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Penulis : Rosalina Tjandrawinata, Eddy, Rafhaela Johanna Halim, Thet Thet Swe, Tansza Setiana Putri

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[DJMKG] Submission Acknowledgement

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Original article

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

Rosalina Tjandrawinata[1]*, Eddy[1], Rafhaela Johanna Halim[2], Thet Thet Swe[3], Tansza Setiana Putri[1]

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ABSTRACT

Background: *Beta-tricalcium phosphate (βTCP) 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 βTCP. These shells, rich in calcium carbonate, provide a cost-effective and environmentally friendly alternative for βTCP synthesis.* ***Purpose:*** *This study aims to fabricate composite blocks from βTCP derived from green mussel shells, mixed with polyacrylic acid (PAA), using a setting reaction and freeze-drying method.* ***Methods:*** *βTCP powder was obtained via wet precipitation, starting with calcium carbonate from green mussel shells, converting it to calcium oxide, and then to βTCP. 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 βTCP had rougher surfaces due to larger particles compared to control blocks made with commercial βTCP (Sigma-Aldrich). Composite blocks with 70% green mussel βTCP and 30% PAA exhibited significantly higher porosity (26.97 ± 2.64%) and DTS (11.76 ± 1.59 MPa) than those made with commercial βTCP (porosity: 13.40 ± 1.56%; DTS: 7.79 ± 1.29 MPa). Reducing βTCP content to 60% resulted in increased porosity (34.22 ± 1.84%) and lower DTS (6.41 ± 0.78 MPa).* ***Conclusion:*** *Composite blocks from green mussel shell-derived βTCP and PAA showed higher porosity and significantly higher DTS than blocks made from commercial βTCP. Decreasing βTCP content increased porosity but decreased DTS.*

Keywords: *β-tricalcium phosphate; polyacrylic acid; freeze-drying; porosity; diametral tensile strength*

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INTRODUCTION

Apart from its osteoconductivity, the higher bioreabsorbability of beta-tricalcium phosphate (βTCP) compared to hydroxyapatite makes it a popular choice in orthopedic and oral surgery applications. This characteristic is expected to promote improved bone formation while replacing the resorbed materials materials.[1–3] The combination of beta-tricalcium phosphate (βTCP) with polymer has been an attractive material of choice for its application for bone substitution.[4] Polyacrylic acid is a synthetic polymer which exhibits notable properties such as biocompatibility, strong adhesion, and biodegradability. In dentistry, particularly in Glass Ionomer Cement (GIC), PAA contributes to dental cement, enhancing mechanical properties and setting reactions of calcium phosphate materials through crosslinking reactions. The chelation reaction between carboxyl groups (–COOH) from PAA and calcium ions from calcium phosphate contributes to scaffold stability.[5–7]

Investigation by Moradi et al.[5] discovered that a composite scaffold consisting of tricalcium phosphate and polyacrylic acid can be fabricated through a crosslinking reaction and freeze-drying process involving various reagents as precursors. Despite successful polymerization and achieving the desired mechanical properties, the research involved a complex mixing procedure with several chemicals. However, Putri et al.[7] suggested a simpler method involving the chelation reaction between calcium ions from βTCP and carboxyl groups from PAA, similar to setting mechanism in Glass Ionomer Cement. The current study will adopt a similar approach to mix βTCP and PAA.

Nonetheless, this study suggests utilizing natural source to obtain βTCP as an alternative to commercial βTCP, considering the availability of materials and cost considerations. Pu'ad et al.[8] claimed that calcium phosphate materials could be derived from natural sources such as mammalian bones, mineral stones, and shellfish, including green mussel shells.[8,9] The significant consumption of green mussels in Southeast Asian regions results in excessive shell waste. The high calcium carbonate content in green mussel shells suggests the potential utilization of this waste for producing calcium phosphate materials, such as βTCP.

Therefore, the objective of this study is to evaluate the feasibility of combining βTCP obtained from green mussel shells with PAA through a setting reaction and freeze-drying method.

MATERIALS AND METHODS

The green mussel shell waste was cleaned and heated to remove any remaining organic components. Afterward, it was 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 βTCP) and calcined at 1,000°C to obtain βTCP.

The βTCP powder was subsequently mixed with a PAA solution (Mw: ~250,000, 35 wt % in H_2O, Sigma Aldrich, USA) at three varying percentage ratios of βTCP to PAA: 60:40, 65:35, and 70:30. 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 βTCP from Sigma Aldrich to mix with PAA.

The porosities of the blocks were measured using the equations below, where the relative density was calculated by dividing the bulk density by the theoretical density of both βTCP (3.07 g/cm³) and PAA (1.15 g/cm³).[10,11] The diametral tensile strength (DTS) of the samples was measured utilizing a universal testing machine (UTM: AGS-X, Shimadzu, 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 (ANOVA) was employed to investigate significant differences among all groups. Following this, post hoc analysis was conducted using Tukey's honestly significant difference (HSD) test to compare significance between each group. The statistical analysis was performed using KaleidaGraph 4.01 software, and the significance level was set at $p < 0.05$.).

RESULTS

The mixtures of βTCP and PAA was successfully set into composite blocks, as illustrated in Figure 1. At macroscopic appearance, sample containing control βTCP displayed a smoother surface compared to other samples (Figure 1A). Moreover, as the quantity of βTCP powder derived from green mussel shells increased, the surface appeared to become rougher (Figure 1B-D).

Figure 2 demonstrates that at the same ratio, composite blocks incorporating βTCP derived from green mussel shells exhibit higher porosity (26.97 ± 2.64 %) compared to those containing commercial βTCP (13.40 ± 1.56 %). An increase in the powder amount from 70 % to 65 % resulted in an increase in porosity (35.00 ± 2.05 %). However, there was no significant difference in porosity observed between blocks containing 60 % (34.22 ± 1.84 %) and 65 % of βTCP.

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

DISCUSSION

The physical difference among all samples can be seen between the blocks containing commercial βTCP and those incorporating βTCP from green mussel shells. The larger particle size of βTCP from green mussel shells (up to 350 μm) and PAA results in a rougher surface in the composite blocks compared to those with commercial βTCP (particle size ranging from 1 to 1.8 μm).[12] This rougher surface has the potential to enhance cell attachment, thereby promoting better penetration of bone cells and ultimately increasing bone ingrowth.[13]

The difference in particle size also affects the porosity of the samples, with blocks containing commercial βTCP showing lower porosity compared to those incorporating βTCP 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, porosity significantly influences the mechanical properties of a material, as demonstrated in prior studies.[13–15] The outcomes of our study suggest that higher porosity in the samples corresponds to higher DTS values (Figure 2,3).

Incorporating a larger quantity of βTCP powder into the solution results in a more densely packed composite material, typically leading to reduced porosity.[11,16] 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 βTCP and PAA, a setting reaction occurred, forming bonds between the carboxylic acid (–COOH) group of PAA and calcium ions of βTCP, thereby creating connections among the βTCP particles. It is assumed that the larger particles in the βTCP would provide a broader surface on each particle, leading to a larger interface area. This, in turn, results in more extensive connections between the carboxylic acid (–COOH) group of PAA and calcium ions of βTCP, contributing to an enhancement in mechanical strength.[7,17]

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 needs to be conducted, including XRD, FTIR, and SEM. These additional physical characterizations are necessary to gain more detailed insights into their correlation with porosity and mechanical properties, which, in turn, would significantly impact the biological activity.

In conclusion, composite block was successfully fabricated by mixing βTCP 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 βTCP powder, the βTCP obtained from green mussel shells showed a significant increase in porosity, accompanied by a significantly higher DTS value. Moreover, a decrease in the amount of βTCP powder resulted in an increase in both porosity and DTS value.

ACKNOWLEDGEMENT

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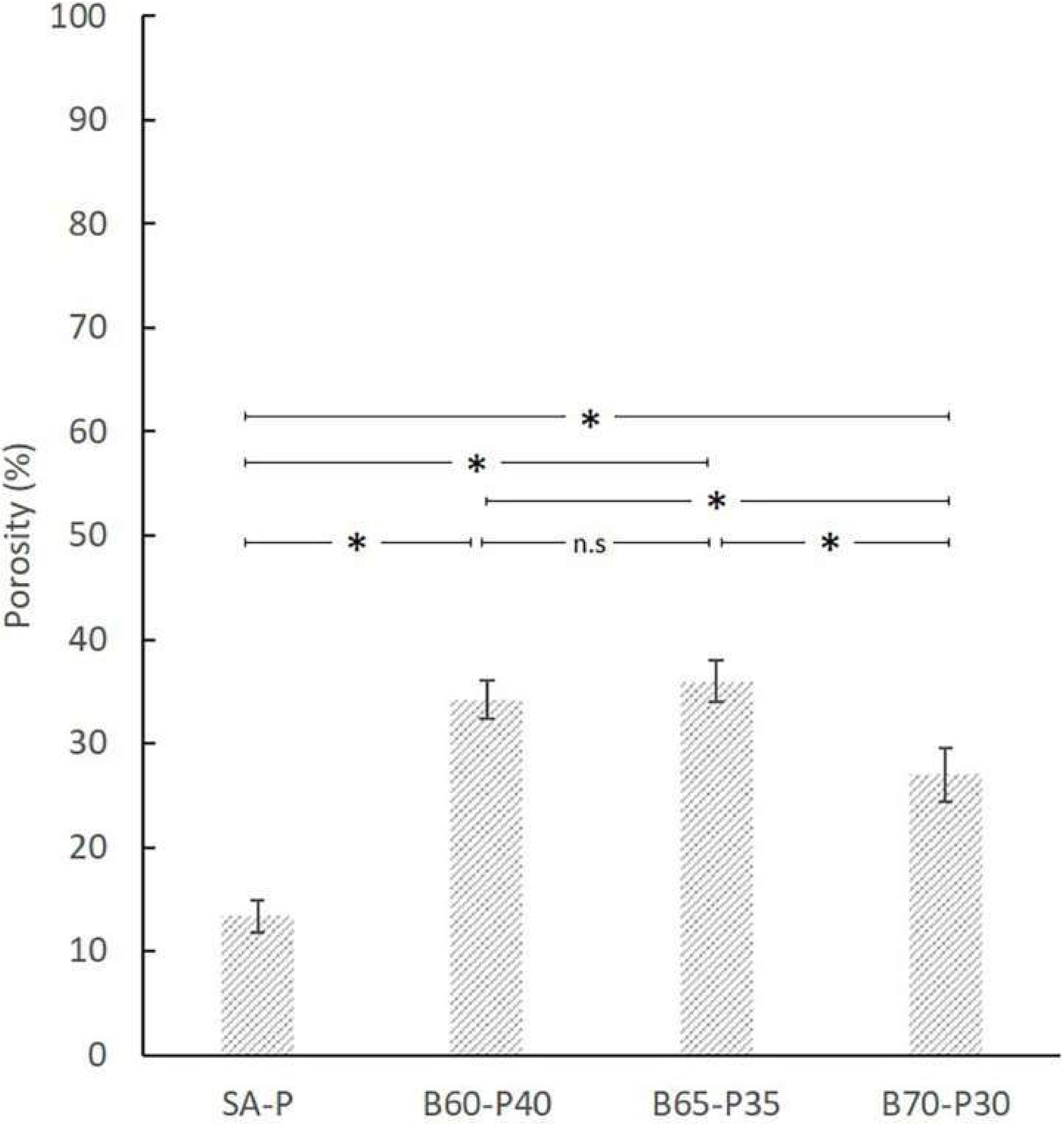
Figure Legends

Figure 1. Composite blocks containing 70 % Sigma-Aldrich βTCP Control with 30 % PAA (A), 60 % βTCP from green mussel shells with 40 % PAA (B), 65 % βTCP from green mussel shells with 35 % PAA (C), and 70 % βTCP from green mussel shells with 30 % PAA composite blocks (D).

Figure 2. Porosities of 70 % Sigma-Aldrich βTCP Control with 30 % PAA (SA-P), 60 % βTCP from green mussel shells with 40 % PAA (B60-P40), 65 % βTCP from green mussel shells with 35 % PAA (B65-P35), and 70 % βTCP from green mussel shells with 30 % PAA (B70-P30) composite blocks (n = 3). (* $p < 0.05$; n.s: not significant)

Figure 3. DTS values of 70 % Sigma-Aldrich βTCP Control with 30 % PAA (BC-PAA), 60 % βTCP from green mussel shells with 40 % PAA (B60-PAA), 65 % βTCP from green mussel shells with 35 % PAA (B65-PAA), and 70 % βTCP from green mussel shells with 30 % PAA (B70-PAA) composite blocks (n = 3). (* $p < 0.05$; n.s: not significant)


(A)
(B)
(C)
(D)
10 mm



Porosity (%)
100
90
80
70
60
50
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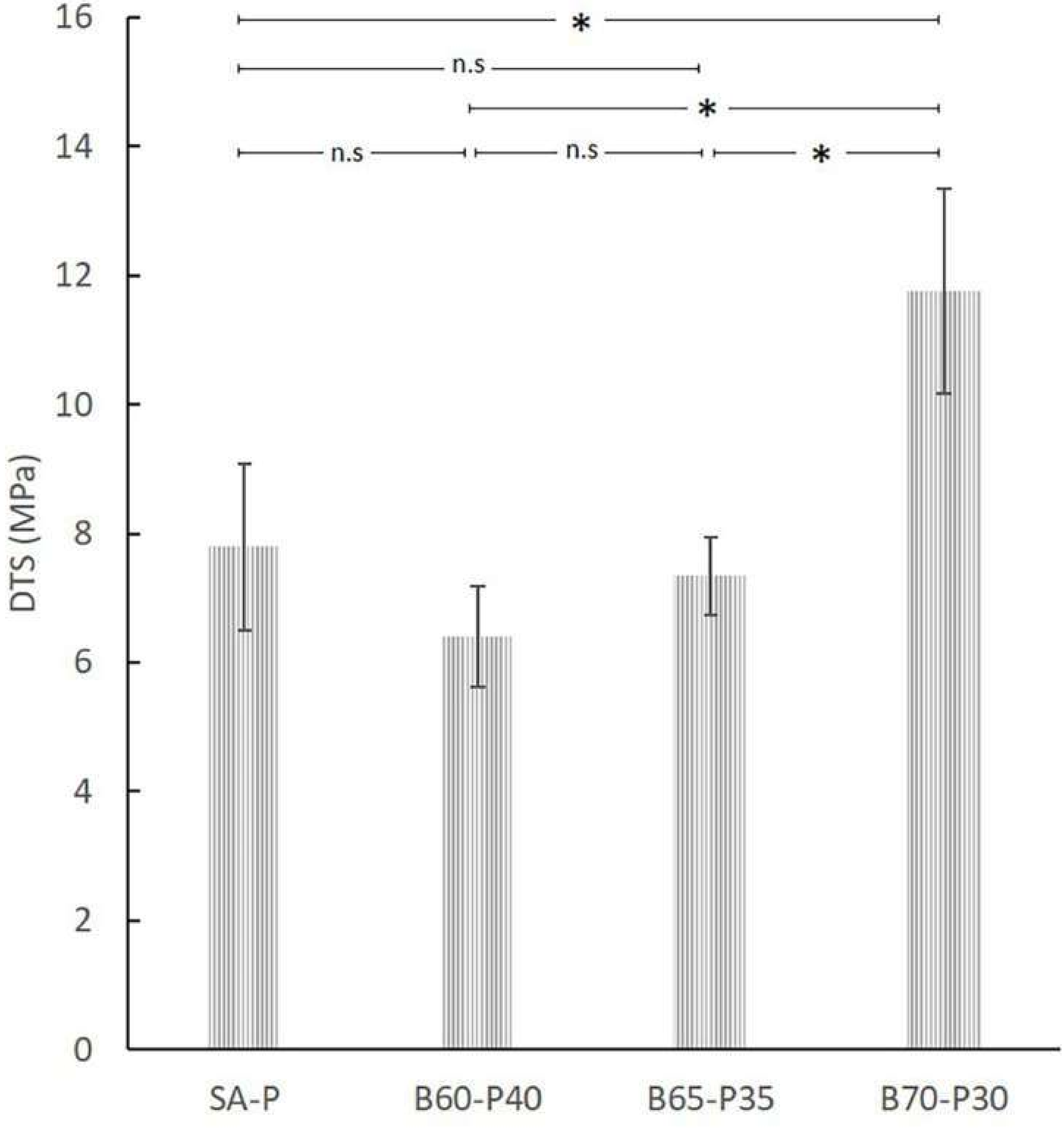
SA-P
B60-P40
B65-P35
B70-P30


