

# The compressive strength and static biodegradation rate of chitosan-gelatin limestone-based carbonate hydroxyapatite composite scaffold

Devi Rianti<sup>1</sup>, Alqomariyah Eka Purnamasari<sup>1</sup>, Rifayinga Ruyani Putri<sup>1</sup>, Noor Zain Salsabilla<sup>1</sup>, Faradillah<sup>1</sup>, Elly Munadzirah<sup>1</sup>, Titien Hary Agustantina<sup>1</sup>, Asti Meizarini<sup>1</sup>, Anita Yuliaty<sup>1</sup>, Ardiyansyah Syahrom<sup>2</sup>

<sup>1</sup>Department of Dental Materials, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia

<sup>2</sup>Medical Devices and Technology centre (MEDITEC), Institute of Human Centered and Engineering (iHumEn), Universiti Teknologi Malaysia (UTM), Johor Bahru, Malaysia

## ABSTRACT

**Background:** One of the main components in tissue engineering is the scaffold, which may serve as a medium to support cell and tissue growth. Scaffolds must have good compressive strength and controlled biodegradability to show biological activities while treating bone defects. This study uses Chitosan-gelatin (C-G) with good flexibility and elasticity and high-strength carbonate hydroxyapatite (CHA), which may be the ideal scaffold for tissue engineering. **Purpose:** To analyze the compressive strength and static biodegradation rate within various ratios of C-G and CHA (C-G:CHA) scaffold as a requirement for bone tissue engineering. **Methods:** The scaffold is synthesized from C-G:CHA with three ratio variations, which are 40:60, 30:70, and 20:80 (weight for weight [w/w]), made with a freeze-drying method. The compressive strengths are then tested. The biodegradation rate is tested by soaking the scaffold in simulated body fluid for 1, 3, 7, 14, and 21 days. Data are analyzed with a one-way ANOVA parametric test. **Results:** The compressive strength of each ratio of C-G:CHA scaffold 40:60 (w/w), 30:70 (w/w), and 20:80 (w/w), consecutively, are 4.2 Megapascals (MPa), 3.3 MPa, 2.2 MPa, and there are no significant differences with the  $p = 0.069$  ( $p > 0.05$ ). The static biodegradation percentage after 21 days on each ratio variation of C-G:CHA scaffold 40:60 (w/w), 30:70 (w/w), and 20:80 (w/w) is 25.98%, 24.67%, and 20.64%. One-way ANOVA Welch test shows the result of the  $p$ -value as  $p < 0.05$ . **Conclusion:** The compressive strength and static biodegradation of the C-G:CHA scaffold with ratio variations of 40:60 (w/w), 30:70 (w/w), and 20:80 (w/w) fulfilled the requirements as a scaffold for bone tissue engineering.

**Keywords:** biodegradation; compressive strength; medicine; scaffold; tissue engineering

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Correspondence: Devi Rianti, Department of Dental Material, Faculty Dental Medicine, Universitas Airlangga. Jl. Mayjen Prof. Dr. Moestopo No. 47, Surabaya, Indonesia. Email: devi-r@fkg.unair.ac.id

## INTRODUCTION

Bone defects may occur in the maxillary and mandibular alveolar bone because of congenital anomaly, trauma, bone deficiency after tumor resection, periodontal diseases, and tooth loss.<sup>1–3</sup> The most common treatment is the application of bone grafts using the concept of tissue engineering, which comprises three fundamental components: cells, scaffolds, and growth factors.<sup>4</sup> Tissue engineering aims to develop new biofunctional tissue to regenerate and repair damaged or diseased tissues.<sup>5,6</sup> In tissue engineering, scaffolds are crucial as they provide support for cell and tissue growth

and can imitate natural bone.<sup>7</sup> The primary characteristics required in a scaffold for tissue engineering include biocompatibility, good mechanical properties, controlled biodegradability, osteoinductivity, osteoconductivity, and non-toxicity.<sup>8</sup>

The scaffold's synthesization involves biomaterials consisting of a natural polymer from chitosan-gelatin (C-G) and a bio-ceramic from carbonate hydroxyapatite (CHA), which display ideal scaffold characteristics.<sup>9</sup> Providing flexibility and elasticity, C-G is an organic material, while CHA is high in crystals, which might contribute to the scaffold's structural strength.<sup>10</sup> Chitosan is

