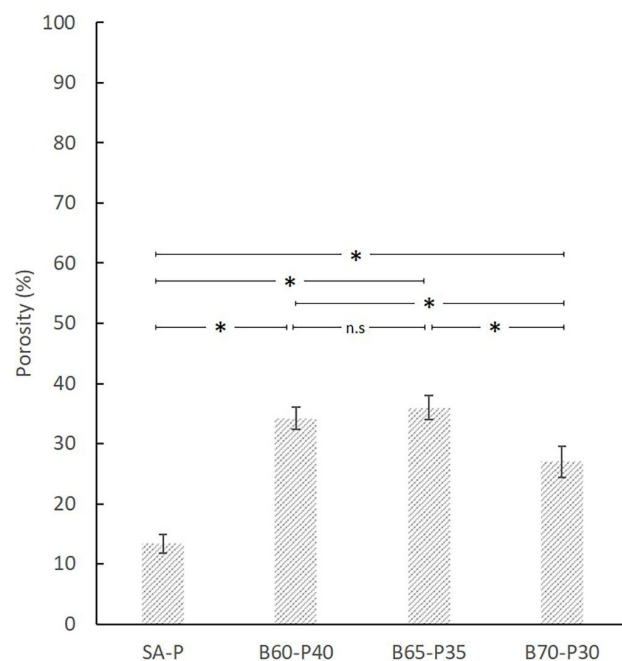


Figure 2. Porosities of composite blocks containing 70% Sigma-Aldrich beta-tricalcium phosphate (β -TCP) control with 30% polyacrylic acid (PAA) (SA-P), 60% green mussel shell-derived β -TCP with 40% PAA (B60-P40), 65% green mussel shell-derived β -TCP with 35% PAA (B65-P35), and 70% green mussel shell-derived β -TCP with 30% PAA (B70-P30) ($n = 3$). (* $p < 0.05$; n.s.: not significant)

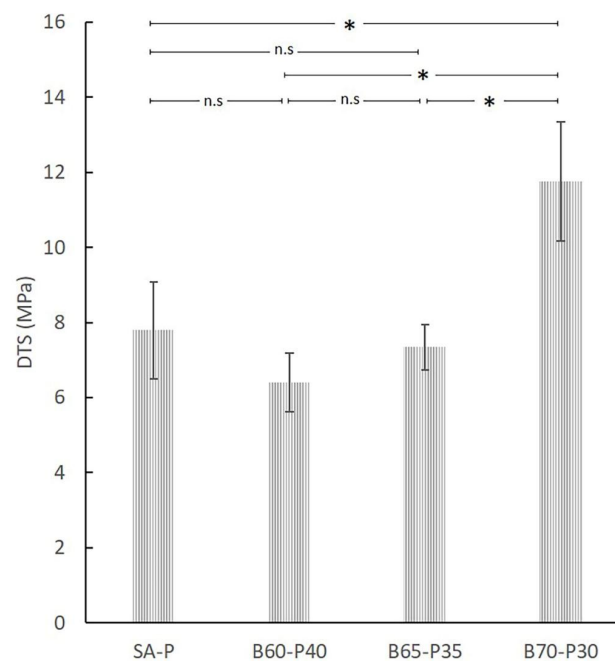


Figure 3. Diametral tensile strength (DTS) values of composite blocks containing 70% Sigma-Aldrich beta-tricalcium phosphate (β -TCP) control with 30% polyacrylic acid (PAA) (SA-P), 60% green mussel shell-derived β -TCP with 40% PAA (B60-P40), 65% green mussel shell-derived β -TCP with 35% PAA (B65-P35), and 70% green mussel shell-derived β -TCP with 30% PAA (B70-P30) ($n = 3$). (* $p < 0.05$; n.s.: not significant.)

DISCUSSION

This study demonstrates that composite blocks made from green mussel shell-derived β -TCP mixed with PAA can be successfully fabricated using a simple setting and freeze-drying process. This approach is novel because it uses a natural, local, and low-cost material as an alternative to expensive commercial β -TCP, which helps reduce waste and cost.^{12,13} Our analysis found important differences in properties between blocks made with green mussel shell-derived β -TCP and those made with commercial β -TCP.