16
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DTS (MPa)
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2. Bukti Review

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15 Mei 2025

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b) The purpose clearly? Comment:		
c) Methods and research design in accordance with the purpose of the study? Comment: **Each ratio is explained using how many grams of BTCP and how many ml or grams of PAA**		
d) The research procedure is described precisely and in detail, thus ensuring internal/external validity? Comment:		

e) The results can answer the research question? Comment:		
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Initial

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

ABSTRACT

Background: *Beta-tricalcium phosphate (βTCP) 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 βTCP. These shells, rich in calcium carbonate, provide a cost-effective and environmentally friendly alternative for βTCP synthesis.* ***Purpose:*** *This study aims to fabricate composite blocks from βTCP derived from green mussel shells, mixed with polyacrylic acid (PAA), using a setting reaction and freeze-drying method.* ***Methods:*** *βTCP powder was obtained via wet precipitation, starting with calcium carbonate from green mussel shells, converting it to calcium oxide, and then to βTCP. 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 βTCP had rougher surfaces due to larger particles compared to control blocks made with commercial βTCP (Sigma-Aldrich). Composite blocks with 70% green mussel βTCP and 30% PAA exhibited significantly higher porosity (26.97 ± 2.64%) and DTS (11.76 ± 1.59 MPa) than those made with commercial βTCP (porosity: 13.40 ± 1.56%; DTS: 7.79 ± 1.29 MPa). Reducing βTCP content to 60% resulted in increased porosity (34.22 ± 1.84%) and lower DTS (6.41 ± 0.78 MPa).* ***Conclusion:*** *Composite blocks from green mussel shell-derived βTCP and PAA showed higher porosity and significantly higher DTS than blocks made from commercial βTCP. Decreasing βTCP content increased porosity but decreased DTS.*

Keywords: *β-tricalcium phosphate; polyacrylic acid; freeze-drying; porosity; diametral tensile strength*

INTRODUCTION

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Therefore, the objective of this study is to evaluate the feasibility of combining βTCP obtained from green mussel shells with PAA through a setting reaction and freeze-drying method.

It is necessary to explain the lacking of the existing βTCP material so we are looking for an alternative βTCP Material from green mussel shells, and it is necessary to add the benefits of this research.

MATERIALS AND METHODS

The green mussel shell waste was cleaned and heated to remove any remaining organic components. Afterward, it was 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 βTCP) and calcined at 1,000°C to obtain βTCP.

The βTCP powder was subsequently mixed with a PAA solution (Mw: ~250,000, 35 wt % in H_2O, Sigma Aldrich, USA) at three varying percentage ratios of βTCP to PAA: 60:40, 65:35, and 70:30. 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 βTCP from Sigma Aldrich to mix with PAA.

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$$\text{Relative density (\%)} = \frac{\text{bulk density}}{\text{theoretical density}} \times 100\ (\%)$$

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

Each ratio is explained using how many grams of BTCP and how many ml or grams of PAA

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

RESULTS

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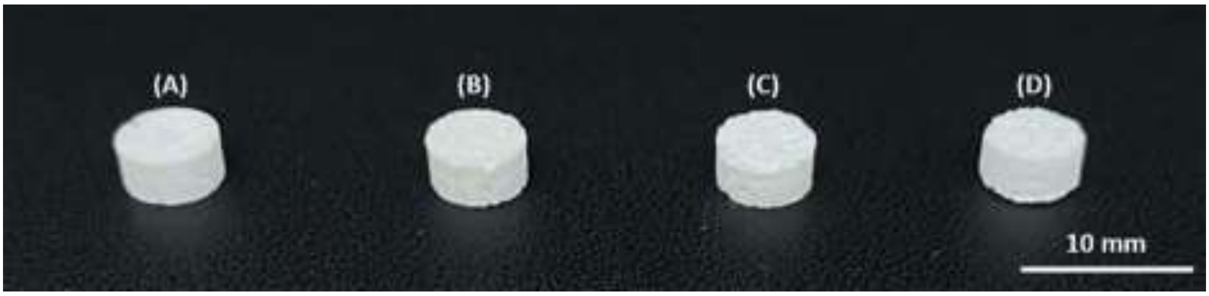

Figure 1. Composite blocks containing 70 % Sigma-Aldrich βTCP Control with 30 % PAA (A), 60 % βTCP from green mussel shells with 40 % PAA (B), 65 % βTCP from green mussel shells with 35 % PAA (C), and 70 % βTCP from green mussel shells with 30 % PAA composite blocks (D).

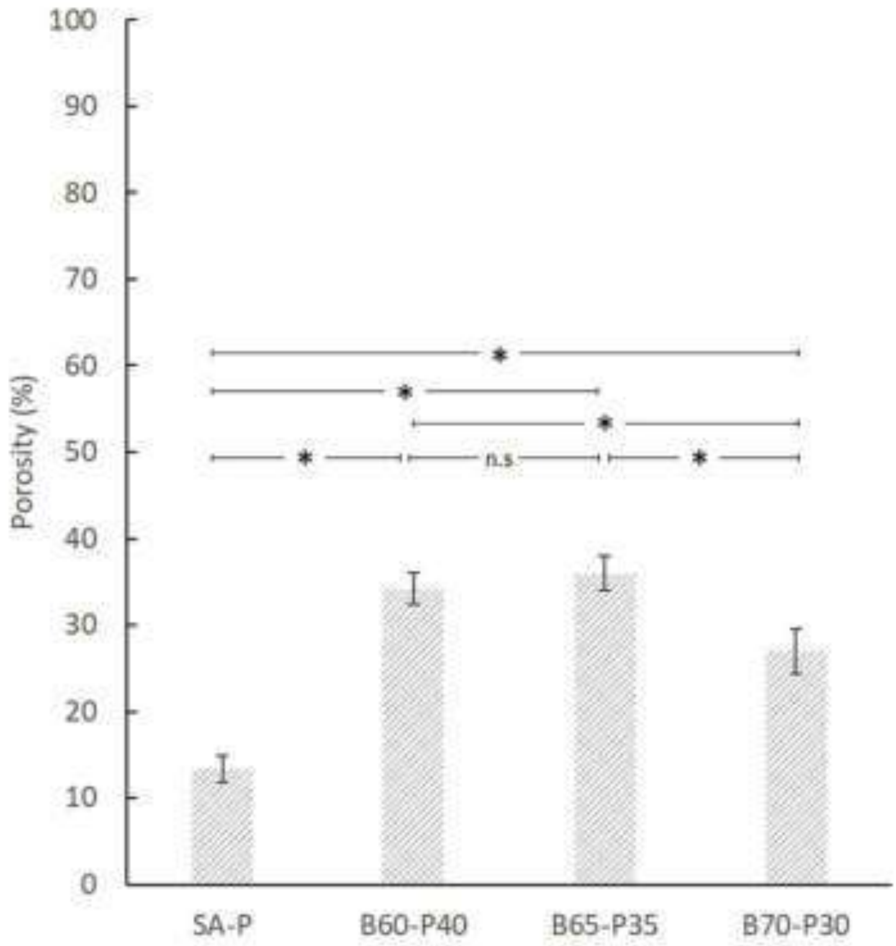

Figure 2. Porosities of 70 % Sigma-Aldrich βTCP Control with 30 % PAA (SA-P), 60 % βTCP from green mussel shells with 40 % PAA (B60-P40), 65 % βTCP from green mussel shells with 35 % PAA (B65-P35), and 70 % βTCP from green mussel shells with 30 % PAA (B70-P30) composite blocks (n = 3). (* $p < 0.05$; n.s: not significant)

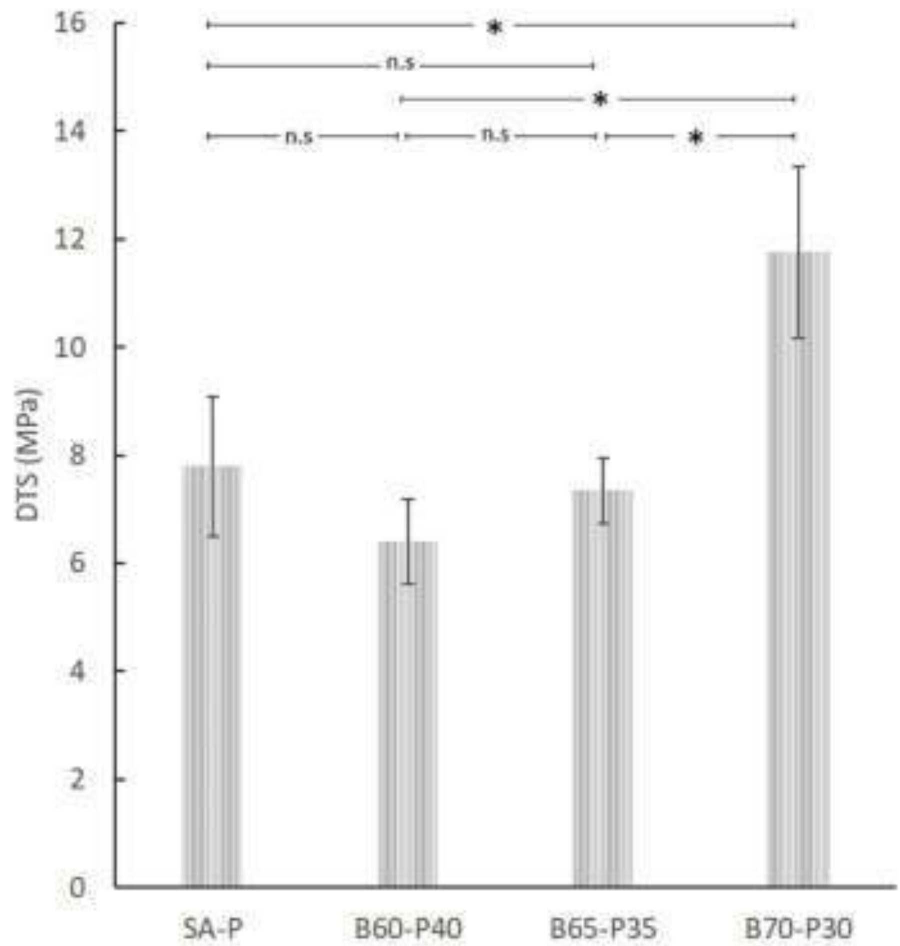

Figure 3. DTS values of 70 % Sigma-Aldrich βTCP Control with 30 % PAA (BC-PAA), 60 % βTCP from green mussel shells with 40 % PAA (B60-PAA), 65 % βTCP from green mussel shells with 35 % PAA (B65-PAA), and 70 % βTCP from green mussel shells with 30 % PAA (B70-PAA) composite blocks (n = 3). (* $p < 0.05$; n.s: not significant)

DISCUSSION

The physical difference among all samples can be seen between the blocks containing commercial βTCP and those incorporating βTCP from green mussel shells. The larger particle size of βTCP from green mussel shells (up to 350 µm) and PAA results in a rougher surface in the composite blocks compared to those with commercial βTCP (particle size ranging from 1 to 1.8 µm).[12] This rougher surface has the potential to enhance cell attachment, thereby promoting better penetration of bone cells and ultimately increasing bone ingrowth.[13] **Please explain why the particle size of βTCP from green mussel shells is larger than commercial βTCP**

The difference in particle size also affects the porosity of the samples, with blocks containing commercial βTCP showing lower porosity compared to those incorporating βTCP 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, porosity significantly influences the mechanical properties of a material, as demonstrated in prior studies.[13–15] The outcomes of our study suggest that higher porosity in the samples corresponds to higher DTS values (Figure 2,3).

Incorporating a larger quantity of βTCP powder into the solution results in a more densely packed composite material, typically leading to reduced porosity.[11,16] 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 βTCP and PAA, a setting reaction occurred, forming bonds between the carboxylic acid (–COOH) group of PAA and calcium ions of βTCP, thereby creating connections among the βTCP particles. It is assumed that the larger particles in the βTCP would provide a broader surface on each particle, leading to a larger interface area. This, in turn, results in more extensive connections between the carboxylic acid (–COOH) group of PAA and calcium ions of βTCP, contributing to an enhancement in mechanical strength.[7,17]

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 needs to be conducted, including XRD, FTIR, and SEM. These additional physical characterizations are necessary to gain more detailed insights into their correlation with porosity and mechanical properties, which, in turn, would significantly impact the biological activity.

In conclusion, **the** composite block was successfully fabricated by mixing βTCP 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 βTCP powder, the βTCP obtained from green mussel shells showed a significant increase in porosity, accompanied by a significantly higher DTS value. Moreover, a decrease in the amount of βTCP powder resulted in an increase in both porosity and DTS value.

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ASSESSMENT FORM FOR MANAGING EDITOR (EDITING LANGUAGE AND FORMATTING SCRIPT)

Manuscript Title: **Fabrication of composite block from β-tricalcium phosphate and polyacrylic acid via freeze-drying method**

Please comments written in English

REVIEW	COMMENT*
FORMAT	
Is the length of the script sufficient? ▪ (10-12 pages, one and a half space, A4 format, 12 pt Times New Roman font)	Yes/ No
▪ Sections of the contents on the script are proportional (The discussion is longer than the introduction	Yes/ No
Title	
▪ In accordance with the problem, purpose and contain the main variable	Yes/ No
▪ Not too long (maximum of 10 words) with the first word starting with a capital letter	Yes/ No
Abstract	
▪ A concise (maximum 250 words), single space, structured in one paragraph	Yes/ No
▪ Keywords correspond to major variables/ main concept in English	Yes/ No
▪ Keywords must be provided with 5 words and/or a phrase and separated by a semicolon (;)	Yes/ No
Figures and Tables	
▪ In accordance with the style of the journal (the truth, the completeness of the title and description/ legend) and included reference	Yes/ No
▪ Number of figure/ tables in the research report and literature review maximum 4	Yes/ No
▪ Number of figure/ tables in the case report maximum 8	Yes/ No
▪ Figures/ tables are written separately with text	Yes/ No
References (*Vancouver superscript no et al format*)	
▪ In accordance with the style of the journal (*Vancouver superscript no et al format*)	Yes/ No
▪ The last 10 years	Yes/ No
▪ Primary reference ≥ 70% (journals, books, patent documents)	Yes/ No
▪ Volume/ number and journal page already listed	Yes/ No

▪ Editions, Publisher, City and book pages are listed	Yes/ No
▪ In sequence on the text	Yes/ No
▪ Author name written all (no et al format)	Yes/ No
▪ Consistency of the author's name increase	Yes/ No
▪ From the internet include website address and access time	Yes/ No
▪ Abbreviate journal title according to the dental index and medical index	Yes/ No
Language	
▪ Not enumerative	Yes/ No
▪ No typos	Yes/ No
▪ Standard spelling	Yes/ No
▪ Standard sentence (subject, predicate, object)	Yes/ No
▪ One paragraph, one subject (more than two sentences)	Yes/ No
ORIGINAL ARTICLE	
FORMAT	
▪ **Abstracts** in original research should consist of **Background:**, **Purpose:**, **Methods:**, **Results:** and **Conclusion:** typed in bold within one paragraph	Yes/ No
▪ Systematic manuscript of the original research consists of the introduction, materials and methods, results, discussion ended conclusion, and references.	Yes/ No
▪ No Sub bab (Subtitle) in each section	Yes/ No
Introduction	
▪ Empirical/ theoretical background	There is/ No
▪ Problems/ goals	There is/ No
▪ Purpose of the study	There is/ No
Materials and Methods	
▪ Design (type, time, place of the study)	There is/ No
▪ Sampling technique	There is/ No
▪ How research works	There is/ No
▪ Data analysis	There is/ No
Results	

▪ Exposure data	There is/ No
▪ Analyze results	There is/ No
Discussion	
▪ Discussion does not repeat the results	Yes/ No
▪ Aligned with the scope of the study and compared with similar research results?	Yes/ No
▪ Explain the meaning of research results and answer the problem	Yes/ No
▪ Conclusion	There is/ No
▪ Suggestions	There is/ No
CASE REPORT	
FORMAT	
▪ Systematic manuscript of case reports consists of introduction, cases, case management, discussion ended conclusion, and references.	Yes/ No
▪ No Sub bab (Subtitle) in each section	Yes/ No
▪ **Abstracts** in case reports should consist of **Background:**, **Purpose:**, **Case(s):**, **Case Management:** and **Conclusion:** typed in bold within one paragraph	Yes/ No
REVIEW ARTICLE	
FORMAT	
▪ Systematic manuscript of literature reviews consists of introduction, the content section (consisting of the subtitle case), and the discussion ended conclusion, and references. ▪ **Abstracts** in literature reviews should be divided into **Background:**, **Purpose:**, **Review:,** and **Conclusion**:, typed in bold within one paragraph	Yes/ No
▪ References (More than 30 references)	Yes/ No

Note:
1. *) Cross the unnecessary ones.
2. If there is no match between the author and the editor should be reconciled to obtain a solution.

