Effect of glutaraldehyde on the characteristics of chitosan–gelatin–β-tricalcium phosphate composite scaffolds

by Devi Rianti

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Effect of glutaraldehyde on the characteristics of chitosan–gelatin–β-tricalcium phosphate composite scaffolds

Tansza Setiana Putri *, Devi Rianti, Priyawan Rachmadi, Anita Yuliati

Dental Materials Science and Technology Division, Faculty of Dental Medicine, Airlangga University, Jl. Prof. Dr. Moestopo No. 47, Surabaya 60132, East Java, Indonesia

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ABSTRACT

A composite scaffold was successfully fabricated using β-tricalcium phosphate (βTCP), which is extracted from limestone by first sintering and then reacting it with phosphoric acid through wet precipitation method. The resultant substance is then mixed with chitosan and gelatin. This novel method utilizes limestone, which is abundant in nature. This study optimizes the composite scaffold fabrication by using βTCP from limestone and evaluating the effect of glutaraldehyde on scaffold characteristics. The freeze-drying method was used to obtain a porous scaffold. The compressive strength of the cross-linked scaffolds (3.3 ± 0.3 MPa) was significantly higher than that of scaffolds without glutaraldehyde (1.7 ± 0.2 MPa). In contrast, the porosity of the cross-linked scaffolds was lower (85.8 ± 0.8 %) than the non-cross-linked scaffolds (89.1 ± 0.4 %). It is clear that the porosity had a considerable impact on the compressive strength, wherein lower porosity led to a higher compressive strength. In conclusion, glutaraldehyde is an effective cross-linker for the fabrication of chitosan–gelatin–βTCP composite scaffolds and significantly improves their compressive strength.

1. Introduction

Chitosan, a polysaccharide biopolymer, when mixed and cross-linked with gelatin, can produce bone scaffolds with improved bioactivity and mechanical properties [1,2]. Glutaraldehyde is prevalently used as a cross-linker for chitosan and gelatin because of its ability to bond to their amine groups [1,2], thereby enhancing their mechanical strength. Lou et al. [2] reported excellent porosities by incorporating chitosan and gelatin through the freeze-drying method. However, the resulting mechanical strength was considered too low for clinical application.

Several reports have noted that adding inorganic materials, such as calcium phosphate materials, could result in higher mechanical strength [1]. The β-tricalcium phosphate (βTCP) has higher solubility than hydroxyapatite leading to rapid degradation and it is expected to be quickly replaced by new bone [3–5].

Pu'ad et al. [6] reported the utilization of natural sources to fabricate calcium phosphate materials such as hydroxyapatite. One of the natural sources used was limestone. Limestone, which mainly contains calcium carbonate and is abundantly available worldwide, could be the calcium source for manufacturing calcium phosphate ceramics, not only hydroxyapatite but also βTCP [6,7]. Thus, the utilization of limestone could be an inexpensive alternative for βTCP preparation. The drawback of using limestone as the precursor is the possibility of contamination by other elements. Ratnasari et al [8] successfully synthesized βTCP powder from limestone through sintering and reaction with phosphoric acid through wet precipitation method. However, the presence of other elements would contaminate the purity of βTCP, which might further affect the properties of the obtained bone scaffold.

Despite the lack of purity in βTCP, because of the massive potential of limestone, the authors expected to evaluate the feasibility of this material during the fabrication of a scaffold. Furthermore, in order to optimize the properties such as porosity and mechanical strength, the authors tried to evaluate the addition of glutaraldehyde as the cross-linker.

The purpose of this study was to fabricate a composite scaffold using chitosan and gelatin, which was incorporated with βTCP extracted from limestone to improve the mechanical property. The effect of glutaraldehyde addition as a cross-linker on the characteristics of the composite scaffolds was also evaluated.

* Corresponding author.
E-mail address: tansza.permata@fkg.unair.ac.id (T.S. Putri).