The physical difference between all samples is evident in the blocks containing commercial β -TCP and those incorporating β -TCP made from green mussel shells. The larger particle size of green mussel shell-derived β -TCP (up to 350 μm) and PAA results in a rougher surface in the composite blocks than in those made with commercial β -TCP (particle size ranging from 1 to 1.8 μm).¹⁶ This difference is due to the synthesis and processing methods. The green mussel shell-derived β -TCP was prepared through manual grinding and calcination, without further milling or sieving to reduce particle size, resulting in coarser particles. By contrast, commercial β -TCP typically undergoes industrial-scale processing with controlled milling and sieving steps to produce finer and more uniform particles. However, the rougher surface on the composite blocks has the potential to enhance cell attachment, thereby promoting better penetration of bone cells and ultimately increasing bone ingrowth.^{6,17,18}

The difference in particle size also affected the porosity of the samples, with blocks containing commercial β -TCP exhibiting lower porosity than those incorporating β -TCP derived from green mussel shells (Figure 2). Porosity plays a crucial role in facilitating cell attachment and penetration during bone regeneration, promoting cell proliferation and differentiation. Additionally, as demonstrated in prior studies, porosity significantly influences the mechanical properties of a material.^{17,19,20} The findings of our study suggest that higher porosity in the samples corresponded to higher DTS values (Figures 2 and 3).

Incorporating a larger quantity of β -TCP powder into the solution results in a more densely packed composite material, typically leading to reduced porosity.^{15,21} This reduction in pores generally enhances structural strength, contributing to an improvement in the DTS value. However, this pattern differs in the current study. Upon mixing β -TCP and PAA, a setting reaction occurred, forming bonds between the $-\text{COOH}$ group of PAA and calcium ions of β -TCP, thereby creating connections among the β -TCP particles. It is assumed that the larger particles in the β -TCP would provide a broader surface on each particle, leading to a larger interface area. This, in turn, results in more extensive connections between the $-\text{COOH}$ group of PAA and calcium ions of β -TCP, contributing to an enhancement in mechanical strength.^{11,22}

However, the phase composition of the samples in the present study is unknown, especially given the use

of green mussel shells as the precursor. Any potential remaining contamination needs to be evaluated. Further characterization should be conducted, including through X-ray diffraction, Fourier transform infrared spectroscopy, and scanning electron microscopy analyses, as well as degradation and swelling property investigations. These additional physical characterizations are needed to gain more detailed insights into their correlation with porosity and mechanical properties, which, in turn, would significantly impact biological activity. These limitations in material characterization make it difficult to fully explain the structure–property relationships observed. Furthermore, no biological tests (e.g., cytotoxicity, cell attachment, or in vivo performance) were performed to confirm the composite's biocompatibility and effectiveness. Moreover, manual grinding without size control may lead to inconsistent particle size, affecting reproducibility. Lastly, only one type of commercial β -TCP was used for comparison, and the setting behavior of PAA with different β -TCP sources may vary.

In conclusion, a composite block was successfully fabricated by mixing β -TCP obtained from green mussel shells with a PAA solution through a setting reaction and freeze-drying process. In comparison to the composite block using Sigma-Aldrich β -TCP powder, the composite fabricated from β -TCP obtained from green mussel shells exhibited a significant increase in porosity, accompanied by a significantly higher DTS value. Moreover, a decrease in the amount of β -TCP powder resulted in an increase in both porosity and DTS value. In future studies, more detailed material testing should be conducted to better understand the structure and composition of the composite. Biological assessments such as cytotoxicity, cell attachment, and in vivo studies are also needed to confirm the composite's safety and effectiveness for bone repair. Controlling the particle size through milling and sieving is recommended to improve consistency. It would also be valuable to study how PAA reacts with different types of β -TCP and compare other natural or synthetic sources. This will help in exploring the extent to which the material can be used for clinical bone regeneration.

