

RESEARCH PAPER

## Fabrication of hydroxyapatite-baghdadite nanocomposite scaffolds coated by PCL/Bioglass with polyurethane polymeric sponge technique

Ebrahim Karamian<sup>1</sup>, Akram Nasehi<sup>1</sup>, Saeed Saber-Samandari<sup>2</sup>, Amirsalar Khandan<sup>3\*</sup>

<sup>1</sup>Advanced Materials Research Center, Faculty of Materials Engineering, Najafabad Branch, Islamic Azad University, Najafabad, Iran

<sup>2</sup>New Technologies Research Center, Amirkabir University of Technology, Tehran, Iran

<sup>3</sup>Young Researchers and Elite Club, Khomeinshahr Branch, Islamic Azad University, Isfahan, Iran

### ABSTRACT

**Objective (s):** Silicate bioceramics like Baghdadite with chemical formula  $\text{Ca}_3\text{ZrSi}_2\text{O}_9$ , has attracted the attention of researchers in biomedical field due to its remarkable in-vitro and in-vivo bioactivity and mechanical properties.

**Materials and Methods:** Therefore, in the current study the baghdadite powder with Sol-Gel method was synthesized. Then, hydroxyapatite/Baghdadite (HA/Bagh) scaffolds were prepared by the replacing the polyurethane polymeric sponge technique. Afterwhile, the ceramic scaffolds were sintered at 1150°C for 3 h. The prepared scaffold was then coated by polycaprolactone/bioglass (PCL/BG) polymer nanocomposite.

**Results:** Bioactivity and biomineralization in the simulated body fluid (SBF) revealed that the nanocomposite scaffolds coated with PCL/BG had significant bioactivity properties. The morphology and microstructure investigation of soaked samples in SBF indicate that bone-like apatite formed on the surfaces. Also, ion release in SBF containing the scaffolds was measured by inductively coupled plasma (ICP) analysis. The nucleation positions of apatite crystals were areas with high silicon containing, Si+4 ion positions.

**Conclusion:** The study indicates that scaffold containing 30 wt. % baghdadite had proper bioactivity behavior due to its ability to form bone-like apatite on the surface of specimens.

**Keywords:** Coating, Nanocomposite, Polymer, Polyurethane polymeric sponge technique, Scaffolds

### How to cite this article

Karamian E, Nasehi A, Saber-Samandari S, Khandan AS. Fabrication of hydroxyapatite-baghdadite nanocomposite scaffolds coated by PCL/Bioglass with polyurethane polymeric sponge technique. *Nanomed J.* 2017; 4(3): 177-183. DOI: 10.22038/nmj.2017.8959

### INTRODUCTION

Developing the bone tissue engineering science and bone graftings methods like autografts, allografts, and xenografts to heal the tissue whereas it was possible to have a trauma or body immune responses in old methods is a great breakthrough in regenerative medicine [1-2]. Lately, scaffolds and biological portions have emerged a new challenging approach to enhance the regeneration of bone tissue engineering methods like using the cells within the biodegradable scaffold [1]. The porosity and cells of tissues play a significant role in bone

regeneration [2]. The repair of bone defects in the musculoskeletal system, such as bone, is a major clinical challenge all around the world [3-4]. Only in the United States, over three million orthopedic surgeries are performed yearly [5-6]. Over half of these procedures involve bone grafting using either an auto graft or an allograft [4-8]. Bone graft have been introduced as current treatment procedures for bone regeneration; However, they have some downsides such as immune rejection, the risk of infection, and tissue necrosis [9-13]. Properties of HA such as bioactivity, biocompatibility, and solubility properties can be tailored over a wide range by

\* Corresponding Author Email: [sas.khandan@iaukhsh.ac.ir](mailto:sas.khandan@iaukhsh.ac.ir)

