Flexural Strength Evaluation of Chitosan-Gelatin-Β-Tricalcium Phosphate-Based Composite Scaffold

Tansza Setiana Putri[1]*, Maab Elsheikh[2]

1. Dental Materials Science and Technology Division, Faculty of Dental Medicine, Airlangga University, Surabaya, East Java, Indonesia.
2. Department of Biomaterials, Faculty of Dental Science, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan.

Abstract

In this study, composite scaffolds of chitosan, gelatin, and β-tricalcium phosphate (βTCP) were fabricated for bone tissue engineering. The flexural strength of scaffolds was compared with and without βTCP. Two types of β-tricalcium phosphate were used: limestone-derived and commercial β-tricalcium phosphates. Scaffolds were fabricated by mixing chitosan solution with gelatin solution, followed by the addition of β-tricalcium phosphate. The mixture was then poured into a mold and lyophilized to obtain a three-dimensional scaffold. The bulk density, porosity, and flexural strength were evaluated. The bulk density of scaffold after βTCP addition significantly increased, with scaffold containing limestone-derived βTCP exhibiting the highest density (0.28 ± 0.06 g/cm3). The scaffold with limestone-derived βTCP had the lowest porosity (87.77 ± 2.49 %).

The flexural strength of chitosan-gelatin-βTCP scaffolds was markedly higher than that of chitosan-gelatin scaffold, with the scaffold containing limestone-derived βTCP exhibiting the highest flexural strength (1.92 ± 0.49 MPa). Although βTCP addition improves the flexural strength of the scaffold, further evaluations are required to consolidate its application in bone regeneration treatment.

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Introduction

Scaffolds in tissue engineering are used as bone matrix replacements in the human body.[1–3] Alloplastic scaffolds have been continuously developed to obtain materials with enhanced physical and mechanical characteristics, which has improved the bone remodeling process. Artificial bone materials must possess properties that affect osteoconduction, including interconnected porosity and good mechanical strength.[1,3,4] Furthermore, materials require an adequate mechanical strength to maintain their structural integrity during bone remodeling.[5–7] A combination of biopolymers and ceramics has often been used to enhance the mechanical properties.[8–13]

Corresponding author:
Tansza Setiana Putri
Dental Materials Science and Technology Division,
Faculty of Dental Medicine, Airlangga University,
Jl. Prof. Dr. Moestopo No. 47, Surabaya 60132,
East Java, Indonesia
E-mail: tansza.permata@fkg.unair.ac.id

Recent studies have evaluated the combination of chitosan, gelatin, and β-tricalcium phosphate (βTCP).[8,10] βTCP has higher solubility than hydroxyapatite, and is a popular ceramic used in scaffold fabrication. However, higher degradability of βTCP is expected to accelerate bone remodeling. The results were promising compared to chitosan and gelatin alone, particularly in terms of mechanical properties.[8,10] Alternative raw materials from natural sources have been explored to obtain βTCP. Limestone is one such widely available, easily obtainable, and inexpensive alternative source of calcium carbonate that can be used for the synthesis of βTCP.[14–16]

Putri et al. studied the feasibility of βTCP addition to chitosan and gelatin scaffold and evaluated the efficacy of glutaraldehyde as a crosslinker for scaffold fabrication.[16] Excellent compressive strength that were comparable to those of cancellous bone was achieved. The study, however, lacked a comparison between limestone-derived βTCP with the commercially available βTCP. In implant-related therapies, other mechanical properties apart from compressive strength, such as flexural strength, need to be evaluated.

Flexural strength is the ability of a material to resist bending stress, which involves both tensile and compression forces.[17] Considering the fracture proneness of human bone, flexural strength analysis of artificial bone materials becomes important due to its correlation to fracture resistance.[18] However, ceramic-based materials exhibit poor flexural strength because of its brittleness and porous structure.[17] Therefore, a combination of ceramics and polymer can enhance the mechanical properties, including flexural strength.

