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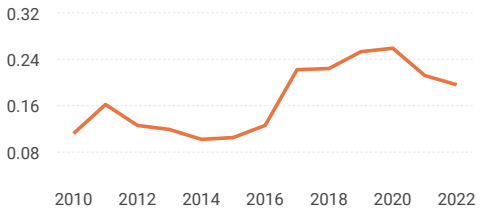

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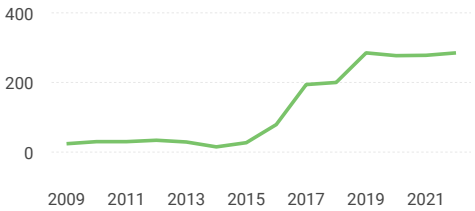

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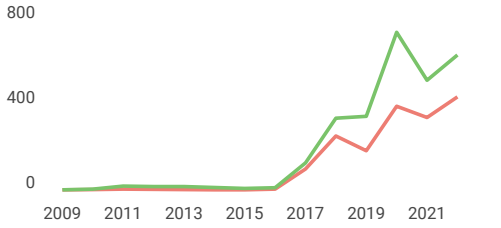

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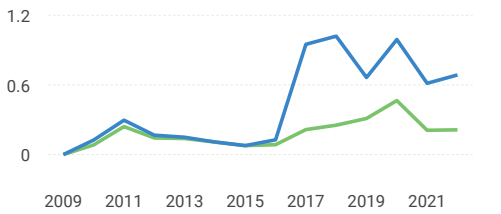

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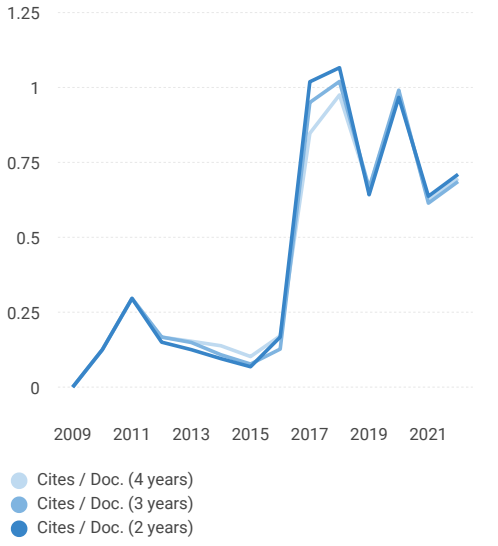