a natural biopolymer derived from chitin with the desirable characteristics of biocompatibility and biodegradability, as well as being antibacterial and non-toxic.<sup>11</sup> Gelatin is a biocompatible, biodegradable, low-toxicity material derived from hydrolyzed and denaturalized collagen, one of the leading organic components in the natural bone.<sup>12,13</sup> The combination of gelatin and chitosan may help improve the bone repair process.<sup>14</sup> With greater homogeneity and fixation ability than hydroxyapatite, CHA is an inorganic compound and is commonly used as a scaffold material for bone repair and replacement due to its bioactive, osteoconductive, and biocompatible characteristics.<sup>15</sup> It is capable of activating cell adhesion differentiation and proliferation with good absorptivity for bone defects, which is essential for tissue engineering.<sup>16,17</sup> CHA may also help increase the ion calcium and phosphate required for new bone formation.<sup>13</sup> In this study, limestone-based CHA from Cirebon, West Java, extracted by the Indonesian Center for Ceramics (BBK Indonesia), has potential application as a bio-ceramic material in the medical field for bone replacement in treating bone defects.

This study uses freeze-drying to synthesize the C-G:CHA scaffold. This method can produce porous 3-dimensional scaffolds with more than 90% porosity and a 20–400 micrometer ( $\mu\text{m}$ ) pore diameter.<sup>18</sup> Combining several natural materials can improve each material's properties to achieve the scaffold's ideal characteristics, particularly good mechanical properties.<sup>19</sup> Chemical cross-linking between the polymer components of C-G and the addition of CHA can affect the mechanical properties of a scaffold.<sup>20</sup>

Scaffolds used as a bone replacement need a 60% to 90% porosity with an average pore size of 150  $\mu\text{m}$  and compressive strength comparable to the cortical bone of 100MPa to 230 MPa or trabecular bone of 1MPa to 12MPa.<sup>21–23</sup> The compressive strength of a scaffold material is mainly studied to determine its maximum load-bearing capacity.<sup>24</sup> The ideal scaffold must have good mechanical properties, including compressive strength to withstand pressure from tissue and maintain space for cell and new bone growth.<sup>25</sup> When a scaffold is implanted into the body, it must maintain its mechanical properties with enough structural integrity, determined by the biodegradability of the biomaterial that it can create space for new bone tissue to grow. Thus, the research aims to determine the mechanical property requirements for a tissue engineering scaffold by testing the compressive strength of the C-G:CHA scaffold composite with ratio variations of 40:60 (w/w), 30:70 (w/w), and 20:80 (w/w) and biodegradation testing to determine how long it takes for the scaffold to degrade into the body completely.<sup>26</sup> A scaffold's controlled and stable degradation process may help regenerate new bone tissue.<sup>27</sup> This study aims to analyze the compressive strength and static degradation rate of the C-G:CHA scaffold composite with specific ratios as requirements for bone regeneration.

## MATERIALS AND METHODS

The materials used in this study were chitosan with a medium molecular weight (Sigma Aldrich 448877, USA), bovine gelatin (Sigma Aldrich G9391, USA), CHA powder made from limestone produced by Indonesian Center for Ceramics (BBK Indonesia), sodium hydroxide (Biomedicine), acetic acid (Merck), distilled water (Duta Farma), and simulated body fluid (SBF Merck). The C-G:CHA 40:60 (w/w) scaffold is prepared by weighing 0.5 grams of chitosan powder, 0.5 grams of gelatin powder, and 1.5 grams of hydroxyapatite carbonate powder. Up to 2% acetic acid is added to the weighed gelatin up to 2 milliliters (ml) and stirred with a magnetic agitator at 50°C until the gelatin powder is homogeneous. The weighed CHA is mixed with 0.94 ml of distilled water and then incorporated with a metal spatula until homogeneous. Then, the dilute CHA is incorporated in the gelatin gel and then stirred until homogeneous, while chitosan powder is added gradually to form a C-G:CHA gel. The C-G:CHA gel was mixed with 0.5 ml of 0.1 molar NaOH to neutralize the acid. The C-G:CHA gel was measured using litmus paper until a pH of 7 was obtained. If a pH >7 (alkaline) was obtained, 0.1 ml of acetic acid solution is added, while if a pH <7 (acidic) is obtained, 0.1 ml of NaOH solution is added. The pH measuring must be done simultaneously to ensure the scaffold's pH is exactly 7. The pH 7 C-G:CHA gel is placed into the 48-well plate using a glass spatula and then compacted using a cement stopper until no hollow spaces are left. The mixture is frozen at -40°C for 2x24 hours and freeze-dried for 2x24 hours.<sup>28</sup> Scaffolds with the ratios of 30:70 (w/w) and 20:80 (w/w) are processed the same way. On the 30:70 (w/w) ratio, 0.375 grams of chitosan powder, 0.375 grams of gelatin powder, and 1.75 grams of CHA powder are used. For the 20:80 (w/w) ratio, 0.25 grams of chitosan powder, 0.25 grams of gelatin powder, and 2 grams of CHA powder are applied.