Note. This manuscript was submitted on May 2, 2017; approved on June 25, 2017

modifying the composition via ionic substitutions [9-12]. Utilizing bioactive ceramic scaffolds to regenerate bone tissue can speed up the healing time, prevent the rejection of implant and improve cell migration, and proliferation [14-16]; however, the main drawback of these scaffolds is their poor brittleness. The best scaffold should have proper interconnected porous structure as well as pore size in the range of 100  $\mu\text{m}$  to allow the cells growth and delivery of nutrient [17-20]. It should be noted that the pore size and mechanical properties have an inverse relationship, as a general rule, increasing the pore size decreases the mechanical properties of the scaffolds [21-23]. Porous bioceramics, most of all calcium-phosphate (CaPs) groups such as HA, have received much interest in tissue engineering application due to the excellent biocompatibility and similar chemical component to the bone [24-26]. In recent decades development of calcium silicate-based ceramics becomes very important because of their superior biocompatibility, bioactivity, and mechanical properties in comparison to hydroxyapatite (HA) [27-31]. The incorporation of some elements such as zirconium (Zr), magnesium (Mg) and zinc (Zn) into the calcium silicate system has performed to control the biological performance and mechanical properties of the scaffolds [32]. The trace elements such as magnesium ( $\text{Mg}^{2+}$ ), zinc ( $\text{Zn}^{2+}$ ), titanium ( $\text{Ti}^{4+}$ ) and zirconium ( $\text{Zr}^{4+}$ ) have been incorporated in the Ca single bond Si microstructure [6]. Resulting minerals, namely  $\text{CaMgSi}_2\text{O}_6$  (diopside),  $\text{Ca}_2\text{MgSi}_2\text{O}_7$  (akermanite) and  $\text{Ca}_3\text{ZrSi}_2\text{O}_9$  (Bagh), have already been tested in vitro and in vivo, and indicate a significant improvement in comparison to simple calcium silicates [32-37]. Bagh is a member of calcium silicate-zirconium group with monoclinic structure. Several types of scaffold fabrication techniques have been introduced to build scaffolds containing gel-casting method, freeze-drying, electro-spinning, space holder methods and spongy method [33-39]. The aim of the current project was to prepare nanostructured HA/Bagh composite scaffolds by replica method using polyurethane foam with apply a PCL/BG coating on the scaffold. Elemental chemical analysis on the scaffolds fabricated was carried out for bioactivity evaluation and investigation of the mechanism of deposited bone-like apatite.

## MATERIALS AND METHODS

### Materials preparation

Baghdadite (Bagh) powder was synthesized by sol-gel technique according to the procedure described in previous work [3, 23, 36]. Briefly, to synthesize baghdadite powder, zirconium nitrate oxide ( $\text{ZrO}(\text{NO}_3)_2$ ), calcium nitrate tetra hydrate ( $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ ) and tetraethyl orthosilicate TEOS, ( $\text{C}_2\text{H}_5\text{O})_4\text{Si}$ ) were selected as starting materials, all materials were purchase from Merck company. TEOS, ethanol, and  $\text{HNO}_3$  (2 M) with the molar ratio of 1:8:0.16 were mixed and were stirred for 30 min. Then  $\text{ZrO}(\text{NO}_3)_2$  and  $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$  were added into the solution with the molar ratio of Zr/Ca/TEOS equals to 1:3:2. In the next step, the prepared solution was stirred for 5 h in the room temperature. Subsequently, the solution was kept at  $60^\circ\text{C}$  for 1 day and dried at  $100^\circ\text{C}$  for 2 days to obtain a dry gel. Finally, the dry gel was annealed at  $1150^\circ\text{C}$  for 3 h. Replica method using polyurethane foam was utilized to produce HA/Bagh nanocomposite scaffolds. The scaffolds were combined with different concentration of Bagh, 10, 20 and 30 wt. %. In addition, 1 wt. % sodium tripolyphosphate (STPP) and 1 wt. % Carboxymethyl cellulose were used as a dispersing and binder agent, respectively, during preparation of the ceramic slurry containing HA/Bagh. Finally, the scaffolds were sintered at  $1350^\circ\text{C}$  for 2 h with the heating and cooling rate of  $3^\circ\text{C}/\text{min}$ . Fig. 1 represent the schematic for the current study.