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Rosalina Tjandrawinata

Fabrication of composite block from beta-tricalcium phosphate and polyacrylic acid via freeze-drying method

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Fabrication of composite block from beta-tricalcium phosphate and polyacrylic acid via freeze-drying method

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ABSTRACT

Background: Beta-tricalcium phosphate (β -TCP) is widely used in bone grafting due to its biocompatibility and bioresorbability. Recently, there has been growing interest in using sustainable materials, such as green mussel shells, as an alternative source for β -TCP. These shells, rich in calcium carbonate, provide a cost-effective and environmentally friendly alternative for β -TCP synthesis. **Purpose:** To fabricate composite blocks from β -TCP derived from green mussel shells, mixed with polyacrylic acid (PAA), using a setting reaction and freeze-drying method. **Methods:** Beta-tricalcium phosphate powder was obtained via wet precipitation, starting with calcium carbonate from green mussel shells, converting it to calcium oxide, and then to β -TCP. The resulting powder was mixed with PAA, set, and freeze-dried to form composite blocks. **Results:** Characterization of the composite blocks for porosity and diametral tensile strength (DTS) showed that blocks made with green mussel shell-derived β -TCP had rougher surfaces due to larger particles than control blocks made with commercial β -TCP. Composite blocks with 70% green mussel shell-derived β -TCP and 30% PAA exhibited significantly higher porosity ($26.97\% \pm 2.64\%$) and DTS (11.76 ± 1.59 MPa) than those made with commercial β -TCP (porosity: $13.40\% \pm 1.56\%$; DTS: 7.79 ± 1.29 MPa). Reducing β -TCP content to 60% resulted in increased porosity ($34.22\% \pm 1.84\%$) and lower DTS (6.41 ± 0.78 MPa). **Conclusion:** Composite blocks made from green mussel shell-derived β -TCP and PAA showed higher porosity and significantly higher DTS than blocks made from commercial β -TCP. Decreasing β -TCP content increased porosity but decreased DTS.

Keywords: beta-tricalcium phosphate; diametral tensile strength; freeze drying; medicine; polyacrylic acid; porosity

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INTRODUCTION

In addition to its osteoconductivity, the higher bioresorbability of beta-tricalcium phosphate (β -TCP) compared with hydroxyapatite makes it a popular choice in orthopedic and oral surgery applications. In this field, β -TCP is widely used as a scaffold material due to its ability to support cell attachment, proliferation, and differentiation, as well as its suitable resorption rate, which matches new bone formation. Clinically, β -TCP is commonly used for bone graft substitutes in dental implants, fracture repair, and spinal fusion, where it provides temporary structural support while promoting new bone growth. These properties make β -TCP an ideal candidate

for use in bone tissue engineering applications, where the balance between mechanical support and biological remodeling is crucial. This characteristic is expected to promote improved bone formation while replacing the resorbed materials.^{1–7} The combination of β -TCP and polymers presents an attractive material of choice for application in bone substitution.⁸ Polyacrylic acid (PAA) is a synthetic polymer that exhibits notable properties, such as biocompatibility, strong adhesion, and biodegradability. In dentistry, particularly in glass ionomer cement (GIC) applications, PAA contributes to dental cement, enhancing mechanical properties and setting reactions of calcium phosphate materials through crosslinking. The chelation reaction between carboxyl groups ($-\text{COOH}$) from PAA and

calcium ions from calcium phosphate contributes to scaffold stability.^{9–11}

An investigation by Moradi et al.⁹ discovered that a composite scaffold consisting of TCP and PAA can be fabricated through a crosslinking reaction and freeze-drying process involving various reagents as precursors. However, although successful polymerization and the desired mechanical properties were achieved, the research involved a complex mixing procedure with several chemicals. Putri et al.¹¹ suggested a simpler method involving the chelation reaction between calcium ions from β -TCP and $-\text{COOH}$ groups from PAA, similar to the setting mechanism in GIC. The current study adopts a similar approach by mixing β -TCP and PAA.

