

Available online at www.synsint.com

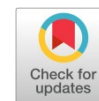
Synthesis and Sintering

ISSN 2564-0186 (Print), ISSN 2564-0194 (Online)



Research article

Innovative fabrication of Zn-doped 45S5 glass-ceramic scaffolds using eggshell: Physico-mechanical properties and bioactivity assessment



Ahmad Norouzi, Sara Banijamali *

Ceramic Department, Materials and Energy Research Center (MERC), Karaj, Iran

ABSTRACT

In the present study, glass-ceramics scaffolds doped with different amounts of zinc oxide were fabricated. In this regard, eggshell, as a natural porogen and environmentally friendly material, was considered to fabricate scaffolds. Physico-mechanical properties, along with bioactivity of the fabricated scaffolds, were evaluated precisely. To this purpose, all glass powders were prepared through melt quenching and subsequent milling. Scaffold specimens were prepared by heat treating mixtures of powdered glass with different amounts of eggshell. Based on the obtained results, the main crystalline phase of the studied scaffolds is sodium calcium silicate. With the increase in the amount of zinc oxide in the glass composition, the sodium zinc silicate phase is also formed. Considering the compressive strength, stability of the scaffold samples, and their physical properties, the optimal amount of eggshell used in scaffold preparation was determined to be 35% by weight. In the most promising specimen, porosity was achieved at 55%. After immersion in simulated body fluid for 28 days, all scaffolds showed apatite formation ability, confirming their acceptable bioactivity.

© 2025 The Authors. Published by Synsint Research Group.

KEYWORDS

Scaffold
45S5 bioactive glass
Eggshell
Zinc oxide
Heat treatment



1. Introduction

In tissue engineering, scaffolds are materials that gradually degrade and transform into soft or hard tissue when exposed to the body's physiological environment [1, 2]. In this field, bioactive glasses are widely used to make scaffolds to replace bone tissues. Among bioactive glasses, 45S5 glass, under the trade name bioglass, was first introduced by Sepulveda et al. [3]. This glass has the highest bioactivity index among bioactive glasses, and its components include oxides of sodium, calcium, phosphorus, and silicon [3–5]. Since its invention, the composition of this glass has undergone many modifications, and many elements have been added to it to induce various biological properties. Zinc is one of the elements whose effect on the properties of 45S5 glass has been of interest. The presence of zinc ions in the structure of 45S5 glass results in properties such as

DNA replication and inhibition of skeletal growth [6–10]. When heated to its crystallization temperature, 45S5 glass transforms into the glass-ceramic product with the dominant crystalline phase of sodium calcium silicate. The formation of crystalline phases in the 45S5 glass-ceramics significantly improves mechanical properties, which is very important in the scaffolding context. Interestingly, despite crystallization, 45S5 glass-ceramics retain their bioactivity to a large extent, which has made them a significant candidate for the fabrication of hard tissue replacement scaffolds [11]. Many efforts have been made to create scaffolds from 45S5 glass, including the use of sacrificial materials that are eliminated during the heat treatment step and leave behind porosity. Recently, much research has been conducted with bio-inspired approaches to fabricate scaffolds used in tissue engineering [12–14]. One of the interesting natural sacrificial materials in scaffold fabrication is eggshell, which is rich in natural materials and leaves a

* Corresponding author. E-mail address: banijamali@merc.ac.ir, banijamal@s@yahoo.com (S. Banijamali)

Received 4 August 2024; Received in revised form 26 June 2025; Accepted 29 June 2025.

Peer review under responsibility of Synsint Research Group. This is an open access article under the CC BY license (<https://creativecommons.org/licenses/by/4.0/>).
<https://doi.org/10.53063/synsint.2025.52239>

porous structure during heat treatment of the scaffold by releasing carbon dioxide gas [15–17].

According to this idea, fabrication of glass-ceramics doped with different amounts of zinc oxide and scaffolding them using eggshells were considered in the present study. In this regard, physico-mechanical properties along with bioactivity of the fabricated scaffolds were evaluated precisely. It is worth mentioning that similar research in this field has mainly focused on measuring biological properties and not on evaluating structural and mechanical properties.