This study aims to evaluate the flexural strength of a scaffold composed of chitosan, gelatin, and limestone-derived βTCP. For comparison, scaffolds based on chitosan-gelatin and chitosan-gelatin-commercial βTCP were also fabricated and analyzed.

Materials and methods

Sample preparation

Chitosan (medium molecular weight), gelatin (Type B), and βTCP were purchased from Sigma Aldrich. Limestone-derived βTCP was produced by the Center for Ceramics (Indonesia). The synthesis of limestone-derived βTCP has been previously reported.[15,16] In brief, calcium carbonate in limestone was converted into calcium hydroxide via sintering and wet milling, followed by reaction with phosphoric acid and sintering to obtain βTCP powder.

The fabrication of the scaffold has been previously reported.[16] Typically, chitosan powder is dissolved in 2% acetic acid solution. Gelatin-in-water solution was then added while maintaining the 1:1 chitosan and gelatin weight ratio. βTCP powder was then added, followed by the addition of glutaraldehyde as crosslinker. The compositional ratio of chitosan-gelatin polymer and βTCP was 30:70 wt%. Chitosan-gelatin mixture without βTCP was also fabricated. The mixtures were then put into a mold (width = 5 mm, height = 3 mm, length = 25 mm), deep-frozen at −80 °C, and lyophilized (VirTis Benchtop K, SP Industries) at temperatures ranging from −90 to −100 °C at 133.3 Pa. Sodium borohydride and sodium hydroxide solutions were used to remove the residual glutaraldehyde and acetic acid, respectively, and the scaffolds were again lyophilized.

Characterization

The bulk density, relative density and porosity of of the samples were calculated using equations (1), (2), and (3), respectively.[19,20]

Bulk density (g/cm^3) = weight/volume (1)

Relative density (%) = bulk density/theoretical density × 100 (2)

Total porosity (%) = 100 − relative density (3)

The flexural strength of the samples was measured using a universal testing machine (Autograph AGS-X, Shimadzu, Japan) at a crosshead speed of 1 mm/min with load capacity of 1000 N. The flexural strength value was determined from the Equation 4,

$$\text{Flexural strength (MPa)} = 3FL / 2wd^2 \quad (4)$$

where F is the applied maximum force, L is the length of the sample, w is the sample width, and d is the sample thickness.

One-way analysis of variance (ANOVA) was used to observe the statistically significant differences between the sample groups, followed by post-hoc analysis of Fisher's least significant difference (LSD) to determine the comparison between each group. The statistical analysis was performed using the KaleidaGraph 4.01 software (Synergy Software) ($p < 0.05$).

Results

The bulk density and porosity values of the samples are shown in Figure 1. Chitosan-gelatin (CG) scaffold exhibited the lowest smallest density among all samples (0.09 ± 0.01 g/cm^3).

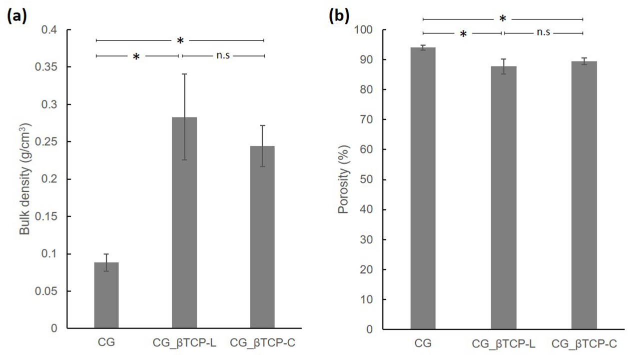

Figure 1. Bulk density and porosity of composite–gelatin (CG), composite–gelatin with βTCP from limestone (CG_βTCP-L), and composite–gelatin with commercial βTCP (CG_βTCP-C) scaffolds (n = 5).

