Nanomed Res J 7(2):205-213, Spring 2022

RESEARCH ARTICLE

Fabrication and characterization of chitosan/gelatin scaffold with bioactive glass reinforcement using PRP to regenerate bone tissue

Farhad Ghomi[1], Azadeh Asefnejad[1*], Morteza Daliri[2], Vahabeddin Godarzi[3], Mehran Hemati[4]

[1] Department of Biomedical Engineering, Science and Research Branch, Islamic Azad University, Tehran, Iran

[2] Department of Animal and Marine Biotechnology, National Institute of Genetic Engineering and Biotechnology, Tehran, Iran

[3] Applied Biotechnology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran

[4] Dental School, Shiraz University of Medical Sciences, Shiraz, Iran

ARTICLE INFO

Article History:
Received 13 February 2022
Accepted 26 April 2022
Published 01 May 2022

Keywords:
Bioglass
Chitosan-Gelatin membrane
Bone tissue
Freeze drying
Platelet-rich plasma

ABSTRACT

In this study, the purpose of this study was to fabricate a porous nanocomposite chitosan-gelatin along with different weight percentages of bioglass using the freeze-drying method. In this study, the Platelet-rich plasma (PRP) technique was used for the first time to strengthen bone grafting. Tensile strength, elastic modulus, and percentage of porosity were evaluated. Also, the samples were examined in simulated body fluid (SBF) and phosphate buffer saline (PBS) to study bone growth and bone dissolution rate. Also, the porosity percentage was about 54% with 39% bone-like apatite formation for the third sample. The early absorption and late absorption rate of bone substitutes and the main volume of bone substitutes can be used for orthopedic approaches. The specimen were abalysized using X-ray diffraction (XRD) and scanning electron microscope (SEM) technique. Porous membranes were examined under SEM tools to estimate pore size and morphological behavior. The pore diameter can be controlled by adjusting the ethanol percentage in the range of 10-30 micrometers. The formation of needle bioglass crystals on the membrane surface after 7 days of immersion in biological solution was also evaluated using SEM images. In general, the third sample can be used to repair the bone tissue of the knee with the PRP technique.

How to cite this article
Ghomi F., Asefnejad A., Daliri M., Godarzi V. Fabrication and characterization of chitosan/gelatin scaffold with bioactive glass reinforcement using PRP to regenerate bone tissue. Nanomed Res J, 2022; 7(2): 205-213. DOI: 10.22034/nmrj.2022.02.010

INTRODUCTION

Tissue engineering is an emerging technology that has been developed to regenerate tissues and organs with increasing interest in the field of biomaterials [1-2]. Bioactive glass (BG) ceramics were made by Hanch et al in the 1960s, as a biomaterial for repairing bone defects. BG is widely used in dentistry and orthopedic applications. Different types of synthetic polymers have been used to make absorbable membranes for bone tissue repairment. The most important of these polymers are known as Polylactice Acid (PLA), poly(glycolic acid) (PGA) and copolymers of these two compounds. PGA has weaker biomechanical properties than PLA, while PGA is more hydrophilic and PLA has hydrophobic properties [3-8]. The elasticity of PGA is much higher than PLA and the adsorption and adsorption rate is slower. Therefore, these two combinations are usually used as a network-like and interwoven microstructure. To improve the mechanical and chemical properties of the copolymer, the two compounds sometimes use a third polymer, such as trimethylene carbonate [6-8]. The main part of the process of decomposition and adsorption of these membranes is a non-enzymatic process that causes the production of PLA and PGA.

Although, researchers believe that the degradation and adsorption stages of these

* Corresponding Author Email: *asefnejad@srbiau.ac.ir*

membranes are associated with the production of acid in the newly formed tissue. There are several studies that have shown the positive performance of these membranes in orthopedic surgeries [9-12]. The adsorption of these membranes can also be adjusted by changing the amount of cross-linking polymer. The complete absorption time of some types of these membranes can last up to about more than a year. It should be noted that collagen membranes are contraindicated in people who are allergic to collagen or animal products such as cattle or pigs. In fact, the importance and value of allografts or xenografts or alloplasts is due to their ability to attach these molecules to their surface [13-15]. In implant osteointegration, the bone is not formed directly on the surface of the titanium alloy, but these cell adhesion molecules are first placed on the surface of the titanium alloy and form the appropriate biological matrix [16-19]. In fact, bone binds through collagen fibrils to materials resembling a natural cement composed of protein and osteopontin located on the surface of the implant fixture. In the field of implant therapies and bone regenerations, bone marrow is considered the gold standard because it has an osteoconductive matrix, together with growth signaling cells and molecules [20-24].

