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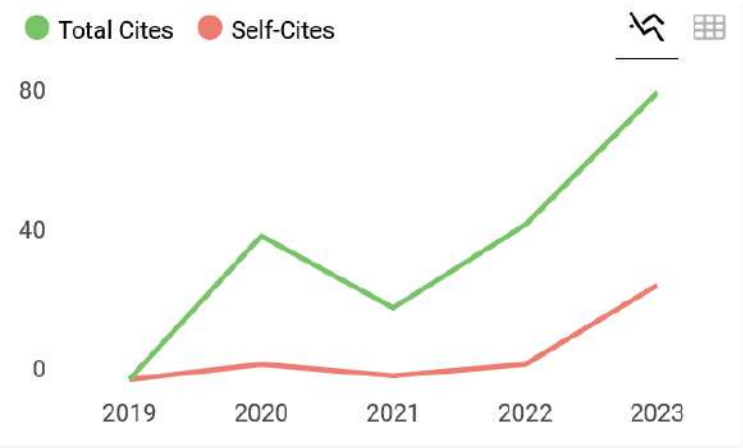
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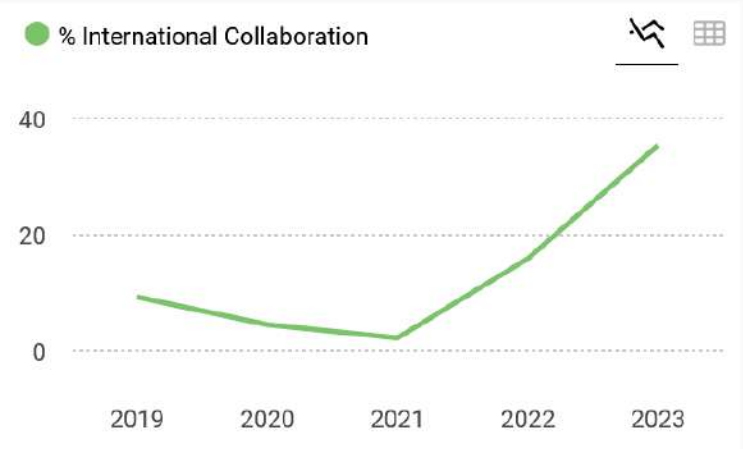
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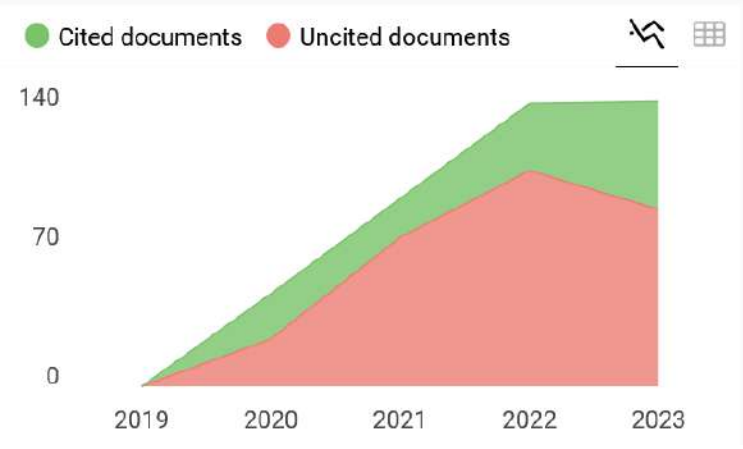
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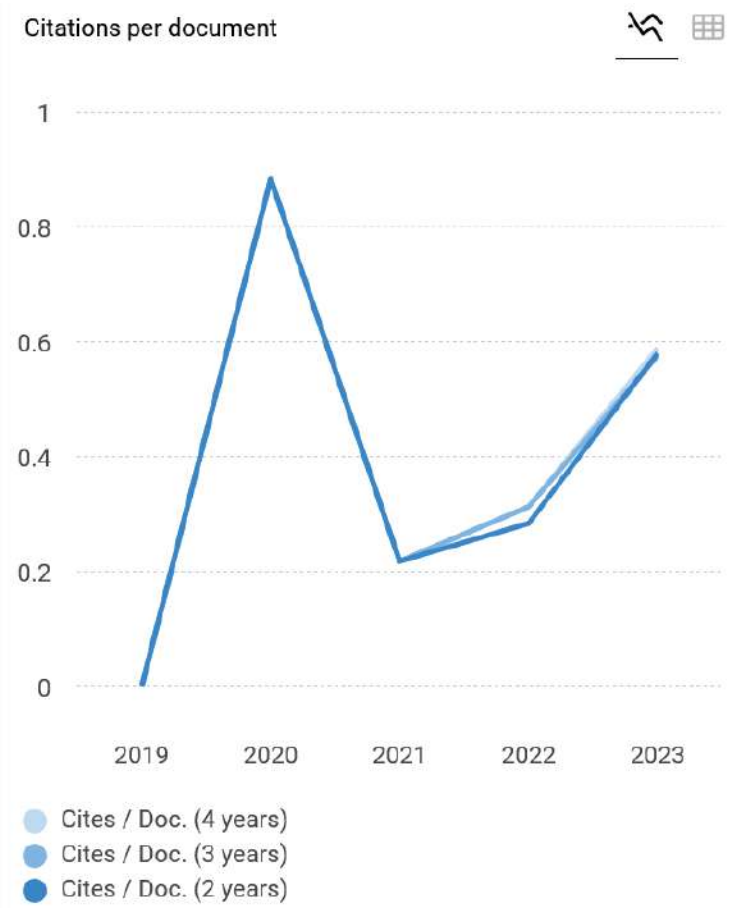
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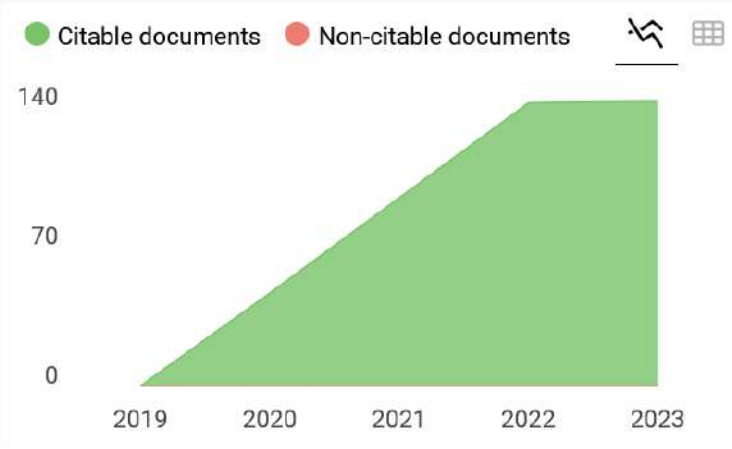