Despite the advantages of β -TCP, its widespread clinical application is limited by factors such as high production costs, limited availability in some regions, and reliance on synthetic or mined sources. These drawbacks are prompting the exploration of alternative, cost-effective, and sustainable sources for producing β -TCP. Natural calcium-rich waste materials, such as mammalian bones, coral, and shellfish, have been reported as potential candidates for synthesizing calcium phosphate materials.¹² Among them are green mussel shells (*Perna viridis*), which are abundantly consumed in Southeast Asia and generate large quantities of shell waste. These shells are primarily composed of calcium carbonate and thus present an excellent renewable resource for calcium-based biomaterials, including β -TCP.^{12,13}

The novelty of the present study lies in the development of a β -TCP–PAA composite block using β -TCP fabricated from green mussel shells through a simple setting and freeze-drying process. This research introduces a new, low-cost source for biomaterials and demonstrates that waste from green mussels can produce materials with similar or better physical and mechanical properties than commercial β -TCP. Compared with other synthetic biomaterials such as hydroxyapatite, β -TCP made from green mussel shells offers improved biodegradability and resorption rates that better match natural bone remodeling. Unlike autologous bone grafts, which have excellent biological compatibility but are limited by donor availability and morbidity, this form of β -TCP provides a cost-effective and abundant alternative without donor-site complications. Additionally, it avoids risks associated with allografts, such as immune rejection and disease transmission, while still supporting osteoconductivity and new bone growth.^{12,13}

When combined with PAA through a setting and freeze-drying process, β -TCP synthesized from green mussel shells produces composite blocks with comparable or improved physical and mechanical properties, such as porosity and diametral tensile strength (DTS), compared with commercial β -TCP-based composites, making it a viable, cost-effective alternative biomaterial for bone tissue engineering. Therefore, this study evaluates the feasibility of combining β -TCP fabricated from green mussel shells with PAA through a setting reaction and freeze-drying method.

MATERIALS AND METHODS

The green mussel shells used in this study were obtained from Muara Angke Port, Jakarta, Indonesia. Only fresh shells were selected, and any shells showing signs of decay, unpleasant odor, visible dirt, or other contamination were excluded to ensure the quality of the raw material. The selected shells were thoroughly cleaned and heated to remove any remaining organic components. Following this, the shells were ground into particles smaller than 350 μm and calcined in a furnace at 1,000°C for 5 hours to obtain calcium oxide (CaO). The resulting CaO powder was reacted with a phosphoric acid solution (calcium/phosphate ratio: 1.5, typical for β -TCP) and calcined at 1,000°C to obtain β -TCP.

The β -TCP powder was subsequently mixed with a PAA solution (Mw: approx. 250,000, 35 wt% in H_2O , Sigma-Aldrich, St. Louis, Missouri, USA) at three varying percentage ratios of β -TCP to PAA: 60:40, 65:35, and 70:30. Each ratio was prepared with a total mixture weight of 2 g. For the 60:40 ratio, 1.2 g of β -TCP was mixed with 0.8 g of PAA solution; for the 65:35 ratio, 1.3 g of β -TCP was mixed with 0.7 g of PAA solution; and for the 70:30 ratio, 1.4 g of β -TCP was mixed with 0.6 g of PAA solution. The resulting mixture was placed into a mold with a diameter of 6 mm and a thickness of 3 mm. The mixture was subjected to deep-freezing at -80°C to facilitate the formation of ice crystals and then underwent the lyophilization process for 48 hours. As a control, we used commercial β -TCP (Sigma-Aldrich, St. Louis, Missouri, USA) for mixing with PAA.

The porosities of the blocks were measured using the equations below. Here, the relative density was calculated by dividing the bulk density by the theoretical density of both β -TCP (3.07 g/cm^3) and PAA (1.15 g/cm^3).^{14,15} The DTS of the samples was measured utilizing a universal testing machine (UTM: AGS-X, Shimadzu, Kyoto, Kyoto Prefecture, Japan) at a crosshead speed of 1 mm/min:

$$\text{Relative density (\%)} = \frac{\text{Bulk density}}{\text{Theoretical density}} \times 100 (\%)$$

$$\text{Total porosity (\%)} = 100 - \text{relative density (\%)}$$

One-way analysis of variance was employed to investigate significant differences among all groups. Following this, post hoc analysis was conducted using Tukey's honestly significant difference test to compare significance between each group. The statistical analysis was performed using KaleidaGraph 4.01 software (Synergy Software, Reading, Pennsylvania, USA), with the significance level set at $p < 0.05$.