2. Experimental procedure

2.1. Materials preparation

Initially, the chemical composition of 45S5 glass was considered the base composition. This composition contained 24.5Na₂O, 24.5CaO, 6P₂O₅, and 45SiO₂ (in wt%). Then, the compositions Z1, Z2, Z3, and Z4 were prepared by adding 1, 2, 3, and 4 weight ratios of zinc oxide to the base composition, respectively. To prepare the relevant glasses, reagent-grade raw materials including sodium carbonate, calcium carbonate, phosphorus oxide, and silica were used. The glasses were melted in an electric furnace at 1300 °C using silica crucibles. The glass melts were converted into frit after quenching in cold water. The corresponding frits were then ground in agate mortar to reach a particle size smaller than 75 microns. Eggshells were also milled to a particle size smaller than 75 microns.

Afterwards, glass frits were mixed with varying amounts of eggshells (2.5–50 in wt%). The mixtures were shaped using a single-axis hydraulic press under the initial pressure of 30 MPa and final pressure of 60 MPa into discs with 13 mm in diameter. Before pressing, each mixture was mixed with 0.1 wt% polyvinyl alcohol as the binding agent. The pressed discs were heat-treated at 1050 °C for 2 h under a heating rate of 10 °C/min. It should be noted that this thermal program was obtained based on the authors' previous research as the optimal sintering conditions for the studied glasses [18].

2.2. Characterization techniques

To evaluate the decomposition temperature of eggshell, a differential thermal analyzer (DTA, Polymer Laboratories 1640) was used in an air atmosphere and heating rate of 10 °C/min. An X-ray fluorescence analysis (XRF, ARL 8420) was also used to identify the components of eggshells. X-ray diffractometer (XRD, Philips-PW3710) with Cu-K_α radiation was used to identify the crystalline phases formed in the scaffold samples. Measurement of physical properties, including bulk density, linear shrinkage, and porosity, was performed based on the standard Archimedes method [19]. The compressive strength of scaffold samples was measured according to the ASTM C773 [20] using samples with a diameter of 10 mm and a height of 20 mm. The number of replicates was 5 to measure the compressive strength of

each specimen. To measure bioactivity, scaffold specimens were immersed in the simulated body fluid (SBF) for 28 days. This solution was prepared according to the Kokubo and Hiroaki instructions [5]. The immersed samples were then subjected to the XRD analysis to determine the possibility of hydroxyapatite formation on the surface.

The microstructures of all scaffolds before and after immersion in SBF solution were examined by a field emission electron microscope (FESEM, Mira 3-Tescan) equipped with an energy dispersive spectroscopy (EDS) detector. The surface of the samples was covered with a thin layer of gold before microstructural evaluation.

3. Results and discussion

To identify the chemical composition of eggshell and its compatibility with 45S5 glass, crushed eggshells were subjected to XRF analysis (see Table 1). It is implied from Table 1 that calcium oxide is the main constituent of eggshell.

The XRD pattern taken from egg shells can be observed in Fig. 1. As expected, calcium carbonate (CaCO₃), as the calcite, is the single crystalline phase of the eggshells.

To determine the decomposition temperature of the utilized eggshell, it was subjected to DTA analysis (see Fig. 2). It is implied from Fig. 2 that the main decomposition of eggshell occurs at about 910 °C.

As mentioned earlier, glass-ceramic scaffolds were prepared by sintering of disc compacts (at 1050 °C for 2 h) composed of starting glasses and different amounts of eggshell. Hereafter, various properties of fabricated scaffolds besides their bioactivity will be discussed.