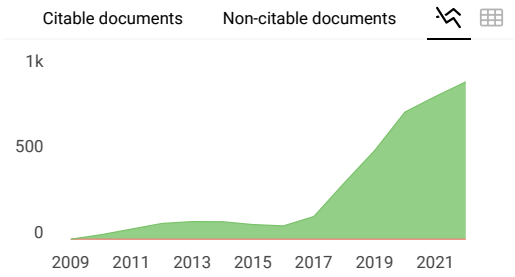

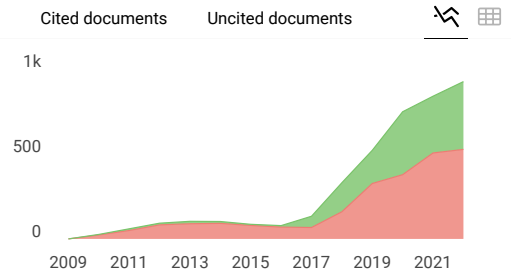

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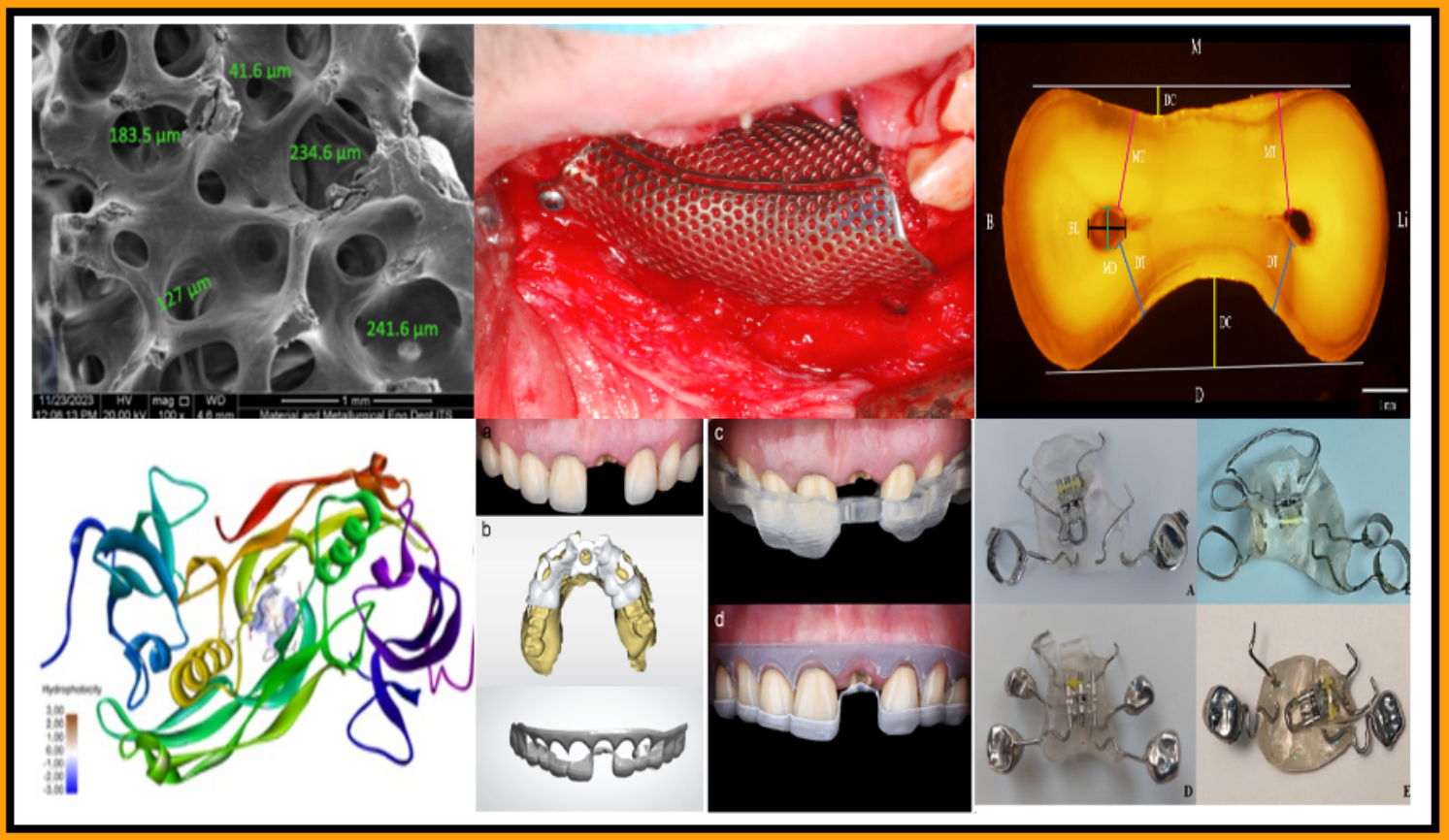

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The Effects of the Freeze-Drying Method on the Characteristics of A B-Tricalcium Phosphate / Polyacrylic Acid Composite Block

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Abstract

A mixture of β-tricalcium phosphate (βTCP) made from green mussel shells and polyacrylic acid (PAA) solution was successfully prepared via a setting reaction and reinforced by a freeze-drying process. The freeze-drying process is a popular method for producing bone substitute materials with porous structures. The sublimation process in freeze-drying eliminates the remaining water or solvent leaving the pores in the materials. The purpose of this study is to evaluate the effect of the freeze-drying method on the porosity and mechanical strength of a βTCP/PAA composite block. Initially, a mixture of βTCP powder with PAA solution was put inside a mold, frozen at low temperature, and dried to obtain a composite block. As a control, the same mixture was stored at 37 °C in an incubator without the freeze-drying process.

Porosity and diametral tensile strength (DTS) were investigated and compared with the control samples obtained without the freeze-drying process. Freeze-dried samples had lower porosity (26.97 ± 2.64%) and higher DTS (11.76 ± 1.59 MPa) than the control sample (porosity: 37.47 ± 4.49%; DTS: 6.19 ± 1.85 MPa).

In conclusion, the freeze-drying process decreased the porosity of the βTCP/PAA composite block and increased the mechanical strength in terms of DTS value.

Experimental article (J Int Dent Med Res 2024; 17(1): 82-84)

Keywords: Freeze-Drying, B-Tricalcium Phosphate, Polyacrylic Acid, Diametral Tensile Strength, Porosity.

