

Available online at www.jourcc.comJournal homepage: www.JOURCC.com

Journal of Composites and Compounds

Production and characterization of PCL (Polycaprolactone) coated TCP/nanoBG composite scaffolds by sponge foam method for orthopedic applications

Jeiran Daraei ^{a*}^a Materials Engineering Department, Najafabad Branch, Islamic Azad University, Isfahan, Iran

ABSTRACT

Bio-ceramics are a set of ceramic materials that possess an important feature called biocompatibility. Bioglass is one of the most applicable ceramic materials contributing to bioactivity improvement due to the presence of Si as an ossification material. Besides, PCL polymer supported by bioglass nanoparticles was used to improve the mechanical properties. In this study, we combined Tricalciumphosphate (TCP) with nano-bioglass (NBG) with four different amounts (10%, 20%, 30%, and 0%) to produce composite scaffolds (lost sponge foam method); then, we coated these composites by Polycaprolactone (PCL) biopolymer, and eventually, evaluated its bioactivity, biodegradability, and mechanical properties. Scanning electron microscopy (SEM) and EDS methods were employed to investigate the morphology and bioactivity of scaffolds. The results of the ion concentration measurement test and SEM and EDS analysis showed the formation of Apatite on the scaffold's surface. The results from SEM indicate that the most bioactivity was observed for composite scaffold containing 20%wt of nano-bioglass presenting it as the preferable sample, in terms of mechanical properties and bioactivity.

©2020 JCC Research Group.

Peer review under responsibility of JCC Research Group

ARTICLE INFORMATION

Article history:

Received 25 January 2020

Received in revised form 23 February 2020

Accepted 28 March 2020

Keywords:

Tissue engineering

Tricalcium phosphate

Nanocomposite scaffold

Nanobioglass

Polycaprolactone

1. Introduction

Pathological degeneration, trauma, tumor resection, and congenital deformities are the main factors affecting orthopedic and dental treatments [1, 2]. One of the most effective ways for bone regeneration is bone tissue engineering (BTE) [3, 4]. To overcome the restrictions of damaged tissues or organs and provide biological alternatives, treatment of BTE has been practiced clinically [5, 6]. Using biomaterials for the production of scaffolds is a promising BTE technique. These scaffolds support new tissue growth by the provision of an artificial extracellular matrix [7, 8]. The role of these scaffolds is to provide daily mastication capability like a natural bone in order to build support for the regeneration region and simultaneously undergo gradual degradation with the regeneration of tissue. Acceptable mechanical strength, suitable physicochemical properties, proper surface morphology and porosity, biocompatibility, controllable biodegradability, and osteoconductivity are the most important properties that a well-functioning scaffold should possess [9-11]. Common scaffolds for BTE are currently various bioactive glass, calcium phosphate, and hydroxyapatite (HA) [12, 13]. Tricalcium phosphate (β -TCP) exhibits promising biodegradability, osteoconductivity, and biocompatibility [14]. On the other hand, this bioceramic also has drawbacks; for instance, its degradation rate is higher than bone regeneration [15, 16]. Additionally, because of having a porous struc-

ture, the mechanical properties of β -TCP as a support for cell growth is relatively poor [17, 18]. As composites materials provide the opportunity to combine the properties of incorporated components [19, 20], several studies have been focused on the production of composite scaffolds consisting of polymers and β -TCP for improving osteoconductivity and the mechanical properties of the scaffold to overcome these shortcomings. Polymer/ceramic composites have offered promising properties due to benefiting from both materials properties [21-23]. Chitosan and collagen are natural polymers that are used to produce composites [24-26], but these natural polymers do not have sufficient mechanical strength [27]. Polylactic acid and poly- ϵ -caprolactone (PCL) are synthetic polymers that have been used for overcoming the disadvantages of natural polymers [28, 29]. Owing to biocompatibility, biodegradability, and good mechanical properties, PCL have been widely investigated [23, 30]. Moreover, bioglass materials with different compositions can be integrated with living bone tissue by creating an intimate bond. The bioglasses that are able to form a bond with the bone are called "bioactive". Several studies investigated the production of scaffolds of PCL coated TCP, but no research focused on PCL coated TCP/nanoBG composite scaffolds synthesized via sponge foam method for orthopedic applications to the best of authors' knowledge. Therefore, in this study, we prepared TCP-bioglass composite scaffolds with different compositions. To enhance the mechanical properties of the scaffolds, they were coated by

* Corresponding author: Jeiran Daraei; E-mail: jeirandaraei20@gmail.comDOR: [10.29252/jcc.2.1.6.4](https://doi.org/10.29252/jcc.2.1.6.4)<https://doi.org/10.29252/jcc.2.1.6>This is an open access article under the CC BY license (<https://creativecommons.org/licenses/by/4.0>)