Compressive strengths are tested using the Mini Autograph Universal Testing Machine's sensor load cell L IP3 Class 0.02 with microcontroller software Phyton 2.7 on the cylindrical-shaped scaffolds. The diameter and height of the scaffolds are measured using vernier calipers to measure their surface area. Scaffold samples are placed in the middle of the pressing machine with their vertical axis perpendicular to the flat plane. Activating the Mini Autograph tool, the suppressor crushes the sample slowly with a pressure load of 400 newtons and 2 mm/minute speed until the samples are distorted and break. The tool will be stopped when the graph in the monitor shows that there is an increase after a decrease. When this occurs, the load no longer pressures the scaffold but is distributed only onto the upper and lower suppressor. Calculations will be made from the graph results, which show displacement and force accepted by the scaffold as the maximum amount of load divided by the surface area of the scaffold sample. The data are then put into the compressive strength formula to calculate the compressive strength value with units of MPa.<sup>29</sup>

Static biodegradation testing is achieved by soaking the samples in 1.5 ml SBF in an Eppendorf container at a temperature of 37°C. Before soaking, the pH measurements of the SBF media are taken by weighing the SBF to determine the initial weight of the scaffold in its dry state (W<sub>0</sub>). The percentage of biodegradation is obtained after calculating the final weight (W<sub>t</sub>) as the scaffolds are dried after soaking for 1, 3, 7, 14, and 21 days. The chosen formula is used to calculate the biodegradation percentage from the scaffolds' W and W<sub>t</sub> data.<sup>30</sup>

$$Biodegradation = \frac{W_0 - W_t}{W_0} \times 100\%$$

Research data are then statistically analyzed using the Kolmogorov–Smirnov test to determine if the data distribution is normal, followed by homogeneity testing

using the Levene test. If the p > 0.05, one-way ANOVA parametric tests are performed to identify the significance of every sample's data results.

## RESULTS

The compressive strength value of the C–G:CHA scaffold is obtained after entering the strength test results from the Mini Autograph Universal Testing Machine's sensor load cell L IP3 Class 0.02 with the Python 2.7 microcontroller software. Results and the standard deviation of the C–G:CHA scaffolds' compressive strength value are shown in Table 1 and Figure 1. The average compressive strength value of the C–G:CHA scaffold appears to increase

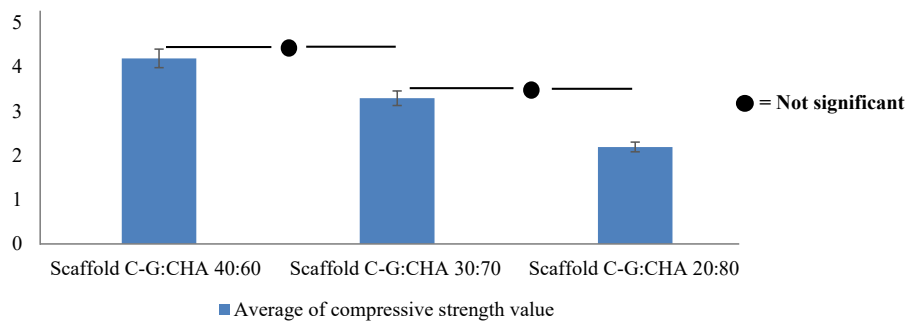
**Table 1.** The compressive strengths of various C–G:CHA scaffold ratios (MPa)

Sample	n	Average of compressive strength value	Standard deviation
C–G:CHA scaffold 40:60	6	4.19	0.79
C–G:CHA scaffold 30:70	6	3.29	0.22
C–G:CHA scaffold 20:80	6	2.19	1.19

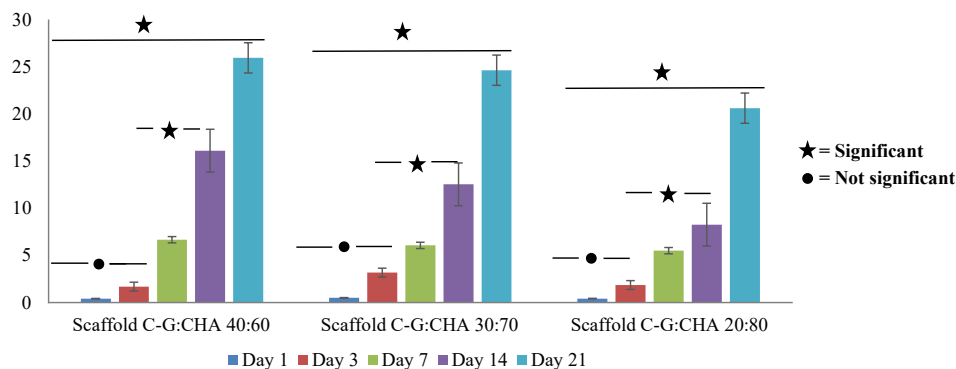
**Table 2.** Static biodegradation test of C–G:CHA scaffold ratios (%)