Recommendation from Managing Editor

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Date: ...24-1-2025...

Managing Editor,

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ASSESSMENT FORM FOR MANAGING EDITOR (EDITING LANGUAGE AND FORMATTING SCRIPT)

Manuscript Title: **Fabrication of composite block from β-tricalcium phosphate and polyacrylic acid via freeze-drying method**

Please comments written in English

REVIEW	COMMENT*
FORMAT	
Is the length of the script sufficient? ▪ (10-12 pages, one and a half space, A4 format, 12 pt Times New Roman font)	No, Too Short
▪ Sections of the contents on the script are proportional (The discussion is longer than the introduction	No, both sections need improvement
Title	
▪ In accordance with the problem, purpose and contain the main variable	Yes
▪ Not too long (maximum of 10 words) with the first word starting with a capital letter	Yes
Abstract	
▪ A concise (maximum 250 words), single space, structured in one paragraph	245
▪ Keywords correspond to major variables/ main concept in English	Yes
▪ Keywords must be provided with 5 words and/or a phrase and separated by a semicolon (;)	Yes, please add SDGs keywords (Medicine)
Figures and Tables	
▪ In accordance with the style of the journal (the truth, the completeness of the title and description/ legend) and included reference	Yes
▪ Number of figure/ tables in the research report and literature review maximum 4	Yes
▪ Number of figure/ tables in the case report maximum 8	Yes
▪ Figures/ tables are written separately with text	Yes
References (*Vancouver superscript no et al format*)	
▪ In accordance with the style of the journal (*Vancouver superscript no et al format*)	Yes
▪ The last 10 years	Yes
▪ Primary reference ≥ 70% (journals, books, patent documents)	Yes

▪ Volume/ number and journal page already listed	Yes
▪ Editions, Publisher, City and book pages are listed	Yes
▪ In sequence on the text	Yes
▪ Author name written all (no et al format)	Yes
▪ Consistency of the author's name increase	Yes
▪ From the internet include website address and access time	Yes
▪ Abbreviate journal title according to the dental index and medical index	Yes
Language	
▪ Not enumerative	Yes
▪ No typos	Yes
▪ Standard spelling	Yes
▪ Standard sentence (subject, predicate, object)	Yes
▪ One paragraph, one subject (more than two sentences)	Yes
ORIGINAL ARTICLE	
FORMAT	
▪ **Abstracts** in original research should consist of **Background:**, **Purpose:**, **Methods:**, **Results:** and **Conclusion:** typed in bold within one paragraph	Yes
▪ Systematic manuscript of the original research consists of the introduction, materials and methods, results, discussion ended conclusion, and references.	Yes
▪ No Sub bab (Subtitle) in each section	Yes
Introduction	
▪ Empirical/ theoretical background	No
▪ Problems/ goals	No
▪ Purpose of the study	There i/
Materials and Methods	
▪ Design (type, time, place of the study)	There is
▪ Sampling technique	There is
▪ How research works	There is
▪ Data analysis	There is

Results	
▪ Exposure data	No
▪ Analyze results	There is
Discussion	
▪ Discussion does not repeat the results	No
▪ Aligned with the scope of the study and compared with similar research results?	No
▪ Explain the meaning of research results and answer the problem	No
▪ Conclusion	There is
▪ Suggestions	No

Note:

1. *) Cross the unnecessary ones.
2. If there is no match between the author and the editor should be reconciled to obtain a solution.

Recommendation from Managing Editor

[.......] 1. The manuscript can be processed without repair by the author.

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Date: .2025.04.16

Managing Editor

NPA

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

ABSTRACT

Background: *Beta-tricalcium phosphate (βTCP) 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 βTCP. These shells, rich in calcium carbonate, provide a cost-effective and environmentally friendly alternative for βTCP synthesis.* ***Purpose:*** *This study aims to fabricate composite blocks from βTCP derived from green mussel shells, mixed with polyacrylic acid (PAA), using a setting reaction and freeze-drying method.* ***Methods:*** *βTCP powder was obtained via wet precipitation, starting with calcium carbonate from green mussel shells, converting it to calcium oxide, and then to βTCP. 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 βTCP had rougher surfaces due to larger particles compared to control blocks made with commercial βTCP (Sigma-Aldrich). Composite blocks with 70% green mussel βTCP and 30% PAA exhibited significantly higher porosity (26.97 ± 2.64%) and DTS (11.76 ± 1.59 MPa) than those made with commercial βTCP (porosity: 13.40 ± 1.56%; DTS: 7.79 ± 1.29 MPa). Reducing βTCP content to 60% resulted in increased porosity (34.22 ± 1.84%) and lower DTS (6.41 ± 0.78 MPa).* ***Conclusion:*** *Composite blocks from green mussel shell-derived βTCP and PAA showed higher porosity and significantly higher DTS than blocks made from commercial βTCP. Decreasing βTCP content increased porosity but decreased DTS.*

Keywords: *β-tricalcium phosphate; polyacrylic acid; freeze-drying; porosity; diametral tensile strength; medicine.*

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INTRODUCTION

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Apart from its osteoconductivity, the higher bioreabsorbability of beta-tricalcium phosphate (βTCP) compared to hydroxyapatite makes it a popular choice in orthopedic and oral surgery applications. This characteristic is expected to promote improved bone formation while replacing the resorbed materials materials.[1–3] The combination of beta-tricalcium phosphate (βTCP) with polymer has been an attractive material of choice for its application for bone substitution.[4] Polyacrylic acid is a synthetic polymer which exhibits notable properties such as biocompatibility, strong adhesion, and biodegradability. In dentistry, particularly in Glass Ionomer Cement (GIC), PAA contributes to dental cement, enhancing mechanical properties and setting reactions of calcium phosphate materials through crosslinking reactions. The chelation reaction between carboxyl groups (–COOH) from PAA and calcium ions from calcium phosphate contributes to scaffold stability.[5–7]

Investigation by Moradi et al.[5] discovered that a composite scaffold consisting of tricalcium phosphate and polyacrylic acid can be fabricated through a crosslinking reaction and freeze-drying process involving various reagents as precursors. Despite successful polymerization and achieving the desired mechanical properties, the research involved a complex mixing procedure with several chemicals. However, Putri et al.[7] suggested a simpler method involving the chelation reaction between calcium ions from βTCP and carboxyl groups from PAA, similar to setting mechanism in Glass Ionomer Cement. The current study will adopt a similar approach to mix βTCP and PAA.

Nonetheless, this study suggests utilizing natural source to obtain βTCP as an alternative to commercial βTCP, considering the availability of materials and cost considerations. Pu'ad et al.[8] claimed that calcium phosphate materials could be derived from natural sources such as mammalian bones, mineral stones, and shellfish, including green mussel shells.[8,9] The significant consumption of green mussels in Southeast Asian regions results in excessive shell waste. The high calcium carbonate content in green mussel shells suggests the potential utilization of this waste for producing calcium phosphate materials, such as βTCP.

Therefore, the objective of this study is to evaluate the feasibility of combining βTCP obtained from green mussel shells with PAA through a setting reaction and freeze-drying method.

MATERIALS AND METHODS

The green mussel shell waste was cleaned and heated to remove any remaining organic components. Afterward, it was 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 βTCP) and calcined at 1,000°C to obtain βTCP.

The βTCP powder was subsequently mixed with a PAA solution (Mw: ~250,000, 35 wt % in H_2O, Sigma Aldrich, USA) at three varying percentage ratios of βTCP to PAA: 60:40, 65:35, and 70:30. The resulting mixture was placed into a mold with a diameter of 6 mm and

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How to treat the sample before the BTCP isolation process?

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 βTCP from Sigma Aldrich to mix with PAA.

The porosities of the blocks were measured using the equations below, where the relative density was calculated by dividing the bulk density by the theoretical density of both βTCP (3.07 g/cm^3) and PAA (1.15 g/cm^3).[10,11] The diametral tensile strength (DTS) of the samples was measured utilizing a universal testing machine (UTM: AGS-X, Shimadzu, 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 (ANOVA) was employed to investigate significant differences among all groups. Following this, post hoc analysis was conducted using Tukey's honestly significant difference (HSD) test to compare significance between each group. The statistical analysis was performed using KaleidaGraph 4.01 software, and the significance level was set at $p < 0.05$.).

RESULTS

The mixtures of βTCP and PAA was successfully set into composite blocks, as illustrated in **Figure 1**. At macroscopic appearance, sample containing control βTCP displayed a smoother surface compared to other samples (**Figure 1A**). Moreover, as the quantity of βTCP powder derived from green mussel shells increased, the surface appeared to become rougher (**Figure 1B-D**).

Figure 2 demonstrates that at the same ratio, composite blocks incorporating βTCP derived from green mussel shells exhibit higher porosity (26.97 ± 2.64 %) compared to those containing commercial βTCP (13.40 ± 1.56 %). An increase in the powder amount from 70 % to 65 % resulted in an increase in porosity (35.00 ± 2.05 %). However, there was no significant difference in porosity observed between blocks containing 60 % (34.22 ± 1.84 %) and 65 % of βTCP.

The DTS values of the samples are shown in Figure 3. At a ratio of 70 % βTCP and 30 % PAA, those containing βTCP from green mussel shells (11.76 ± 1.59 MPa) displayed a significant increase in DTS value compared to those with commercial βTCP (7.79 ± 1.29 MPa).

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However, reducing the powder amount to 65 % resulted in a significant decrease in DTS value (7.35 ± 0.61 MPa). Nonetheless, there was no significant difference observed between blocks with 60 % (6.41 ± 0.78 MPa) and 65 % βTCP.

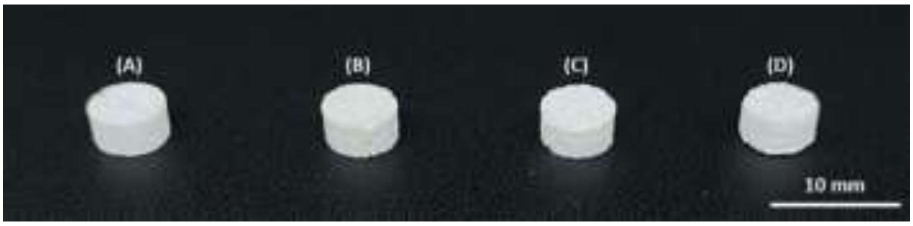

Figure 1. Composite blocks containing 70 % Sigma-Aldrich βTCP Control with 30 % PAA (A), 60 % βTCP from green mussel shells with 40 % PAA (B), 65 % βTCP from green mussel shells with 35 % PAA (C), and 70 % βTCP from green mussel shells with 30 % PAA composite blocks (D).

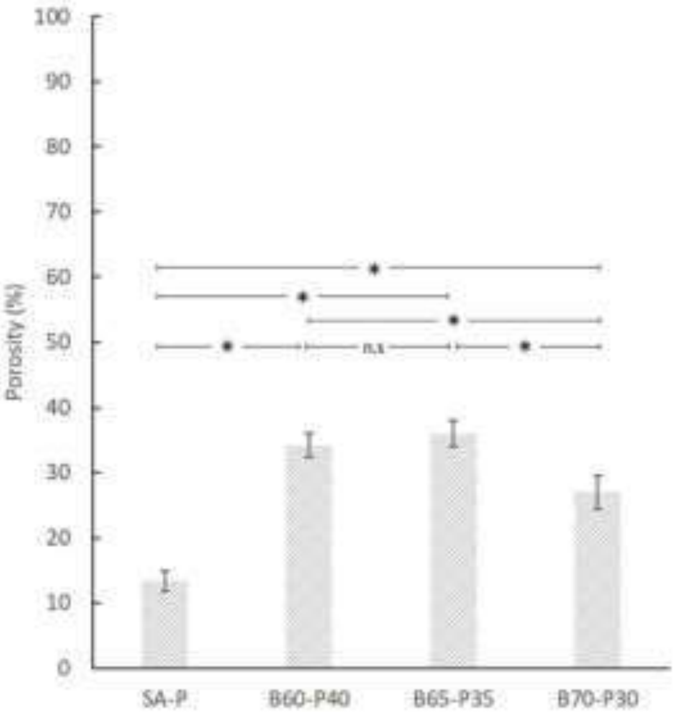

Figure 2. Porosities of 70 % Sigma-Aldrich βTCP Control with 30 % PAA (SA-P), 60 % βTCP from green mussel shells with 40 % PAA (B60-P40), 65 % βTCP from green mussel shells with 35 % PAA (B65-P35), and 70 % βTCP from green mussel shells with 30 % PAA (B70-P30) composite blocks (n = 3). (* $p < 0.05$; n.s: not significant)

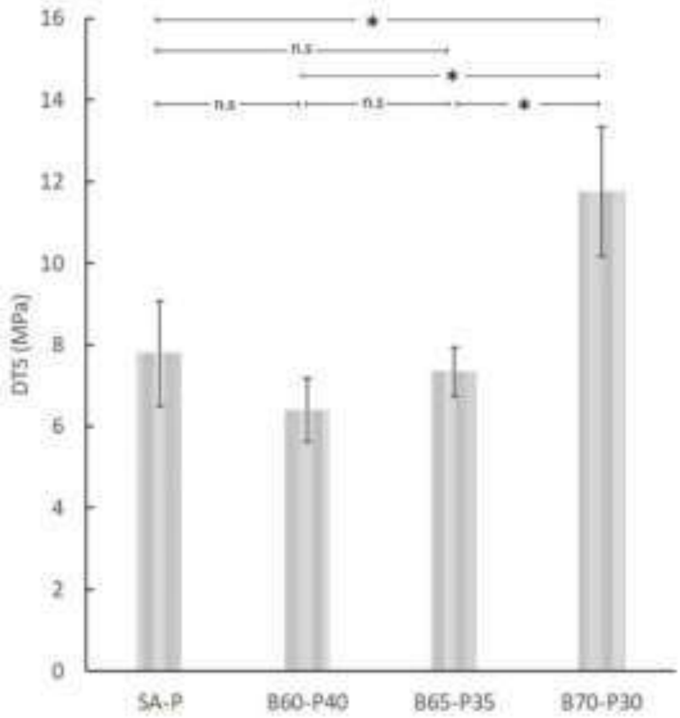

Figure 3. DTS values of 70 % Sigma-Aldrich βTCP Control with 30 % PAA (BC-PAA), 60 % βTCP from green mussel shells with 40 % PAA (B60-PAA), 65 % βTCP from green mussel shells with 35 % PAA (B65-PAA), and 70 % βTCP from green mussel shells with 30 % PAA (B70-PAA) composite blocks (n = 3). (* $p < 0.05$; n.s: not significant)

DISCUSSION

The physical difference among all samples can be seen between the blocks containing commercial βTCP and those incorporating βTCP from green mussel shells. The larger particle size of βTCP from green mussel shells (up to 350 μm) and PAA results in a rougher surface in the composite blocks compared to those with commercial βTCP (particle size ranging from 1 to 1.8 μm).[12] This rougher surface has the potential to enhance cell attachment, thereby promoting better penetration of bone cells and ultimately increasing bone ingrowth.[13]

The difference in particle size also affects the porosity of the samples, with blocks containing commercial βTCP showing lower porosity compared to those incorporating βTCP from green mussel shells (Figure 2). Porosity plays a crucial role in facilitating cell attachment

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Incorporating a larger quantity of βTCP powder into the solution results in a more densely packed composite material, typically leading to reduced porosity.[11,16] 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 βTCP and PAA, a setting reaction occurred, forming bonds between the carboxylic acid (–COOH) group of PAA and calcium ions of βTCP, thereby creating connections among the βTCP particles. It is assumed that the larger particles in the βTCP would provide a broader surface on each particle, leading to a larger interface area. This, in turn, results in more extensive connections between the carboxylic acid (–COOH) group of PAA and calcium ions of βTCP, contributing to an enhancement in mechanical strength.[7,17]

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 needs to be conducted, including XRD, FTIR, and SEM. These additional physical characterizations are necessary to gain more detailed insights into their correlation with porosity and mechanical properties, which, in turn, would significantly impact the biological activity.