3.1. Characterization of physico-mechanical properties

Fig. 3 depicts the XRD patterns of glass-ceramic scaffolds after heat treatment. As can be observed, the presence of sodium calcium silicate crystalline phase with two stoichiometries of Na₂CaSiO₄ and Na₂Ca₃Si₂O₈ is evident in all specimens. In the case of Z3 and Z4 specimens, the occurrence of sodium zinc silicate (Na₂ZnSiO₄) is also detectable. The formation of the aforementioned phase is logical given the higher amounts of zinc oxide in the relevant samples. On the other hand, by further increasing ZnO content, the intensity of crystalline phases is increased. Since all specimens were heat-treated at the same temperature (1050 °C), the increase in peak lines means their enhanced crystallinity.

Fig. 4 shows the changes in the physical properties (bulk density, linear shrinkage, and porosity content) of the scaffolds produced depending on the amount of eggshell used. According to the figure, the lowest bulk density and linear shrinkage were obtained for 35% by weight of eggshell. Based on this figure, Scaffolds made from this amount of eggshells contain approximately 50% porosity. The highest amount of porosity was formed in scaffold Z2 at 55%.

Table 1. XRD analysis taken from powdered eggshells (wt%).

Fe ₂ O ₃	CaO	Na ₂ O	MgO	K ₂ O	TiO ₂	MnO	P ₂ O ₅	LOI (loss on ignition)
0.5	55	0.3	0.7	0.06	0.03	0.01	0.4	43

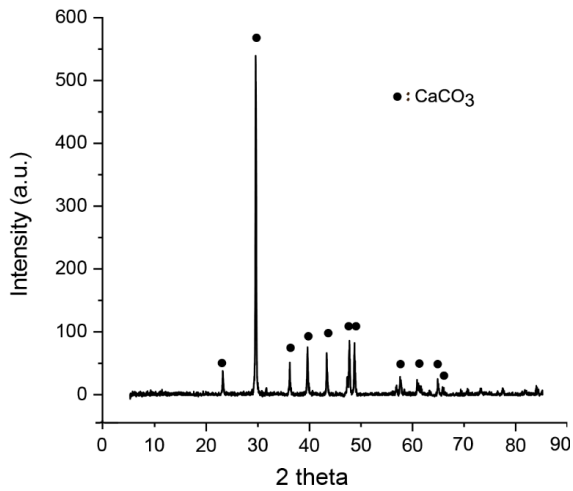


Fig. 1. XRD pattern of powdered eggshells.

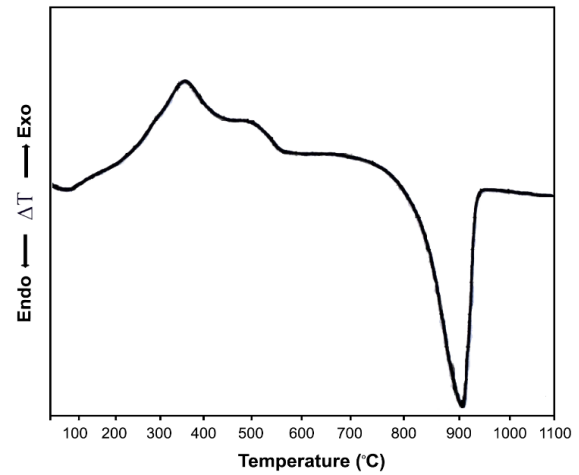


Fig. 2. DTA thermograph taken from powdered eggshell.

Considering Fig. 4, 35% by weight was selected as the optimal amount of eggshell for use in the fabrication of glass-ceramic scaffolds, and the relevant samples were further examined. Fig. 5 indicates FESEM micrographs taken from the microstructure of all scaffolds.

According to Fig. 5, all samples have porosity in the range of 50 to 100 microns. The results obtained confirm the significant performance of eggshell in creating structural porosity. Fig. 6 shows the compressive strength values of the fabricated scaffolds compared to the amount of porosity present in them. Interestingly, the sample without zinc oxide (base), despite having the lowest porosity, also has the lowest compressive strength. This result shows that zinc oxide, in addition to increasing the porosity in the scaffold microstructure, also increased the compressive strength, probably due to the enhanced

crystallinity of the zinc oxide-containing samples compared to the base sample.