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Introduction

The fabrication of a composite material comprising calcium phosphate and a polymer mixture through freeze-drying is an innovative method for creating artificial bone substitutes. This method aims to create a scaffold with enhanced mechanical strength, biocompatibility, and bioactivity that is suitable for bone tissue regeneration. Several studies have reported the successful fabrication of calcium phosphate materials, such as hydroxyapatite and β-tricalcium phosphate (βTCP; $Ca_3(PO_4)_2$) mixed with various kinds of polymer using the freeze-drying method.[1–3] However, most of these experiments used chemically synthetic reagents as the precursor, which are costly and limited in availability, especially in developing countries.

Pu'ad et al. stated that natural sources can be useful in the fabrication of calcium phosphate materials, such as hydroxyapatite and tricalcium phosphate.[4] One of the natural sources commonly used in the production of calcium phosphate materials is shellfish. In Indonesia, the green mussel is one of the most frequently consumed shellfish, resulting in a substantial amount of green mussel shell waste.[4–6] The calcium carbonate ($CaCO_3$) content in the shells is beneficial in the synthesis of calcium phosphate, particularly βTCP. Compared to hydroxyapatite, βTCP is more bioresorbable, allowing the gradual replacement of βTCP with new bone tissue, which eventually facilitates bone remodelling.[7–9] This study employed green mussel shells in the synthesis of βTCP as the inorganic component.

The fabrication of pure βTCP blocks through a setting reaction between βTCP and polyacrylic acid (PAA: $(C_3H_4O_2)n$) followed by a

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sintering process has been reported by Putri et al.[10] The current study adopts the binding mechanism between βTCP and PAA in which the carboxyl group (–COOH) in PAA bonds with calcium ions in βTCP to produce a composite block. However, in this method, water could possibly remain inside the block. Thus, a freeze-drying process was employed to eliminate the remaining water.

Therefore, the purpose of this study is to evaluate the diametral tensile strength (DTS) of a βTCP/PAA composite block obtained through the freeze-drying method.

Materials and methods

Preparation of βTCP powder was initiated by heating green mussel shells at 110 °C for 5 h and crushing them into powder. The powder was then sintered at 1,000 °C to convert it to calcium oxide. The powder was then reacted with a phosphoric acid solution with a calcium phosphate (Ca/P) ratio of 1.5 to obtain βTCP.

The βTCP powder was hand-mixed with a PAA solution (Mw: ~250,000, 35 wt% in H_2O, Sigma Aldrich, USA) on a paper pad with a spatula to produce a viscous paste, which was then put inside a mold with a diameter and thickness of 6 mm and 3 mm, respectively. The weight ratio of βTCP to PAA was 70 to 30. The mixture was deep-freezed at –80 °C to convert the remaining water into the ice crystal phase, followed by freeze-drying for the lyophilization process for 48 h. As a control, the mixture was only kept at 37 °C for 24 h in an incubator without the freeze-drying process to set.

The composite block was measured for porosity by subtracting the density from 100%, where density was calculated from the sample's weight and volume (Eqs. 1 and 2).[11,12]

$$\textbf{(1)}\quad \text{Relative density (\%)} = \frac{\text{bulk density}}{\text{theoretical density}} \times 100\ (\%)$$

$$\textbf{(2)}\quad \text{Total porosity (\%)} = 100 - \text{relative density (\%)}$$

The DTS value was evaluated using a universal testing machine (UTM: AGS-X, Shimadzu, Japan) with a crosshead speed of 1 mm/min.

Statistical analysis was performed with KaleidaGraph 4.01 software (Synergy Software) using one-way analysis of variance (ANOVA) and Fisher's least significant difference (LSD) post-hoc analysis to evaluate significant differences with a significance level set to $p < 0.05$.

Results

The appearance of the composite block is shown in Figure 1. The mixture of βTCP with PAA was successfully set into blocks. There were slight visible differences between the samples: the composite block synthesized without the freeze-drying process (Sample A) seemed to expand at the top compared to the block synthesized with the freeze-drying process (Sample B). This was confirmed by calculating the block volume, with Sample A having a higher volume (0.11 ± 0.01 cm^3) compared to Sample B (0.08 ± 0.01 cm^3).