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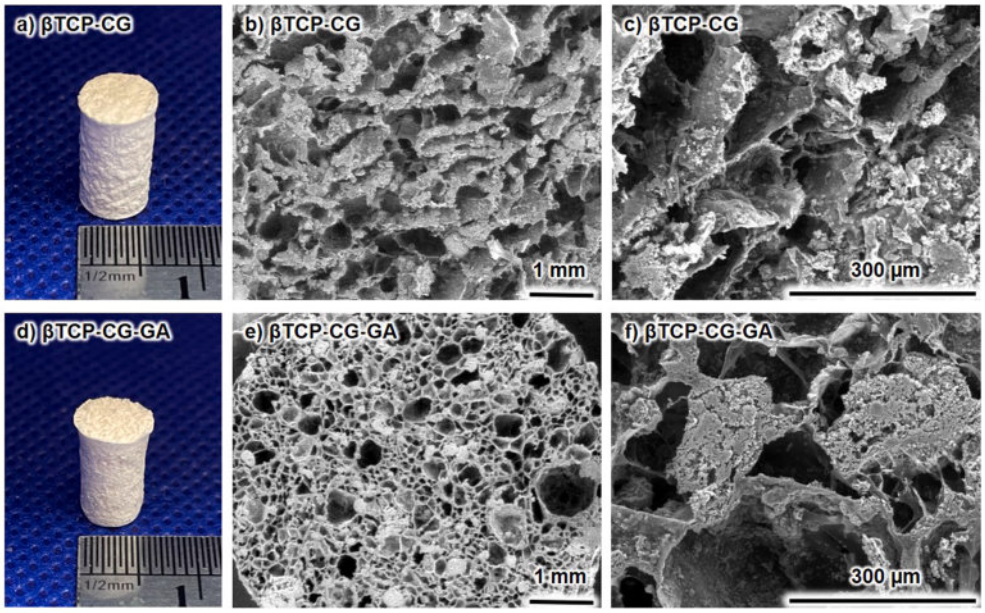

Fig. 1. The photographs of (a) βTCP-CG and (d) βTCP-CG-GA scaffolds. SEM images of (b, c) βTCP-CG and (e, f) βTCP-CG-GA scaffolds in low and high magnification.

2. Material and methods

2.1. Sample preparation

The βTCP powder used to fabricate the scaffolds were made by the Center for Ceramics in Indonesia via a method previously reported [7]. Briefly, calcium hydroxide was made from limestone through sintering and wet milling, then mixed with phosphoric acid through wet precipitation and sintered at 1000 °C to obtain the βTCP powder.

Chitosan powder (medium molecular weight; Sigma Aldrich, USA) dissolved in 2% acetic acid solution and stirred at 45 °C for 10 min. Then, gelatin (Type B; Sigma Aldrich, USA) was dissolved in distilled water (W/P = 2), added to the chitosan solution and stirred, followed by the addition of βTCP, which was manually mixed. One group of samples was treated with 0.25% glutaraldehyde (βTCP-CG-GA) at a concentration of 5 vol% relative to the total mixture, and the other was without glutaraldehyde (βTCP-CG). The composition was chitosan:gelatin:βTCP = 15:15:70%. After mixing, the slurry was poured into a mold (diameter = 6 mm, height = 11 mm) and freeze-dried (Freeze-dryer; VirTis Benchtop K, SP Industries, USA). The scaffolds were then washed and neutralized using sodium borohydride and sodium hydroxide solutions, respectively.

2.2. Characterization

Samples were analyzed using X-ray diffractometry (XRD; X'Pert PRO PANanalytical: X'Pert3MRD, Malvern Panalytical, United Kingdom) at 40 kV and 30 mA and scanned over the diffraction angle (2θ) range from 15° to 40°. Fourier-transform infrared (FT-IR; Nicolet iS10 FTIR Spectrometer, Thermo Fisher Scientific, USA) spectroscopy was conducted to determine the functional groups. Scaffolds were coated with Au/Pd, and observed by scanning electron microscopy (SEM; FEI Inspect™ S50, FEI, USA).

The compressive strength was measured using a universal testing machine (Autograph; AGS-X, Shimadzu, Japan) with a crosshead speed of 1 mm/min (n = 6). The percentage of the sample porosity was calculated by subtracting the relative density (%) from 100%. The relative density was obtained by calculating the sample bulk density and theoretical density of βTCP, chitosan, and gelatin (Eqs. (1) and (2))[8].