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In conclusion, composite block was successfully fabricated by mixing βTCP 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 βTCP powder, the βTCP obtained from green mussel shells showed a significant increase in porosity, accompanied by a significantly higher DTS value. Moreover, a decrease in the amount of βTCP powder resulted in an increase in both porosity and DTS value.

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REFERENCES

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Original article

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

ABSTRACT

Background: *Beta-tricalcium phosphate (βTCP) 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 βTCP. These shells, rich in calcium carbonate, provide a cost-effective and environmentally friendly alternative for βTCP synthesis.* ***Purpose:*** *This study aims to fabricate composite blocks from βTCP derived from green mussel shells, mixed with polyacrylic acid (PAA), using a setting reaction and freeze-drying method.* ***Methods:*** *βTCP powder was obtained via wet precipitation, starting with calcium carbonate from green mussel shells, converting it to calcium oxide, and then to βTCP. 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 βTCP had rougher surfaces due to larger particles compared to control blocks made with commercial βTCP (Sigma-Aldrich). Composite blocks with 70% green mussel βTCP and 30% PAA exhibited significantly higher porosity (26.97 ± 2.64%) and DTS (11.76 ± 1.59 MPa) than those made with commercial βTCP (porosity: 13.40 ± 1.56%; DTS: 7.79 ± 1.29 MPa). Reducing βTCP content to 60% resulted in increased porosity (34.22 ± 1.84%) and lower DTS (6.41 ± 0.78 MPa).* ***Conclusion:*** *Composite blocks from green mussel shell-derived βTCP and PAA showed higher porosity and significantly higher DTS than blocks made from commercial βTCP. Decreasing βTCP content increased porosity but decreased DTS.*

Keywords: *β-tricalcium phosphate; polyacrylic acid; freeze-drying; porosity; diametral tensile strength; medicine.*

INTRODUCTION

Apart from its osteoconductivity, the higher bioreabsorbability of beta-tricalcium phosphate (βTCP) compared to hydroxyapatite makes it a popular choice in orthopedic and oral surgery applications. In this field, βTCP 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 that matches new bone formation. Clinically, βTCP 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 βTCP 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 materials.[1–3] The combination of beta-tricalcium phosphate (βTCP) with polymer has been an attractive material of choice for its application for bone substitution.[4] Polyacrylic acid (PAA) is a synthetic polymer which exhibits notable properties such as biocompatibility, strong adhesion, and biodegradability. In dentistry, particularly in Glass Ionomer Cement (GIC), PAA contributes to dental cement, enhancing mechanical properties and setting reactions of calcium phosphate materials through crosslinking reactions. The chelation reaction between carboxyl groups (–COOH) from PAA and calcium ions from calcium phosphate contributes to scaffold stability.[5–7]

Investigation by Moradi et al.[5] discovered that a composite scaffold consisting of tricalcium phosphate and polyacrylic acid can be fabricated through a crosslinking reaction and freeze-drying process involving various reagents as precursors. Despite successful polymerization and achieving the desired mechanical properties, the research involved a complex mixing procedure with several chemicals. However, Putri et al.[7] suggested a simpler method involving the chelation reaction between calcium ions from βTCP and carboxyl groups from PAA, similar to setting mechanism in Glass Ionomer Cement. The current study will adopt a similar approach to mix βTCP and PAA.

Despite the advantages of βTCP, its widespread clinical application is limited by factors such as high production cost, limited availability in some regions, and reliance on synthetic or mined sources. These drawbacks motivate the exploration of alternative, cost-effective, and sustainable sources for producing βTCP. Natural calcium-rich waste materials such as mammalian bones, coral, and shellfish have been reported as potential candidates for synthesizing calcium phosphate materials.[8] Among them, green mussel shells (Perna viridis), which are abundantly consumed in Southeast Asia, 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 βTCP.[8,9]

The novelty of this study is the development of a βTCP–PAA composite block using βTCP made from green mussel shells through a simple setting and freeze-drying process. This research introduces a new, low-cost source for biomaterials and shows that waste from green mussels can produce materials with similar or better physical and mechanical properties than commercial βTCP. Compared to other synthetic biomaterials like hydroxyapatite, βTCP 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 βTCP 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.[8,9]

βTCP synthesized from green mussel shells, when combined with polyacrylic acid (PAA) through a setting and freeze-drying process, will produce composite blocks with comparable or improved physical and mechanical properties, such as porosity and diametral tensile strength, compared to commercial βTCP based composites, making it a viable, cost-effective alternative biomaterial for bone tissue engineering. Therefore, the objective of this study is to evaluate the feasibility of combining βTCP obtained 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. Afterward, it was 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 βTCP) and calcined at 1,000°C to obtain βTCP.

The βTCP powder was subsequently mixed with a PAA solution (Mw: ~250,000, 35 wt % in H_2O, Sigma Aldrich, St. Louis, Missouri, USA) at three varying percentage ratios of βTCP to PAA: 60:40, 65:35, and 70:30. Each ratio was prepared with a total mixture weight of 2 grams. For the 60:40 ratio, 1.2 grams of βTCP was mixed with 0.8 grams of PAA solution; for the 65:35 ratio, 1.3 grams of βTCP was mixed with 0.7 grams of PAA solution; and for the 70:30 ratio, 1.4 grams of βTCP was mixed with 0.6 grams 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 βTCP (Sigma Aldrich, St. Louis, Missouri, USA) to mix with PAA.

The porosities of the blocks were measured using the equations below, where the relative density was calculated by dividing the bulk density by the theoretical density of both βTCP (3.07 g/cm³) and PAA (1.15 g/cm³).[10,11] The diametral tensile strength (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.

$$\text{Relative density (\%)} = \frac{\text{bulk density}}{\text{theoretical density}} \times 100 \text{ (\%)}$$

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

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

RESULTS

The mixtures of βTCP and PAA was successfully set into composite blocks, as illustrated in Figure 1. At macroscopic appearance, sample containing control βTCP displayed a smoother surface compared to other samples (**Figure 1A**). Moreover, as the quantity of βTCP powder derived from green mussel shells increased, the surface appeared to become rougher (**Figure 1B-D**).

Figure 2 demonstrates that at the same ratio, composite blocks incorporating βTCP derived from green mussel shells exhibit higher porosity (26.97 ± 2.64 %) compared to those containing commercial βTCP (13.40 ± 1.56 %). An increase in the powder amount from 70 % to 65 % resulted in an increase in porosity (35.00 ± 2.05 %). However, there was no significant difference in porosity observed between blocks containing 60 % (34.22 ± 1.84 %) and 65 % of βTCP.

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

DISCUSSION

This study shows that composite blocks made from βTCP derived from green mussel shells mixed with polyacrylic acid (PAA) can be successfully made using a simple setting and freeze-drying process. This is new because it uses a natural, local, and low-cost material as an alternative to expensive commercial βTCP, which helps reduce waste and cost.[8,9] Our results found important differences in properties between blocks made with green mussel βTCP and those made with commercial βTCP.

The physical difference among all samples can be seen between the blocks containing commercial βTCP and those incorporating βTCP from green mussel shells. The larger particle size of βTCP from green mussel shells (up to 350 μm) and PAA results in a rougher surface in the composite blocks compared to those with commercial βTCP (particle size ranging from 1 to 1.8 μm).[12] This difference is due to the synthesis and processing methods. The green mussel-derived βTCP was prepared through manual grinding and calcination, without further milling or sieving to reduce particle size, resulting in coarser particles. In contrast, commercial βTCP typically undergoes industrial-scale processing with controlled milling and sieving steps to produce finer and more uniform particles. However, the rougher surface resulted on the composite blocks has the potential to enhance cell attachment, thereby promoting better penetration of bone cells and ultimately increasing bone ingrowth.[13]

The difference in particle size also affects the porosity of the samples, with blocks containing commercial βTCP showing lower porosity compared to those incorporating βTCP 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, porosity significantly influences the mechanical properties of a material, as demonstrated in prior studies.[13–15] The outcomes of our study suggest that higher porosity in the samples corresponds to higher DTS values (Figure 2,3).

Incorporating a larger quantity of βTCP powder into the solution results in a more densely packed composite material, typically leading to reduced porosity.[11,16] 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 βTCP and PAA, a setting reaction occurred, forming bonds between the carboxylic acid (–COOH) group of PAA and calcium ions of βTCP, thereby creating connections among the βTCP particles. It is assumed that the larger particles in the βTCP would provide a broader surface on each particle, leading to a larger interface area. This, in turn, results in more extensive connections between the carboxylic acid (–COOH) group of PAA and calcium ions of βTCP, contributing to an enhancement in mechanical strength.[7,17]

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 needs to be conducted, including XRD, FTIR, SEM, and degradation as well as swelling properties. These additional physical characterizations are necessary to gain more detailed insights into their correlation with porosity and mechanical properties, which, in turn, would significantly impact the biological activity. These limitations in material characterization make it difficult to fully explain the structure–property relationships observed. On the other hand, no biological tests (such as cytotoxicity, cell attachment, or *in vivo* performance) were performed to confirm its biocompatibility and effectiveness. Also, manual grinding without size control may lead to inconsistent particle size, affecting reproducibility. Lastly, only one type of commercial βTCP was used for comparison, and the setting behavior of PAA with different βTCP sources may vary.

In conclusion, the composite block was successfully fabricated by mixing βTCP 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 βTCP powder, the βTCP obtained from green mussel shells showed a significant increase in porosity, accompanied by a significantly higher DTS value. Moreover, a decrease in the amount of βTCP powder resulted in an increase in both porosity and DTS value. In future studies, more detailed material testing should be done to better understand the structure and composition of the composite. Biological tests like cytotoxicity, cell attachment, and *in vivo* studies are also needed to confirm its safety and effectiveness for bone repair. Controlling the particle size through milling and sieving is recommended to improve consistency. It would also be useful to study how PAA reacts with different types of βTCP and compare other natural or synthetic sources. This will help explore how widely the material can be used for clinical bone regeneration.

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Figure Legend

Figure 1. Composite blocks containing 70 % Sigma-Aldrich βTCP Control with 30 % PAA (A), 60 % βTCP from green mussel shells with 40 % PAA (B), 65 % βTCP from green mussel shells with 35 % PAA (C), and 70 % βTCP from green mussel shells with 30 % PAA composite blocks (D).

Figure 2. Porosities of 70 % Sigma-Aldrich βTCP Control with 30 % PAA (SA-P), 60 % βTCP from green mussel shells with 40 % PAA (B60-P40), 65 % βTCP from green mussel shells with 35 % PAA (B65-P35), and 70 % βTCP from green mussel shells with 30 % PAA (B70-P30) composite blocks (n = 3). (* $p < 0.05$; n.s: not significant)

Figure 3. DTS values of 70 % Sigma-Aldrich βTCP Control with 30 % PAA (BC-PAA), 60 % βTCP from green mussel shells with 40 % PAA (B60-PAA), 65 % βTCP from green mussel shells with 35 % PAA (B65-PAA), and 70 % βTCP from green mussel shells with 30 % PAA (B70-PAA) composite blocks (n = 3). (* $p < 0.05$; n.s: not significant)

Dear Editor and Reviewers of DJMKG,

Thank you for giving me the opportunity to submit a revised draft of my manuscript titled **Fabrication of composite block from β-tricalcium phosphate and polyacrylic acid via freeze-drying method** to Dental Journal (Majalah Kedokteran Gigi). I/We appreciate the time and effort that you and the reviewers have dedicated to providing your valuable feedback on my manuscript. I am/We are grateful to the reviewers for their insightful comments on my paper. I/We have been able to incorporate changes to reflect most of the suggestions provided by the reviewers. I/We have highlighted the changes within the manuscript.

Here is a point-by-point response to the reviewers' comments and concerns.

No.	Reviewer comments	Before revision	Revision	Notes
Revision-1				
Reviewer 1 (RV1)				
1.	Methods: Each ratio is explained using how many grams of BTCP and how many ml or grams of PAA		Each formulation was prepared with a total mixture weight of 2 grams. For the 60:40 ratio, 1.2 grams of βTCP was mixed with 0.8 grams of PAA solution; for the 65:35 ratio, 1.3 grams of βTCP was mixed with 0.7 grams of PAA solution; and for the 70:30 ratio, 1.4 grams of βTCP was mixed with 0.6 grams of PAA solution.	Page: 4 Paragraph: 2
2.	Discussion: In first paragraph, please explain why the particle size of βTCP from green mussel shells is larger than commercial βTCP		This difference is due to the synthesis and processing methods. The green mussel-derived βTCP was prepared through manual grinding and calcination, without further milling or sieving to reduce particle size, resulting in coarser particles. In contrast, commercial βTCP typically undergoes industrial-scale processing with controlled milling and sieving steps to produce finer and more uniform particles.	Page: 5 Paragraph: 4
3.	(PAA)		revised	Page: 2 Paragraph: 1
4.	It is necessary to explain the lacking of the existing βTCP material so we are looking for an alternative βTCP Material from green mussel shells, and it is		Despite the advantages of βTCP, its widespread clinical application is limited by factors such as high production cost, limited availability in some regions, and reliance on synthetic or	Page: 2 Paragraph: 3

4.	necessary to add the benefits of this research.		mined sources. These drawbacks motivate the exploration of alternative, cost-effective, and sustainable sources for producing βTCP. Natural calcium-rich waste materials such as mammalian bones, coral, and shellfish have been reported as potential candidates for synthesizing calcium phosphate materials.8 Among them, green mussel shells (Perna viridis), which are abundantly consumed in Southeast Asia, 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 βTCP.	
5.	the		revised	Page: 6 Paragraph: 4
Managing Editor (ME)				
1.	The length of the script too short		revised	Page: Paragraph:
2.	SDG keywords (Medicine)		revised	Page: 1 Paragraph: 1
3.	Please improve this section by providing the novelty of this study or the research gap.		The novelty of this study is the development of a βTCP–PAA composite block using βTCP made from green mussel shells through a simple setting and freeze-drying process. This research introduces a new, low-cost source for biomaterials and shows that waste from green mussels can produce materials with similar or better physical and mechanical properties than commercial βTCP.	Page: 3 Paragraph: 2
4.	Please give your insight about BTCP in term of Tissue Engineering		In this field, βTCP is widely used as a scaffold material due to its ability to support cell attachment, proliferation,	Page: 2 Paragraph: 1

			and differentiation, as well as its suitable resorption rate that matches new bone formation. Clinically, βTCP 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 βTCP an ideal candidate for use in bone tissue engineering applications, where the balance between mechanical support and biological remodeling is crucial.	
5.	Please add the clinical use of BTCP		Clinically, βTCP 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.	Page: 2 Paragraph: 1
	Please expand this abbreviation	PAA	revised	Page: 2 Paragraph: 1
	Compare your BTCP with another synthetic biomaterial or even a autologous and allograft		Compared to other synthetic biomaterials like hydroxyapatite, βTCP 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 βTCP 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.	Page: 3 Paragraph: 2