Although not significant, the chitosan-gelatin with a limestone-derived βTCP (CG_βTCP-L) scaffold had a higher density (0.28 ± 0.06 g/cm^3) than the chitosan-gelatin with a commercial βTCP (CG_βTCP-C) scaffold (0.24 ± 0.03 g/cm^3). By contrast, CG had the highest porosity (93.99 ± 0.78 %), whereas the porosity of CG_βTCP-L (87.77 ± 2.49 %) was only slightly lower than that of CG_βTCP-C (89.43 ± 1.19 %).

Figure 2 shows the flexural strength values of the scaffold samples. CG_βTCP-L exhibited the highest flexural strength (1.92 ± 0.49 MPa), which was higher than that of CG_βTCP-C (1.33 ± 0.05 MPa) and CG (1.08 ± 0.33 MPa) scaffolds.

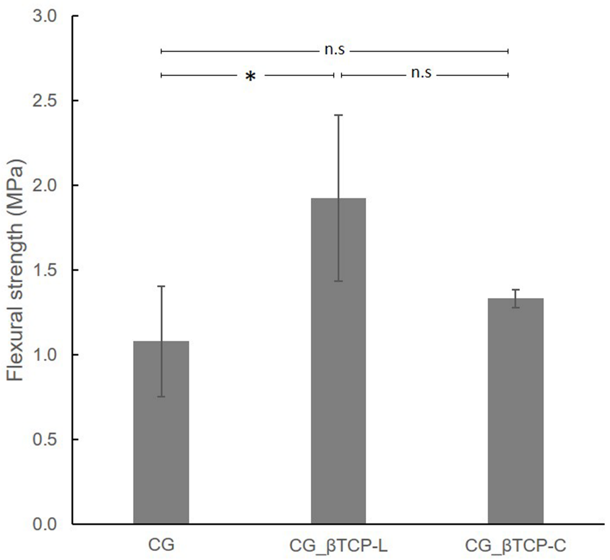

Figure 2. Flexural strength of composite–gelatin (CG), composite–gelatin with βTCP from limestone (CG_βTCP-L), and composite–gelatin with commercial βTCP (CG_βTCP-C) scaffolds (n = 5).

Discussion

Porosity and pore interconnectivity are essential for cells and tissue penetration, supporting cell proliferation and differentiation, whereas adequate mechanical properties are required to provide structural integrity for the materials to support bone regeneration. Moreover, porosity also affects the mechanical properties of a material.[1,5,6,7,12]

A distinct difference was observed between the chitosan-gelatin scaffolds with and without βTCP. The composite scaffolds containing βTCP had higher flexural strength and lower porosity. The addition of βTCP increased the bulk density by increasing the viscosity of the mixture during the fabrication of scaffolds. The higher viscosity led to the formation of firm solid structure, which would maintained its shape when solid and liquid phases was separated through lyophilization.[16] This resulted in a denser material, which also led to a decreased porosity and increased flexural strength. The denser the material, the higher is its resistance against deformation or fracture. However, even though βTCP is brittle, the scaffolds with βTCP managed to resist high bending stress compared to the chitosan-gelatin scaffold. This could be due to the strong bonds through chemical interaction between the calcium and phosphate ions from βTCP and the functional groups from chitosan and gelatin.[8]

The limestone-derived βTCP afforded no significant advantage in terms of flexural strength compared to commercial βTCP, even though the value was slightly higher. Its higher density and lower porosity clearly affected the flexural strength, as previously explained. However, this needs to be further evaluated. Impurities in each kind of βTCP may influence the properties of the corresponding fabricated scaffold, including mechanical property.[16] Moreover, morphology and particle size also affects the mechanical properties, although morphology and particle size analysis is outside the scope of this study.

The future scope of this study will involve further characterizations that would explain the increased flexural strength, such as FTIR analysis to confirm the bonding between the components, particle size analysis, and elemental analysis of each scaffold. Composition optimization is also required to obtain the best performance. At present, the flexural strength of these scaffolds is still too low for bone defect replacement. Furthermore, biological response studies are also required.

In conclusion, the addition of βTCP increased the flexural strength of the scaffold. However, further optimization and characterizations are required for potential application in bone tissue engineering.

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Declaration of Interest

The authors declare no conflict of interest.

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