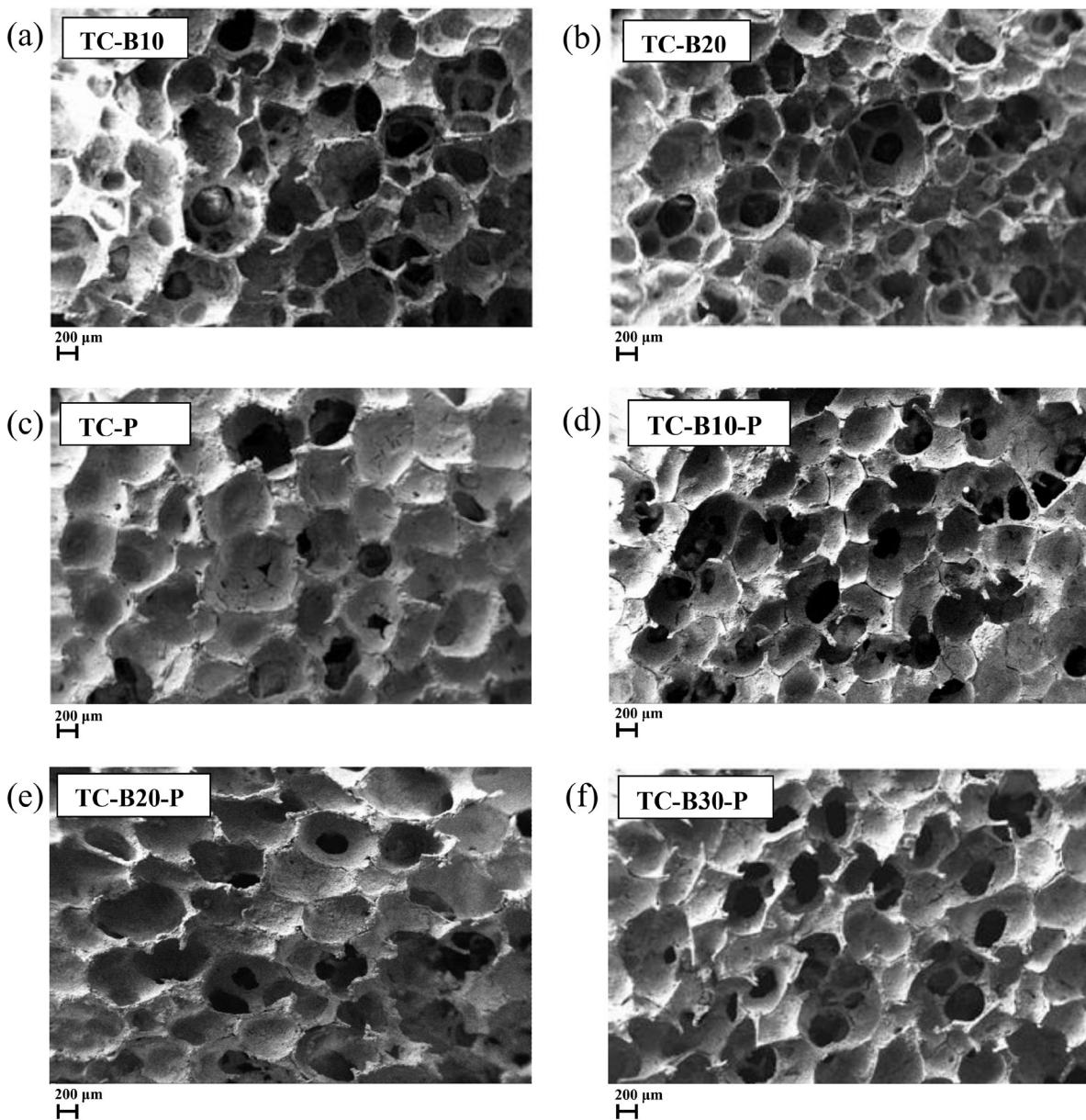


Fig. 1. SEM images TCP/bioglass/PCL composite scaffolds of (a) TC-B10, (b) TC-B20, (c) TC-P, (d) TC-B10-P, (e) TC-B20-P, and (f) TC-B30-P

a polycaprolactone biopolymer, and their mechanical, bioactivity, and biodegradation were evaluated.

2. Experimental

2.1. Materials

In this study, calcium nitrate tetrahydrate ($\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$), TEOS ($(\text{C}_2\text{H}_5\text{O})_4\text{Si}$), PCL polymer (molecular weight of 80,000) and tetraethyl phosphate ($(\text{C}_2\text{H}_5\text{O})_3\text{PO}$) were bought from Aldrich company. Simulated body fluid (SBF), sodium tripolyphosphate solvent, carboxymethyl cellulose powder, nitric acid (63%), and chloroform (99.5%) were obtained from Merck company, Germany.