In many bone diseases such as Multiple Myeloma, Fibrous Dysplasin, Paget's disease, or diseases such as uncontrolled diabetes that affects bone repair, bone grafts or osteointegration of dental and orthopedic implants. Natural polymers including collagen, alginate, agarose, fibrin, and chitosan is widely used in the field of bone and neuro tissue science [25-34]. These materials are known as biocompatible and show similar environment to natural tissues [26-38], blood vessels [23] and nerve. However, the main problems of natural polymers are poor mechanical properties, lack of cellular interaction and uncontrolled degradation [39-45]. To enhance the mechanical properties, some polymer composites have been developed for bone tissue engineering. Gelatin is added to the membrane system to increase its physical and mechanical properties [45-52]. Chitosan-gelatin membrane bioactivity must be improved for specific tissues like many polymers. To improve membrane bioactivity, it is often combined with other bioactive substances [53-57]. Chitosan-gelatin membranes can be molded into various shapes and by freeze drying technique, it can create a porous microstructure [58-59]. In this paper, we describe the preparation of bioactive glass cement and chitosan-gelatin membranes and their properties related to the engineering applications of bone tissue.

MATERIALS AND METHODS

The materials used in this article is bioactive glass (BG) cement derived from sol-gel and chitosan-gelatin membrane. BG cement was synthesized by combining SiO_2 (64%), CaO (26%), P_2O_5 (8%) and $AgNO_3$ (2%) by sol-gel method in which that the solution was prepared using specific amount of Tetraethyl orthosilicate to 0.1 mol nitric and then mixed to continue for 4 hours at room temperature for hydrolysis. Triethyl phosphate and calcium nitrate tetrahydrate were combined to the solution and stirred for 1 hour. The solution was then kept at 37°C for 10 days to gelatinize and for removing the water, the gel was heated at 70°C for 3 days. The dried gel was then heated to 700°C at 3°C/min and reminded for 1 day at 700°C for stabilization. Finally, BG powder was prepared in a high energy ball mill for 30 minutes. To prepare the chitosan-gelatin membrane, 2 wt% of chitosan-gelatin (mass ratio 70 to 30) was added to 20 ml of distilled water and stirred on magnetic stirrer. Two samples were obtained by adding ethanol 1% and 2% to the solution. The samples were then frozen and the samples were insert into the freeze-drying machine at 0.01 mbar and -45°C. The samples were tested using tensile strength by Hounsfield machine with 0.2 mm/min speed rate. The stress-strain diagram presents the tensile strength and elastic modulus of the four samples. The samples then soaked in the simulated body fluid and phosphate buffer saline for bioactivity analysis and biodegrdatuin rate after 21 days in water bath.

X-ray diffraction analysis

The samples microstructure containing various amount of BG was analyzed using X-ray diffraction (XRD) method by Philips PW 3710. The XRD voltage of 30 kV and Cu-Kα radiation was applied.

Scanning electron microscopy analysis

The samples containimg various amount of BG in CHI-GN membrane were coated with a thin layer of gold (EMITECH K450X, England) and after that the morphological investigation of the prepared specimen membranes were observed using a scanning electron microscope (SEM; STEREOSCAN S 360).

RESULT AND DISCUSSION

The characterization of nanocomposite sample was performed with XRD tools. The XRD pattern shows in Fig. 1. It shows the XRD pattern of bioactive glass which present the straight base line and sharp diffraction peaks of the sample as a well crystallized nanoparticle. The result of XRD for bioglass nanoparticles is shown in Fig. 1, which are shown at angles of 20, 25 and 32°.

The use of BG nanoparticles to enhance the mechanical properties of bone scaffolds is proposed for knee joint repair with PRP technique which can improve cell growth. The XRD pattern shows the crystal structure is similar to that of human bone. Fig. 2 shows the first and second samples with a lower bioglass weight percentage to evaluate the lower tensile strength compared to the third and fourth samples. In general, it can be said that the elastic modulus for the fourth sample is about 1.1 MPa which increased 0.3 MPa compared to the pure sample. Fig. 3 indicates the porosity of the samples and the bone growth rate using the results of SEM images for four samples.