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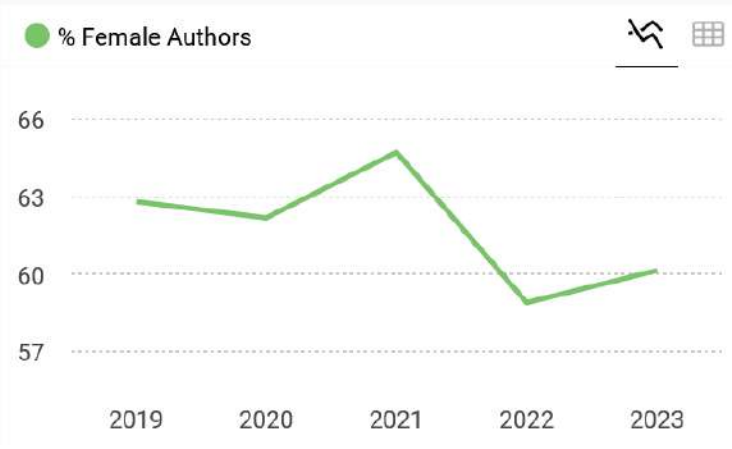
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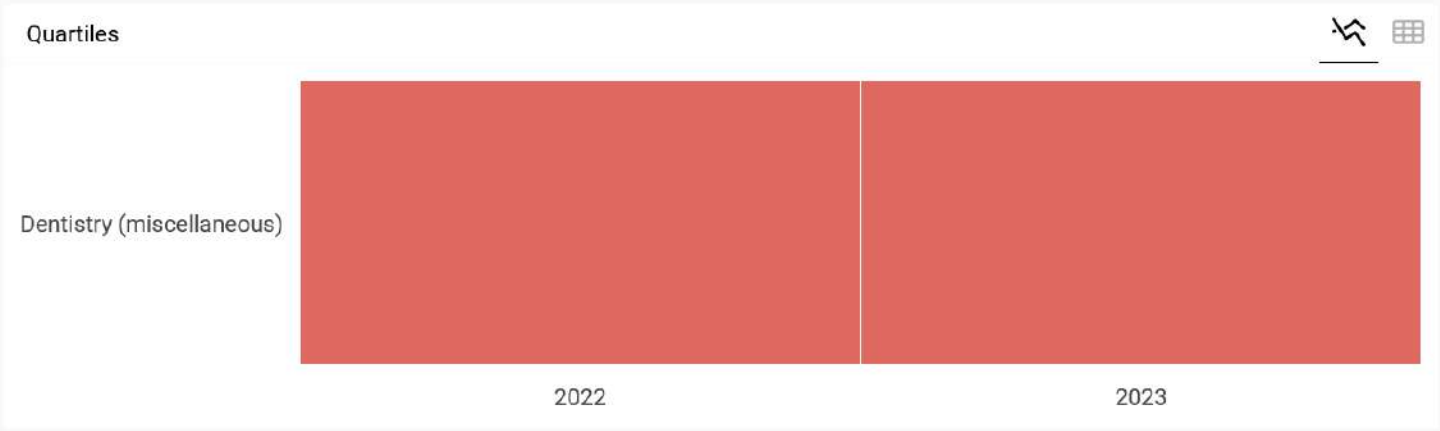
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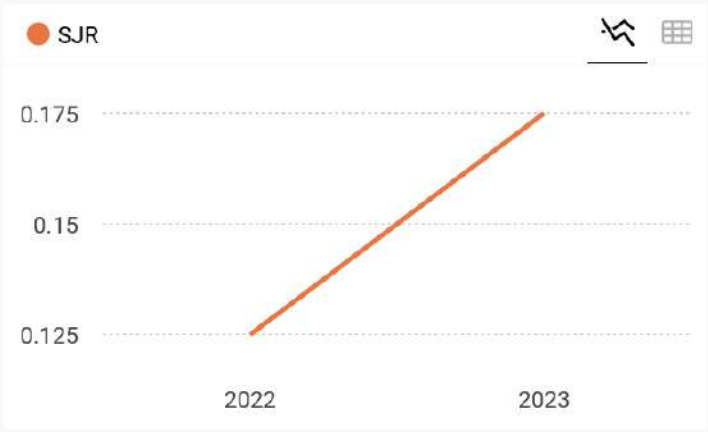
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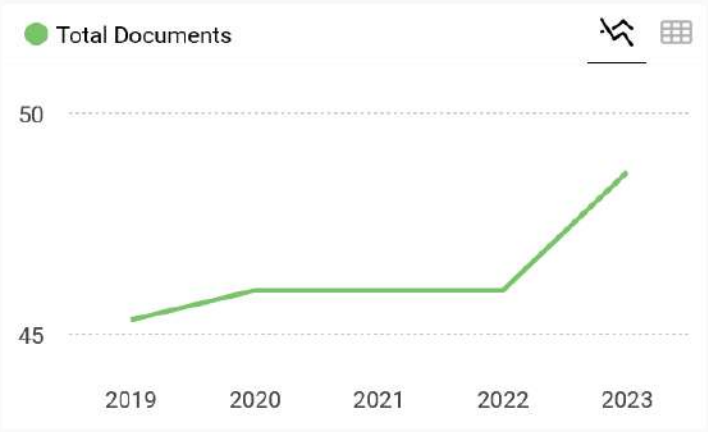
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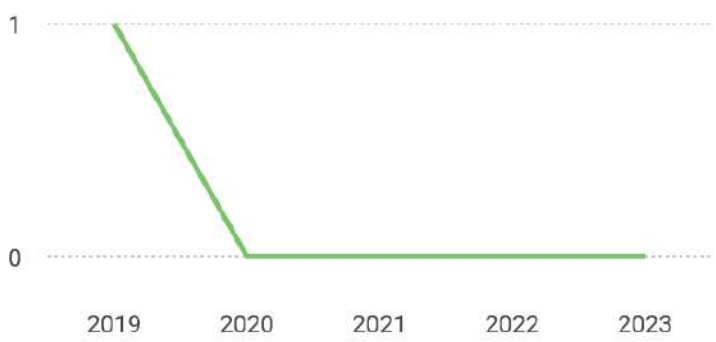