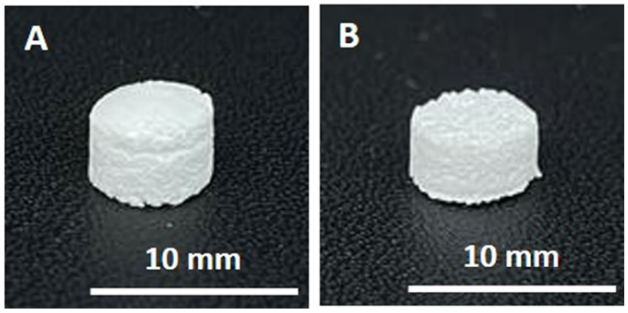

Figure 1. Photograph image of composite blocks containing βTCP mixed with PAA through (A) incubation and (B) freeze-drying method.

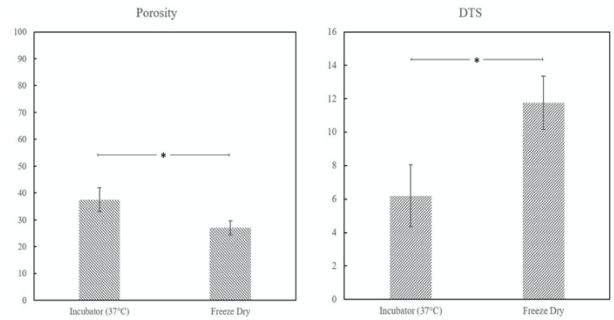

Figure 2. Porosity and DTS values of composite blocks containing βTCP mixed with PAA through incubation and freeze-drying method (n = 3).

The porosity and DTS values of the samples are displayed in Figure 2. The composite block fabricated through freeze-drying had significantly lower porosity (26.97 ± 2.64%) and higher DTS (11.76 ± 1.59 MPa) compared to the control block (porosity: 37.47 ± 4.49%; DTS: 6.19 ± 1.85 MPa).

Discussion

The composite block prepared through the freeze-drying method has a lower volume than the one set only by incubation at 37°C. This was due to the elimination of remaining water inside the block, which resulted in shrinkage. The freeze-drying process began with a freezing stage at a low temperature in which the remaining solvent or water turned into ice crystals. Upon drying, a sublimation process took place, leading to a decrease in the sample's volume. This was confirmed by a previous study by Moradi et al.[13] Haugh et al[14] also investigated the decrease in pore size of a scaffold due to the freeze-drying process. This study has proven that freeze-drying decreases a sample's volume, as well as its porosity.

Porous structures in bone substitute materials are crucial in facilitating the penetration of bone cells and tissue into the materials and promote new bone formation. Additionally, porosity influences the mechanical strength of a material. This coincides with the findings of this research, which indicate that lower porosity in the samples resulted in increased values of DTS.

The bonding mechanism between βTCP and PAA also plays a role in increasing mechanical strength. Carboxyl groups (–COOH) contained in PAA bind with calcium ions (Ca^{2+}) in the βTCP through chelating reactions, leading to the setting of the mixture. The bonding between these two reinforces the structure, which contributes to improved mechanical strength.[10,15] In the current study, both samples went through the bonding reaction. However, the freeze-dried samples were reinforced by decreased porosity, which means higher density and higher mechanical strength.

Conclusions

A composite block fabricated from βTCP and PAA through a freeze-drying method was found to have lower porosity and higher mechanical strength compared to a composite block synthesized without a freeze-drying process. However, the findings in this study need further characterization, such as FTIR observation, to confirm the bonding between the two materials. Other weight ratios of βTCP and PAA also need to be investigated.

Acknowledgements

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Declaration of Interest

The authors report no conflict of interest.

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The Effects of the Freeze-Drying Method on the Characteristics of A B-Tricalcium Phosphate / Polyacrylic Acid Composite Block

by Rosalina Tjandrawinata FKG

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Abstract

A mixture of β-tricalcium phosphate (βTCP) made from green mussel shells and polyacrylic acid (PAA) solution was successfully prepared via a setting reaction and reinforced by a freeze-drying process. The freeze-drying process is a popular method for producing bone substitute materials with porous structures. The sublimation process in freeze-drying eliminates the remaining water or solvent leaving the pores in the materials. The purpose of this study is to evaluate the effect of the freeze-drying method on the porosity and mechanical strength of a βTCP/PAA composite block. Initially, a mixture of βTCP powder with PAA solution was put inside a mold, frozen at low temperature, and dried to obtain a composite block. As a control, the same mixture was stored at 37 °C in an incubator without the freeze-drying process.

Porosity and diametral tensile strength (DTS) were investigated and compared with the control samples obtained without the freeze-drying process. Freeze-dried samples had lower porosity (26.97 ± 2.64%) and higher DTS (11.76 ± 1.59 MPa) than the control sample (porosity: 37.47 ± 4.49%; DTS: 6.19 ± 1.85 MPa).