	Whats the study hypothesis?		βTCP synthesized from green mussel shells, when combined with polyacrylic acid (PAA) through a setting and freeze-drying process, will produce composite blocks with comparable or improved physical and mechanical properties, such as porosity and diametral tensile strength, compared to commercial βTCP based composites, making it a viable, cost-effective alternative biomaterial for bone tissue engineering.	Page: 3 Paragraph: 3
	This section is too short, please improve and more detail		revised	Page: 3 Paragraph: 4
	Where you obtain the sample? Are there any characterization or inclusion criteria of the sample? How to treat the sample before the BTCP isolation process?		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.	Page: 3 Paragraph: 4
	The result of this study is too simple, it would be better the authors add qualitative and quantitative, more data with other methods such as Degradation, Swelling, and etc.		We appreciate the suggestion to include additional analysis such as degradation and swelling. However, this study focuses on initial physical and mechanical characterizations, such as porosity and mechanical testing, in line with our main objective. Other data such as degradation and swelling are indeed necessary and are planned as part of our future work. This has been noted in the revised manuscript.	
	If its possible please add the SEM-EDX or TEM with FITR analysis		We appreciate the suggestion to include additional analysis such as SEM-EDX or TEM with	

			FTIR. These characterizations are indeed necessary and are being prepared for a separate publication focusing on more detailed material analysis.	
...	The authors failed to present the novelty found in this study. There is no comparison with previous study, in line or contradiction. Please highlight your finding and improve discussion section into 750-1000 words		revised	Page: 5 Paragraph: 3
	Add the limitation of this study.		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 needs to be conducted, including XRD, FTIR, SEM, and degradation as well as swelling properties. These additional physical characterizations are necessary to gain more detailed insights into their correlation with porosity and mechanical properties, which, in turn, would significantly impact the biological activity. These limitations in material characterization make it difficult to fully explain the structure–property relationships observed. On the other hand, no biological tests (such as cytotoxicity, cell attachment, or in vivo performance) were	Page: 6 Paragraph: 3

			performed to confirm its biocompatibility and effectiveness. Also, manual grinding without size control may lead to inconsistent particle size, affecting reproducibility. Lastly, only one type of commercial βTCP was used for comparison, and the setting behavior of PAA with different βTCP sources may vary.	
	Whats the suggestion or your plan for future study?		In future studies, more detailed material testing should be done to better understand the structure and composition of the composite. Biological tests like cytotoxicity, cell attachment, and in vivo studies are also needed to confirm its safety and effectiveness for bone repair. Controlling the particle size through milling and sieving is recommended to improve consistency. It would also be useful to study how PAA reacts with different types of βTCP and compare other natural or synthetic sources. This will help explore how widely the material can be used for clinical bone regeneration.	Page: 6 Paragraph: 4
Revision-2				
Reviewer				
1.				Page: Paragraph:
2.				Page: Paragraph:
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4.				
5.				
...				

In addition to the above comments, all spelling and grammatical errors pointed out by the reviewers have been corrected. We look forward to hearing from you in due time regarding our submission and to respond to any further questions and comments you may have.

15 May 2025
Sincerely,
Authors

3. Bukti revisi kedua, respon kepada reviewer

20 Juni 2025

UNIVERSITAS TRISAKTI

Rosalina Tjandrawinata <rosalina@trisakti.ac.id>

[DJMKG] Editor Decision: Revision for the next round

1 message

Alexander Patera Nugraha <dental_journal@fkg.unair.ac.id> Fri, Jun 20, 2025 at 1:53 PM
To: Rosalina Tjandrawinata <rosalina@trisakti.ac.id>, Eddy <eddydrg@trisakti.ac.id>, Rafhaela Johanna Halim <rafhaela.lim2731@gmail.com>, Thet Thet Swe <thetthetswe@uy.edu.mm>, Tansza Setiana Putri <tansza@trisakti.ac.id>

Dear Rosalina Tjandrawinata, Eddy, Rafhaela Johanna Halim, Thet Thet Swe, Tansza Setiana Putri,

We have reached a decision regarding your submission to Dental Journal, "Fabrication of composite block from β-tricalcium phosphate and polyacrylic acid via freeze-drying method".

Our decision is to: Revise your manuscript to review in the next round

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Original article

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

ABSTRACT

Background: *Beta-tricalcium phosphate (βTCP) 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 βTCP. These shells, rich in calcium carbonate, provide a cost-effective and environmentally friendly alternative for βTCP synthesis.* ***Purpose:*** *This study aims to fabricate composite blocks from βTCP derived from green mussel shells, mixed with polyacrylic acid (PAA), using a setting reaction and freeze-drying method.* ***Methods:*** *βTCP powder was obtained via wet precipitation, starting with calcium carbonate from green mussel shells, converting it to calcium oxide, and then to βTCP. 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 βTCP had rougher surfaces due to larger particles compared to control blocks made with commercial βTCP (Sigma-Aldrich). Composite blocks with 70% green mussel βTCP and 30% PAA exhibited significantly higher porosity (26.97 ± 2.64%) and DTS (11.76 ± 1.59 MPa) than those made with commercial βTCP (porosity: 13.40 ± 1.56%; DTS: 7.79 ± 1.29 MPa). Reducing βTCP content to 60% resulted in increased porosity (34.22 ± 1.84%) and lower DTS (6.41 ± 0.78 MPa).* ***Conclusion:*** *Composite blocks from green mussel shell-derived βTCP and PAA showed higher porosity and significantly higher DTS than blocks made from commercial βTCP. Decreasing βTCP content increased porosity but decreased DTS.*

Keywords: *β-tricalcium phosphate; polyacrylic acid; freeze-drying; porosity; diametral tensile strength; medicine.*

INTRODUCTION

Apart from its osteoconductivity, the higher bioreabsorbability of beta-tricalcium phosphate (βTCP) compared to hydroxyapatite makes it a popular choice in orthopedic and oral surgery applications. In this field, βTCP 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 that matches new bone formation. Clinically, βTCP 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 βTCP 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 materials.[1–3] The combination of beta-tricalcium phosphate (βTCP) with polymer has been an attractive material of choice for its application for bone substitution.[4] Polyacrylic acid (PAA) is a synthetic polymer which exhibits notable properties such as biocompatibility, strong adhesion, and biodegradability. In dentistry, particularly in Glass Ionomer Cement (GIC), PAA contributes to dental cement, enhancing mechanical properties and setting reactions of calcium phosphate materials through crosslinking reactions. The chelation reaction between carboxyl groups (–COOH) from PAA and calcium ions from calcium phosphate contributes to scaffold stability.[5–7]

Investigation by Moradi et al.[5] discovered that a composite scaffold consisting of tricalcium phosphate and polyacrylic acid can be fabricated through a crosslinking reaction and freeze-drying process involving various reagents as precursors. Despite successful polymerization and achieving the desired mechanical properties, the research involved a complex mixing procedure with several chemicals. However, Putri et al.[7] suggested a simpler method involving the chelation reaction between calcium ions from βTCP and carboxyl groups from PAA, similar to setting mechanism in Glass Ionomer Cement. The current study will adopt a similar approach to mix βTCP and PAA.

Despite the advantages of βTCP, its widespread clinical application is limited by factors such as high production cost, limited availability in some regions, and reliance on synthetic or mined sources. These drawbacks motivate the exploration of alternative, cost-effective, and sustainable sources for producing βTCP. Natural calcium-rich waste materials such as mammalian bones, coral, and shellfish have been reported as potential candidates for synthesizing calcium phosphate materials.[8] Among them, green mussel shells (Perna viridis), which are abundantly consumed in Southeast Asia, 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 βTCP.[8,9]

The novelty of this study is the development of a βTCP–PAA composite block using βTCP made from green mussel shells through a simple setting and freeze-drying process. This research introduces a new, low-cost source for biomaterials and shows that waste from green mussels can produce materials with similar or better physical and mechanical properties than commercial βTCP. Compared to other synthetic biomaterials like hydroxyapatite, βTCP 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 βTCP 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.[8,9]

βTCP synthesized from green mussel shells, when combined with polyacrylic acid (PAA) through a setting and freeze-drying process, will produce composite blocks with comparable or improved physical and mechanical properties, such as porosity and diametral tensile strength, compared to commercial βTCP based composites, making it a viable, cost-effective alternative biomaterial for bone tissue engineering. Therefore, the objective of this study is to evaluate the feasibility of combining βTCP obtained 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. Afterward, it was 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 βTCP) and calcined at 1,000°C to obtain βTCP.

The βTCP powder was subsequently mixed with a PAA solution (Mw: ~250,000, 35 wt % in H_2O, Sigma Aldrich, USA) at three varying percentage ratios of βTCP to PAA: 60:40, 65:35, and 70:30. Each ratio was prepared with a total mixture weight of 2 grams. For the 60:40 ratio, 1.2 grams of βTCP was mixed with 0.8 grams of PAA solution; for the 65:35 ratio, 1.3 grams of βTCP was mixed with 0.7 grams of PAA solution; and for the 70:30 ratio, 1.4 grams of βTCP was mixed with 0.6 grams 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 βTCP (from Sigma Aldrich, USA) to mix with PAA.

The porosities of the blocks were measured using the equations below, where the relative density was calculated by dividing the bulk density by the theoretical density of both βTCP (3.07 g/cm³) and PAA (1.15 g/cm³).[10,11] The diametral tensile strength (DTS) of the samples was measured utilizing a universal testing machine (UTM: AGS-X, Shimadzu, 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 (ANOVA) was employed to investigate significant differences among all groups. Following this, post hoc analysis was conducted using Tukey's honestly significant difference (HSD) test to compare significance between each group. The statistical analysis was performed using KaleidaGraph 4.01 software, and the significance level was set at $p < 0.05$.).

RESULTS

The mixtures of βTCP and PAA was successfully set into composite blocks, as illustrated in Figure 1. At macroscopic appearance, sample containing control βTCP displayed a smoother surface compared to other samples (**Figure 1A**). Moreover, as the quantity of βTCP powder derived from green mussel shells increased, the surface appeared to become rougher (**Figure 1B-D**).

Figure 2 demonstrates that at the same ratio, composite blocks incorporating βTCP derived from green mussel shells exhibit higher porosity (26.97 ± 2.64 %) compared to those containing commercial βTCP (13.40 ± 1.56 %). An increase in the powder amount from 70 % to 65 % resulted in an increase in porosity (35.00 ± 2.05 %). However, there was no significant difference in porosity observed between blocks containing 60 % (34.22 ± 1.84 %) and 65 % of βTCP.

The DTS values of the samples are shown in **Figure 3.** At a ratio of 70 % βTCP and 30 % PAA, those containing βTCP from green mussel shells (11.76 ± 1.59 MPa) displayed a significant increase in DTS value compared to those with commercial βTCP (7.79 ± 1.29 MPa). However, reducing the powder amount to 65 % resulted in a significant decrease in

DTS value (7.35 ± 0.61 MPa). Nonetheless, there was no significant difference observed between blocks with 60 % (6.41 ± 0.78 MPa) and 65 % βTCP.

DISCUSSION

This study shows that composite blocks made from βTCP derived from green mussel shells mixed with polyacrylic acid (PAA) can be successfully made using a simple setting and freeze-drying process. This is new because it uses a natural, local, and low-cost material as an alternative to expensive commercial βTCP, which helps reduce waste and cost.[8,9] Our results found important differences in properties between blocks made with green mussel βTCP and those made with commercial βTCP.

The physical difference among all samples can be seen between the blocks containing commercial βTCP and those incorporating βTCP from green mussel shells. The larger particle size of βTCP from green mussel shells (up to 350 μm) and PAA results in a rougher surface in the composite blocks compared to those with commercial βTCP (particle size ranging from 1 to 1.8 μm).[12] This difference is due to the synthesis and processing methods. The green mussel-derived βTCP was prepared through manual grinding and calcination, without further milling or sieving to reduce particle size, resulting in coarser particles. In contrast, commercial βTCP typically undergoes industrial-scale processing with controlled milling and sieving steps to produce finer and more uniform particles. However, the rougher surface resulted on the composite blocks has the potential to enhance cell attachment, thereby promoting better penetration of bone cells and ultimately increasing bone ingrowth.[13]

The difference in particle size also affects the porosity of the samples, with blocks containing commercial βTCP showing lower porosity compared to those incorporating βTCP 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, porosity significantly influences the mechanical properties of a material, as demonstrated in prior studies.[13–15] The outcomes of our study suggest that higher porosity in the samples corresponds to higher DTS values (Figure 2,3).

Incorporating a larger quantity of βTCP powder into the solution results in a more densely packed composite material, typically leading to reduced porosity.[11,16] 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 βTCP and PAA, a setting reaction occurred, forming bonds between the carboxylic acid (–COOH) group of PAA and calcium ions of βTCP, thereby creating connections among the βTCP particles. It is assumed that the larger particles in the βTCP would provide a broader surface on each particle, leading to a larger interface area. This, in turn, results in more extensive connections between the carboxylic acid (–COOH) group of PAA and calcium ions of βTCP, contributing to an enhancement in mechanical strength.[7,17]

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 needs to be conducted, including XRD, FTIR, SEM, and degradation as well as swelling properties. These additional physical characterizations are necessary to gain more detailed insights into their correlation with porosity and mechanical properties, which, in turn, would significantly impact the biological activity. These limitations in material characterization make it difficult to fully explain the structure–property relationships observed. On the other hand, no biological tests (such as cytotoxicity, cell attachment, or *in vivo* performance) were performed to confirm its biocompatibility and effectiveness. Also, manual grinding without size control may lead to inconsistent particle size, affecting reproducibility. Lastly, only one type of commercial βTCP was used for comparison, and the setting behavior of PAA with different βTCP sources may vary.

In conclusion, the composite block was successfully fabricated by mixing βTCP 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 βTCP powder, the βTCP obtained from green mussel shells showed a significant increase in porosity, accompanied by a significantly higher DTS value. Moreover, a decrease in the amount of βTCP powder resulted in an increase in both porosity and DTS value. In future studies, more detailed material testing should be done to better understand the structure and composition of the composite. Biological tests like cytotoxicity, cell attachment, and *in vivo* studies are also needed to confirm its safety and effectiveness for bone repair. Controlling the particle size through milling and sieving is recommended to improve consistency. It would also be useful to study how PAA reacts with different types of βTCP and compare other natural or synthetic sources. This will help explore how widely the material can be used for clinical bone regeneration.

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Figure Legend

Figure 1. Composite blocks containing 70 % Sigma-Aldrich βTCP Control with 30 % PAA (A), 60 % βTCP from green mussel shells with 40 % PAA (B), 65 % βTCP from green mussel shells with 35 % PAA (C), and 70 % βTCP from green mussel shells with 30 % PAA composite blocks (D).