### Characterizations

#### XRD analysis

The phase characterization of the HA and HA/Bagh 20 wt.% nanocomposite was done X-ray diffractometer (X'pert Philips) with  $\text{CuK}\alpha$  radiation ( $\lambda = 0.154 \text{ nm}$ ) (IAUN, Isfahan, Iran). The XRD traces were recorded in the  $2\theta$  range of  $10-90^\circ$ . The result has shown in Fig. 2.

#### SEM analysis

To investigate the pore morphology and detecting the apatite on the surface of the scaffold, scanning electron microscopy (SEM, Philips XL30) at an acceleration voltage of 30 kV were used. Also, the Energy Dispersive X-Ray Analysis (EDX) analysis was utilized for more precise comparison of the amount of apatite formation in the sintered samples soaked in the SBF for certain period of time.

**Bioactivity test analysis**

In order to evaluate the apatite formation ability of the scaffolds, simulated body fluid (SBF) was used which is described other works [23-26]. Cubic scaffolds  $1 \times 1 \times 1 \text{ cm}^3$  (height  $\times$  width  $\times$  depth) were soaked in 12 cc SBF in the falcon tube for 28 days and kept in benmarray bath with circulatory water at  $37^\circ\text{C}$ .

**Chemical changes analysis**

The pH of the solution was measured every two days by a pH meter. In addition, changes in element concentration of SBF were also measured by inductively coupled plasma (ICP) method (Equipment Model, Zaies).

**RESULTS AND DISCUSSIONS**

**Phase characterization**

Fig. 2 shows the XRD patterns of nanocrystalline hydroxyapatite (NHA) powder Which was utilized in the scaffolds. In the current work, the sample just heated at  $850^\circ\text{C}$  to fabricate the scaffolds. As

depicted in Fig 2 all the peaks belong to HA phase and there is not any extra phase as impurities. Fig 2 represented the XRD pattern of nanostructure HA/Bagh nanocomposite powder containing 20 wt.% baghdadite, HB20 sample for preparation of scaffolds. As it is seen, there are both phases' peaks, HA phase and baghdadite, in the XRD pattern. Also, in the other word, Fig. 2 depicts the XRD patterns related to the complete synthesis of baghdadite at  $1150\text{-}1200^\circ\text{C}$ . As can be seen in this Fig, there is no impurity in Bagh product.

**Morphology characterization**

Fig. 3 illustrates the SEM micrographs of the scaffolds after soaking for 28 days in SBF solution. In higher magnification, plenty of bright precipitations (cauliflower) were observed on the surface of the scaffolds. As it is seen, by increasing baghdadite amount in the HA/Bagh nanocomposite samples, Fig. (3-d) is the comparison with the HA sample that shows increasing density of bright precipitations. It means that bioactivity has been increased by



Fig. 1. Schematic of HA-Bagh nanocomposite coated with BG/PCL reinforcement preparation

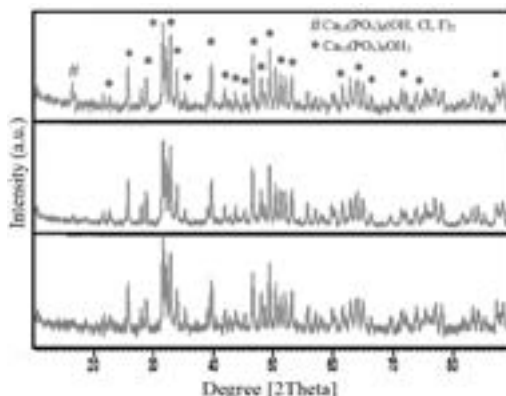


Fig. 2. XRD patterns of natural HA powder using in the scaffolds

increasing of concentration of baghdadite and the ability to form and precipitate bone-like apatite as the bright precipitations increased (Fig 3 (a-c)). EDX point analysis has been done on the point A which shows in the SEM micrographs. It indicates that the bright precipitations are composition of Ca and P, Ca-P compounds. In fact, it illustrates that these are probably bone-like apatite. In addition, the presence of Si and Zr elements along with Ca and P in the EDX results indicates that these sediments formed and deposited on the baghdadite particles (See Fig. 4b). In fact, nucleation of the bright precipitations carries out on the baghdadite particles. Also, the result proved that  $Si^{+4}$  ions have very important role