RESULTS

The β -TCP and PAA mixtures were successfully set into composite blocks, as illustrated in Figure 1. At the macroscopic level, the sample containing the control

β -TCP displayed a smoother surface than the other samples (Figure 1A). Moreover, as the quantity of β -TCP powder derived from green mussel shells was increased, the surface appeared to become rougher (Figure 1B–D).

As Figure 2 shows, at the same ratio, composite blocks incorporating β -TCP derived from green mussel shells exhibited higher porosity ($26.97\% \pm 2.64\%$) than those containing commercial β -TCP ($13.40\% \pm 1.56\%$). An increase in the powder amount from 70% to 65% resulted in an increase in porosity ($35.00\% \pm 2.05\%$). However, there was no significant difference in porosity observed

between blocks containing 60% ($34.22\% \pm 1.84\%$) and 65% β -TCP.

The DTS values of the samples are shown in Figure 3. At a ratio of 70% β -TCP and 30% PAA, the samples containing β -TCP fabricated from green mussel shells (11.76 ± 1.59 MPa) displayed a significant increase in DTS value compared with those containing commercial β -TCP (7.79 ± 1.29 MPa). However, reducing the amount of powder to 65% resulted in a significant decrease in DTS value (7.35 ± 0.61 MPa). There was no significant difference between blocks containing 60% (6.41 ± 0.78 MPa) and 65% β -TCP.



Figure 1. Composite blocks containing (A) 70% Sigma-Aldrich beta-tricalcium phosphate (β -TCP) control with 30% polyacrylic acid (PAA) (SA-P), (B) 60% green mussel shell-derived β -TCP with 40% PAA (B60-P40), (C) 65% green mussel shell-derived β -TCP with 35% PAA (B65-P35), and (D) 70% green mussel shell-derived β -TCP with 30% PAA (B70-P30).