Figure 2. Porosities of 70 % Sigma-Aldrich βTCP Control with 30 % PAA (SA-P), 60 % βTCP from green mussel shells with 40 % PAA (B60-P40), 65 % βTCP from green mussel shells with 35 % PAA (B65-P35), and 70 % βTCP from green mussel shells with 30 % PAA (B70-P30) composite blocks (n = 3). (* $p < 0.05$; n.s: not significant)

Figure 3. DTS values of 70 % Sigma-Aldrich βTCP Control with 30 % PAA (BC-PAA), 60 % βTCP from green mussel shells with 40 % PAA (B60-PAA), 65 % βTCP from green mussel shells with 35 % PAA (B65-PAA), and 70 % βTCP from green mussel shells with 30 % PAA (B70-PAA) composite blocks (n = 3). (* $p < 0.05$; n.s: not significant)

Original article

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

ABSTRACT

Background: *Beta-tricalcium phosphate (βTCP) 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 βTCP. These shells, rich in calcium carbonate, provide a cost-effective and environmentally friendly alternative for βTCP synthesis.* ***Purpose:*** *This study aims to fabricate composite blocks from βTCP derived from green mussel shells, mixed with polyacrylic acid (PAA), using a setting reaction and freeze-drying method.* ***Methods:*** *βTCP powder was obtained via wet precipitation, starting with calcium carbonate from green mussel shells, converting it to calcium oxide, and then to βTCP. 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 βTCP had rougher surfaces due to larger particles compared to control blocks made with commercial βTCP (Sigma-Aldrich). Composite blocks with 70% green mussel βTCP and 30% PAA exhibited significantly higher porosity (26.97 ± 2.64%) and DTS (11.76 ± 1.59 MPa) than those made with commercial βTCP (porosity: 13.40 ± 1.56%; DTS: 7.79 ± 1.29 MPa). Reducing βTCP content to 60% resulted in increased porosity (34.22 ± 1.84%) and lower DTS (6.41 ± 0.78 MPa).* ***Conclusion:*** *Composite blocks from green mussel shell-derived βTCP and PAA showed higher porosity and significantly higher DTS than blocks made from commercial βTCP. Decreasing βTCP content increased porosity but decreased DTS.*

Keywords: *β-tricalcium phosphate; polyacrylic acid; freeze-drying; porosity; diametral tensile strength; medicine.*

INTRODUCTION

Apart from its osteoconductivity, the higher bioreabsorbability of beta-tricalcium phosphate (βTCP) compared to hydroxyapatite makes it a popular choice in orthopedic and oral surgery applications. In this field, βTCP 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 that matches new bone formation. Clinically, βTCP 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 βTCP 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 materials.[1–7] The combination of beta-tricalcium phosphate (βTCP) with polymer has been an attractive material of choice for its application for bone substitution.[8] Polyacrylic acid (PAA) is a synthetic polymer which exhibits notable properties such as biocompatibility, strong adhesion, and biodegradability. In dentistry, particularly in Glass Ionomer Cement (GIC), PAA contributes to dental cement, enhancing mechanical properties and setting reactions of calcium phosphate materials through crosslinking reactions. The chelation reaction between carboxyl groups (–COOH) from PAA and calcium ions from calcium phosphate contributes to scaffold stability.[9–11]

Investigation by Moradi et al.[9] discovered that a composite scaffold consisting of tricalcium phosphate and polyacrylic acid can be fabricated through a crosslinking reaction and freeze-drying process involving various reagents as precursors. Despite successful polymerization and achieving the desired mechanical properties, the research involved a complex mixing procedure with several chemicals. However, Putri et al.[11] suggested a simpler method involving the chelation reaction between calcium ions from βTCP and carboxyl groups from PAA, similar to setting mechanism in Glass Ionomer Cement. The current study will adopt a similar approach to mix βTCP and PAA.

Despite the advantages of βTCP, its widespread clinical application is limited by factors such as high production cost, limited availability in some regions, and reliance on synthetic or mined sources. These drawbacks motivate the exploration of alternative, cost-effective, and sustainable sources for producing βTCP. 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, green mussel shells (*Perna viridis*), which are abundantly consumed in Southeast Asia, 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 βTCP.[12,13]

The novelty of this study is the development of a βTCP–PAA composite block using βTCP made from green mussel shells through a simple setting and freeze-drying process. This research introduces a new, low-cost source for biomaterials and shows that waste from green mussels can produce materials with similar or better physical and mechanical properties than commercial βTCP. Compared to other synthetic biomaterials like hydroxyapatite, βTCP 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 βTCP 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]

βTCP synthesized from green mussel shells, when combined with polyacrylic acid (PAA) through a setting and freeze-drying process, will produce composite blocks with comparable or improved physical and mechanical properties, such as porosity and diametral tensile strength, compared to commercial βTCP based composites, making it a viable, cost-effective alternative biomaterial for bone tissue engineering. Therefore, the objective of this study is to evaluate the feasibility of combining βTCP obtained 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. Afterward, it was 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 βTCP) and calcined at 1,000°C to obtain βTCP.

The βTCP powder was subsequently mixed with a PAA solution (Mw: ~250,000, 35 wt % in H_2O, Sigma Aldrich, St. Louis, Missouri, USA) at three varying percentage ratios of βTCP to PAA: 60:40, 65:35, and 70:30. Each ratio was prepared with a total mixture weight of 2 grams. For the 60:40 ratio, 1.2 grams of βTCP was mixed with 0.8 grams of PAA solution; for the 65:35 ratio, 1.3 grams of βTCP was mixed with 0.7 grams of PAA solution; and for the 70:30 ratio, 1.4 grams of βTCP was mixed with 0.6 grams 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 βTCP (Sigma Aldrich, St. Louis, Missouri, USA) to mix with PAA.

The porosities of the blocks were measured using the equations below, where the relative density was calculated by dividing the bulk density by the theoretical density of both βTCP (3.07 g/cm³) and PAA (1.15 g/cm³).[14,15] The diametral tensile strength (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 (ANOVA) was employed to investigate significant differences among all groups. Following this, post hoc analysis was conducted using Tukey's honestly significant difference (HSD) test to compare significance between each group. The statistical analysis was performed using KaleidaGraph 4.01 software (Synergy Software, Reading, Pennsylvania, USA) and the significance level was set at $p < 0.05$.).

RESULTS

The mixtures of βTCP and PAA was successfully set into composite blocks, as illustrated in Figure 1. At macroscopic appearance, sample containing control βTCP displayed a smoother surface compared to other samples (**Figure 1A**). Moreover, as the quantity of βTCP powder derived from green mussel shells increased, the surface appeared to become rougher (**Figure 1B-D**).

Figure 2 demonstrates that at the same ratio, composite blocks incorporating βTCP derived from green mussel shells exhibit higher porosity (26.97 ± 2.64 %) compared to those containing commercial βTCP (13.40 ± 1.56 %). An increase in the powder amount from 70 % to 65 % resulted in an increase in porosity (35.00 ± 2.05 %). However, there was no significant difference in porosity observed between blocks containing 60 % (34.22 ± 1.84 %) and 65 % of βTCP.

The DTS values of the samples are shown in **Figure 3.** At a ratio of 70 % βTCP and 30 % PAA, those containing βTCP from green mussel shells (11.76 ± 1.59 MPa) displayed a significant increase in DTS value compared to those with commercial βTCP (7.79 ± 1.29 MPa). However, reducing the powder amount to 65 % resulted in a significant decrease in

DTS value (7.35 ± 0.61 MPa). Nonetheless, there was no significant difference observed between blocks with 60 % (6.41 ± 0.78 MPa) and 65 % βTCP.

DISCUSSION

This study shows that composite blocks made from βTCP derived from green mussel shells mixed with polyacrylic acid (PAA) can be successfully made using a simple setting and freeze-drying process. This is new because it uses a natural, local, and low-cost material as an alternative to expensive commercial βTCP, which helps reduce waste and cost.[12,13] Our results found important differences in properties between blocks made with green mussel βTCP and those made with commercial βTCP.

The physical difference among all samples can be seen between the blocks containing commercial βTCP and those incorporating βTCP from green mussel shells. The larger particle size of βTCP from green mussel shells (up to 350 µm) and PAA results in a rougher surface in the composite blocks compared to those with commercial βTCP (particle size ranging from 1 to 1.8 µm).[16] This difference is due to the synthesis and processing methods. The green mussel-derived βTCP was prepared through manual grinding and calcination, without further milling or sieving to reduce particle size, resulting in coarser particles. In contrast, commercial βTCP typically undergoes industrial-scale processing with controlled milling and sieving steps to produce finer and more uniform particles. However, the rougher surface resulted 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 affects the porosity of the samples, with blocks containing commercial βTCP showing lower porosity compared to those incorporating βTCP 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, porosity significantly influences the mechanical properties of a material, as demonstrated in prior studies.[17,19,20] The outcomes of our study suggest that higher porosity in the samples corresponds to higher DTS values (Figure 2,3).

Incorporating a larger quantity of βTCP 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 βTCP and PAA, a setting reaction occurred, forming bonds between the carboxylic acid (–COOH) group of PAA and calcium ions of βTCP, thereby creating connections among the βTCP particles. It is assumed that the larger particles in the βTCP would provide a broader surface on each particle, leading to a larger interface area. This, in turn, results in more extensive connections between the carboxylic acid (–COOH) group of PAA and calcium ions of βTCP, 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 needs to be conducted, including XRD, FTIR, SEM, and degradation as well as swelling properties. These additional physical characterizations are necessary to gain more detailed insights into their correlation with porosity and mechanical properties, which, in turn, would significantly impact the biological activity. These limitations in material characterization make it difficult to fully explain the structure–property relationships observed. On the other hand, no biological tests (such as cytotoxicity, cell attachment, or *in vivo* performance) were performed to confirm its biocompatibility and effectiveness. Also, manual grinding without size control may lead to inconsistent particle size, affecting reproducibility. Lastly, only one type of commercial βTCP was used for comparison, and the setting behavior of PAA with different βTCP sources may vary.

In conclusion, the composite block was successfully fabricated by mixing βTCP 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 βTCP powder, the βTCP obtained from green mussel shells showed a significant increase in porosity, accompanied by a significantly higher DTS value. Moreover, a decrease in the amount of βTCP powder resulted in an increase in both porosity and DTS value. In future studies, more detailed material testing should be done to better understand the structure and composition of the composite. Biological tests like cytotoxicity, cell attachment, and *in vivo* studies are also needed to confirm its safety and effectiveness for bone repair. Controlling the particle size through milling and sieving is recommended to improve consistency. It would also be useful to study how PAA reacts with different types of βTCP and compare other natural or synthetic sources. This will help explore how widely the material can be used for clinical bone regeneration.

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Figure Legend

Figure 1. Composite blocks containing (A) 70 % Sigma-Aldrich βTCP Control with 30 % PAA (SA-P), (B) 60 % βTCP from green mussel shells with 40 % PAA (B60-P40), (C) 65 % βTCP from green mussel shells with 35 % PAA (B65-P35), and (D) 70 % βTCP from green mussel shells with 30 % PAA composite blocks (B70-B30).

Figure 2. Porosities of 70 % Sigma-Aldrich βTCP Control with 30 % PAA (SA-P), 60 % βTCP from green mussel shells with 40 % PAA (B60-P40), 65 % βTCP from green mussel shells with 35 % PAA (B65-P35), and 70 % βTCP from green mussel shells with 30 % PAA (B70-P30) composite blocks (n = 3). (* $p < 0.05$; n.s: not significant)

Figure 3. DTS values of 70 % Sigma-Aldrich βTCP Control with 30 % PAA (SA-P), 60 % βTCP from green mussel shells with 40 % PAA (B60-P40), 65 % βTCP from green mussel shells with 35 % PAA (B65-B35), and 70 % βTCP from green mussel shells with 30 % PAA (B70-P30) composite blocks (n = 3). (* $p < 0.05$; n.s: not significant)

Dear Editor and Reviewers of DJMKG,

Thank you for giving me the opportunity to submit a revised draft of my manuscript titled **Fabrication of composite block from β-tricalcium phosphate and polyacrylic acid via freeze-drying method** to Dental Journal (Majalah Kedokteran Gigi). I/We appreciate the time and effort that you and the reviewers have dedicated to providing your valuable feedback on my manuscript. I am/We are grateful to the reviewers for their insightful comments on my paper. I/We have been able to incorporate changes to reflect most of the suggestions provided by the reviewers. I/We have highlighted the changes within the manuscript.

Here is a point-by-point response to the reviewers' comments and concerns.

No.	Reviewer comments	Before revision	Revision	Notes
Revision-1				
Reviewer 1 (RV1)				
1.	Methods: Each ratio is explained using how many grams of BTCP and how many ml or grams of PAA		Each formulation was prepared with a total mixture weight of 2 grams. For the 60:40 ratio, 1.2 grams of βTCP was mixed with 0.8 grams of PAA solution; for the 65:35 ratio, 1.3 grams of βTCP was mixed with 0.7 grams of PAA solution; and for the 70:30 ratio, 1.4 grams of βTCP was mixed with 0.6 grams of PAA solution.	Page: 4 Paragraph: 2
2.	Discussion: In first paragraph, please explain why the particle size of βTCP from green mussel shells is larger than commercial βTCP		This difference is due to the synthesis and processing methods. The green mussel-derived βTCP was prepared through manual grinding and calcination, without further milling or sieving to reduce particle size, resulting in coarser particles. In contrast, commercial βTCP typically undergoes industrial-scale processing with controlled milling and sieving steps to produce finer and more uniform particles.	Page: 5 Paragraph: 4
3.	(PAA)		revised	Page: 2 Paragraph: 1
4.	It is necessary to explain the lacking of the existing βTCP material so we are looking for an alternative βTCP Material from green mussel shells, and it is		Despite the advantages of βTCP, its widespread clinical application is limited by factors such as high production cost, limited availability in some regions, and reliance on synthetic or	Page: 2 Paragraph: 3

4.	necessary to add the benefits of this research.		mined sources. These drawbacks motivate the exploration of alternative, cost-effective, and sustainable sources for producing βTCP. Natural calcium-rich waste materials such as mammalian bones, coral, and shellfish have been reported as potential candidates for synthesizing calcium phosphate materials.8 Among them, green mussel shells (Perna viridis), which are abundantly consumed in Southeast Asia, 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 βTCP.	
5.	the		revised	Page: 6 Paragraph: 4
	Managing Editor (ME)			
1.	The length of the script too short		revised	Page: Paragraph:
2.	SDG keywords (Medicine)		revised	Page: 1 Paragraph: 1
3.	Please improve this section by providing the novelty of this study or the research gap.		The novelty of this study is the development of a βTCP–PAA composite block using βTCP made from green mussel shells through a simple setting and freeze-drying process. This research introduces a new, low-cost source for biomaterials and shows that waste from green mussels can produce materials with similar or better physical and mechanical properties than commercial βTCP.	Page: 3 Paragraph: 2
4.	Please give your insight about BTCP in term of Tissue Engineering		In this field, βTCP is widely used as a scaffold material due to its ability to support cell attachment, proliferation,	Page: 2 Paragraph: 1

			and differentiation, as well as its suitable resorption rate that matches new bone formation. Clinically, βTCP 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 βTCP an ideal candidate for use in bone tissue engineering applications, where the balance between mechanical support and biological remodeling is crucial.	
5.	Please add the clinical use of BTCP		Clinically, βTCP 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.	Page: 2 Paragraph: 1
	Please expand this abbreviation	PAA	revised	Page: 2 Paragraph: 1
	Compare your BTCP with another synthetic biomaterial or even a autologous and allograft		Compared to other synthetic biomaterials like hydroxyapatite, βTCP 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 βTCP 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.	Page: 3 Paragraph: 2

	Whats the study hypothesis?		βTCP synthesized from green mussel shells, when combined with polyacrylic acid (PAA) through a setting and freeze-drying process, will produce composite blocks with comparable or improved physical and mechanical properties, such as porosity and diametral tensile strength, compared to commercial βTCP based composites, making it a viable, cost-effective alternative biomaterial for bone tissue engineering.	Page: 3 Paragraph: 3
	This section is too short, please improve and more detail		revised	Page: 3 Paragraph: 4
	Where you obtain the sample? Are there any characterization or inclusion criteria of the sample? How to treat the sample before the BTCP isolation process?		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.	Page: 3 Paragraph: 4
	The result of this study is too simple, it would be better the authors add qualitative and quantitative, more data with other methods such as Degradation, Swelling, and etc.		We appreciate the suggestion to include additional analysis such as degradation and swelling. However, this study focuses on initial physical and mechanical characterizations, such as porosity and mechanical testing, in line with our main objective. Other data such as degradation and swelling are indeed necessary and are planned as part of our future work. This has been noted in the revised manuscript.	
	If its possible please add the SEM-EDX or TEM with FITR analysis		We appreciate the suggestion to include additional analysis such as SEM-EDX or TEM with	

			FTIR. These characterizations are indeed necessary and are being prepared for a separate publication focusing on more detailed material analysis.	
...	The authors failed to present the novelty found in this study. There is no comparison with previous study, in line or contradiction. Please highlight your finding and improve discussion section into 750-1000 words		revised	Page: 5 Paragraph: 3
	Add the limitation of this study.		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 needs to be conducted, including XRD, FTIR, SEM, and degradation as well as swelling properties. These additional physical characterizations are necessary to gain more detailed insights into their correlation with porosity and mechanical properties, which, in turn, would significantly impact the biological activity. These limitations in material characterization make it difficult to fully explain the structure–property relationships observed. On the other hand, no biological tests (such as cytotoxicity, cell attachment, or in vivo performance) were	Page: 6 Paragraph: 3

3.			performed to confirm its biocompatibility and effectiveness. Also, manual grinding without size control may lead to inconsistent particle size, affecting reproducibility. Lastly, only one type of commercial βTCP was used for comparison, and the setting behavior of PAA with different βTCP sources may vary.	
	What is the suggestion or your plan for future study?		In future studies, more detailed material testing should be done to better understand the structure and composition of the composite. Biological tests like cytotoxicity, cell attachment, and in vivo studies are also needed to confirm its safety and effectiveness for bone repair. Controlling the particle size through milling and sieving is recommended to improve consistency. It would also be useful to study how PAA reacts with different types of βTCP and compare other natural or synthetic sources. This will help explore how widely the material can be used for clinical bone regeneration.	Page: 6 Paragraph: 4
Revision-2				
Reviewer				
1.	Add city and state	Sigma Aldrich, USA	Sigma Aldrich, St. Louis, Missouri, USA	Page: 3 Paragraph: 4
2.	Add city and state	UTM: AGS-X, Shimadzu, Japan	UTM: AGS-X, Shimadzu, Kyoto, Kyoto Prefecture, Japan	Page: 4 Paragraph: 1
3.	Add detail of this software, company, city, state, country	KaleidaGraph 4.01 software	KaleidaGraph 4.01 software (Synergy Software, Reading, Pennsylvania, USA)	Page: 4 Paragraph: 2

4.	Please add kindly cite this reference:		added	References no 4, 5, 6, 7, 18
5.	Please add all abbreviation in the footnote of your each figure		added	
...				