at the bright precipitations formation. Therefore,  $Si^{+4}$  ions leads to the formation of silanol (...Si-OH) group in the surface layer, a pH increase, and finally the production of a negatively charged surface with the functional group (Si-O). Karamian et al. [25] show that natural fluor-hydroxyapatite (FNHA)-TiO<sub>2</sub> nanocomposite ceramic with unique biocompatibility and sufficient chemical stability can be synthesized completely after 8 h of high energy ball milling. They showed that TiO<sub>2</sub> can significantly influence on the chemical stability of FNHA. They indicated that with increasing the incorporation of F into the HA structure thermal stability is considerably increased. By changing the

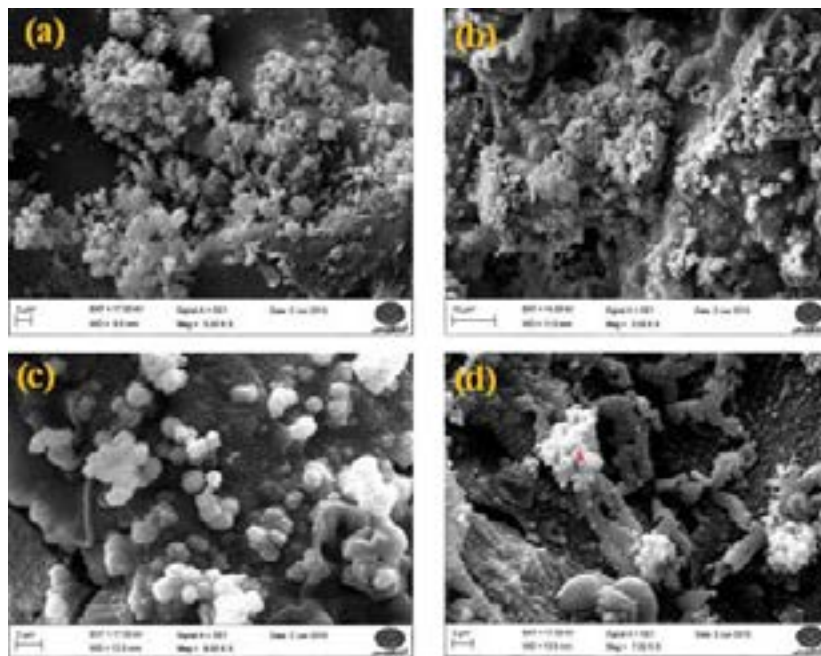


Fig. 3. SEM micrographs of HA/Bagh scaffolds after soaking in SBF with different concentration of (a) 0 wt. % (b) 10 wt. % (c) 20 wt. % and (d) 30 wt.% Bagh

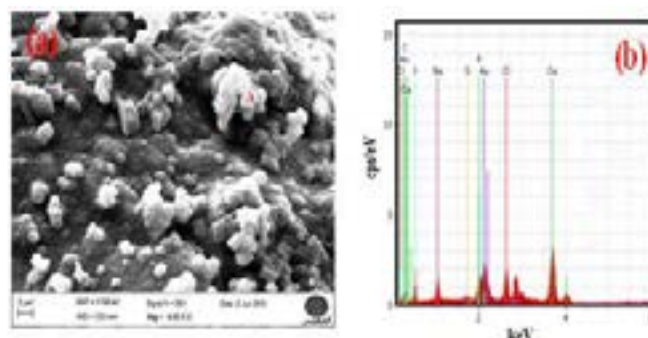


Fig. 4. (a) SEM micrographs and (b) EDS analysis of HA/20 wt. % Bagh scaffolds, after soaking in SBF for 28 days