C–G:CHA scaffold ratios	n	Day 1	Day 3	Day 7	Day 14	Day 21
		x + SD	x + SD	x + SD	x + SD	x + SD
40:60	6	0.41±0.06	1.69±0.69	6.67±1.64	16.13±5.43	25.98±2.74
30:70	6	0.50±0.13	3.18±1.35	6.07±0.52	12.55±2.03	24.67±3.77
20:80	6	0.42±0.09	1.86±0.80	5.51±0.79	8.27±1.51	20.64±6.40

Notes: x: average biodegradation percentage; SD: standard deviation



**Figure 1.** Graph of the average compressive strength of C–G:CHA scaffolds.



**Figure 2.** The graph shows the average static biodegradation rate of the C–G:CHA scaffolds on days 1, 3, 7, 14, and 21.

with an increase in the C–G ratio and a decrease in the CHA ratio.

Initial and final weights are calculated on each sample at every time stamp during the research. Time-stamp variations for the biodegradation tests are 1, 3, 7, 14, and 21 days. Data from the initial and final weights are used to calculate the degradation percentage of the C–G:CHA scaffolds with various ratios. Results and the standard deviation of the C–G:CHA scaffolds' degradation are shown in Table 2 and Figure 2.

## DISCUSSION

The scaffold's mechanical properties are essential in the manufacturing process. Developing a porous structured scaffold compatible with bone is a central issue in tissue engineering.<sup>31,32</sup> The porous structure of the scaffold is to facilitate cell attachment and proliferation, but then it must also have the sufficient mechanical strength to enhance biostability.<sup>33</sup>

Based on the results, the average compressive strength value increases when the CHA ratio decreases, and the C–G ratio rises. A one-way ANOVA statistical analysis showed no significant difference in the compressive strength values of the three variations of the C–G:CHA scaffold ratios of 20:80 (w/w), 30:70 (w/w), and 40:60 (w/w). This result means that the average compressive strength value increase was insignificant. In previous studies, the C–G:CHA scaffold had been tested by FTIR, SEM-EDX, and XRD, and the results contained a phosphate group ( $\text{PO}_4^{3-}$ ), a carbonate group ( $\text{CO}_3^{2-}$ ), and a hydroxyl group ( $\text{OH}^-$ ). Chitosan has several hydroxyl groups ( $-\text{OH}$ ) and amine groups ( $-\text{NH}_2$ ) in its chain, while gelatin has active hydroxyl groups ( $-\text{OH}$ ), carboxylic groups ( $-\text{COOH}$ ), and amine groups ( $-\text{NH}_2$ ). The groups in chitosan and gelatin form hydrogen bonds between the amine and carboxylic groups or with phosphate and carbonate groups.<sup>33</sup>

Bonds between carboxyl groups in gelatin and amine groups in chitosan also produce ionic bonds, which cause the formation of a scaffold with denser properties.<sup>34</sup> Bonds in chitosan and gelatin will form intermolecular hydrogen bonds. Hydrogen bonds will be created due to bonds with  $-\text{NH}_2$  and carbonates hydroxyapatite or interactions between  $-\text{COOH}$  and carbonates apatite. Combining these 3 materials will form crystalline particles, which are dominantly formed from CHA material containing the elements O, Ca, and P and has crystalline particles.<sup>33</sup> During the freeze-drying process, the mixture of the three materials will produce crystals and amorphs, which will balance the crystallinity of the scaffold so that the addition of the C–G ratio can increase the bond to the 3 materials.

The above confirms the opinion that chemical cross-linking between polymer components, namely C–G, can affect the mechanical properties of the scaffold.<sup>20</sup> The compressive strength value in this study is still in the range of compressive strength values in trabecular bone of

0.1 to 16 MPa. Engineering scaffold tissue must possess sufficient mechanical properties to support new bone tissue at the implantation site and maintain good integrity for cells in vitro and in vivo.<sup>35-38</sup> Thus, it is vital for a bone scaffold to have identical mechanical properties as trabecular bone.<sup>23</sup>