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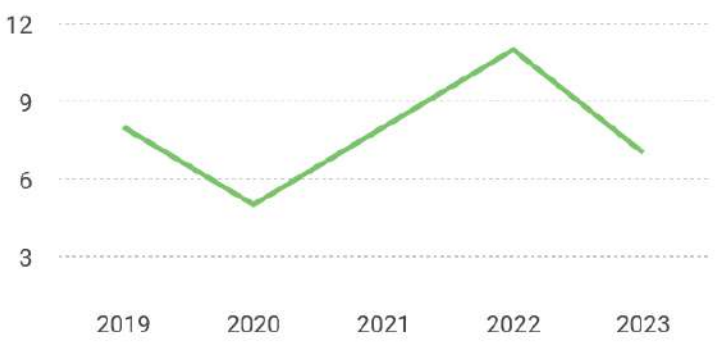

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Original article

Degradation of chitosan–gelatin and chitosan–gelatin–β-tricalcium phosphate scaffolds

Tansza Setiana Putri[1], Deviyanti Pratiwi[1], Dewi Liliany Margaretta[1], Rosalina Tjandrawinata[1], Khairul Anuar Shariff[2]
[1]Department of Dental Materials, Faculty of Dentistry, Universitas Trisakti, Jakarta, Indonesia
[2]Biomaterials Niche Area, School of Materials and Mineral Resources Engineering, Universiti Sains Malaysia, Pulau Pinang, Malaysia

ABSTRACT

Background: *Fabrication of the composite scaffold was carried out by combining chitosan, gelatin, and β-tricalcium phosphate (βTCP) derived from limestone. The extraction of βTCP was based on the abundance of limestone containing calcium carbonate, which can be a source of βTCP synthesis.* ***Purpose:*** *This study evaluates the degradation of the combination of chitosan–gelatin (ChG) and chitosan–gelatin–βTCP (ChG-βTCP) composite scaffolds.* ***Methods:*** *The freeze-drying method was used to obtain the composite scaffold, which was a mixture of chitosan, gelatin, and βTCP. Degradation was measured by immersing the samples in a simulated body fluid solution at 37°C for 3, 7, 14, and 21 days. For statistical analysis, one-way analysis of variance (ANOVA) and post hoc Fisher's least significant difference were performed.* ***Results:*** *The ChG scaffold shows better degradability than the ChG-βTCP scaffold. The ChG scaffold shows higher weight degradation than the ChG-βTCP scaffold up to 21 days.* ***Conclusion:*** *In conclusion, the scaffold containing βTCP has lower degradation than the ChG scaffold.*