In addition to the above comments, all spelling and grammatical errors pointed out by the reviewers have been corrected. We look forward to hearing from you in due time regarding our submission and to respond to any further questions and comments you may have.

21 June 2025
Sincerely,
Authors

4. Bukti konfirmasi artikel accepted

23 Juni 2025

Rosalina Tjandrawinata <rosalina@trisakti.ac.id>

[DJMKG] Editor Decision: Letter of Acceptance (LoA)

1 message

Alexander Patera Nugraha <dental_journal@fkg.unair.ac.id> Mon, Jun 23, 2025 at 1:26 PM
To: Rosalina Tjandrawinata <rosalina@trisakti.ac.id>, Eddy <eddydrg@trisakti.ac.id>, Rafhaela Johanna Halim <rafhaela.lim2731@gmail.com>, Thet Thet Swe <thetthetswe@uy.edu.mm>, Tansza Setiana Putri <tansza@trisakti.ac.id>

Dear Rosalina Tjandrawinata, Eddy, Rafhaela Johanna Halim, Thet Thet Swe, Tansza Setiana Putri,

We have reached a decision regarding your submission to Dental Journal, "Fabrication of composite block from β-tricalcium phosphate and polyacrylic acid via freeze-drying method".

Our decision is to: Accept your manuscript

Manuscript URL: https://e-journal.unair.ac.id/MKG/authorDashboard/submission/68781

Articles will go through the process of copyediting (including re-check of similarity) and production (proofreading, galley layout and publishing).
Determination of the publication of articles on the issue will be notified further.

Thank you for your submission. Your next manuscript is very welcome.

Best Regards,

Editor in Chief

Dental Journal (Majalah Kedokteran Gigi)
https://e-journal.unair.ac.id/MKG/index

5. Bukti konfirmasi kelanjutan produksi menuju proofreading

18 September 2025

Rosalina Tjandrawinata <rosalina@trisakti.ac.id>

[DJMKG] Editor Decision: Send to production

1 message

Alexander Patera Nugraha <dental_journal@fkg.unair.ac.id> Thu, Sep 18, 2025 at 3:29 PM
To: Rosalina Tjandrawinata <rosalina@trisakti.ac.id>, Eddy <eddydrg@trisakti.ac.id>, Rafhaela Johanna Halim <rafhaela.lim2731@gmail.com>, Thet Thet Swe <thetthetswe@uy.edu.mm>, Tansza Setiana Putri <tansza@trisakti.ac.id>

Dear Rosalina Tjandrawinata, Eddy, Rafhaela Johanna Halim, Thet Thet Swe, Tansza Setiana Putri,

The editing of your manuscript submission, "Fabrication of composite block from beta-tricalcium phosphate and polyacrylic acid via freeze-drying method," is complete. We are now sending it to production including proofreading, galley proof and publishing.

Submission URL: https://e-journal.unair.ac.id/MKG/authorDashboard/submission/68781

Regards,

Editor

Dental Journal (Majalah Kedokteran Gigi)
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6. Bukti konfirmasi copy editing, publikasi dan pembayaran APC

27 Oktober 2025

Rosalina Tjandrawinata <rosalina@trisakti.ac.id>

[DJMKG] Editor Decision: Letter of Acceptance (LoA)

Alexander Patera Nugraha <dental_journal@fkg.unair.ac.id> Mon, Oct 27, 2025 at 10:26 AM
To: Rosalina Tjandrawinata <rosalina@trisakti.ac.id>, Eddy <eddydrg@trisakti.ac.id>, Rafhaela Johanna Halim <rafhaela.lim2731@gmail.com>, Thet Thet Swe <thetthetswe@uy.edu.mm>, Tansza Setiana Putri <tansza@trisakti.ac.id>

Dear Rosalina Tjandrawinata, Eddy, Rafhaela Johanna Halim, Thet Thet Swe, Tansza Setiana Putri,

We have reached a decision regarding your submission to Dental Journal (Majalah Kedokteran Gigi), "Fabrication of composite block from beta-tricalcium phosphate and polyacrylic acid via freeze-drying method".

Our decision is to: Accept your manuscript

Manuscript URL: https://e-journal.unair.ac.id/MKG/authorDashboard/submission/68781

Articles will go through the process of copyediting (including re-check of similarity) and production (proofreading, galley layout and publishing).

Based on queue, your article scheduled to be published on issue 1 2026.

Dental Journal plan to adjust the APC fee starting next year (2026), which will apply to articles published in 2027. Therefore, accepted articles which scheduled for publication in 2026 will continue to use the old rules based on Airlangga University Chancellor's Decree No. 44 concerning service rates, with the APC fee remaining at USD 100. **For this reason, we ask the corresponding author to complete the payment of the APC fee of 100 USD which must be paid within a week.**

Thank you for your submission. Your next manuscript is very welcome.

Best Regards,

Editor in Chief

Dental Journal (Majalah Kedokteran Gigi)
https://e-journal.unair.ac.id/MKG/index

Rosalina Tjandrawinata <rosalina@trisakti.ac.id>

[DJMKG] Editor Decision: Letter of Acceptance (LoA)

Rosalina Tjandrawinata <rosalina@trisakti.ac.id> Mon, Oct 27, 2025 at 7:37 PM
To: Alexander Patera Nugraha <dental_journal@fkg.unair.ac.id>

Dear Dr. Alexander Patera Nugraha,

Thank you very much for the acceptance information. We are delighted to receive the news. Hereby, I enclose the Article Processing Charge transfer.

Sincerely yours,
Rosa
[Quoted text hidden]

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Original article

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% ± 2.64%) and DTS (11.76 ± 1.59 MPa) than those made with commercial β-TCP (porosity: 13.40% ± 1.56%; DTS: 7.79 ± 1.29 MPa). Reducing β-TCP content to 60% resulted in increased porosity (34.22% ± 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 (−COOH) from PAA and

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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 −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/cm³) and PAA (1.15 g/cm³).[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:

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

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

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

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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% ± 2.64%) than those containing commercial β-TCP (13.40% ± 1.56%). An increase in the powder amount from 70% to 65% resulted in an increase in porosity (35.00% ± 2.05%). However, there was no significant difference in porosity observed between blocks containing 60% (34.22% ± 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.

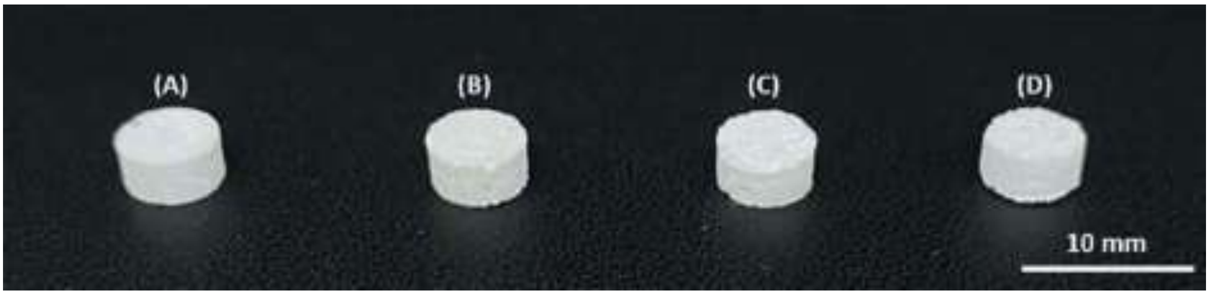

Figure 1. Composite blocks containing (A) 70% Sigma-Aldrich beta-tricalcium phosphate (β-TCP) control with 30% polyacrylic acid (PAA) (SA–P), (B) 60% green mussel shell-derived β-TCP with 40% PAA (B60–P40), (C) 65% green mussel shell-derived β-TCP with 35% PAA (B65–P35), and (D) 70% green mussel shell-derived β-TCP with 30% PAA (B70–B30).

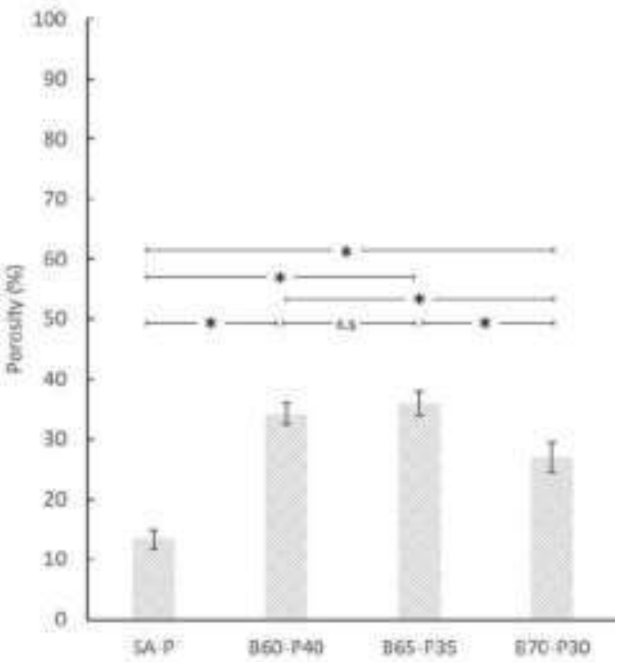

Figure 2. Porosities of composite blocks containing 70% Sigma-Aldrich beta-tricalcium phosphate (β-TCP) control with 30% polyacrylic acid (PAA) (SA–P), 60% green mussel shell-derived β-TCP with 40% PAA (B60–P40), 65% green mussel shell-derived β-TCP with 35% PAA (B65–P35), and 70% green mussel shell-derived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

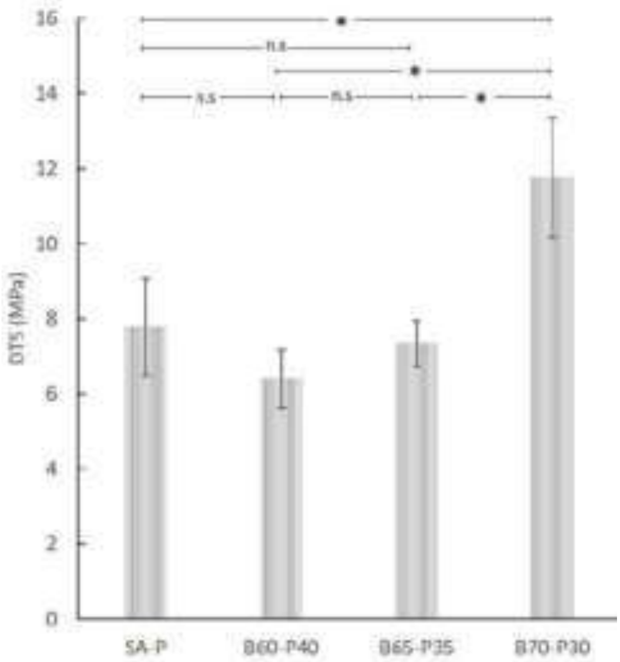

Figure 3. Diametral tensile strength (DTS) values of composite blocks containing 70% Sigma-Aldrich beta-tricalcium phosphate (β-TCP) control with 30% polyacrylic acid (PAA) (SA–P), 60% green mussel shell-derived β-TCP with 40% PAA (B60-P40), 65% green mussel shell-derived β-TCP with 35% PAA (B65–B35), and 70% green mussel shell-derived β-TCP with 30% PAA (B70–P30) (n = 3). (*$p < 0.05$; n.s: not significant.)