3.2. Bioactivity assessment

In order to investigate the bioactivity and the possibility of hydroxyapatite formation, the fabricated scaffolds were placed in a simulated body solution (SBF) for 28 days. Fig. 7 shows the XRD patterns of the corresponding samples after immersion in SBF solution. According to Fig. 7, the formation of two phases of hydroxyapatite ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) and calcium phosphate ($\text{Ca}_2\text{P}_2\text{O}_7$) is evident on the surface of all samples, indicating the bioactivity of the fabricated scaffolds. In addition, it is seen that the intensity of the calcium phosphate phase decreases with increasing zinc oxide content in the samples.

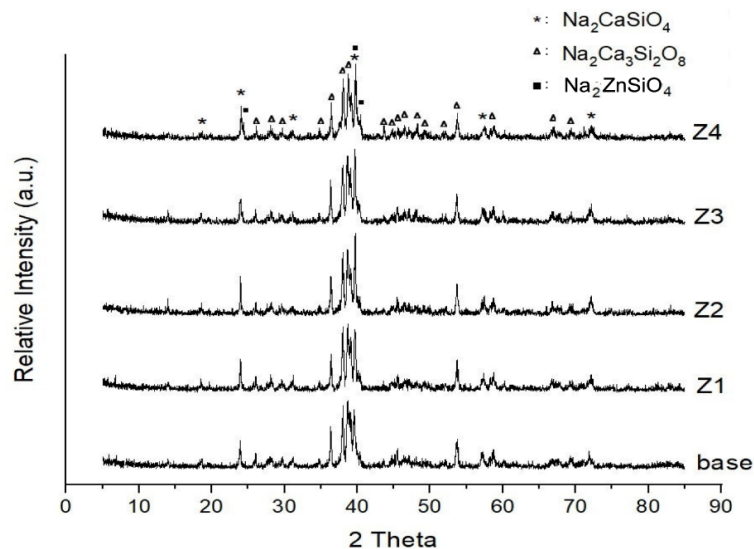


Fig. 3. XRD patterns of glass-ceramic scaffolds after heat treatment.

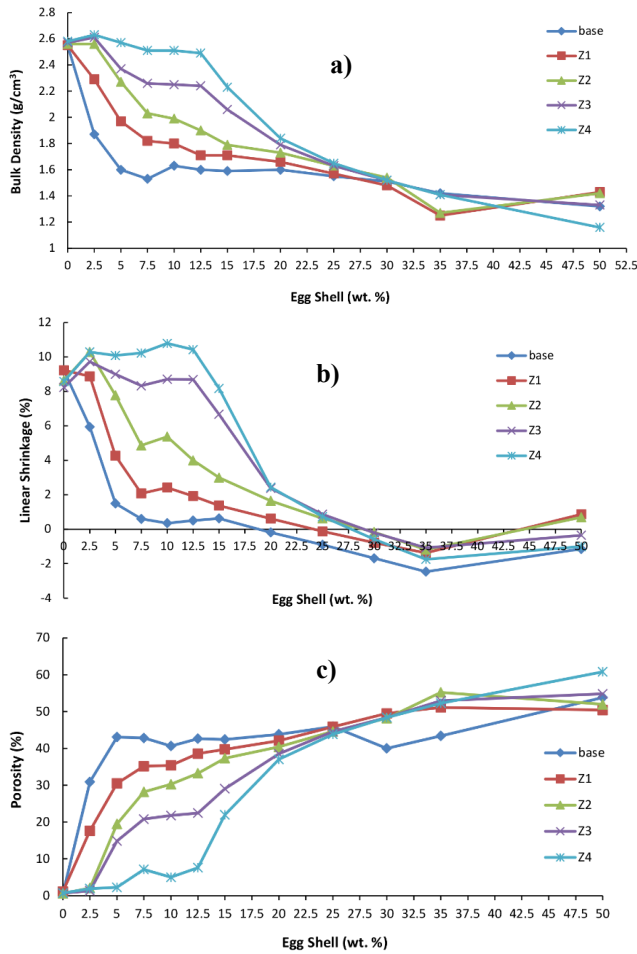


Fig. 4. Changes in physical properties of fabricated scaffolds versus eggshell amount: a) bulk density, b) linear shrinkage, and c) porosity.