Fig. 4 shows that with the increase of bioglass

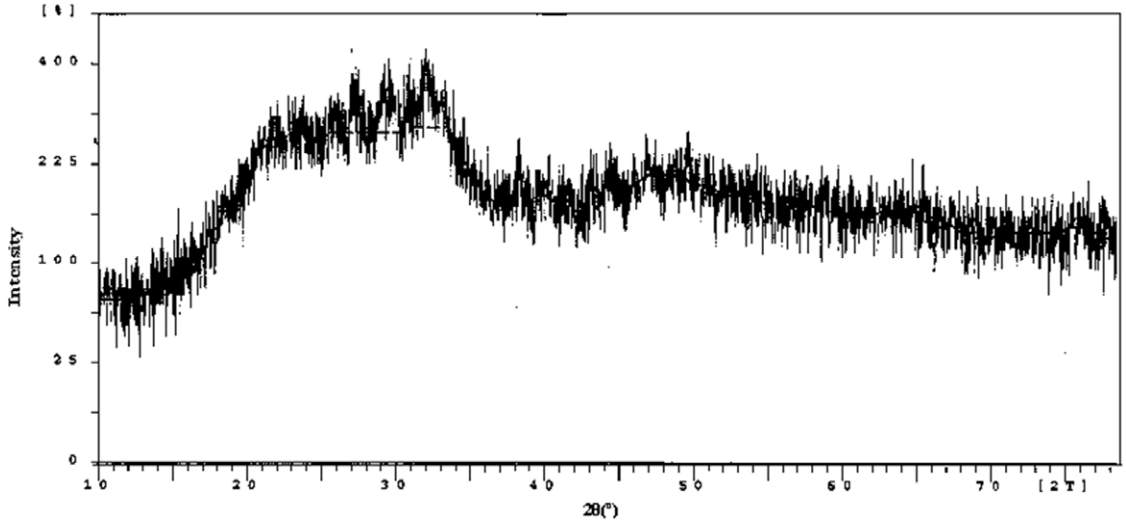

Fig. 1. X-ray diffraction image of bioglass nanoparticles added to chitosan-gelatin structure for PRP treatment

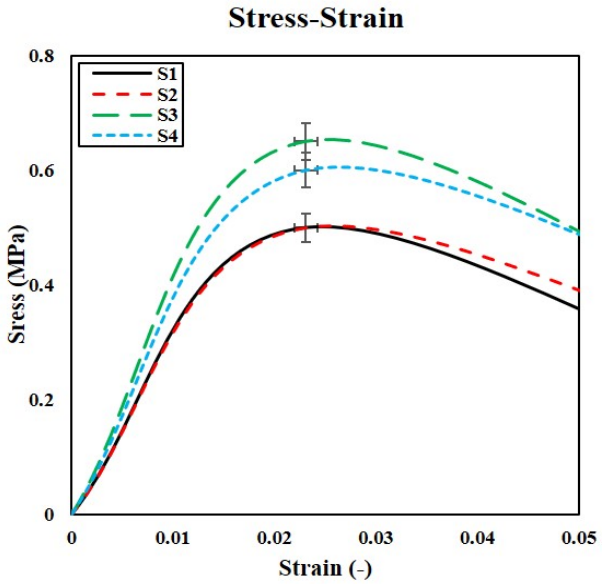

Fig. 2. Stress-strain diagram of samples containing different bioglass nanoparticles added to chitosan-gelatin structure for PRP treatment

Table 1. The materials and methods used for preparation of porous scaffold containing chitosan with various applications