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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 −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 −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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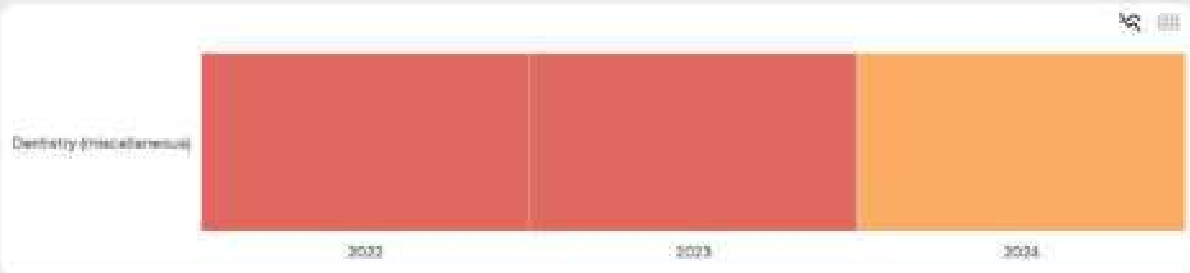

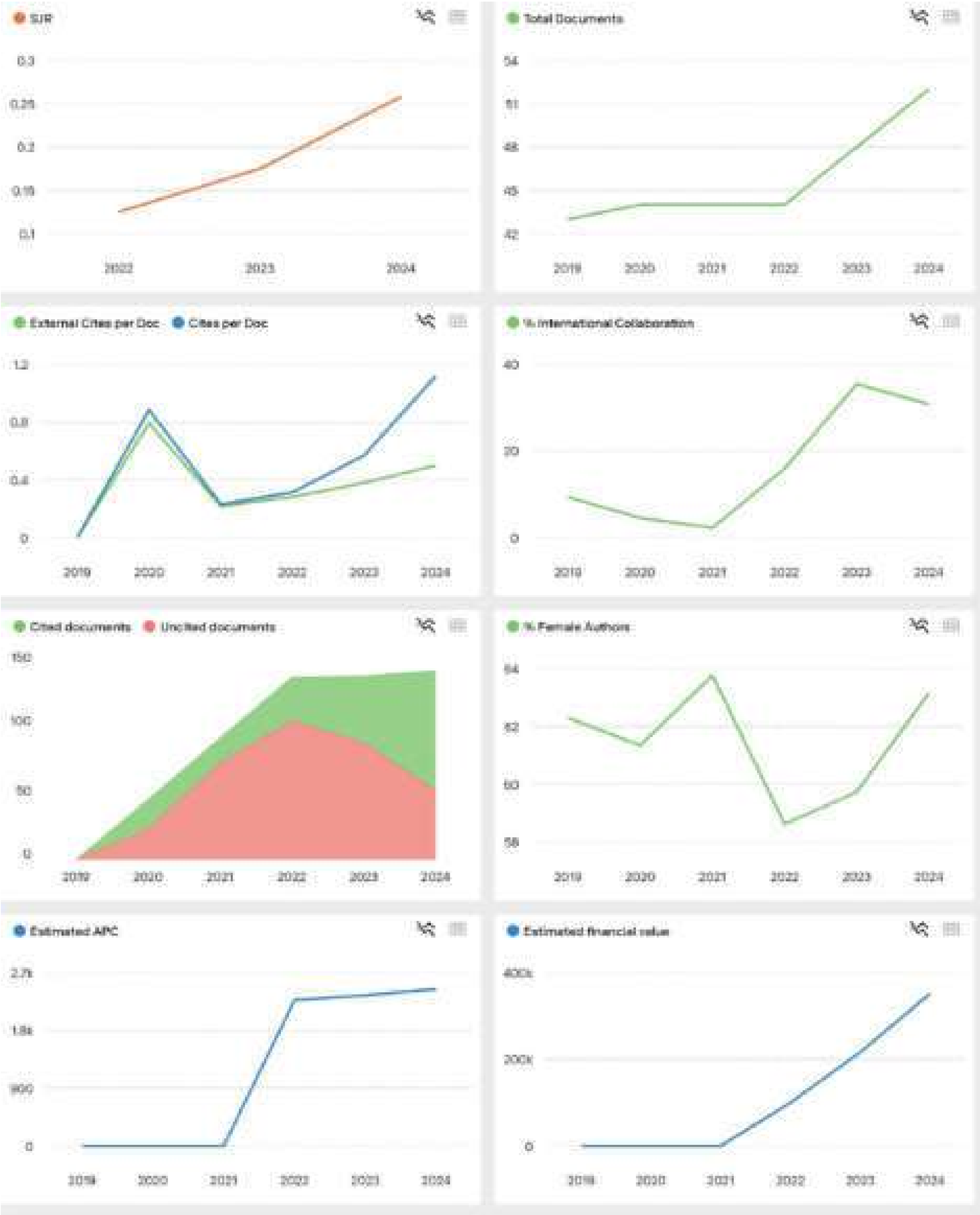
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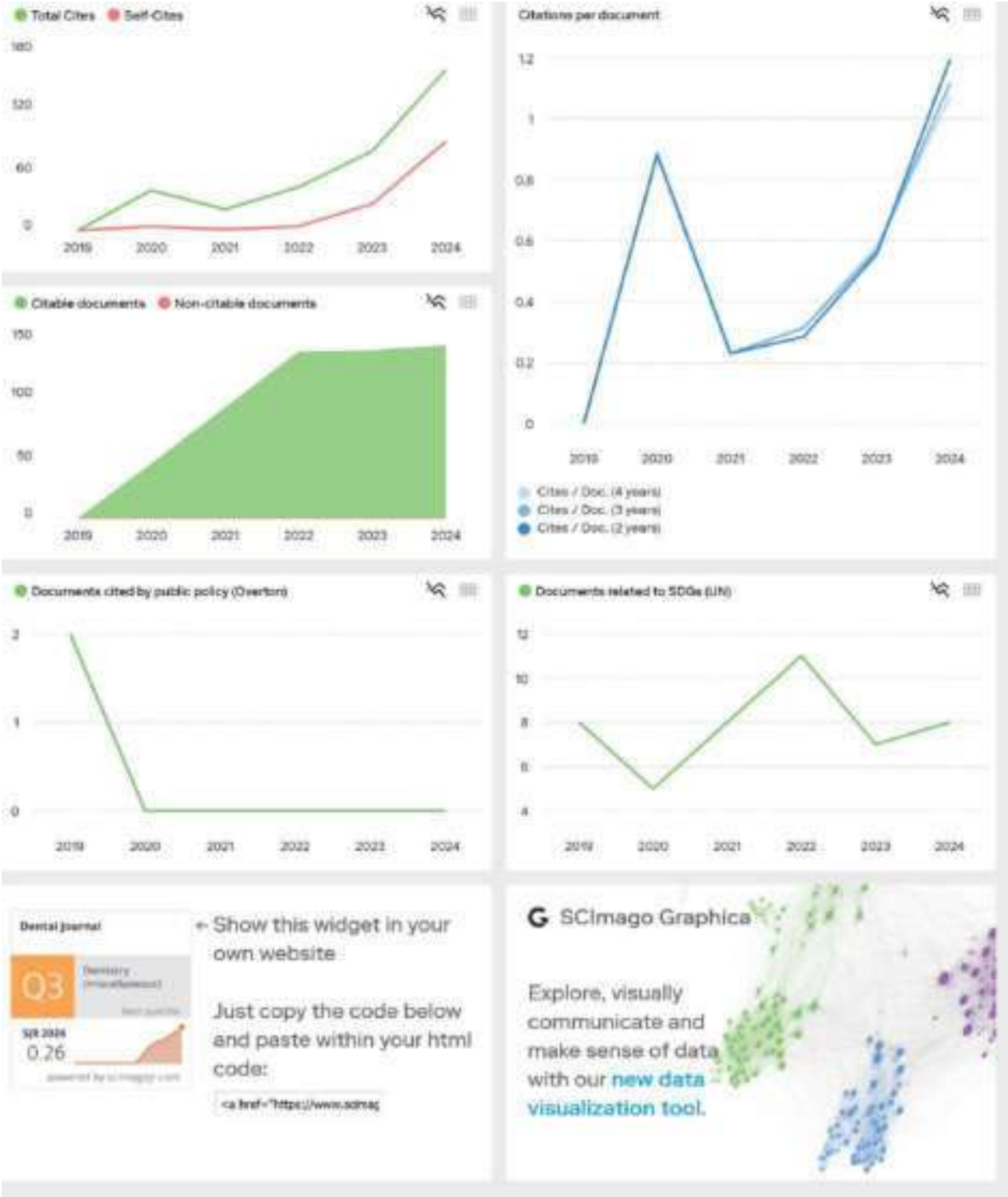

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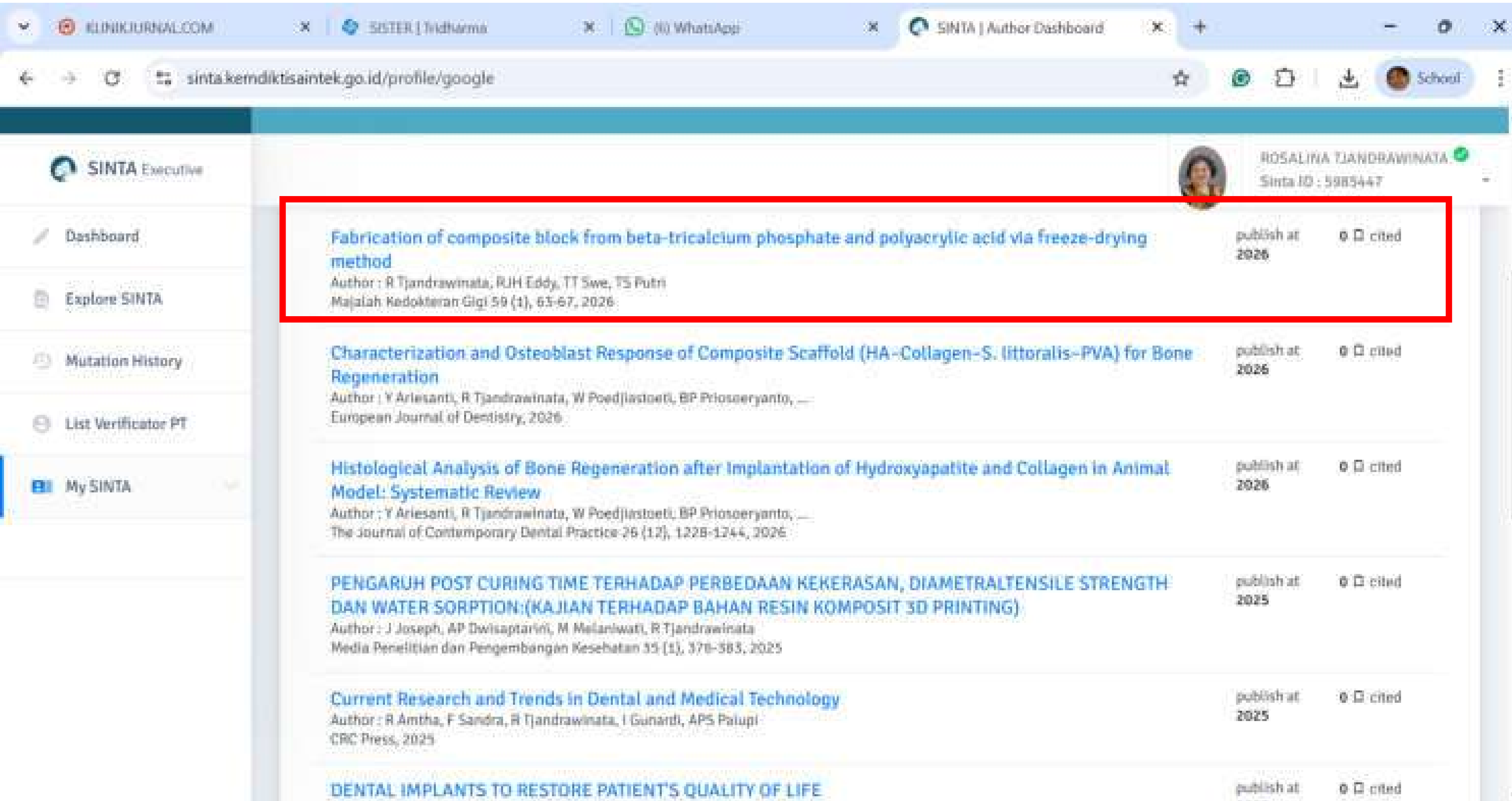
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Original article

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% ± 2.64%) and DTS (11.76 ± 1.59 MPa) than those made with commercial β-TCP (porosity: 13.40% ± 1.56%; DTS: 7.79 ± 1.29 MPa). Reducing β-TCP content to 60% resulted in increased porosity (34.22% ± 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 (−COOH) from PAA and

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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 −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/cm³) and PAA (1.15 g/cm³).[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:

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

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

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

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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% ± 2.64%) than those containing commercial β-TCP (13.40% ± 1.56%). An increase in the powder amount from 70% to 65% resulted in an increase in porosity (35.00% ± 2.05%). However, there was no significant difference in porosity observed between blocks containing 60% (34.22% ± 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.

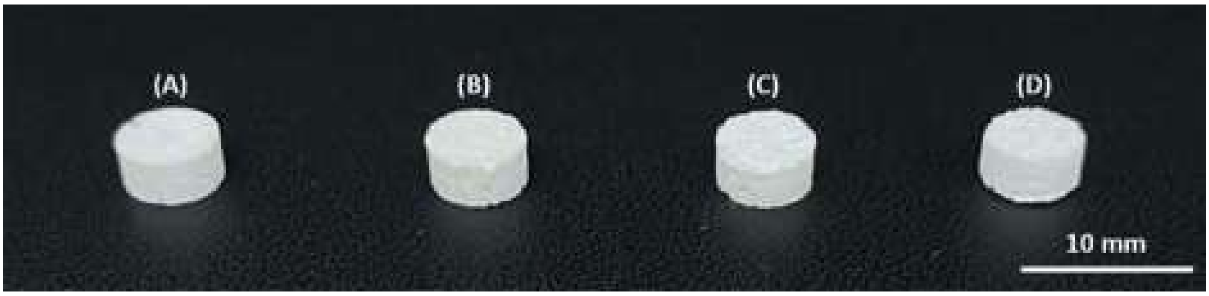

Figure 1. Composite blocks containing (A) 70% Sigma-Aldrich beta-tricalcium phosphate (β-TCP) control with 30% polyacrylic acid (PAA) (SA–P), (B) 60% green mussel shell-derived β-TCP with 40% PAA (B60–P40), (C) 65% green mussel shell-derived β-TCP with 35% PAA (B65–P35), and (D) 70% green mussel shell-derived β-TCP with 30% PAA (B70–B30).

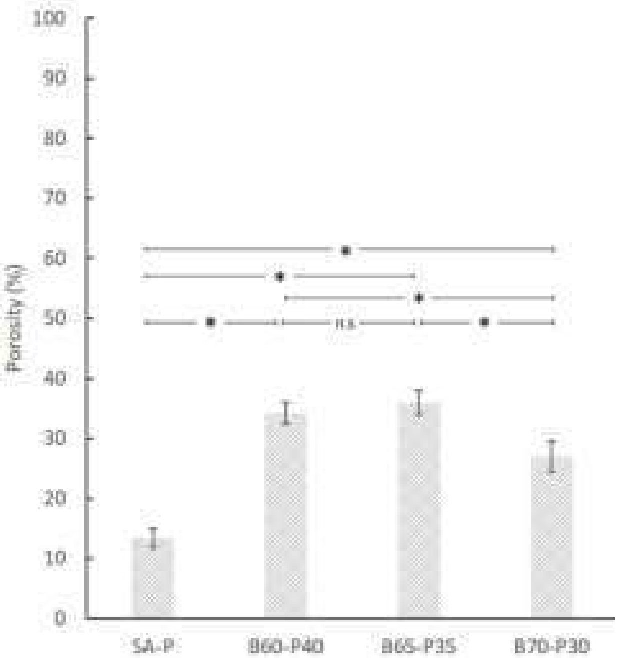

Figure 2. Porosities of composite blocks containing 70% Sigma-Aldrich beta-tricalcium phosphate (β-TCP) control with 30% polyacrylic acid (PAA) (SA–P), 60% green mussel shell-derived β-TCP with 40% PAA (B60–P40), 65% green mussel shell-derived β-TCP with 35% PAA (B65–P35), and 70% green mussel shell-derived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

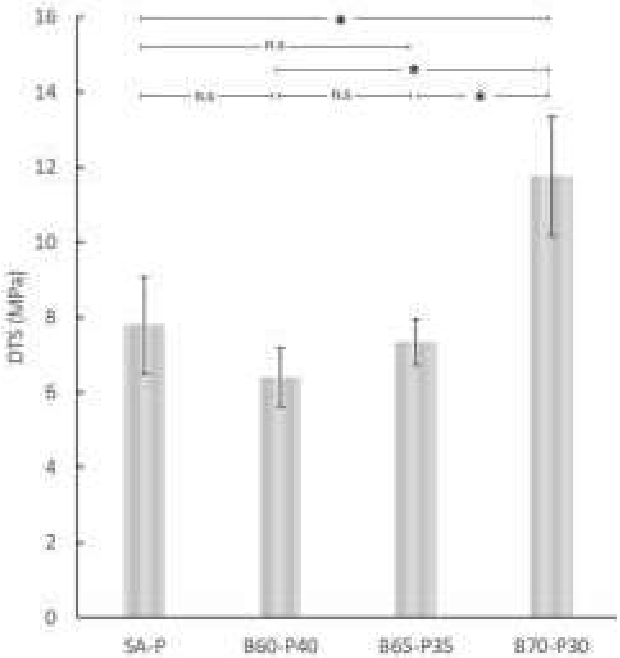

Figure 3. Diametral tensile strength (DTS) values of composite blocks containing 70% Sigma-Aldrich beta-tricalcium phosphate (β-TCP) control with 30% polyacrylic acid (PAA) (SA–P), 60% green mussel shell-derived β-TCP with 40% PAA (B60-P40), 65% green mussel shell-derived β-TCP with 35% PAA (B65–B35), and 70% green mussel shell-derived β-TCP with 30% PAA (B70–P30) (n = 3). (*$p < 0.05$; n.s: not significant.)

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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 −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 −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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