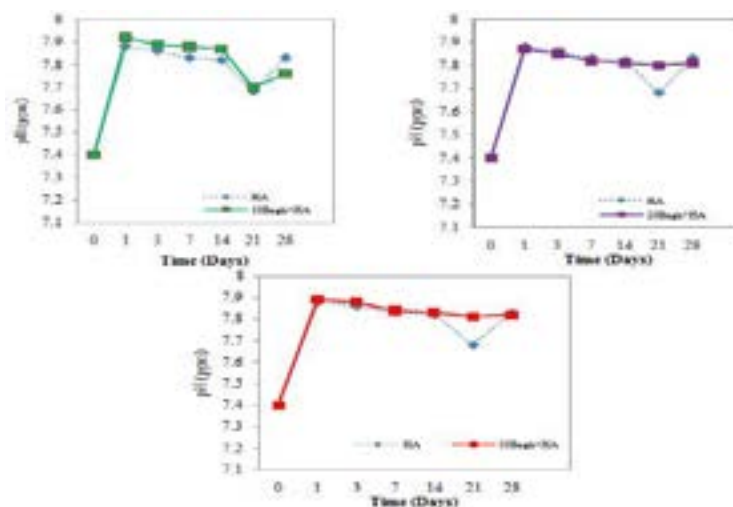


Fig. 5. Change of pH value in SBF solutions of the samples in the during 28 days

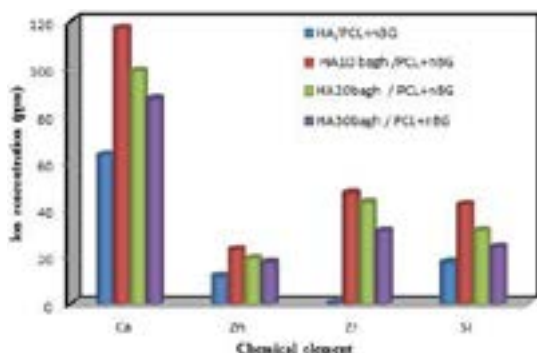


Fig. 6. Elemental chemical (ICP results) evolution of SBF solution after 28 days

amount of Fluoride substitutions the solubility, as well as a biological lifetime, can be fine-tuned [25]. Notwithstanding the weak mechanical properties of HA and its composites, their unique biological properties guide us to consider about working on developing their properties alternately of simply replacing them by other materials [25].

In the current work, baghdadite also improve the mechanical strength and bioactivity of HA as one of the common calcium phosphates. In another study Khandan et al. [22] indicate that spark plasma sintered (SPSed) NHA and baghdadite composite have enhanced mechanical properties compare to pure NHA and without sintering process.

#### Bioactivity evaluation

In order to evaluate the bioactivity of produced nanostructure baghdadite scaffolds, the samples were immersed in SBF solution for 28 days. Differences in pH of SBF as a function

of 7.92) which can be ascribed to the release of  $\text{Ca}^{2+}$  ions into the SBF (see Table 1).

On the other hand, Si ions in the bagh can form Si-OH groups on the surface of the scaffold (see Fig. 5). The formation of silanol groups induces a negative charge on the surface. Subsequently, the existing  $\text{Ca}^{2+}$  ions in the SBF can be adsorbed on the surface and consequently result in the deposition of calcium ions and the formation of an appetite layer on the surface of the scaffolds. These results are in a good agreement with the results obtained from the SEM micrographs of bagh scaffolds after soaking in SBF solution (Fig 4a-b). In a recent study, the bending strength, hardness, and fracture toughness of dense Bagh with 0.5% porosity were reported to be  $98 \pm 16$  MPa,  $7.9 \pm 0.2$  GPa and  $1.3 \pm 0.1$  MPa.m<sup>1/2</sup>, respectively [7], which is close to the mechanical properties of cortical bone. Furthermore, baghdadite is recognized as a bioceramic with excellent biocompatibility as well as potential to enhance osteoblast and osteoclast proliferation [33].