Keywords: *β-tricalcium phosphate; chitosan; gelatin; degradation; bone tissue engineering*
Article history: *Received 10 March 2023; Revised 23 June 2023; Accepted 10 July 2023; Published 1 June 2024*

Correspondence: Tansza Setiana Putri, Department of Dental Materials, Faculty of Dentistry, Universitas Trisakti. Jl. Kyai Tapa 260, Jakarta 11440, Indonesia. Email: tansza@trisakti.ac.id

INTRODUCTION

A combination of chitosan and gelatin (ChG) has recently become an alternative in bone regeneration treatment. Upon *in vitro* and *in vivo* evaluation, this combination exhibits excellent biocompatibility.[1–4] Few studies have successfully fabricated a scaffold containing ChG through the freeze-drying method and showed an excellent interconnected porosity.[1,2,5] On the other hand, a combination of ChG could be optimized in terms of degradability by regulating the porosity. However, the ChG scaffold also shows limited mechanical strength.[1,2]

Inorganic materials could be added to increase the mechanical strength of the scaffold.[6–9] ChG would act as the organic component or matrix that bonds the inorganic solid.[6,7] Most inorganic materials used in orthopedic and dental treatment contain calcium phosphate. β-tricalcium phosphate (βTCP) has a higher degradability compared to hydroxyapatite, which would later facilitate the new bone formation and eventually lead to appropriate bone remodeling.[6,7,10–15]

However, purchasing βTCP remains expensive, especially in developed countries. Researchers have attempted to develop βTCP from natural sources, such as limestone.[16,17] Limestone has a main component of calcium carbonate, which makes it a potential source of calcium in fabricating βTCP.[17–19] Putri et al.,[18–20] successfully fabricated a composite scaffold from ChG with the addition of βTCP derived from limestone. However, the degradation of the scaffold has not yet been evaluated. One of the crucial parameters in fabricating biomaterials for bone regeneration application is to analyze the degradation in order to determine whether the materials will degrade in time with new bone formation in the bone remodeling cycle. Therefore, this study evaluates and compares the degradation of the ChG scaffold and the ChG scaffold with the addition of βTCP derived from limestone (ChG–βTCP). Materials with a suitable degradation rate are applicable

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in bone regeneration treatment because, with appropriate degradation, new bone formation is expectedly to occur simultaneously, leading to proper bone remodeling.

MATERIALS AND METHODS

βTCP powder was produced from limestone at the Center for Ceramics in Indonesia as the precursor.[17] Calcium carbonate contained in limestone was sintered to calcium oxide at 1,000°C and converted to calcium hydroxide through wet milling. Afterward, the calcium hydroxide was mixed with phosphoric acid through the wet precipitation method and then sintered at 1,000°C to acquire βTCP.

Chitosan solutions were prepared by dissolving chitosan powder (medium molecular weight; Sigma Aldrich) in a 2% acetic acid solution and mixed at 45°C for 10 minutes. A gelatin-in-water solution (W/P=2) was added into the chitosan solution and mixed for another 10 minutes at 45°C, followed by the addition of the obtained βTCP powder and 0.25% glutaraldehyde, which was then manually mixed until the mixture was homogenous. The composition of chitosan:gelatin:βTCP was 15:15:70 (ChG–βTCP). One group of samples was obtained without the addition of βTCP (ChG). The mixture was then put inside a 6 mm 11 mm mold (diameter height) and deep-frozen at –80°C for 24 hours, followed by the freeze-drying process (Freeze-dryer; VirTis Benchtop K, SP Industries). The samples were washed using sodium borohydride and sodium hydroxide solutions.

Degradation of the samples was evaluated by immersing the samples in a simulated body fluid (SBF) solution at 37°C for 3, 7, 14, and 21 days. After immersion, the samples were freeze-dried. The degradation percentage was calculated using Equation 1:

$$Wd\ (\%) = \frac{W1 - W2}{W1} x\ 100$$

where *Wd* is the degraded weight in percentage and *W1* and *W2* are the sample weights before and after immersion in the SBF solution, respectively. The number of samples in each immersion duration was three (n=3).

The percentage of both the remaining materials was analyzed by one-way analysis of variance (ANOVA). A *post hoc* Fisher's least significant difference (LSD) test was also performed using Kaleidagraph version 4.01 (Synergy Software, Reading, PA, USA). The level of significance was $p < 0.05$.

RESULTS

Two groups of scaffolds (ChG and ChG–βTCP) were successfully fabricated through the freeze-drying method (Figure 1). The scaffold with the addition of βTCP had an opaque, whitish appearance, whereas the scaffold without βTCP had a more translucent and yellowish appearance.

Figure 2 shows the degradation percentage of the scaffolds in the SBF solution. Since the third day, both

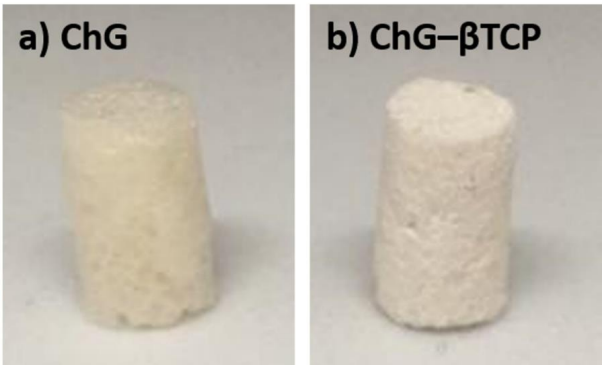

Figure 1. The photographs of (a) ChG and (b) ChG–βTCP scaffolds.