In the results of previous studies, the trabecular bone mechanical properties have a value of compressive strength of at least 1 MPa.<sup>39</sup> A study by Waletzko-Hellwig showed that the compressive strength of trabecular bone is 2 to 48 MPa. In comparison, a study by Mohaghegh suggested a compressive strength of 1.5 to 45 MPa, and research by Gerhardt and Boccaccini showed a compressive strength of 0.1 to 16 MPa.<sup>37,40,41</sup> This indicates that trabecular bone has a highly anisotropic and heterogeneous structure whose mechanical properties depend highly on anatomical location.<sup>42</sup> In addition, the stiffness level of the bone scaffold must not be too low to provide mechanical stability and not too high to prevent stress shielding, resulting in friction under continuous pressure and damage to the surrounding bones.<sup>43</sup> This can affect bone remodeling.<sup>44</sup> Previous research on Balai Besar Keramik's hydroxyapatite (HABBK) scaffold, combined with C–G, proved the HABBK:C–G scaffold composite with a ratio of 60:40 (w/w) had the highest average compressive strength value, i.e. 0.81 MPa, less than the compressive strength value of this study using CHA.<sup>29,45</sup> This study shows that all ratios meet the requirements as a scaffold in tissue engineering: the compressive strength in the C–G:CHA scaffold with the ratio of 40:60 (w/w) is 4.19 MPa; the C–G:CHA 30:70 (w/w) is 3.29 MPa; and the C–G:CHA scaffold 20:80 (w/w) is 2.19 MPa. All these values are still within the range of compressive strength values of the trabecular bone. Developing chitosan, gelatin, and CHA materials into a three-dimensional structural scaffold with good mechanical strength and biological function will increase the recovery of bone defects. It can be used as a good candidate for designing biomimetic bone scaffolds.

Table 2 and Figure 2 show that the average value of the static biodegradation rate of the C–G:CHA scaffold in each ratio variation increased during the study from day 1 to day 21. The test results for the highest average degradation rate value of the C–G:CHA scaffold sample for each ratio variation were obtained on the 21<sup>st</sup> day. In the C–G:CHA scaffold with a variation of the ratio of 40:60 (w/w), the static biodegradation rate is faster compared to the other ratio variations. One of the ideal properties of biomaterials in tissue engineering is biodegradability. Biomaterials used for scaffolds have an essential role in the success of tissue engineering. Tissue engineering in a scaffold must support the tissue growth process that acts as a temporary extracellular matrix during the cell attachment and adhesion processes. This matrix must be made of biodegradable materials capable of being metabolized by the body and eventually gradually degraded when cells begin to undergo a process of proliferation and differentiation.<sup>46,47</sup>



The static biodegradation rate of the 40:60, 30:70, and 20:80 scaffolds throughout 1, 3, 7, 14, and 21 days increased due to bonds between the scaffold components and calcium and phosphate ions from the SBF solution. This bond gradually damages the scaffold components and causes a decrease in the weight of the scaffold, increasing the degradation rate due to the interaction between the scaffold and the SBF media. In the degradation process of the C–G:CHA scaffold in the SBF, the increasing weight loss was affected by the bond between the scaffold components with  $\text{Ca}^{2+}$  ions and  $\text{PO}_4^{3-}$  ions originating from the SBF solution. The interaction of the scaffold and the SBF also caused the degradation of chitosan in the form of an amide complex such as  $\text{NH}_2$  in the SBF. This process will cause the SBF to gradually damage the scaffold components and increase the percentage of scaffold weight loss as the static immersion time rises to 21 days.<sup>33,48</sup>

The static biodegradation test, the gold standard, is used in this study.<sup>49</sup> According to previous research, the reduction in scaffold weight should not be too fast or slow, following the bone remodeling process of around 3–6 months.<sup>50,51</sup> In another opinion, the minimum degradation process is between 1–2 weeks because this period is the bone repair stage, starting with the elimination of damaged cells and replacing the weak fibrin clot with a more mechanically stable structure called a callus.<sup>52</sup>

The biodegradation rate of the C–G:CHA scaffold is 20% to 25.98% on day 21, the largest ratio variation in the study. Based on previous research, the trabecular bone regeneration process takes place for about 2–3 months.<sup>53</sup> According to other studies, bone regeneration in the trabecular bone takes about 200 days (6 months).<sup>54</sup> In this study, the degradation rate of C–G:CHA scaffold of 20% to 25.98% for 21 days is expected to lead to complete degradation within 3–6 months, showing that the C–G:CHA scaffold has a biodegradation rate suitable for the bone regeneration process. All of the C–G:CHA scaffold ratios demonstrate biodegradable properties. Over 1, 3, 7, 14, and 21 days, the scaffold underwent in vitro bone regeneration, or it could be said that the scaffold was degraded. Therefore, the C–G:CHA scaffold with the ratios of 40:60 (w/w), 30:70 (w/w), and 20:80 (w/w) all have reasonable degradation rates for a scaffold in tissue engineering. This study has limitations because it has not been able to observe complete biodegradation, which will occur in the future. It is also necessary to undertake further research using dynamic degradation techniques by simulating the movement of bone marrow in the trabecular bone.