#### Chemical changes evaluation

Fig. 6 shows the results of the ICP, elemental chemical results, of SBF containing the scaffolds. Decreasing of the percentage of Si ion in solutions containing more baghdadite powder samples indicates the presence of higher amount of Si element and it works as actively for formation Ca/P compounds precipitations.

In fact, this element, Si ions, role very important at formation Ca/P compounds precipitations on the surface scaffolds. CaPs have an interesting

property with potential orthopedic and dental applications like good mechanical properties, osteoconductivity, bioactivity, and bone bonding ability. HA is not biodegradable and can be removed and remodeled in the host. If implanted directly, it may dislocate within the tissue [39-41]. In fact, the nucleation was grown on baghdadite particles. As,  $\text{Si}^{+4}$  ions in the baghdadite, can form silanol groups (...Si-OH) on the surface of the scaffolds and it induces a negative charge on the surface with the functional group (Si-O). It is the reason to absorb positive ions,  $\text{Ca}^{+2}$ , and finally the formation Ca/P compounds precipitations.

## CONCLUSION

Baghdadite scaffolds with a highly porous structure and interconnected porosity were successfully fabricated by the replica method. The results of in vitro study demonstrated the apatite formation ability of baghdadite scaffold. In fact, formation ability of bone-like apatite increase by increasing of Baghdadite concentration on HA/bagh composite scaffolds surface.  $\text{Si}^{+4}$  ions in the Bagh, is a member of calcium silicate – zirconium group, can form silanol groups (...Si-OH) on the surface of the scaffolds. The formation of silanol groups induces a negative charge on the surface with the functional group (Si-O). It leading to absorb positive ions,  $\text{Ca}^{+2}$ , and finally the formation Ca/P compounds precipitations on the surface 3D scaffolds. The maximum change in pH was observed at the first day of soaking of the scaffold (7.4 up to 7.92) which can be ascribed to the release of  $\text{Ca}^{2+}$  and  $\text{Zr}^{4+}$  ions into the SBF solution.

## ACKNOWLEDGEMENTS

The authors would like to extend their gratitude for the support provided by the Najafabad branches of Islamic Azad University, Isfahan, and Iran.

## CONFLICT OF INTEREST

The authors confirm that this article content has not any conflicts of interest.