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

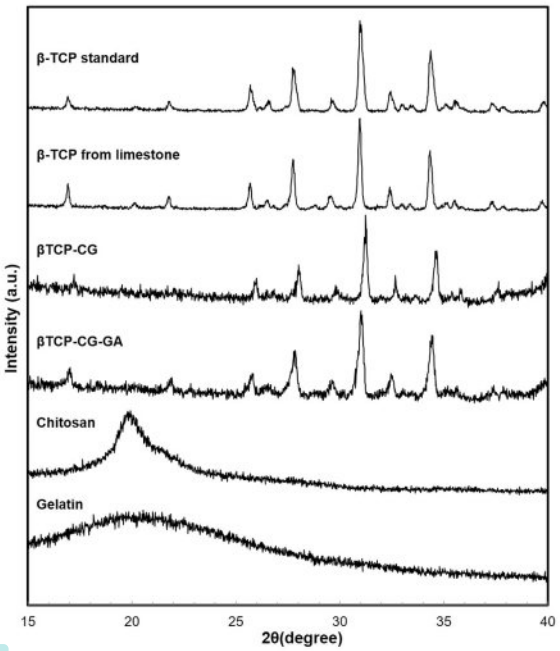

Fig. 2. The XRD patterns of βTCP standard, βTCP from limestone, βTCP-CG scaffold, βTCP-CG-GA scaffold, chitosan, and gelatin reference.

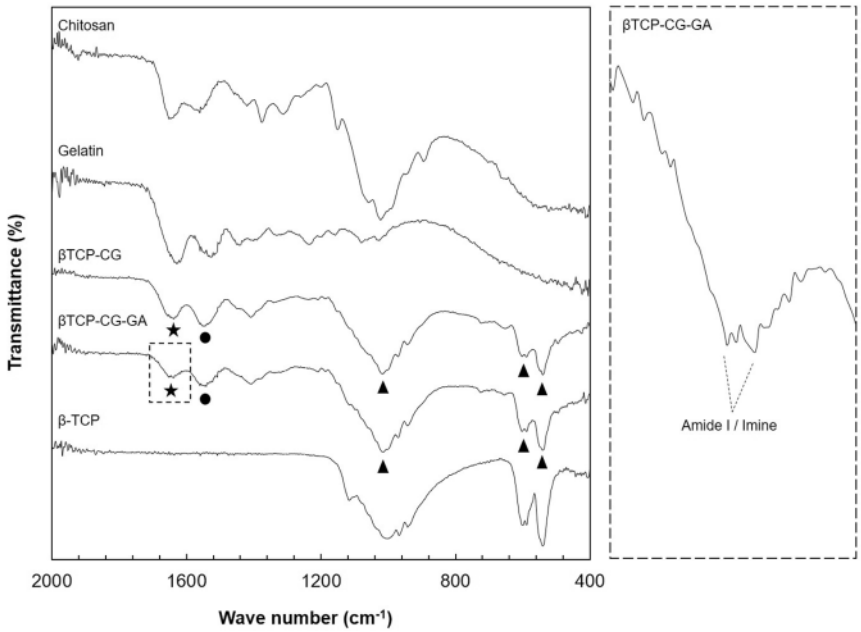

Fig. 3. The FT-IR spectra of chitosan and gelatin reference, βTCP-CG, βTCP-CG-GA scaffolds, and βTCP reference. (▲: phosphate; ★: amide I; ●: amide II).

Table 1
Compressive strength and porosity of the βTCP-CG and βTCP-CG-GA scaffolds.

Sample	Compressive strength (MPa)			Porosity (%)		
	Mean	SD	p-value	Mean	SD	p-value
βTCP-CG	1.7	0.2	< 0.0001	89.1	0.4	<0.0001
βTCP-CG-GA	3.3	0.3		85.8	0.8	

n = 6.

$$Total\ porosity(\%) = 100 - relative\ density(\%) \quad (2)$$

For statistical analysis, one-way analysis of variance (ANOVA) with *post hoc* test of Fisher's least significant difference (LSD) was performed using the Kaleidagraph Version 4.01 software (Synergy Software, Reading, PA, USA) ($p < 0.05$).