Materials	Method	Application	References
chitosan/nano-hydroxyapatite/nano-copper–zinc	freeze drying	bone tissue engineering	(Anjali Tripathi 2012)
Chitosan/PMMA- co -PHEMA/Nano- Hydroxyapatite	blend	Bone	(Arundhati Bhowmick 2013)
chitosan/hydroxyapatite	wet spinning	Bone	(Beom-Su Kim 2012)
Nano-hydroxyapatite /chitosan	solvent casting and evaporation methods	bone guided regeneration	(Cheng Xianmiao 2009)
alginate, chitosan, collagen and hydroxyapatite	Electrospinning techniques	bone tissue engineering	(Chia-Cherng Yu 2013)
hydroxyapatite/tussah silk fibroin/chitosan	--	Bone	(Cui 2012)
Chitosan Glutamate and Hydroxyapatite	--	--	(D. P. Mukherjee 2003)
carboxymethyl–chitosan/gelatin/nano-hydroxyapatite	--	bone tissue engineering	(Debasish Mishra 2011)
chitosan/hydroxyapatite	electrospinning technique	bone tissue engineering	(Doan Van Hong Thien 2013)
collagen–chitosan–hydroxyapatite		bone grafting	(Dongmei Luo 2011)
chitosan/nano-hydroxyapatite nano-hydroxyapatite/chitosan	Freeze drying co-precipitation method	bone tissue engineering bone cement	(Lijun Kong, 2006) (Qin Zou, 2008)
chitosan/hydroxyapatite and sustained releasing icariin	freeze-drying	bone repair	(WU Tao, 2009)
nano-hydroxyapatite /chitosan/ carboxymethyl cellulose	freeze-drying	bone tissue engineering	(Jiang Liuyun, 2009)
chitosan/hydroxyapatite	in situ precipitation method	bone tissue engineering	(Jiazhen Zhang, 2014)

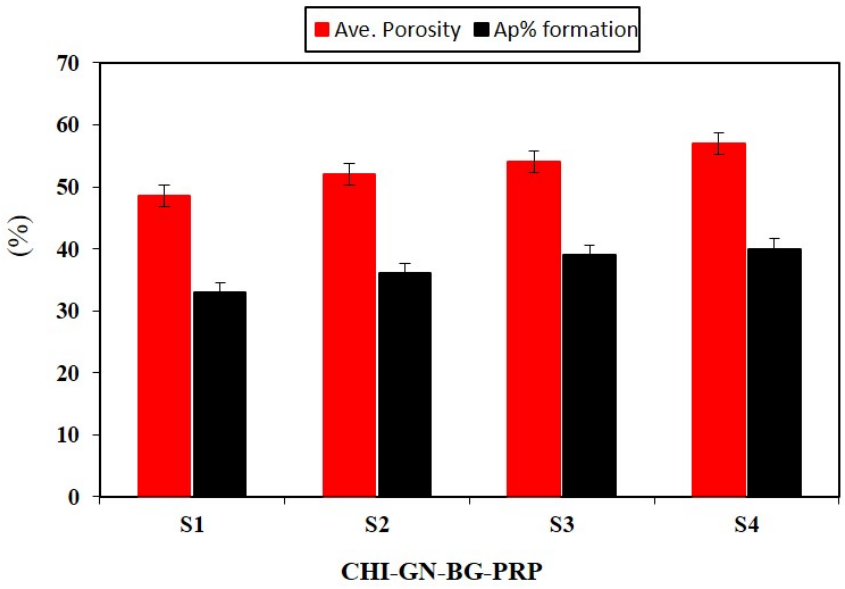

Fig. 3. Comparison of porosity percentage results with apatite growth rate of samples of chitosan-gelatin structure containing different weight percentages of bioglass for PRP treatment

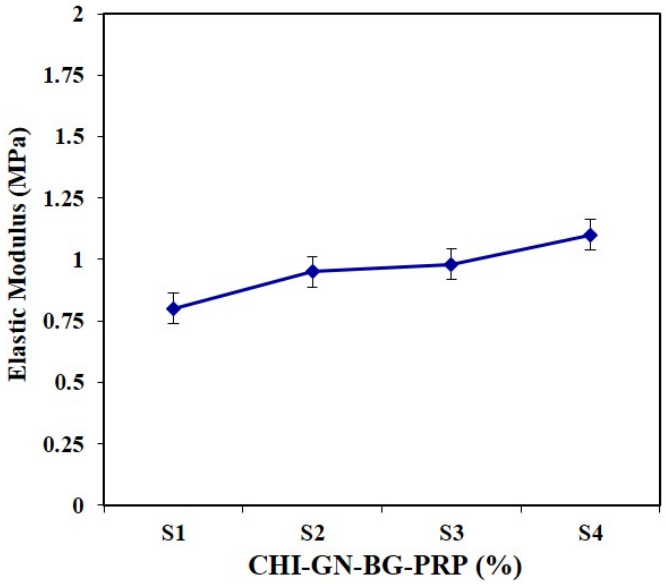

Fig. 4. Hardness modulus result of chitosan-gelatin structure samples containing different weight percent of bioglass for PRP treatment