2.2. Sample preparation

2.2.1. Production of TCP particles

After boiling the cow thigh in water for 2 hours to remove residual tissue, the bone was kept at 60 °C for 24 hours in an oven to dry. Then, the bone pieces were burnt by fire flame at about 400 °C for 3 h to de-

grade the organic components of the bone. The obtained black ash was heated at 900 °C for 2 h to produce hydroxyapatite powder.

2.2.2. Synthesis of bioglass nanoparticles (nanoBG)

For the preparation of bioactive glass, raw materials including calcium nitrate (28 mol. %), TEOS (63 mol. %), and tetraethyl phosphate (9 mol. %) were used. Ethanol and hydrochloric acid were also used as a solvent and a catalyst, respectively. To prepare the sol, the solution of deionized water and hydrochloric acid was stirred by a magnetic stirrer for 30 min. After adding TEOS and ethanol with a molar ratio of 1, the solution was stirred for another 30 minutes following the addition of tetraethyl phosphate and stirring for 20 min. Finally, it was stirred at medium speed for 2 hours with the addition of calcium nitrate. After gelation of the initial solution, the formed gel was dried at 60 °C for 10 h. To eliminate excess gases, the gel was heat-treated for 15 h at 130 °C. Finally, by heating the dried gel at 900 °C for 2 h, bioglass was obtained as the final product of the operation.