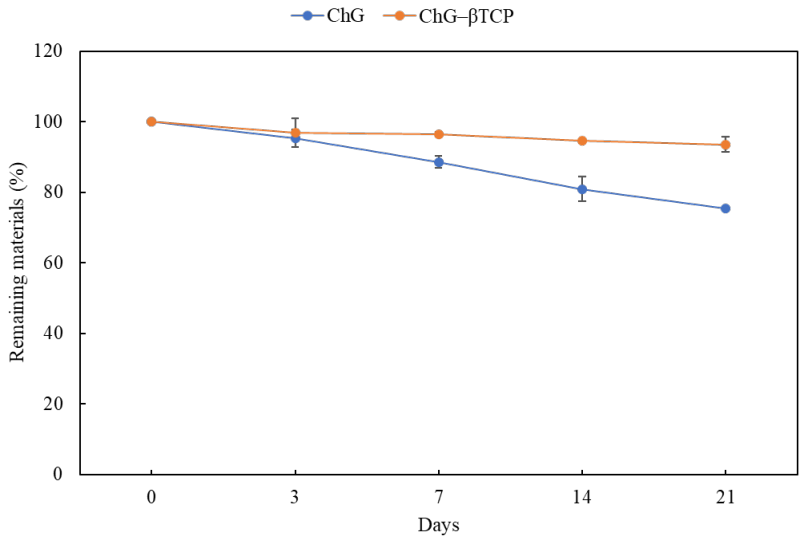

Figure 2. The percentage of the remaining scaffolds after immersion in the SBF solution.

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Table 1. Statistical analysis with a *post hoc* Fisher's LSD test

Comparison	Mean Difference	|t|	*p*
ChG (3d) vs ChG (7d)	6.6591	1.8434	0.1025
ChG (3d) vs ChG (14d)	14.3224	3.9649	0.0041*
ChG (3d) vs ChG (21d)	19.7597	5.4701	0.0006*
ChG (7d) vs ChG (14d)	7.6633	2.1214	0.0667
ChG (7d) vs ChG (21d)	13.1006	3.6266	0.0067*
ChG (14d) vs ChG (21d)	5.4373	1.5052	0.1707
ChG-βTCP (3d) vs ChG-βTCP (7d)	0.0423	0.0117	0.9909
ChG-βTCP (3d) vs ChG-βTCP (14d)	2.0545	0.5688	0.5851
ChG-βTCP (3d) vs ChG-βTCP (21d)	3.0538	0.8454	0.4225
ChG-βTCP (7d) vs ChG-βTCP (14d)	2.0968	0.5805	0.5776
ChG-βTCP (7d) vs ChG-βTCP (21d)	3.0961	0.8571	0.4163
ChG-βTCP (14d) vs ChG-βTCP (21d)	0.9993	0.2766	0.7891
ChG (3d) vs ChG-βTCP (3d)	1.3983	0.3871	0.7088
ChG (7d) vs ChG-βTCP (7d)	8.0997	2.2422	0.0552
ChG (14d) vs ChG-βTCP (14d)	13.6662	3.7832	0.0054*
ChG (21d) vs ChG-βTCP (21d)	18.1042	5.0118	0.001*

*$p < 0.05$

samples' weights decreased until day 21. However, the ChG scaffold shows a significant decrease at every time interval, while the ChG–βTCP scaffold is more stable and the weight decrease is not significant. Until day 21, ChG has 24.55% weight loss, while ChG–βTCP has only 6.45% weight loss.

Table 1 exhibits the significant difference between the ChG scaffold and the ChG–βTCP scaffold at each time interval. There was no significant weight decrease on the ChG–βTCP scaffold until day 21. However, on the ChG scaffold, there was significant weight loss from day 3 to day 14, from day 3 to day 21, and from day 7 to day 21. Additionally, at day 14 and day 21, ChG shows significantly more weight loss compared to ChG–βTCP.

DISCUSSION

We fabricated a composite scaffold from chitosan, gelatin, and βTCP derived from limestone and a ChG scaffold as a control (Figure 1). In contrast to the translucent ChG scaffold, the ChG–βTCP scaffold had an opaque appearance, which is given by the typically white powder of bioceramics materials (in this case, βTCP). The scaffold fabricated in this study consisted of 15% chitosan, 15% gelatin, and 70% βTCP. The 70:30 composition between βTCP and the polymers mimics the composition of inorganic material and organic component in bone,[21] while the ChG scaffold employed a 50:50 composition as the control sample.

Chitosan and gelatin are biodegradable, and both polymers can be dissolved in water.[1,22] Salati et al.[22] evaluated the degradation of the ChG composite scaffold at various compositions. It was found that the density of hydrophilic groups in the structure affected the degradation of the scaffold. Putri et al.[18] revealed that the porosity of the ChG scaffold is higher than the scaffold with the addition of βTCP. Higher porosity means less density; thus, ChG has higher degradation. This is confirmed by the decrease in weight on the ChG sample in this study.