from 0 wt% increase the porosity percentage from 48.5% to 57% which has a significant effect on the apatite formation of the porous scaffold. Therefore, the apatite formation increases from 33% to 40% by addition of PRP. The obtained results show that the increase in porosity has the same effect on an increase in apatite formation. In general, it can be said that with increasing porosity, bone growth, calcium and phosphorus deposition increase which are the most important apatite formation and created inside porous cavities. Fig. 4 demonstrate the elastic modulus of the porous samples so that the elastic modulus has increased from 0.8 MPa to 1.3 MPa due to the addition of BG nanoparticles which have a spherical microstructure with high mechanical strength.

The SEM images was used to examine chitosan-gelatin membranes containing 0, 1 and 2% ethanol and to observe their morphology, especially needle HA nanocrystals in samples taken in ringer solution and to estimate the shape and diameter of porosity. The bone's response to a simple fracture differs from that in which a gap is created at a distance between two pieces of bone. Therefore, the bone response is different according to the type of injury [55-58]. A significant proportion of advanced implant therapies are clearly associated with maxillofacial bone surgery. Therefore, correct understanding and sufficient knowledge of bone and effective factors in bone response to treatment are the background of advanced implant therapies. Bone is made by osteoblasts or osteoplasts and bone-forming cells are derived from osteogenic progenitor cells during a complex evolutionary pathway, and osteogenic progenitor cells either directly by cell differentiation in mesenchymal stem cells (MSCs) [57-64].

With increasing ethanol percentage, SEM results showed an increase in porous size. Meanwhile, as can be seen, the porosity forms become spherical with increasing percentage of ethanol. The SEM images showed the formation of nano-sized needle-like hydroxyapatite crystals on the membrane surface after 7 days of immersion.

Fig. 5 demonstarte a SEM image for 4 samples immersed in a SBF after 21 days which shows by decreasing the weight percentage of BG, the microstructure and chemical stability decrease and the dissolution rate increases, which causes a corrolation between bone formation and bone dissolution. Since it is possible to use collagen membranes in two or three layers during the surgical phase, the surgeon can extend the presence and function of the membrane by increasing the number of layers of collagen membrane, depending on the required treatment. At present, the maximum time for complete absorption of collagen membranes is about 6 to 8 months (26 to 28 weeks). It should be noted that these images do not indicate how long the gelatin membrane has