In conclusion, the freeze-drying process decreased the porosity of the βTCP/PAA composite block and increased the mechanical strength in terms of DTS value.

Experimental article (J Int Dent Med Res 2024; 17(1): 82-84)

Keywords: Freeze-Drying, B-Tricalcium Phosphate, Polyacrylic Acid, Diametral Tensile Strength, Porosity.

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Introduction

The fabrication of a composite material comprising calcium phosphate and a polymer mixture through freeze-drying is an innovative method for creating artificial bone substitutes. This method aims to create a scaffold with enhanced mechanical strength, biocompatibility, and bioactivity that is suitable for bone tissue regeneration. Several studies have reported the successful fabrication of calcium phosphate materials, such as hydroxyapatite and β-tricalcium phosphate (βTCP; $Ca_3(PO_4)_2$) mixed with various kinds of polymer using the freeze-drying method.[1–3] However, most of these experiments used chemically synthetic reagents as the precursor, which are costly and limited in availability, especially in developing countries.

Pu'ad et al. stated that natural sources can be useful in the fabrication of calcium phosphate materials, such as hydroxyapatite and tricalcium phosphate.[4] One of the natural sources commonly used in the production of calcium phosphate materials is shellfish. In Indonesia, the green mussel is one of the most frequently consumed shellfish, resulting in a substantial amount of green mussel shell waste.[4–6] The calcium carbonate ($CaCO_3$) content in the shells is beneficial in the synthesis of calcium phosphate, particularly βTCP. Compared to hydroxyapatite, βTCP is more bioresorbable, allowing the gradual replacement of βTCP with new bone tissue, which eventually facilitates bone remodelling.[7–9] This study employed green mussel shells in the synthesis of βTCP as the inorganic component.

The fabrication of pure βTCP blocks through a setting reaction between βTCP and polyacrylic acid (PAA: $(C_3H_4O_2)n$) followed by a

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sintering process has been reported by Putri et al.[10] The current study adopts the binding mechanism between βTCP and PAA in which the carboxyl group (–COOH) in PAA bonds with calcium ions in βTCP to produce a composite block. However, in this method, water could possibly remain inside the block. Thus, a freeze-drying process was employed to eliminate the remaining water.

Therefore, the purpose of this study is to evaluate the diametral tensile strength (DTS) of a βTCP/PAA composite block obtained through the freeze-drying method.

Materials and methods

Preparation of βTCP powder was initiated by heating green mussel shells at 110 °C for 5 h and crushing them into powder. The powder was then sintered at 1,000 °C to convert it to calcium oxide. The powder was then reacted with a phosphoric acid solution with a calcium phosphate (Ca/P) ratio of 1.5 to obtain βTCP.

The βTCP powder was hand-mixed with a PAA solution (Mw: ~250,000, 35 wt% in H_2O, Sigma Aldrich, USA) on a paper pad with a spatula to produce a viscous paste, which was then put inside a mold with a diameter and thickness of 6 mm and 3 mm, respectively. The weight ratio of βTCP to PAA was 70 to 30. The mixture was deep-freezed at –80 °C to convert the remaining water into the ice crystal phase, followed by freeze-drying for the lyophilization process for 48 h. As a control, the mixture was only kept at 37 °C for 24 h in an incubator without the freeze-drying process to set.

The composite block was measured for porosity by subtracting the density from 100%, where density was calculated from the sample's weight and volume (Eqs. 1 and 2).[11,12]

$$\textbf{(1)}\quad \text{Relative density (\%)} = \frac{\text{bulk density}}{\text{theoretical density}} \times 100\ (\%)$$

$$\textbf{(2)}\quad \text{Total porosity (\%)} = 100 - \text{relative density (\%)}$$

The DTS value was evaluated using a universal testing machine (UTM: AGS-X, Shimadzu, Japan) with a crosshead speed of 1 mm/min.

Statistical analysis was performed with KaleidaGraph 4.01 software (Synergy Software) using one-way analysis of variance (ANOVA) and Fisher's least significant difference (LSD) post-hoc analysis to evaluate significant differences with a significance level set to $p < 0.05$.

Results

The appearance of the composite block is shown in Figure 1. The mixture of βTCP with PAA was successfully set into blocks. There were slight visible differences between the samples: the composite block synthesized without the freeze-drying process (Sample A) seemed to expand at the top compared to the block synthesized with the freeze-drying process (Sample B). This was confirmed by calculating the block volume, with Sample A having a higher volume (0.11 ± 0.01 cm^3) compared to Sample B (0.08 ± 0.01 cm^3).