## REFERENCES

- Karamian E, Khandan A, Eslami M, Gheisari H, Rafiaei N. Investigation of HA nanocrystallite size crystallographic characterizations in NHA, BHA and HA pure powders and their influence on biodegradation of HA. *Adv Mat Res*. 2014; 829: 314-318.
- Boudriot U, Goetz B, Dersch R, Greiner A, Wendorff JH. Role of electrospun nanofibers in stem cell technologies and tissue engineering. *Macromol Chem Phys*. 2005; 225 (1): 9-16.
- Kariem H, Pastrama MI, Roohani-Esfahani SI, Pivonka P, Zreiqat H, Hellmich C. Micro-poro-elasticity of baghdadite-based bone tissue engineering scaffolds: a unifying approach based on ultrasonics, nanoindentation, and homogenization theory. *Mat Sci Eng C-Mater*. 2015; 46: 553-564.
- Sheridan MH, Shea LD, Peters MC, Mooney DJ. Bioabsorbable polymer scaffolds for tissue engineering capable of sustained growth factor delivery. *J Control Release*. 2000; 64(1): 91-102.
- Laurencin CT, Nair LS, editors. *Nanotechnology and regenerative engineering: the scaffold*. CRC Press; 2014.
- Pangon A, Saesoo S, Saengkrit N, Ruktanonchai U, Intasanta V. Hydroxyapatite-hybridized chitosan/chitin whisker bionanocomposite fibers for bone tissue engineering applications. *Carbohydr Polym*. 2016; 144: 419-427.
- Ramaswamy Y, Wu C, Zhou H, Zreiqat H. Biological response of human bone cells to zinc-modified Ca-Si-based ceramics. *Acta Biomater*. 2008; 4(5): 1487-1497.
- Kwong FN, Harris MB. Recent developments in the biology of fracture repair. *J Am Acad Orthop Sur*. 2008; 16(11): 619-625.
- Tabata Y. Recent progress in tissue engineering. *Drug discov today*. 2001; 6(9):483-487.
- Chu SJ, Salama MA, Garber DA, Salama H, Sarnachiaro GO, Sarnachiaro E, Gotta SL, Reynolds MA, Saito H, Tarnow DP. Flapless postextraction socket implant placement, part 2: the effects of bone grafting and provisional restoration on peri-implant soft tissue height and thickness-a retrospective study. *Int J Periodontics Restorative Dent*. 2015; 35(6): 803-809.
- Giannoudis PV, Dinopoulos H, Tsiridis E. Bone substitutes: an update. *Injury*. 2005; 36(3): S20-7.
- Hutmacher DW, Schantz JT, Lam CX, Tan KC, Lim TC. State of the art and future directions of scaffold-based bone engineering from a biomaterials perspective. *J tissue eng regen m*. 2007; 1(4): 245-260.
- Kumar PR, Sreenivasan K, Kumary TV. Alternate method for grafting thermoresponsive polymer for transferring in vitro cell sheet structures. *J Appl Polym Sci*. 2007; 105(4): 2245-2251.
- Karamian E, Motamedi MR, Khandan A, Soltani P, Maghsoudi S. An in vitro evaluation of novel NHA/zircon plasma coating on 316L stainless steel dental implant. *Prog Nat Sci-Mater*. 2014; 24(2): 150-156.
- Karamian E, Khandan A, Kalantar Motamedi MR, Mirmohammadi H. Surface characteristics and bioactivity of a novel natural HA/zircon nanocomposite coated on dental implants. *Biomed Res Int*. 2014.
- Khandan A, Abdellahi M, Ozada N, Ghayour H. Study of the bioactivity, wettability and hardness behaviour of the bovine hydroxyapatite-diopside bio-nanocomposite coating. *J Taiwan Inst Chem E*. 2016; 60: 538-546.
- Park HJ, Yu SJ, Yang K, Jin Y, Cho AN, Kim J, Lee B, Yang HS, Im SG, Cho SW. Paper-based bioactive scaffolds for stem cell-mediated bone tissue engineering. *Biomaterials*. 2014; 35(37): 9811-9823.
- Komlev VS, Barinov SM. Porous hydroxyapatite ceramics of bi-modal pore size distribution. *J Mater Sci-Mater M*. 2002; 13(3): 295-299.
- Baino F, Caddeo S, Novajra G, Vitale-Brovarone C. Using porous bioceramic scaffolds to model healthy and osteoporotic bone. *J Eur Ceram Soc*. 2016; 36(9): 2175-