FESEM images taken from the surface of the scaffolds after immersion show the formation of crystalline phases with cauliflower morphology on the surface of all samples (see Fig. 8). According to the EDS pattern taken from Z4 scaffolds, the blade-shaped crystals forming the cauliflower morphology are related to the formation of hydroxyapatite and calcium pyrophosphate crystalline phases. It should be noted that

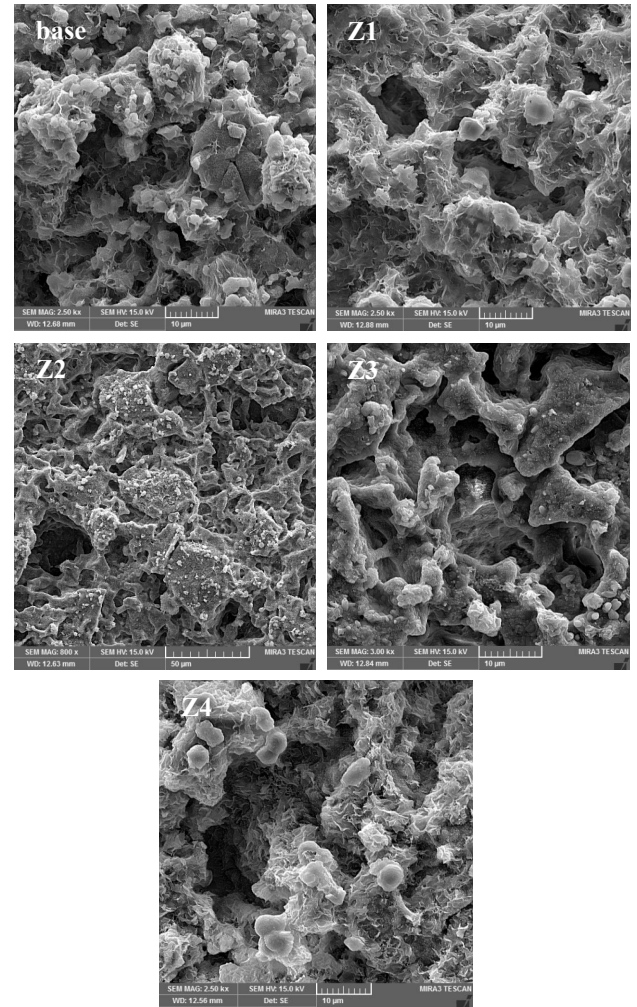


Fig. 5. Microstructural images of fabricated glass-ceramic scaffolds.

precise quantification of Ca/P ratio according to the EDS was not possible due to the small size of clustered blade-like crystals subjected to the EDS analysis.

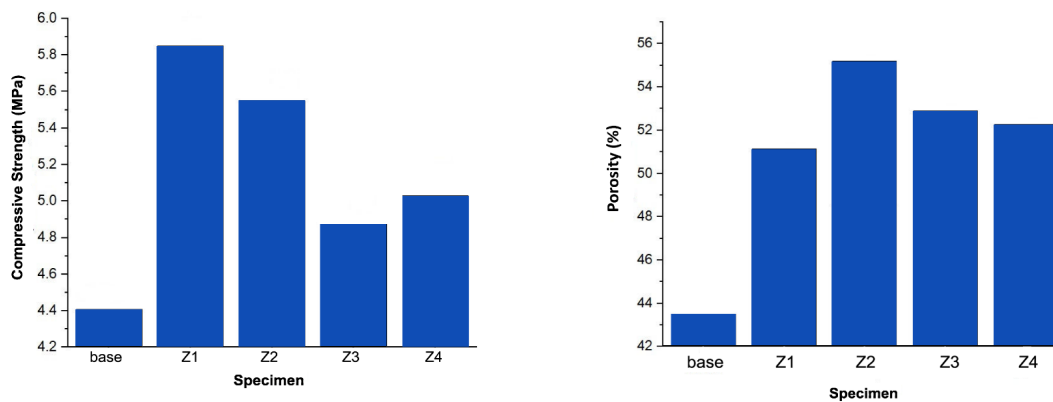


Fig. 6. Compressive strength and porosity content of the fabricated glass-ceramic scaffolds.