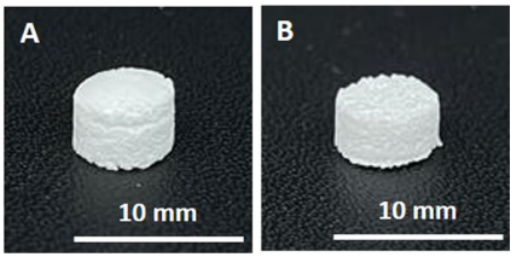

Figure 1. Photograph image of composite blocks containing βTCP mixed with PAA through (A) incubation and (B) freeze-drying method.

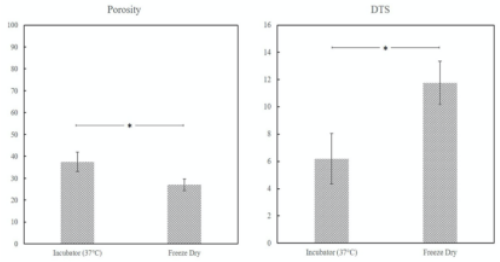

Figure 2. Porosity and DTS values of composite blocks containing βTCP mixed with PAA through incubation and freeze-drying method (n = 3).

The porosity and DTS values of the samples are displayed in Figure 2. The composite block fabricated through freeze-drying had significantly lower porosity (26.97 ± 2.64%) and higher DTS (11.76 ± 1.59 MPa) compared to the control block (porosity: 37.47 ± 4.49%; DTS: 6.19 ± 1.85 MPa).

Discussion

The composite block prepared through the freeze-drying method has a lower volume than the one set only by incubation at 37°C. This was due to the elimination of remaining water inside the block, which resulted in shrinkage. The freeze-drying process began with a freezing stage at a low temperature in which the remaining solvent or water turned into ice crystals. Upon drying, a sublimation process took place, leading to a decrease in the sample's volume. This was confirmed by a previous study by Moradi et al.[13] Haugh et al[14] also investigated the decrease in pore size of a scaffold due to the freeze-drying process. This study has proven that freeze-drying decreases a sample's volume, as well as its porosity.

Porous structures in bone substitute materials are crucial in facilitating the penetration of bone cells and tissue into the materials and promote new bone formation. Additionally, porosity influences the mechanical strength of a material. This coincides with the findings of this research, which indicate that lower porosity in the samples resulted in increased values of DTS.

The bonding mechanism between βTCP and PAA also plays a role in increasing mechanical strength. Carboxyl groups (–COOH) contained in PAA bind with calcium ions (Ca^{2+}) in the βTCP through chelating reactions, leading to the setting of the mixture. The bonding between these two reinforces the structure, which contributes to improved mechanical strength.[10,15] In the current study, both samples went through the bonding reaction. However, the freeze-dried samples were reinforced by decreased porosity, which means higher density and higher mechanical strength.

Conclusions

A composite block fabricated from βTCP and PAA through a freeze-drying method was found to have lower porosity and higher mechanical strength compared to a composite block synthesized without a freeze-drying process. However, the findings in this study need further characterization, such as FTIR observation, to confirm the bonding between the two materials. Other weight ratios of βTCP and PAA also need to be investigated.

Acknowledgements

The authors would like to thank Universitas Trisakti for supporting this research (reference number: 0604/PUF/FKG/2023-2024).

Declaration of Interest

The authors report no conflict of interest.

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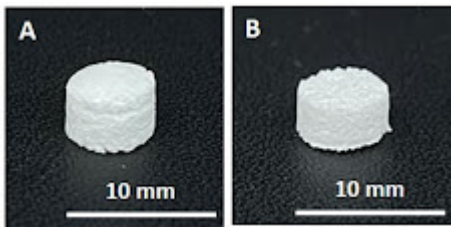

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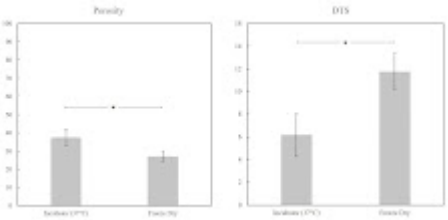

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Dear Editor,

I have already found our published article. Thank you for the opportunity to publish in your journal. I apologize for the late reply.
However, is it possible for us to request another page with the editorial board?

I apologize for any inconvenience.

Thank you very much.

Sincerely,
Tansza Setiana Putri

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