Serra et al.[7] explains that the structure of the ChG scaffold contains a high amount of hydrophilic groups, such as amine and hydroxyl groups. This causes the scaffold to easily dissolve in water. The addition of βTCP powder into the ChG mixture increases the viscosity of the mixture, which then creates a firmer and denser scaffold. In addition, the bond between chitosan, gelatin, and βTCP consumes some hydrophilic groups, which further inhibits the molecules to hydrolyze.[6,7] βTCP enhances the stability of the network and increases the bond strength, causing the degradation of the ChG–βTCP scaffold to decrease. This result is in accordance with the research conducted by Maji et al.,[6] where the addition of βTCP decreased the degradation rate of the scaffold.

Putri et al.[18] found that the scaffold containing βTCP has lower porosity compared to the ChG scaffold. This also corresponds to the result of this study. The lower porosity in the ChG–βTCP scaffold indicates a denser structure, which causes lower degradation. On the other hand, the higher porosity in the ChG scaffold facilitates the penetration of liquid into the materials, which enables the materials to dissolve better and, in turn, increases its degradation.

In conclusion, the scaffold containing βTCP has a more stable structure and is more resistant to degradation compared to the ChG scaffold. This result indicates that ChG-βTCP could be a candidate for bone substitution due to its ability to maintain its structure and facilitate the bone remodeling process. Other variables correlated with the materials' degradation such as bioactivity and biomineralization need to be evaluated.

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Original article

Degradation of chitosan–gelatin and chitosan–gelatin–β-tricalcium phosphate scaffolds

Tansza Setiana Putri[1], Deviyanti Pratiwi[1], Dewi Liliany Margaretta[1], Rosalina Tjandrawinata[1], Khairul Anuar Shariff[2]
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ABSTRACT

Background: *Fabrication of the composite scaffold was carried out by combining chitosan, gelatin, and β-tricalcium phosphate (βTCP) derived from limestone. The extraction of βTCP was based on the abundance of limestone containing calcium carbonate, which can be a source of βTCP synthesis.* ***Purpose:*** *This study evaluates the degradation of the combination of chitosan–gelatin (ChG) and chitosan–gelatin–βTCP (ChG-βTCP) composite scaffolds.* ***Methods:*** *The freeze-drying method was used to obtain the composite scaffold, which was a mixture of chitosan, gelatin, and βTCP. Degradation was measured by immersing the samples in a simulated body fluid solution at 37°C for 3, 7, 14, and 21 days. For statistical analysis, one-way analysis of variance (ANOVA) and post hoc Fisher's least significant difference were performed.* ***Results:*** *The ChG scaffold shows better degradability than the ChG-βTCP scaffold. The ChG scaffold shows higher weight degradation than the ChG-βTCP scaffold up to 21 days.* ***Conclusion:*** *In conclusion, the scaffold containing βTCP has lower degradation than the ChG scaffold.*

Keywords: *β-tricalcium phosphate; chitosan; gelatin; degradation; bone tissue engineering*
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INTRODUCTION

A combination of chitosan and gelatin (ChG) has recently become an alternative in bone regeneration treatment. Upon *in vitro* and *in vivo* evaluation, this combination exhibits excellent biocompatibility.[1–4] Few studies have successfully fabricated a scaffold containing ChG through the freeze-drying method and showed an excellent interconnected porosity.[1,2,5] On the other hand, a combination of ChG could be optimized in terms of degradability by regulating the porosity. However, the ChG scaffold also shows limited mechanical strength.[1,2]

Inorganic materials could be added to increase the mechanical strength of the scaffold.[6–9] ChG would act as the organic component or matrix that bonds the inorganic solid.[6,7] Most inorganic materials used in orthopedic and dental treatment contain calcium phosphate. β-tricalcium phosphate (βTCP) has a higher degradability compared to hydroxyapatite, which would later facilitate the new bone formation and eventually lead to appropriate bone remodeling.[6,7,10–15]

However, purchasing βTCP remains expensive, especially in developed countries. Researchers have attempted to develop βTCP from natural sources, such as limestone.[16,17] Limestone has a main component of calcium carbonate, which makes it a potential source of calcium in fabricating βTCP.[17–19] Putri et al.,[18–20] successfully fabricated a composite scaffold from ChG with the addition of βTCP derived from limestone. However, the degradation of the scaffold has not yet been evaluated. One of the crucial parameters in fabricating biomaterials for bone regeneration application is to analyze the degradation in order to determine whether the materials will degrade in time with new bone formation in the bone remodeling cycle. Therefore, this study evaluates and compares the degradation of the ChG scaffold and the ChG scaffold with the addition of βTCP derived from limestone (ChG–βTCP). Materials with a suitable degradation rate are applicable

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in bone regeneration treatment because, with appropriate degradation, new bone formation is expectedly to occur simultaneously, leading to proper bone remodeling.