In conclusion, the compressive strength value of the C–G:CHA scaffold composite with ratio variations of 40:60 (w/w), 30:70 (w/w), and 20:80 (w/w) met the requirements of a scaffold in tissue engineering. The variation of the ratio of the C–G:CHA scaffold composite 40:60 (w/w), 30:70 (w/w), and 20:80 (w/w) increased throughout 1, 3, 7, and 14 days, with the highest percentage of biodegradation on day 21 of 25.98% on the C–G:CHA scaffold with a ratio variation of 40:60 (w/w). With good flexibility and

elasticity, C–G combined with high-strength CHA, is an ideal scaffold for tissue engineering.

## REFERENCES

- Prasadh S, Wong RCW. Unraveling the mechanical strength of biomaterials used as a bone scaffold in oral and maxillofacial defects. *Oral Sci Int.* 2018; 15(2): 48–55.
- Ibrahim A. 3D bioprinting bone. In: *3D Bioprinting for Reconstructive Surgery.* Elsevier; 2018. p. 245–75.
- Shukla S, Chug A, Mahesh L, Singh S, Singh K. Optimal management of intrabony defects: current insights. *Clin Cosmet Investig Dent.* 2019; 11: 19–25.
- Ghassemi T, Shahroodi A, Ebrahimzadeh MH, Mousavian A, Movaffagh J, Moradi A. Current concepts in scaffolding for bone tissue engineering. *Arch Bone Jt Surg.* 2018; 6(2): 90–9.
- Collins MN, Ren G, Young K, Pina S, Reis RL, Oliveira JM. Scaffold fabrication technologies and structure/function properties in bone tissue engineering. *Adv Funct Mater.* 2021; 31(21): 2010609.
- Witzler M, Ottensmeyer PF, Gericke M, Heinze T, Tobiasch E, Schulze M. Non-cytotoxic agarose/hydroxyapatite composite scaffolds for drug release. *Int J Mol Sci.* 2019; 20(14): 3565.
- Samadian H, Farzamfar S, Vaez A, Ehterami A, Bit A, Alam M, Goodarzi A, Darya G, Salehi M. A tailored polylactic acid/polycaprolactone biodegradable and bioactive 3D porous scaffold containing gelatin nanofibers and Taurine for bone regeneration. *Sci Rep.* 2020; 10(1): 13366.
- Darus F, Jaafar M. Enhancement of carbonate apatite scaffold properties with surface treatment and alginate and gelatine coating. *J Porous Mater.* 2020; 27(3): 831–42.
- Safarzadeh M, Chee CF, Ramesh S, Fauzi MNA. Effect of sintering temperature on the morphology, crystallinity and mechanical properties of carbonated hydroxyapatite (CHA). *Ceram Int.* 2020; 46(17): 26784–9.
- Li J, Jansen JA, Walboomers XF, van den Beucken JJ. Mechanical aspects of dental implants and osseointegration: A narrative review. *J Mech Behav Biomed Mater.* 2020; 103: 103574.
- Ghiasi B, Sefidbakht Y, Rezaei M. Hydroxyapatite for biomedicine and drug delivery. In: *Nanomaterials for advanced biological applications.* Springer; 2019. p. 85–120.
- Zheng X, Liu Y, Liu Y, Pan Y, Yao Q. Novel three-dimensional bioglass functionalized gelatin nanofibrous scaffolds for bone regeneration. *J Biomed Mater Res Part B Appl Biomater.* 2021; 109(4): 517–26.
- Noor Khairiyah Hanisah H, Muhammad Ridhwan R, Nurazreena A. Synthesis of porous carbonate apatite/gelatin scaffolds via freeze drying method. *J Phys Conf Ser.* 2018; 1082: 012004.
- Oryan A, Alidadi S, Bigham-Sadegh A, Moshiri A. Comparative study on the role of gelatin, chitosan and their combination as tissue engineered scaffolds on healing and regeneration of critical sized bone defects: an in vivo study. *J Mater Sci Mater Med.* 2016; 27(10): 155.
- Iancu L, Ion R-M, Grigorescu RM, Ghioca PN, Spurcaci B, David ME, Andrei RE, Ghiurea M, Stirbescu RM, Bucurica A. Carbonated hydroxyapatite substituted with magnesium for stone consolidation. In: *The 16th International Symposium "Priorities of Chemistry for a Sustainable Development" PRIOCHEM.* Basel Switzerland: MDPI; 2020. p. 59.
- He J, Hu X, Cao J, Zhang Y, Xiao J, Peng L, Chen D, Xiong C, Zhang L. Chitosan-coated hydroxyapatite and drug-loaded poly(trimethylene carbonate)/polylactic acid scaffold for enhancing bone regeneration. *Carbohydr Polym.* 2021; 253: 117198.
- Januariyasa IK, Yusuf Y. Porous carbonated hydroxyapatite-based scaffold using simple gas foaming method. *J Asian Ceram Soc.* 2020; 8(3): 634–41.
- Fereshteh Z. Freeze-drying technologies for 3D scaffold engineering. In: *Functional 3D Tissue Engineering Scaffolds.* Elsevier; 2018. p. 151–74.