2.2.3. Preparation of ceramic slurry and scaffolds

The obtained tricalcium phosphate powders with a mean particle

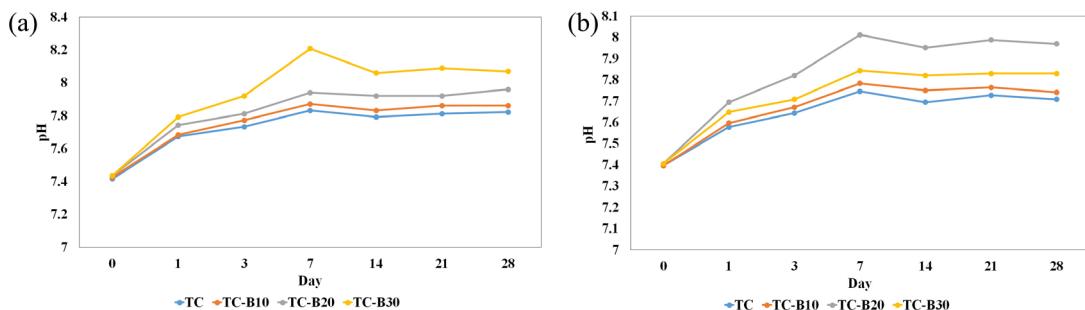


Fig. 2. PH changes in a) uncoated scaffolds and b) coated scaffolds

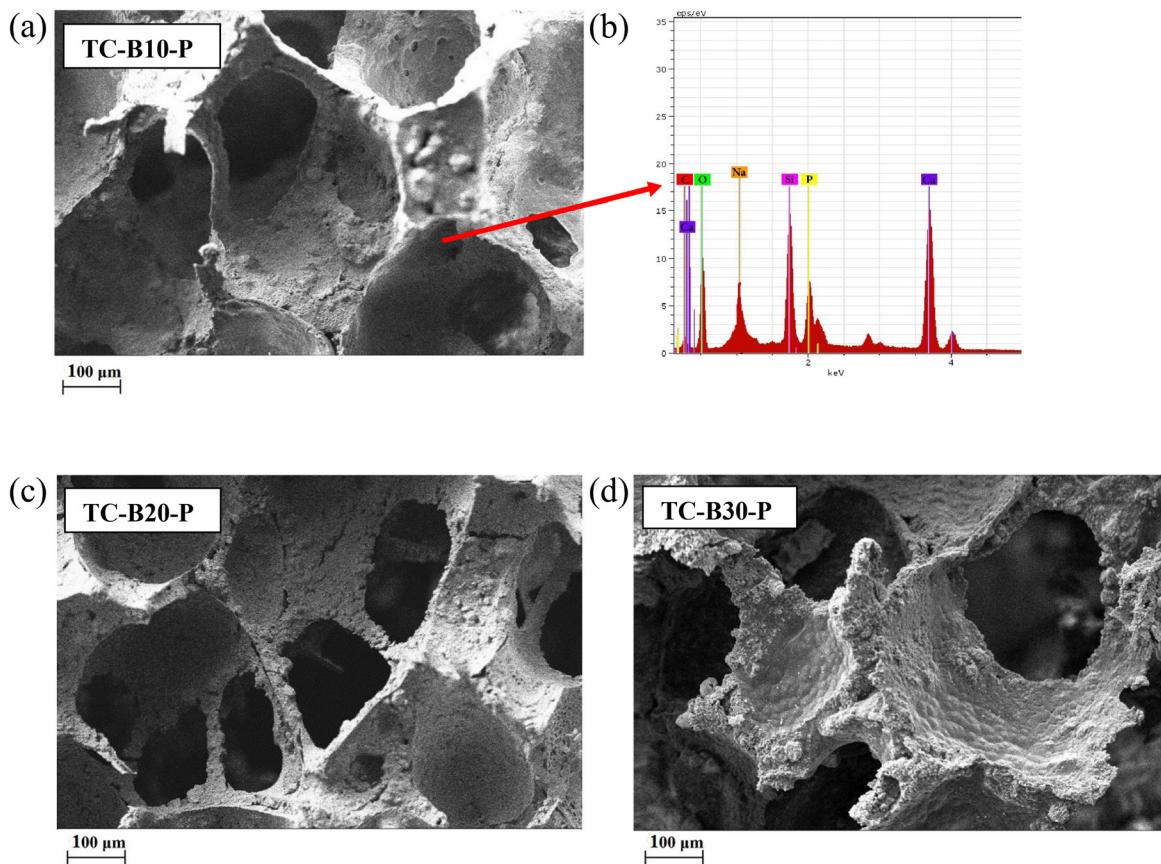


Fig. 3. SEM images of the coated scaffolds of (a) TC-B10-P (b) with its EDX analysis, (c) TC-B20-P, and (d) TC-B30-P after 28 days of immersion in SBF

size of 85 nm were used as the base material to fabricate the scaffold. Hydroxyapatite and nanoBG powders with 0, 10, 20, and 30 wt. % bioglass were added slowly to deionized water. After homogenization of the mixture, 1 wt. % sodium tripolyphosphate (STPP) was used to increase the solid loading on the sponge disks. The slurry was then stirred for 30 min at 300 rpm and then 1 wt. % of carboxymethyl cellulose was added as the binder. Finally, stirring was continued at 60 °C until complete homogenization.

Commercial polyurethane foam was cut into 10×10×10 mm cubes and immersed slowly into the ceramic slurry. Then, the samples were dried in a vacuum for 24 hours to prevent sponge pores from closing. The samples were subjected to heat treatment in the furnace during four stages to prepare the scaffolds: 1) heated at 600 °C for an hour to completely burn the polymer foam (heating rate of 3 °C/min), 2) heating from 600 °C to 1350 °C with a heating rate of 5 °C/min, 3) keeping the samples at 1350 °C for 2 hours, and 4) cooling the samples in the oven to room temperature with a 5 °C/min cooling rate. The uncoated scaffolds containing 10, 20, and 30 wt. % bioglass is noted as TC-B10, TC-B20,

and TC-B30, and P denotes the polymer coating in composites scaffolds.

2.2.4. Composite coatings on scaffolds

The PCL polymer ($M_w = 80000$) was dissolved in chloroform (10% (w/v)) and stirred for 30 min. 10 wt. % of nanoBG particles was sonicated in 10 cm³ chloroform for 15 minutes and then added to the PCL solution. The final solution was then stirred at room temperature for further homogenization for 24 h. After sterilizing the surface of the scaffold with acetone and ethanol, the scaffolds were soaked in the prepared solution for 1 minute. Finally, the scaffolds were dried in an oven for 7 days at 37 °C.

2.3. Characterization

2.3.1. Measurement of scaffold porosity and density

For the calculation of density and porosity of scaffolds, the liquid displacement method was employed. In this study, ethanol 96% was used to diffuse into scaffold porosities. The scaffold density was calcu-

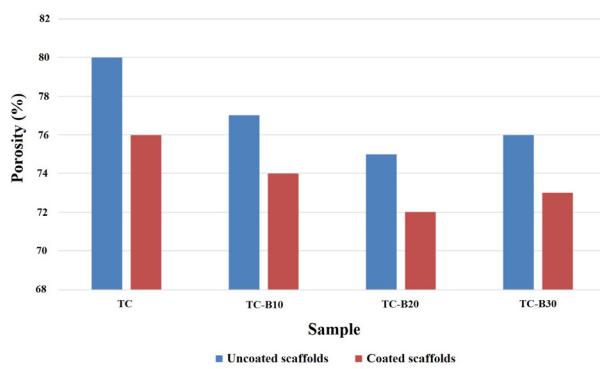


Fig. 4. Porosity percentage of the scaffolds

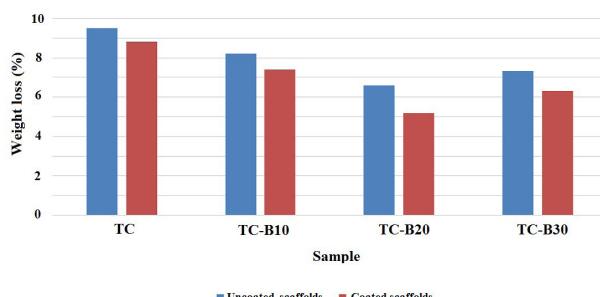


Fig. 6. Weight loss of the scaffolds after 28 days of immersion in SBF

lated from the following equation:

$$\rho = W/(V_2 - V_3) \quad (1)$$

moreover, the amount of open porosity of the scaffold is obtained from the following relationship:

$$\varepsilon = (V_1 - V_3)/(V_2 - V_3) \quad (2)$$

where W , V_1 , V_2 , and V_3 denote the scaffold weight, the volume of ethanol contained in the cylinder, volume of ethanol after immersion of the scaffold in the cylinder for 5 min, and the volume of ethanol remaining after removal of the scaffold from the cylinder, respectively. The amount of ε and ρ represent the volume of scaffold and adsorbed ethanol, respectively. Thus, the final volume of the scaffold is calculated through the equation 3.

$$V = (V_1 - V_2)/(V_1 - V_3) \quad (3)$$

2.3.2. SEM and EDS analysis

The microstructure and morphology of the prepared scaffolds were investigated via Zeiss 00947B scanning electron microscope and EDS analysis. Prior to microscopic observations, the surface of the composite scaffolds was gold-coated.

2.3.3. Measurement of pH in SBF solution

After immersion of the scaffolds in the SBF solution, the pH of the solution was measured by Aqbus Model 2000 pH meter after 2, 3, 7, 14, 21, and 28 days of immersion.

2.3.4. Bioactivity assessment

In vitro bioactivity evaluation was performed by immersion in SBF for 28 days. The cubic scaffolds were immersed in SBS solution and incubated at 37 °C and after 1, 7, 14, and 21 days, the composite scaffolds were taken out, washed with water, and dried in a vacuum oven at 60°C and taken for further characterization.

2.3.5. Plasma coupling optical emission spectroscopy (ICP)

ICP method was used to measure the number of ions released from

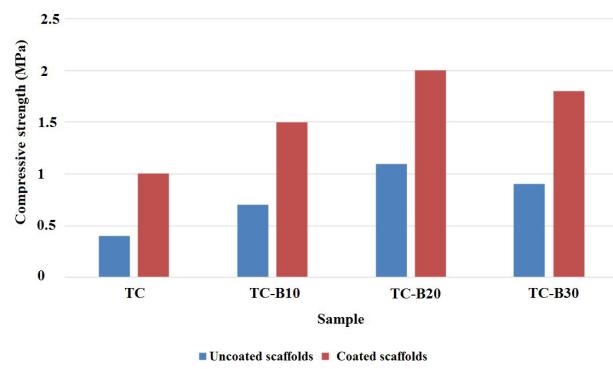


Fig. 5. Compressive strength of the composite scaffolds

the scaffold immersed in SBF solution. In this study, a Zaires 110394 device was used.

3. Results and discussion

3.1. Microstructural study of the composite scaffolds

The SEM images of the TCP/bioglass/PCL scaffolds are shown in Figure 1. As seen in SEM images, the cross-section of the synthesized scaffold shows interconnected porosities. The mean pore size of the scaffolds appears to be about 300-550 μ m, which is appropriate for cells to infiltrate into the channels and pores of the scaffold. Ideal scaffolds are required to possess a high volume of open and interconnected pores in order to obtain high cell seeding density in the scaffolds, and to facilitate transporting of oxygen as well as nutrients for cell differentiation and cell proliferation. Scaffolds with porosity dimensions of 100 to 500 microns are considered for cell culture, soft and hard tissue growth, and vascularization. In bone tissue engineering, the pore size larger than 300 microns is required. It should be noted that the increase in porosity size leads to a decrease in the mechanical strength of the scaffold; therefore, the balance between these parameters should be considered. As can be seen in the figure, PCL has been successfully coated on the scaffolds.

3.2. Measurement of pH and Released ions in SBF solution

Figure 2 shows the pH variations of coated and uncoated scaffolds in the SBF solution within 28 days. According to the results, the pH change is between 7.4 and 8, which is acceptable for a physiologic environment to prevent damages to cells and tissues.

SBF solution was analyzed after 28 days to measure the released amount of ions from the scaffolds. The obtained results for uncoated samples are presented in Table 1. As shown in the table, most of the calcium and phosphorus ions were absorbed by the sample surface, and the concentration of these ions in SBF decreased, showing the apatite formation. The lowest concentration of Ca and P ions is related to the TC-B20 sample, which means the apatite formation is more; in other words, the bioactivity of this scaffold is higher than that of other scaffolds.