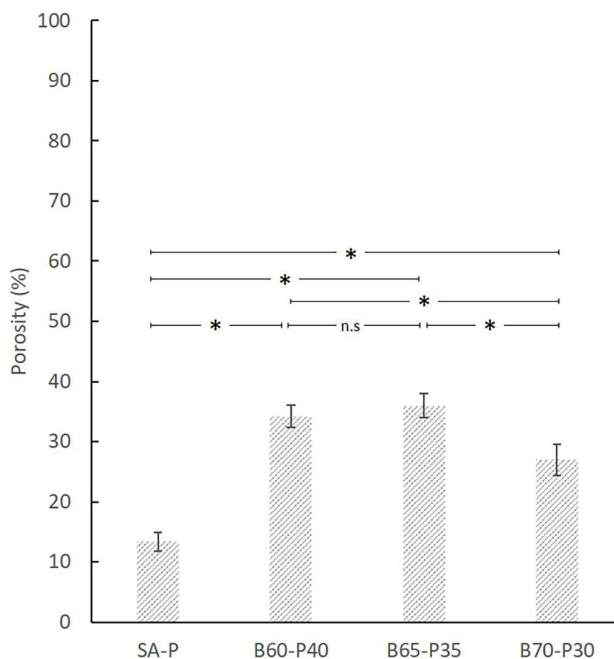


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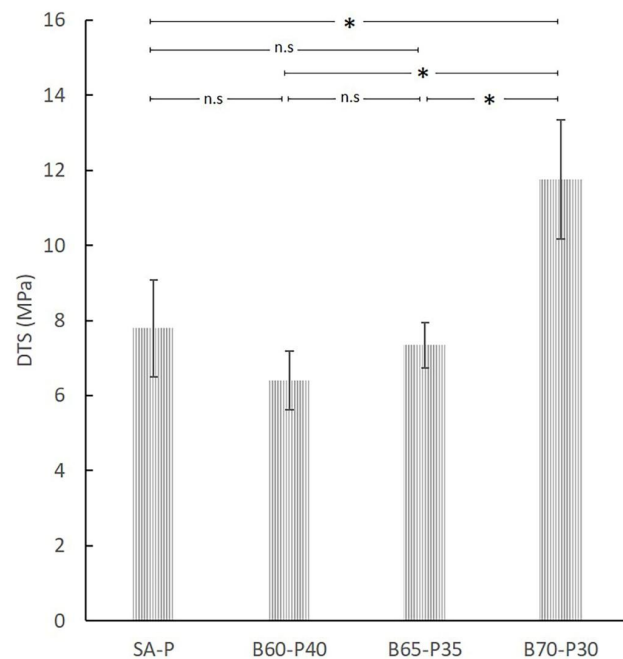


Figure 3. Diametral tensile strength (DTS) values of composite blocks containing 70% Sigma-Aldrich beta-tricalcium phosphate (β -TCP) control with 30% polyacrylic acid (PAA) (SA-P), 60% green mussel shell-derived β -TCP with 40% PAA (B60-P40), 65% green mussel shell-derived β -TCP with 35% PAA (B65-P35), and 70% green mussel shell-derived β -TCP with 30% PAA (B70-P30) ($n = 3$). (* $p < 0.05$; n.s.: not significant.)

DISCUSSION

This study demonstrates that composite blocks made from green mussel shell-derived β -TCP mixed with PAA can be successfully fabricated using a simple setting and freeze-drying process. This approach is novel because it uses a natural, local, and low-cost material as an alternative to expensive commercial β -TCP, which helps reduce waste and cost.^{12,13} Our analysis found important differences in properties between blocks made with green mussel shell-derived β -TCP and those made with commercial β -TCP.

The physical difference between all samples is evident in the blocks containing commercial β -TCP and those incorporating β -TCP made from green mussel shells. The larger particle size of green mussel shell-derived β -TCP (up to 350 μ m) and PAA results in a rougher surface in the composite blocks than in those made with commercial β -TCP (particle size ranging from 1 to 1.8 μ m).¹⁶ This difference is due to the synthesis and processing methods. The green mussel shell-derived β -TCP was prepared through manual grinding and calcination, without further milling or sieving to reduce particle size, resulting in coarser particles. By contrast, commercial β -TCP typically undergoes industrial-scale processing with controlled milling and sieving steps to produce finer and more uniform particles. However, the rougher surface on the composite blocks has the potential to enhance cell attachment, thereby promoting better penetration of bone cells and ultimately increasing bone ingrowth.^{6,17,18}

The difference in particle size also affected the porosity of the samples, with blocks containing commercial β -TCP exhibiting lower porosity than those incorporating β -TCP derived from green mussel shells (Figure 2). Porosity plays a crucial role in facilitating cell attachment and penetration during bone regeneration, promoting cell proliferation and differentiation. Additionally, as demonstrated in prior studies, porosity significantly influences the mechanical properties of a material.^{17,19,20} The findings of our study suggest that higher porosity in the samples corresponded to higher DTS values (Figures 2 and 3).

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In conclusion, a composite block was successfully fabricated by mixing β -TCP obtained from green mussel shells with a PAA solution through a setting reaction and freeze-drying process. In comparison to the composite block using Sigma-Aldrich β -TCP powder, the composite fabricated from β -TCP obtained from green mussel shells exhibited a significant increase in porosity, accompanied by a significantly higher DTS value. Moreover, a decrease in the amount of β -TCP powder resulted in an increase in both porosity and DTS value. In future studies, more detailed material testing should be conducted to better understand the structure and composition of the composite. Biological assessments such as cytotoxicity, cell attachment, and in vivo studies are also needed to confirm the composite's safety and effectiveness for bone repair. Controlling the particle size through milling and sieving is recommended to improve consistency. It would also be valuable to study how PAA reacts with different types of β -TCP and compare other natural or synthetic sources. This will help in exploring the extent to which the material can be used for clinical bone regeneration.

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