19. Re F, Sartore L, Moulisova V, Cantini M, Almici C, Bianchetti A, Chinello C, Dey K, Agnelli S, Manferdini C, Bernardi S, Lopomo NF, Sardini E, Borsani E, Rodella LF, Savoldi F, Paganelli C, Guizzi P, Lisignoli G, Magni F, Salmeron-Sanchez M, Russo D. 3D gelatin-chitosan hybrid hydrogels combined with human platelet lysate highly support human mesenchymal stem cell proliferation and osteogenic differentiation. *J Tissue Eng.* 2019; 10: 204173141984585.
20. Georgopoulou A, Papadogiannis F, Batsali A, Marakis J, Alpentaki K, Eliopoulos AG, Pontikoglou C, Chatzinikolaïdou M. Chitosan/gelatin scaffolds support bone regeneration. *J Mater Sci Mater Med.* 2018; 29(5): 59.
21. Roohani-Esfahani S-I, Newman P, Zreiqat H. Design and fabrication of 3D printed scaffolds with a mechanical strength comparable to cortical bone to repair large bone defects. *Sci Rep.* 2016; 6(1): 19468.
22. Sutthi R, Kaewwinud N, Chindaprasit P, Mutoh Y, Laonapakula T. Effect of curing temperature and time on the mechanical properties of hydroxyapatite/calcined kaolin. *ScienceAsia.* 2018; 44(6): 397.
23. Kim T-R, Kim M-S, Goh TS, Lee JS, Kim YH, Yoon S-Y, Lee C-S. Evaluation of structural and mechanical properties of porous artificial bone scaffolds fabricated via advanced TBA-based freeze-gel casting technique. *Appl Sci.* 2019; 9(9): 1965.
24. Mondal S, Nguyen TP, Pham VH, Hoang G, Manivasagan P, Kim MH, Nam SY, Oh J. Hydroxyapatite nano bioceramics optimized 3D printed poly lactic acid scaffold for bone tissue engineering application. *Ceram Int.* 2020; 46(3): 3443–55.
25. Chi H, Song X, Song C, Zhao W, Chen G, Jiang A, Wang X, Yu T, Zheng L, Yan J. Chitosan-gelatin scaffolds incorporating decellularized platelet-rich fibrin promote bone regeneration. *ACS Biomater Sci Eng.* 2019; 5(10): 5305–15.
26. Bose S, Roy M, Bandyopadhyay A. Recent advances in bone tissue engineering scaffolds. *Trends Biotechnol.* 2012; 30(10): 546–54.
27. Maji K, Dasgupta S, Kundu B, Bissoyi A. Development of gelatin-chitosan-hydroxyapatite based bioactive bone scaffold with controlled pore size and mechanical strength. *J Biomater Sci Polym Ed.* 2015; 26(16): 1190–209.
28. Fanny G. Uji karakteristik scaffold kitosan gelatin karbonat apatit batu kapur Balai Besar Keramik. Thesis. Universitas Airlangga. Surabaya; 2020. p. 22–34.
29. Karina RY. Compressive strength scaffold komposit hidroksiapatit Balai Besar Keramik dengan berbagai rasio. Thesis. Universitas Airlangga. Surabaya; 2020. p. 22–4.
30. Wang X, Yu T, Chen G, Zou J, Li J, Yan J. Preparation and characterization of a chitosan/gelatin/extracellular matrix scaffold and its application in tissue engineering. *Tissue Eng Part C Methods.* 2017; 23(3): 169–79.
31. Shahbazi S, Zamanian A, Pazouki M, Jafari Y. Introducing an attractive method for total biomimetic creation of a synthetic biodegradable bioactive bone scaffold based on statistical experimental design. *Mater Sci Eng C.* 2018; 86: 109–20.
32. Tamburaci S, Tihminlioglu F. Biosilica incorporated 3D porous scaffolds for bone tissue engineering applications. *Mater Sci Eng C.* 2018; 91: 274–91.
33. Rianti D, Fanny G, Nathania RV, Purnamasari AE, Putri RR, Soekartono H, Soebagio S, Yuliati A, Syahrom A. The characteristics, swelling ratio and water content percentage of chitosan-gelatin/limestone-based carbonate hydroxyapatite composite scaffold. *Int J Integr Eng.* 2022; 14(2): 13–23.
34. Aufan MR. Sintesis scaffold alginat-kitosan-karbonat apatit sebagai bone graft menggunakan metode freeze drying. *J Biofisika.* 2012; 8(1): 16–24.