MATERIALS AND METHODS

βTCP powder was produced from limestone at the Center for Ceramics in Indonesia as the precursor.[17] Calcium carbonate contained in limestone was sintered to calcium oxide at 1,000°C and converted to calcium hydroxide through wet milling. Afterward, the calcium hydroxide was mixed with phosphoric acid through the wet precipitation method and then sintered at 1,000°C to acquire βTCP.

Chitosan solutions were prepared by dissolving chitosan powder (medium molecular weight; Sigma Aldrich) in a 2% acetic acid solution and mixed at 45°C for 10 minutes. A gelatin-in-water solution (W/P=2) was added into the chitosan solution and mixed for another 10 minutes at 45°C, followed by the addition of the obtained βTCP powder and 0.25% glutaraldehyde, which was then manually mixed until the mixture was homogenous. The composition of chitosan:gelatin:βTCP was 15:15:70 (ChG–βTCP). One group of samples was obtained without the addition of βTCP (ChG). The mixture was then put inside a 6 mm 11 mm mold (diameter height) and deep-frozen at –80°C for 24 hours, followed by the freeze-drying process (Freeze-dryer; VirTis Benchtop K, SP Industries). The samples were washed using sodium borohydride and sodium hydroxide solutions.

Degradation of the samples was evaluated by immersing the samples in a simulated body fluid (SBF) solution at 37°C for 3, 7, 14, and 21 days. After immersion, the samples were freeze-dried. The degradation percentage was calculated using Equation 1:

$$Wd\ (\%) = \frac{W1 - W2}{W1} x\ 100$$

where *Wd* is the degraded weight in percentage and *W1* and *W2* are the sample weights before and after immersion in the SBF solution, respectively. The number of samples in each immersion duration was three (n=3).

The percentage of both the remaining materials was analyzed by one-way analysis of variance (ANOVA). A *post hoc* Fisher's least significant difference (LSD) test was also performed using Kaleidagraph version 4.01 (Synergy Software, Reading, PA, USA). The level of significance was $p < 0.05$.

RESULTS

Two groups of scaffolds (ChG and ChG–βTCP) were successfully fabricated through the freeze-drying method (Figure 1). The scaffold with the addition of βTCP had an opaque, whitish appearance, whereas the scaffold without βTCP had a more translucent and yellowish appearance.

Figure 2 shows the degradation percentage of the scaffolds in the SBF solution. Since the third day, both

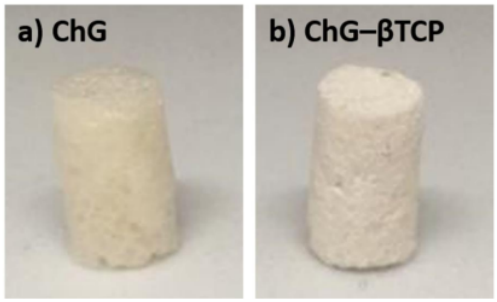

Figure 1. The photographs of (a) ChG and (b) ChG–βTCP scaffolds.

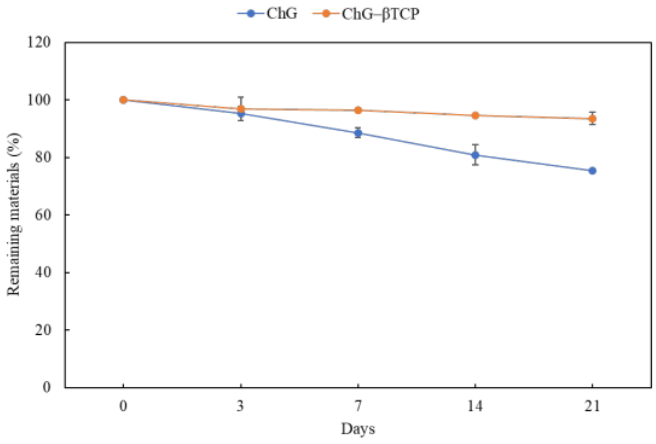

Figure 2. The percentage of the remaining scaffolds after immersion in the SBF solution.

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Table 1. Statistical analysis with a *post hoc* Fisher's LSD test

Comparison	Mean Difference	|t|	*p*
ChG (3d) vs ChG (7d)	6.6591	1.8434	0.1025
ChG (3d) vs ChG (14d)	14.3224	3.9649	0.0041*
ChG (3d) vs ChG (21d)	19.7597	5.4701	0.0006*
ChG (7d) vs ChG (14d)	7.6633	2.1214	0.0667
ChG (7d) vs ChG (21d)	13.1006	3.6266	0.0067*
ChG (14d) vs ChG (21d)	5.4373	1.5052	0.1707
ChG-βTCP (3d) vs ChG-βTCP (7d)	0.0423	0.0117	0.9909
ChG-βTCP (3d) vs ChG-βTCP (14d)	2.0545	0.5688	0.5851
ChG-βTCP (3d) vs ChG-βTCP (21d)	3.0538	0.8454	0.4225
ChG-βTCP (7d) vs ChG-βTCP (14d)	2.0968	0.5805	0.5776
ChG-βTCP (7d) vs ChG-βTCP (21d)	3.0961	0.8571	0.4163
ChG-βTCP (14d) vs ChG-βTCP (21d)	0.9993	0.2766	0.7891
ChG (3d) vs ChG-βTCP (3d)	1.3983	0.3871	0.7088
ChG (7d) vs ChG-βTCP (7d)	8.0997	2.2422	0.0552
ChG (14d) vs ChG-βTCP (14d)	13.6662	3.7832	0.0054*
ChG (21d) vs ChG-βTCP (21d)	18.1042	5.0118	0.001*

*$p < 0.05$

samples' weights decreased until day 21. However, the ChG scaffold shows a significant decrease at every time interval, while the ChG–βTCP scaffold is more stable and the weight decrease is not significant. Until day 21, ChG has 24.55% weight loss, while ChG–βTCP has only 6.45% weight loss.