- 2182.
20. Esfahani SR, Tavangarian F, Emadi R. Nanostructured bioactive glass coating on porous hydroxyapatite scaffold for strength enhancement. *Mater Lett.* 2008; 62(19): 3428-3430.
  21. Karageorgiou V, Kaplan D. Porosity of 3D biomaterial scaffolds and osteogenesis. *Biomaterials.* 2005; 26(27): 5474-5491.
  22. Khandan A, Karamian E, Mehdikhani-Nahrkhalaji M, Mirmohammadi H, Farzadi A, Ozada N, Heidarshenas B, Zamani K. Influence of spark plasma sintering and baghdadite powder on mechanical properties of hydroxyapatite. *Proc Mat Sci.* 2015; 11: 183-189.
  23. Khandan A, Abdellahi M, Barenji RV, Ozada N, Karamian E. Introducing natural hydroxyapatite-diopside (NHA-Di) nano-bioceramic coating. *Ceram Int.* 2015; 41(9): 12355-12363.
  24. Khandan A, Karamian E, Bonakdarchian M. Mechanochemical synthesis evaluation of nanocrystalline bone-derived bioceramic powder using for bone tissue engineering. *Dent Hypo.* 2014; 5(4): 155.
  25. Karamian E, Abdellahi M, Khandan A, Abdellah S. Introducing the fluorine doped natural hydroxyapatite-titania nanobiocomposite ceramic. *J Alloy Compd.* 2016; 679: 375-83.
  26. Sadeghpour S, Amirjani A, Hafezi M, Zamanian A. Fabrication of a novel nanostructured calcium zirconium silicate scaffolds prepared by a freeze-casting method for bone tissue engineering. *Ceram Int.* 2014; 40(10): 16107-16114.
  27. Staiger MP, Pietak AM, Huadmai J, Dias G. Magnesium and its alloys as orthopedic biomaterials: a review. *Biomaterials.* 2006; 27(9): 1728-1734.
  28. Sopyan I, Mel M, Ramesh S, Khalid KA. Porous hydroxyapatite for artificial bone applications. *Sci Technol Adv Mat.* 2007; 8(1): 116-123.
  29. Heydary HA, Karamian E, Poorazizi E, Heydaripour J, Khandan A. Electrospun of polymer/bioceramic nanocomposite as a new soft tissue for biomedical applications. *J As Cer S.* 2015; 3(4): 417-425.
  30. An SH, Matsumoto T, Miyajima H, Nakahira A, Kim KH, Imazato S. Porous zirconia/hydroxyapatite scaffolds for bone reconstruction. *Dent Mater.* 2012; 28(12): 1221-1231.
  31. De Aza PN, Luklinska ZB, Martinez A, Anseau MR, Guitian F, De Aza S. Morphological and structural study of pseudowollastonite implants in bone. *J Microsc-Oxford.* 2000; 197(1): 60-67.
  32. Sadeghzade S, Emadi R, Tavangarian F. Combustion assisted synthesis of hardystonite nanopowder. *Ceram Int.* 2016; 42(13): 14656-14660.
  33. Wang G, Lu Z, Dwarte D, Zreiqat H. Porous scaffolds with tailored reactivity modulate in-vitro osteoblast responses. *Mat Sci Eng C-Mater.* 2012; 32(7): 1818-1826.
  34. Sadeghzade S, Emadi R, Labbaf S. Formation mechanism of nano-hardystonite powder prepared by mechanochemical synthesis. *Adv Powder Technol.* 2016; 27(5): 2238-2244.
  35. Abdellahi M, Najafinejad AA, Ghayour H, Saber-Samandari S, Khandan A. Preparing diopside scaffolds via space holder method: Simulation of the compressive strength and porosity. *J MECH BEHAV BIOMED.* 2017; 72: 171-181.
  36. Najafinezhad A, Abdellahi M, Ghayour H, Soheily A, Chami A, Khandan A. A comparative study on the synthesis mechanism, bioactivity and mechanical properties of three silicate bioceramics. *Mat Sci Eng C-Mater.* 2017; 72: 259-267.
  37. Khandan A, Ozada N, Karamian E. Novel Microstructure Mechanical Activated Nano Composites for Tissue Engineering Applications. *J of Bioeng & Biomed Sci.* 2015; 5(1):1.
  38. Doustgani A. The effect of electrospun poly (lactic acid) and nanohydroxyapatite nanofibers' diameter on proliferation and differentiation of mesenchymal stem cells. *Nanomed J.* 2016; 3(4): 217-222.
  39. Saber-Samandari S, Gross KA. Micromechanical properties of single crystal hydroxyapatite by nanoindentation. *Acta Biomater.* 2009; 5(6): 2206-2212.
  40. Saber-Samandari S, Gross KA. Nanoindentation reveals mechanical properties within thermally sprayed hydroxyapatite coatings. *Surf Coat Tech.* 2009; 203(12): 1660-1664.
  41. Saber-Samandari S, Alamara K, Saber-Samandari S. Calcium phosphate coatings: Morphology, micro-structure and mechanical properties. *Ceram Int.* 2014; 40(1): 563-572.