3. Results

The cross-linked composite scaffolds exhibited visible shrinkage compared to the non-cross-linked scaffolds (Fig. 1a,d). Interconnected porosity was confirmed by the cross-sectional SEM images of the scaffolds (Fig. 1b, c, e, f). The morphologies of the two samples were different: the cross-linked scaffolds had a sturdier structure (Fig. 1e, f) than the non-cross-linked scaffolds (Fig. 1b, c). The average pore size of the cross-linked scaffolds (302.2 μm) was lower than that of the non-cross-linked ones (328.4 μm).

Fig. 2 shows the XRD patterns of both βTCP-CG and βTCP-CG-GA, which correspond to the βTCP reference. The broad peaks corresponding to chitosan or gelatin were absent in the XRD patterns of the composite scaffolds, which may be due to their small amounts.

The FT-IR spectra (Fig. 3) of both the samples showed the presence of amide I (C═O) at 1637 cm^{-1} (βTCP-CG) and 1638 cm^{-1} (βTCP-CG-GA), and amide II (N—H) bands at 1545 cm^{-1} (βTCP-CG) and 1541 cm^{-1} (βTCP-CG-GA) corresponding to chitosan and gelatin. In magnified spectra of βTCP-CG-GA, a peak indicating an imine (C═N) band was present and merging with the amide I band. In addition, phosphate bands with the highest peaks at 1017 cm^{-1} (βTCP-CG) and 1016 cm^{-1} (βTCP-CG) corresponding to the tricalcium phosphate compound were also observed.

Table 1 shows the compressive strength and porosity of the samples. The compressive strength of βTCP-CG-GA was significantly higher than that of βTCP-CG. On the contrary, the porosity of the samples with glutaraldehyde was lower than of those without glutaraldehyde.

4. Discussion

The scaffold used in this study was composed of 70% βTCP derived from limestone as the inorganic material mixed with 30% chitosan and gelatin as the biopolymers. This study employed a 1:1 ratio of chitosan and gelatin. However, differences were noted when glutaraldehyde was introduced as a cross-linker. Composite scaffold containing glutaraldehyde have significant better mechanical property and better structural form in terms of porous structure.

The overlapping peaks of the imine (C═N) band and amide I band (Fig. 3) may indicate the cross-linking reaction in the βTCP-CG-GA scaffold. The presence of imine is a result of the cross-linking reaction between chitosan and gelatin, where the aldehyde groups of glutaraldehyde create a bridge that bonds with the amine groups of chitosan and gelatin [1].

Bonds created by cross-linking also increase the viscosity of the mixture, leading to the formation of a firm solid structure. Thus, the solid structure was not damaged during the separation of the solid and liquid phases upon freezing and resulting in smaller macropore sizes and smaller porosity value, unlike the non-cross-linked scaffolds. However, as bonds were formed, the structure was found to shrink, and the volume of the scaffolds was found to decrease. In addition, strong bonds distinctly increased the compressive strength, which was significantly higher for the cross-linked scaffolds (3.3 ± 0.3 MPa) than the non-cross-linked scaffolds (1.7 ± 0.2 MPa), which is comparable to the compressive strength of cancellous bone (2–12 MPa) [9].

Besides the functional group bonding, porosity also affects the mechanical strength. A higher density and thicker walls on the firm cross-linked scaffolds result in a higher compressive strength. It is observed

that more uniformed pores and a smaller pore size result in a higher resistance to the compression force than the less firm and less uniform pores of the non-cross-linked scaffolds.

In conclusion, a composite scaffold was successfully fabricated using chitosan, gelatin, and limestone-extracted βTCP. The addition of glutaraldehyde as a cross-linker significantly decreased the porosity and increased the compressive strength of the scaffold making it suitable for bone tissue engineering.

CRediT authorship contribution statement

Tansza Setiana Putri: Conceptualization, Methodology, Investigation, Writing - original draft. **Devi Rianti:** Resources, Investigation. **Priyawan Rachmadi:** Writing - review & editing. **Anita Yuliati:** Conceptualization, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.matlet.2021.130672.

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