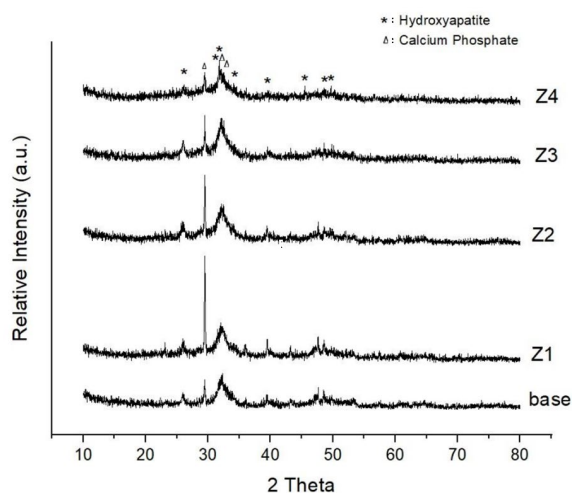


Fig. 7. XRD patterns of fabricated scaffolds after 28 immersions in SBF solution.

4. Conclusions

It was found that sodium calcium silicate precipitated as the main crystalline phase in the fabricated scaffolds. Sodium zinc silicate phase also crystallized in specimens with higher amounts of zinc oxide in the chemical composition. The optimized amount of eggshell in scaffold

fabrication was found to be 35% by weight, with respect to the compressive strength and physical properties of relevant scaffolds. All scaffolds had porosities in the range of 50 to 100 microns, and in the case of the most promising specimen (scaffold Z2), porosity was achieved at 55%. After immersion in simulated body fluid for 28 days, all scaffolds showed apatite formation ability, confirming their acceptable bioactivity.