As observed in Figure 3, the bright precipitates formed on the surface

Table 1.

The concentration of ions released in SBF

Sample	Ca (ppm)	P (ppm)	Si (ppm)
TC	24	22	0
TC-B10	20	20	50
TC-B20	18	14	40
TC-30	20	19	44
SBF	100	31	0

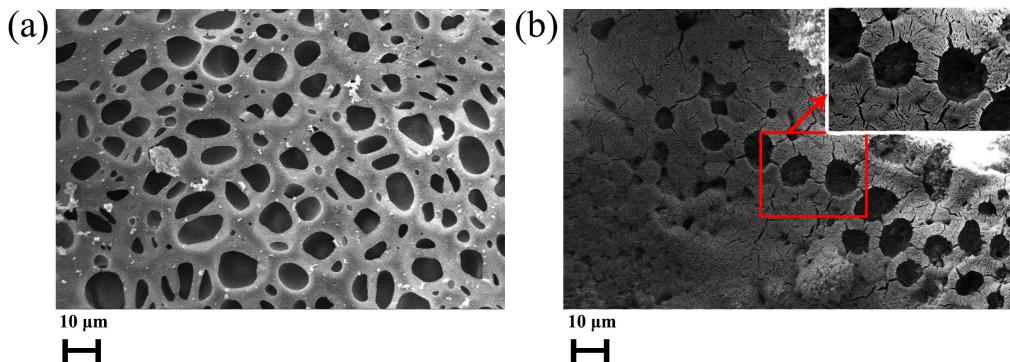


Fig. 7. SEM images of TC-B20-P before and after immersion in SBF for 28 days

of the scaffolds indicate the deposition and formation of apatite particles. In addition, the result of point analysis by EDS on the bright spots of the TC-B10-P scaffold shows the presence of P and Ca, revealing that these precipitates are calcium phosphate. This also indicates the bioactive behavior of this sample.

3.3. Porosity and mechanical properties of the scaffolds

In general, well-functioning implants for tissue engineering are required to have high pore density, good biocompatibility, and sufficient mechanical strength. The porosity percentages of the samples are shown in Figure 4. As shown evidently, polymer coating decreased the porosity of the scaffolds. The porosity of all the prepared samples is in the range of 75-80%, which is in the acceptable range for tissue engineering scaffolds. It can be concluded from the compressive strength results (Figure 5) that the polymer coating inside and on the surface of the porosities caused enhancement of adhesion strength. TC-B20-P shows the highest value of compressive strength. The polymer coating on the surface and inside the pores of the scaffold results in the improvement of the scaffold strength so that in some cases, the strength is multiplied. PCL penetrates the ceramics scaffold, preventing stress concentration and cracking in the scaffold, thus increasing its strength. On the other hand, the highest mechanical strength is related to the sample containing 20 wt. % of bioglass. The bioglass nanoparticles in this sample are homogeneously dispersed in the space between the beta-tricalcium phosphate particles, resulting in higher strength.

3.4. Biodegradation

Weight loss of the scaffolds after soaking in SBF for 28 days is depicted in Figure 6. The sample TC-B20 shows the best performance in terms of biodegradation. In the case of coated specimens, the rate of degradation is relatively lower than that of the coated specimens but similar to the coated specimens, TC-B20-P has the lowest degradation. The SEM images of TC-B20-P before and after degradation are shown in Figure 7.

4. Conclusions

In this research, TCP/nano-bioglass/PCL composite scaffolds with different compositions were produced. Adding 20% bioglass improved the mechanical strength more than 2 times (from 0.4 MPa to 1.1 MPa). The coating of the scaffolds also enhanced the mechanical properties as the strength of the TC-B20 sample increased from 1.1 MPa to 2 MPa. All scaffold samples contain 75-80% porosity, which is acceptable for bone scaffolds. The result of the elemental analysis of ICP showed that the highest bioactivity behavior was related to the TC-B20 sample. Evaluation of elemental analysis results of SBF solution on coated scaffold samples showed the positive effect of coating and improved bioavailability. Finally, the degradation results showed that the degradation improved in TC-B20 and the enhancement was observed by coating.

Acknowledgments

The authors thank the Islamic Azad University, Najafabad Branch, Isfahan, Iran, for its financial and other support.

Conflict of Interest

All authors declare no conflicts of interest in this paper.