35. Dressler M, Dombrowski F, Simon U, Börnstein J, Hodoroaba VD, Feigl M, Grunow S, Gildenhaar R, Neumann M. Influence of gelatin coatings on compressive strength of porous hydroxyapatite ceramics. *J Eur Ceram Soc.* 2011; 31(4): 523–9.
36. Bello AB, Kim D, Kim D, Park H, Lee S-H. Engineering and functionalization of gelatin biomaterials: from cell culture to medical applications. *Tissue Eng Part B Rev.* 2020; 26(2): 164–80.
37. Gerhardt L-C, Boccaccini AR. Bioactive glass and glass-ceramic scaffolds for bone tissue engineering. *Materials (Basel).* 2010; 3(7): 3867–910.
38. Escobar-Sierra DM, Martins J, Ossa-Orozco CP. Chitosan/hydroxyapatite scaffolds for tissue engineering manufacturing method effect comparison. *Rev Fac Ing Univ Antioquia.* 2015; (75): 24–35.
39. Levensgood SKL, Zhang M. Chitosan-based scaffolds for bone tissue engineering. *J Mater Chem B.* 2014; 2(21): 3161.
40. Waletzko-Hellwig J, Saemann M, Schulze M, Frerich B, Bader R, Dau M. Mechanical characterization of human trabecular and formed granulate bone cylinders processed by high hydrostatic pressure. *Materials (Basel).* 2021; 14(5): 1069.
41. Mohaghegh S, Hosseini SF, Rad MR, Khojasteh A. 3D printed composite scaffolds in bone tissue engineering: a systematic review. *Curr Stem Cell Res Ther.* 2022; 17(7): 648–709.
42. Oftadeh R, Perez-Viloria M, Villa-Camacho JC, Vaziri A, Nazarian A. Biomechanics and mechanobiology of trabecular bone: a review. *J Biomech Eng.* 2015; 137(1): 010802.
43. Pei P, Wei D, Zhu M, Du X, Zhu Y. The effect of calcium sulfate incorporation on physicochemical and biological properties of 3D-printed mesoporous calcium silicate cement scaffolds. *Microporous Mesoporous Mater.* 2017; 241: 11–20.
44. Bahraminasab M. Challenges on optimization of 3D-printed bone scaffolds. *Biomed Eng Online.* 2020; 19(1): 69.
45. Ghouse S, Reznikov N, Boughton OR, Babu S, Ng KCG, Blunn G, Cobb JP, Stevens MM, Jeffers JRT. The design and in vivo testing of a locally stiffness-matched porous scaffold. *Appl Mater Today.* 2019; 15: 377–88.
46. Yuliati A, Kartikasari N, Munadzirah E, Rianti D. The profile of crosslinked bovine hydroxyapatite gelatin chitosan scaffolds with 0.25% glutaraldehyde. *J Int Dent Med Res.* 2017; 10(1): 151–5.
47. Widayansari NKS. Biodegradasi komposit scaffold berbasis hidroksiapatit Balai Besar Keramik. Dissertation. Universitas Airlangga. Surabaya; 2020.
48. Shuai C, Zhou Y, Yang Y, Feng P, Liu L, He C, Zhao M, Yang S, Gao C, Wu P. Biodegradation resistance and bioactivity of hydroxyapatite enhanced Mg-Zn composites via selective laser melting. *Materials (Basel).* 2017; 10(3): 307.
49. Md. Saad AP, Jasmawati N, Harun MN, Abdul Kadir MR, Nur H, Hermawan H, Syahrom A. Dynamic degradation of porous magnesium under a simulated environment of human cancellous bone. *Corros Sci.* 2016; 112: 495–506.
50. Zhang K, Fan Y, Dunne N, Li X. Effect of microporosity on scaffolds for bone tissue engineering. *Regen Biomater.* 2018; 5(2): 115–24.
51. Yadav N, Srivastava P. In vitro studies on gelatin/hydroxyapatite composite modified with osteoblast for bone bioengineering. *Heliyon.* 2019; 5(5): e01633.
52. Rodriguez I. Tissue engineering composite biomimetic gelatin sponges for bone regeneration. Thesis. Virginia Commonwealth University: Virginia; 2013. p. 23–7, 35–56.
53. Irish J, Virdi AS, Sena K, McNulty MA, Sumner DR. Implant placement increases bone remodeling transiently in a rat model. *J Orthop Res.* 2013; 31(5): 800–6.
54. Eriksen EF. Cellular mechanisms of bone remodeling. *Rev Endocr Metab Disord.* 2010; 11(4): 219–27.