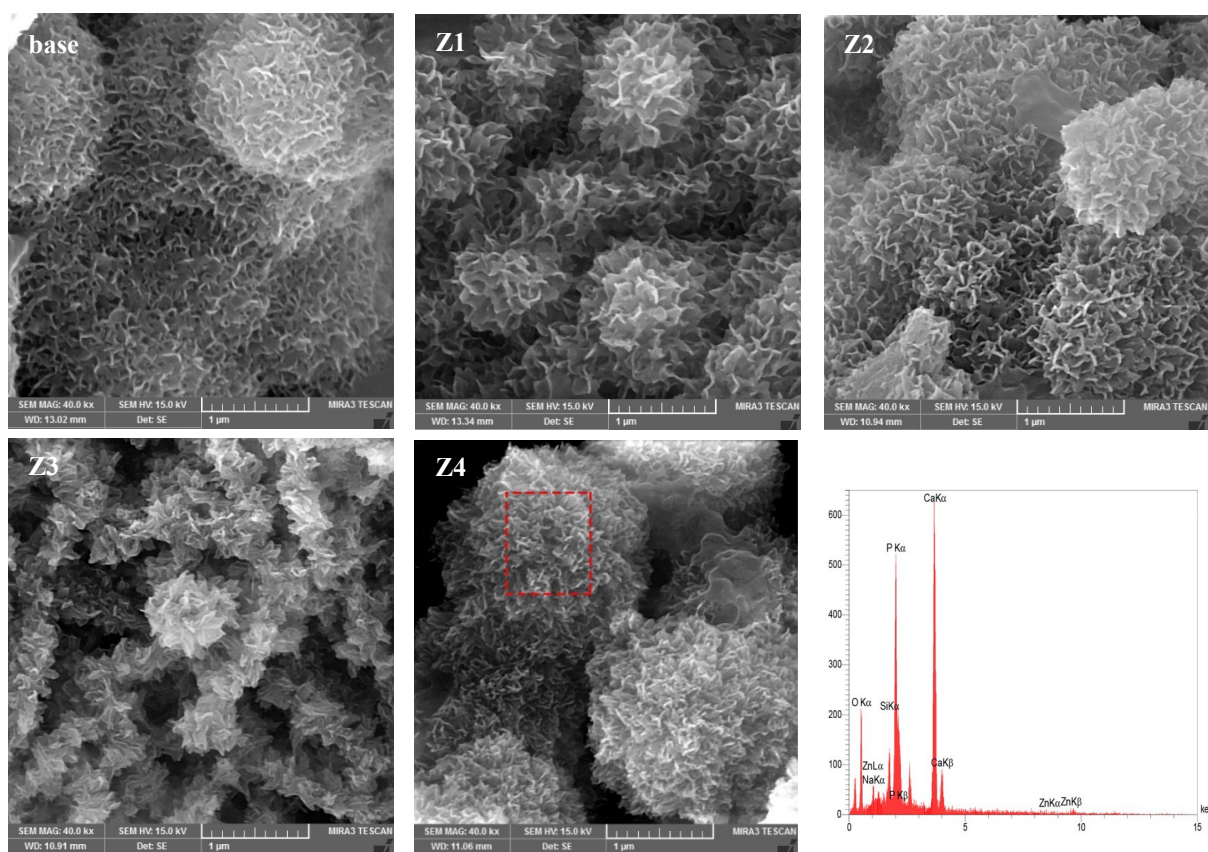


Fig. 8. FESEM images of the prepared scaffolds along with the EDS pattern (taken from the selected area of Z4) after immersion in SBF solution for 28 days.

CRediT authorship contribution statement

Ahmad Norouzi: Writing – original draft, Investigation, Methodology, Software.

Sara Banijamali: Writing – review & editing, Supervision, Project administration, Resources.

Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

Declaration of competing interest

The authors declare no competing interests.

Funding and acknowledgment

The authors would like to thank the Materials and Energy Research Center (MERC) for financial support.