REFERENCES

- [1] S. Rahimi, F. SharifianJazi, A. Esmailkhani, M. Moradi, A.H. Safi Samghabadi, Effect of SiO_2 content on $\text{Y-TZP}/\text{Al}_2\text{O}_3$ ceramic-nanocomposite properties as potential dental applications, *Ceramics International* 46(8, Part A) (2020) 10910-10916.
- [2] E. Jabbarzadeh, T. Starnes, Y.M. Khan, T. Jiang, A.J. Wirtel, M. Deng, Q. Lv, L.S. Nair, S.B. Doty, C.T. Laurencin, Induction of angiogenesis in tissue-engineered scaffolds designed for bone repair: a combined gene therapy-cell transplantation approach, *Proceedings of the National Academy of Sciences* 105(32) (2008) 11099-11104.
- [3] A. Moghanian, A. Ghorbanoghi, M. Kazem-Rostami, A. Pazhouheshgar, E. Salari, M. Saghafi Yazdi, T. Alimardani, H. Jahani, F. Sharifian Jazi, M. Tahriri, Novel antibacterial Cu/Mg-substituted 58S-bioglass: Synthesis, characterization and investigation of in vitro bioactivity, *International Journal of Applied Glass Science* 11(4) (2020) 685-698.
- [4] L. Bazli, H. Nargesi khoramabadi, A. Modarresi Chahardehi, H. Arsal, B. Malekpouri, M. Asgari Jazi, N. Azizabadi, Factors influencing the failure of dental implants: a systematic review, *Journal of Composites and Compounds* 2(2) (2020) 18-25.
- [5] F. Sharifianjazi, N. Parvin, M. Tahriri, Formation of apatite nano-needles on novel gel derived $\text{SiO}_2\text{-P}_2\text{O}_5\text{-CaO-SrO-Ag}_2\text{O}$ bioactive glasses, *Ceramics International* 43(17) (2017) 15214-15220.
- [6] C. Gao, Y. Deng, P. Feng, Z. Mao, P. Li, B. Yang, J. Deng, Y. Cao, C. Shuai, S. Peng, Current progress in bioactive ceramic scaffolds for bone repair and regeneration, *International journal of molecular sciences* 15(3) (2014) 4714-4732.
- [7] U. Kneser, D.J. Schaefer, E. Polykandriotis, R.E. Horch, Tissue engineering of bone: the reconstructive surgeon's point of view, *Journal of cellular and molecular medicine* 10(1) (2006) 7-19.
- [8] L. Lu, Q. Zhang, D. Wootton, R. Chiou, D. Li, B. Lu, P. Lelkes, J. Zhou, Bio-compatibility and biodegradation studies of PCL/ β -TCP bone tissue scaffold fabricated by structural porogen method, *Journal of Materials Science: Materials in Medicine* 23(9) (2012) 2217-2226.
- [9] M.S.N. Shahrabak, F. Sharifianjazi, D. Rahban, A. Salimi, A Comparative Investigation on Bioactivity and Antibacterial Properties of Sol-Gel Derived 58S Bioactive Glass Substituted by Ag and Zn, *Silicon* 11(6) (2019) 2741-2751.
- [10] J.L. Drury, D.J. Mooney, Hydrogels for tissue engineering: scaffold design variables and applications, *Biomaterials* 24(24) (2003) 4337-4351.
- [11] C.-F. Wang, B. Hu, H.-H. Yi, W.-B. Li, The effect of PS porosity on the structure, optical and electrical properties of ZnS/PS, *Optics and Spectroscopy* 116(3) (2014) 427-430.
- [12] F. Sharifianjazi, N. Parvin, M. Tahriri, Synthesis and characteristics of sol-gel bioactive $\text{SiO}_2\text{-P}_2\text{O}_5\text{-CaO-Ag}_2\text{O}$ glasses, *Journal of Non-Crystalline Solids* 476 (2017) 108-113.
- [13] Z. Goudarzi, N. Parvin, F. Sharifianjazi, Formation of hydroxyapatite on surface of $\text{SiO}_2\text{-P}_2\text{O}_5\text{-CaO-SrO-ZnO}$ bioactive glass synthesized through sol-gel route, *Ceramics International* 45(15) (2019) 19323-19330.
- [14] A. Esmailkhani, F. Sharifianjazi, A. Abouchenari, A. Rouhani, N. Parvin,

M. Irani, Synthesis and characterization of natural nano-hydroxyapatite derived from turkey femur-bone waste, *Applied biochemistry and biotechnology* 189(3) (2019) 919-932.

[15] B. Liu, D.x. Lun, Current application of β -tricalcium phosphate composites in orthopaedics, *Orthopaedic surgery* 4(3) (2012) 139-144.

[16] S.H. Kwon, Y.K. Jun, S.H. Hong, I.S. Lee, H.E. Kim, Y.Y. Won, Calcium phosphate bioceramics with various porosities and dissolution rates, *Journal of the American Ceramic Society* 85(12) (2002) 3129-3131.