Table 1 exhibits the significant difference between the ChG scaffold and the ChG–βTCP scaffold at each time interval. There was no significant weight decrease on the ChG–βTCP scaffold until day 21. However, on the ChG scaffold, there was significant weight loss from day 3 to day 14, from day 3 to day 21, and from day 7 to day 21. Additionally, at day 14 and day 21, ChG shows significantly more weight loss compared to ChG–βTCP.

DISCUSSION

We fabricated a composite scaffold from chitosan, gelatin, and βTCP derived from limestone and a ChG scaffold as a control (Figure 1). In contrast to the translucent ChG scaffold, the ChG–βTCP scaffold had an opaque appearance, which is given by the typically white powder of bioceramics materials (in this case, βTCP). The scaffold fabricated in this study consisted of 15% chitosan, 15% gelatin, and 70% βTCP. The 70:30 composition between βTCP and the polymers mimics the composition of inorganic material and organic component in bone,[21] while the ChG scaffold employed a 50:50 composition as the control sample.

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Participants

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Alexander Patera Nugraha (pateraha)

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Messages

Note	From
Dear Tansza Setiana Putri, Deviyanti Pratiwi, Dewi Liliany Margaretta, Rosalina Tjandrawinata, Khairul Anuar Shariff, We have reached a decision regarding your submission to Dental Journal, "Degradation of chitosan–gelatin and chitosan–gelatin–β-tricalcium phosphate scaffolds". Our decision is to: Accept your manuscript Manuscript URL: https://e-journal.unair.ac.id/MKG/authorDashboard/submission/44064 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 Alexander Patera Nugraha	pateraha 2023-07-10 08:40 AM
Dear Tansza Setiana Putri, Deviyanti Pratiwi, Dewi Liliany Margaretta, Rosalina Tjandrawinata, Khairul Anuar Shariff, We have reached a decision regarding your submission to Dental Journal, "Degradation of chitosan–gelatin and chitosan–gelatin–β-tricalcium phosphate scaffolds". Our decision is to: Accept your manuscript Manuscript URL: https://e-journal.unair.ac.id/MKG/authorDashboard/submission/44064 It will be published by Dental Journal (Majalah Kedokteran Gigi) on volume 57, issue 2 – June 2024 Articles will go through the process of copyediting (including re-check of similarity) and production (proofreading, galley layout and publishing). Thank you for your submission. Your next manuscript is very welcome. Best Regards, Editor in Chief	pateraha 2024-01-10 09:40 AM

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Copyediting

Participants

Tansza Permata Setiana Putri (tanstri)

Alexander Patera Nugraha (pateraha)

Messages

Note	From
Dear Tansza Setiana Putri, Deviyanti Pratiwi, Dewi Liliany Margaretta, Rosalina Tjandrawinata, Khairul Anuar Shariff, The editing of your manuscript submission, "Degradation of chitosan–gelatin and chitosan–gelatin–β-tricalcium phosphate scaffolds," 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/44064 Regards, Editor	pateraha 2024-03-05 09:15 AM

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Proofreading

Participants

Tansza Permata Setiana Putri (tanstri)

Alexander Patera Nugraha (pateraha)

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Note	From
Dear Dr Tansza, Please find the proofreading article attached below. Please complete or answer the proofreader's questions in the comments section using the 'CleanCopy__' file. **Please reply to this message if you have already answered the proofreader's comments**. Best wishes, Editor CleanCopy-Tansza Putri_Degradation of chitosangelatin and chitosangelatinβ-tricalcium-274359.docx TrackChanges-Tansza Putri_Degradation of chitosangelatin and chitosangelatinβ-tricalcium-274359.docx	pateraha 2024-03-13 09:14 AM
Dear Editor, Please find the revised proofreading article in te attachment. Thank you. Best regards, Tansza CleanCopy-Tansza+Putri_Degradation+of+chitosangelatin+and+chitosangelatinβ-tricalcium-274359.docx	tanstri 2024-03-18 09:29 AM

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Galley proof

Participants

Tansza Permata Setiana Putri (tanstri)

Alexander Patera Nugraha (pateraha)

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Note	From
Dear Tansza Permata Setiana Putri, Please give approval for the attached galley proof of the article. If there is any correction please do not hesitate to contact us and reply to this message. Regards, Editor DJMKG 57(2)2024_Galley proof 1_Tansza Putri et al.pdf	pateraha 2024-03-28 08:24 AM
Dear Editor, The authors have approved the galley proof. Thank you very much. Best regards, Tansza	tanstri 2024-03-28 12:49 PM

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