References

- [1] A. Saeedi, S. Banijamali, M. Heydari, Cobalt doped bioactive glasses: Sinterability, crystallization trend, and biodegradation assessment of relevant glass-ceramic scaffolds, *Iran. J. Mater. Sci. Eng.* 21 (2024) 1–23. <https://doi.org/10.22068/ijmse.3592>.
- [2] A. Saeedi, M. Heydari, S. Banijamali, Densification-crystallization behavior of biodegradable copper-doped modified 45S5 glasses, *Synth. Sinter.* 3 (2023) 192–199. <https://doi.org/10.53063/synsint.2023.33174>.
- [3] P. Sepulveda, J.R. Jones, L.L. Hench, Bioactive sol-gel foams for tissue repair, *J. Biomed. Mater. Res.* 59 (2002) 340–348. <https://doi.org/10.1002/jbm.1250>.
- [4] L. Gerhardt, A.R. Boccaccini, Bioactive glass and glass-ceramic scaffolds for bone tissue Engineering, *Materials.* 3 (2010) 3867–3910. <https://doi.org/10.3390/ma3073867>.
- [5] T. Kokubo, H. Hiroaki, How useful is SBF in predicting in vivo bone bioactivity?, *Biomaterials.* 27 (2006) 2907–2915. <https://doi.org/10.1016/j.biomaterials.2006.01.017>.
- [6] E. Fiume, C. Migneco, S. Kargozar, E. Verné, F. Baino, Processing of bioactive glass scaffolds for bone tissue engineering, *Bioactive Glasses and Glass-Ceramics: Fundamentals and Applications*, John Wiley & Sons, Inc. (2022) 119–145. <https://doi.org/10.1002/9781119724193>.
- [7] H.R. Fernandes, A. Gaddam, A. Rebelo, D. Brazete, G.E. Stan, J.M.F. Ferreira, Bioactive glasses and glass-ceramics for healthcare applications in bone regeneration and tissue engineering, *Materials.* 11 (2018) 2530. <https://doi.org/10.3390/ma11122530>.
- [8] D. Galusková, H. Kaňková, A. Švančárková, L. Buňová, D. Galusek, Direct monitoring of immediate release of Zn from zinc-doped bioactive glass, *Int. J. App. Glass Sci.* 13 (2022) 476–483. <https://doi.org/10.1111/ijag.16568>.
- [9] V. Anand, K. J. Singh, K. Kaur, Evaluation of zinc and magnesium doped 45S5 mesoporous bioactive glass system for the growth of hydroxyl apatite layer, *J. Non-Cryst. Solids.* 406 (2014) 88–95. <https://doi.org/10.1016/j.jnoncrysol.2014.09.050>.
- [10] C. Ozel, C.B. Cevlik, A.C. Ozarslan, C. Emir, Y.B. Elalmis, S. Yusel, Evaluation of biocomposite putty with strontium and zinc co-doped 45S5 bioactive glass and sodium hyaluronate, *Int. J. Biol. Macromol.* 242 (2023) 124901. <https://doi.org/10.1016/j.jbiomac.2023.124901>.
- [11] M.C. Crovace, M.T. Souza, C.R. Chinaglia, O. Peitl, E.D. Zanotto, Biosilicate®- A multipurpose, highly bioactive glass-ceramic. In vitro, in vivo and clinical trials, *J. Non-Cryst. Solids.* 432 (2016) 90–110. <https://doi.org/10.1016/j.jnoncrysol.2015.03.022>.
- [12] Y. Peng, Y. Zhuang, Y. Liu, H. Le, D. Li, et al., Bioinspired gradient scaffolds for osteochondral tissue engineering, *Exploration.* 3 (2023) 20210043. <https://doi.org/10.1002/EXP.20210043>.
- [13] M.M. Fernandes, D.M. Correia, C. Ribeiro, N. Castro, V. Correia, S.L. Mendez, Bioinspired three-dimensional magnetoactive scaffolds for bone tissue engineering, *ACS Appl. Mater. Interfaces.* 11 (2019) 45265–45275. <https://doi.org/10.1021/acsami.9b14001>.
- [14] E. Fiume, J. Barberi, E. Verne, F. Baion, Bioactive glasses: From parent 45S5 composition to scaffold-assisted tissue-healing therapies, *J. Funct. Biomater.* 9 (2018) 24. <https://doi.org/10.3390/jfb9010024>.
- [15] X. Wu, O. Gauntlett, T. Zhang, S. Suvarnapathaki, C. McCarthy, et al., Eggshell microparticle reinforced scaffolds for regeneration of critical sized cranial defects, *ACS Appl. Mater. Interfaces.* 13 (2021) 60921–60932. <https://doi.org/10.1021/acsami.1c19884>.
- [16] N.E. Putra, J. Zhou, A.A. Zadpoor, Sustainable sources of raw materials for additive manufacturing of bone substituting biomaterials, *Adv. Healthcare Mater.* 13 (2024) 2301837. <https://doi.org/10.1002/adhm.202301837>.
- [17] M.E.T. Gouveia, C. Mihans, M. Gezek, G. Camsi-Unal, Eggshell-based unconventional biomaterials for medical application, *Adv. NanoBiomed. Res.* 5 (2024) 2400120. <https://doi.org/10.1002.anbr.202400120>.
- [18] A. Norouzi, S. Banijamali, A. Keshavarzi, Sinter-crystallization, phase development and microstructural evaluations of ZnO containing 45S5® glass-ceramic, *Mater. Today. Proc.* 5 (2018) 15696–15701. <https://doi.org/10.1016/matpr.2018.04.180>.
- [19] Standard test method for water absorption, bulk density, apparent porosity, and apparent specific gravity of fired whiteware products, ASTM International, Designation: C 373-88 (Reapproved 2006).
- [20] Standard test method for compressive (crushing) strength of fired whiteware materials, ASTM International, Designation: C 773-88 (Reapproved 2006).