[17] S. Bose, J. Darsell, M. Kintner, H. Hosick, A. Bandyopadhyay, Pore size and pore volume effects on alumina and TCP ceramic scaffolds, *Materials Science and Engineering: C* 23(4) (2003) 479-486.

[18] C. Young, P. Ladd, C. Browning, A. Thompson, J. Bonomo, K. Shockley, C. Hart, Release, biological potency, and biochemical integrity of recombinant human platelet-derived growth factor-BB (rhPDGF-BB) combined with AugmentTM Bone Graft or GEM 21S beta-tricalcium phosphate (β -TCP), *Journal of controlled release* 140(3) (2009) 250-255.

[19] L. Bazli, M. Siavashi, A. Shiravi, A review of carbon nanotube/TiO₂ composite prepared via sol-gel method, *Journal of Composites and Compounds* 1(1) (2019) 1-9.

[20] S. Saadi, B. Nazari, Recent developments and applications of nanocomposites in solar cells: a review, *Journal of Composites and Compounds* 1(1) (2019) 41-50.

[21] L. Bazli, A. Khavandi, M.A. Boutorabi, M. Karrabi, Correlation between vis-coelastic behavior and morphology of nanocomposites based on SR/EPDM blends compatibilized by maleic anhydride, *Polymer* 113 (2017) 156-166.

[22] L. Bazli, A. Khavandi, M.A. Boutorabi, M. Karrabi, Morphology and visco-elastic behavior of silicone rubber/EPDM/Cloisite 15A nanocomposites based on Maxwell model, *Iranian Polymer Journal* 25(11) (2016) 907-918.

[23] D. Rohner, D.W. Hutmacher, T.K. Cheng, M. Oberholzer, B. Hammer, In vivo efficacy of bone-marrow-coated polycaprolactone scaffolds for the reconstruction of orbital defects in the pig, *Journal of Biomedical Materials Research Part B: Applied Biomaterials: An Official Journal of The Society for Biomaterials, The Japanese Society for Biomaterials, and The Australian Society for Biomaterials and the Korean Society for Biomaterials* 66(2) (2003) 574-580.

[24] M.C. Phipps, W.C. Clem, J.M. Grunda, G.A. Cline, S.L. Bellis, Increasing the pore sizes of bone-mimetic electrospun scaffolds comprised of polycaprolactone, collagen I and hydroxyapatite to enhance cell infiltration, *Biomaterials* 33(2) (2012) 524-534.

[25] S.I. Yoon, Y.K. Lee, K.N. Kim, S. Kim, H. Son, J. Kwak, J. Kim, H. Choi, A comparison of the bone-like apatite formation potency between Hydroxyapatite and β -Tricalcium phosphate in Glass ionomer dental luting cement, *Key Engineering Materials*, Trans Tech Publ, 2006, pp. 885-890.

[26] A. Ibara, H. Miyaji, B. Fugetsu, E. Nishida, H. Takita, S. Tanaka, T. Sugaya, M. Kawanami, Osteoconductivity and biodegradability of collagen scaffold coated with nano- β -TCP and fibroblast growth factor 2, *Journal of Nanomaterials* 43 (2013).

[27] M. Ngiam, S. Liao, A.J. Patil, Z. Cheng, C.K. Chan, S. Ramakrishna, The fabrication of nano-hydroxyapatite on PLGA and PLGA/collagen nanofibrous composite scaffolds and their effects in osteoblastic behavior for bone tissue engineering, *Bone* 45(1) (2009) 4-16.

[28] Y. Yang, Y. Zhao, G. Tang, H. Li, X. Yuan, Y. Fan, In vitro degradation of porous poly (l-lactide-co-glycolide)/ β -tricalcium phosphate (PLGA/ β -TCP) scaffolds under dynamic and static conditions, *Polymer Degradation and Stability* 93(10) (2008) 1838-1845.

[29] W. Xue, A. Bandyopadhyay, S. Bose, Polycaprolactone coated porous tricalcium phosphate scaffolds for controlled release of protein for tissue engineering, *Journal of Biomedical Materials Research Part B: Applied Biomaterials: An Official Journal of The Society for Biomaterials, The Japanese Society for Biomaterials, and The Australian Society for Biomaterials and the Korean Society for Biomaterials* 91(2) (2009) 831-838.

[30] X. Zhang, W. Chang, P. Lee, Y. Wang, M. Yang, J. Li, S.G. Kumbar, X. Yu, Polymer-ceramic spiral structured scaffolds for bone tissue engineering: effect of hydroxyapatite composition on human fetal osteoblasts, *PloS one* 9(1) (2014).