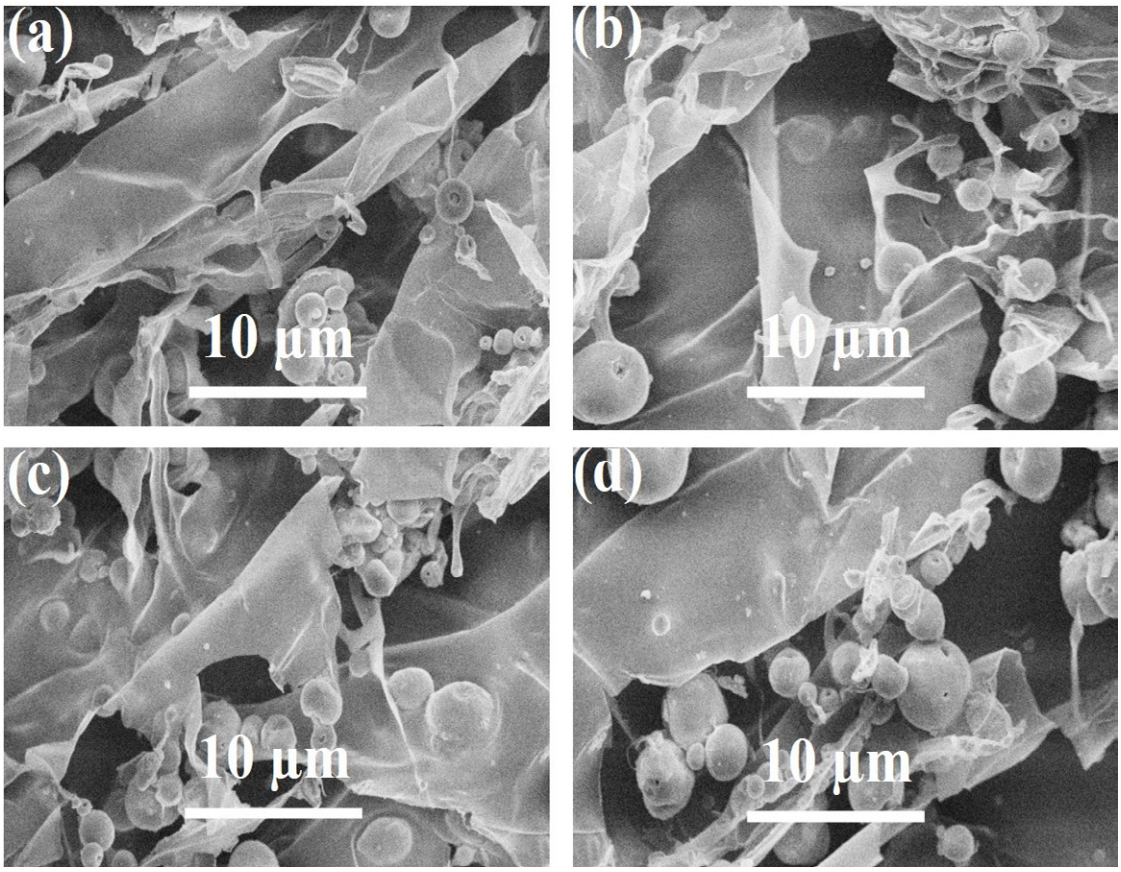

Fig. 5. SEM images of chitosan-gelatin nanocomposite containing different amount of BG nanoparticlrs and PRP treatment

membrane function. It seems that as the gelatin membrane maintains its membrane integrity for one to two months.

CONCLUSION

The use of platelet-rich plasma due to growth factor in the lesion with bioactive glass causes faster healing, repair, very high biocompatibility as anti-inflammatory. The bioactive glass and chitosan/gelatin membrane can be suitable choice for *in vivo* tests for bone lesions and regenerates the jaw. The mean pore size of chitosan-gelatin membrane with 2% ethanol was 30 ± 6.3 µm. The membrane with the optimal percentage of ethanol is very suitable for tissue engineering with sufficient pore size for cell penetration. One of the disadvantages of collagen as a membrane is that the materials completely degraded before the process of bone formation and the process of its decomposition and absorption is formed along with partial inflammation in the tissue.

ACKLOWDGEMENT

The authors of the article are grateful for the valuable guidance of the professors at the Islamic Azad University of Research Sciences who supported the preparation of this research.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCE

1) Khandan, A., Abdellahi, M., Ozada, N., & Ghayour, H. (2016). Study of the bioactivity, wettability and hardness behaviour of the bovine hydroxyapatite-diopside bio-nanocomposite coating. Journal of the Taiwan Institute of Chemical Engineers, 60, 538-546.
2) Karamian, E., Motamedi, M. R. K., Khandan, A., Soltani, P., & Maghsoudi, S. (2014). An in vitro evaluation of novel NHA/zircon plasma coating on 316L stainless steel dental implant. Progress in Natural Science: Materials International, 24(2), 150-156.
3) Karamian, E., Abdellahi, M., Khandan, A., & Abdellah, S. (2016). Introducing the fluorine doped natural

hydroxyapatite-titania nanobiocomposite ceramic. Journal of Alloys and Compounds, 679, 375-383.
4) Najafinezhad, A., Abdellahi, M., Ghayour, H., Soheily, A., Chami, A., & Khandan, A. (2017). A comparative study on the synthesis mechanism, bioactivity and mechanical properties of three silicate bioceramics. Materials Science and Engineering: C, 72, 259-267.
5) Ghayour, H., Abdellahi, M., Ozada, N., Jabbrzare, S., & Khandan, A. (2017). Hyperthermia application of zinc doped nickel ferrite nanoparticles. Journal of Physics and Chemistry of Solids, 111, 464-472.
6) Kazemi, A., Abdellahi, M., Khajeh-Sharafabadi, A., Khandan, A., & Ozada, N. (2017). Study of in vitro bioactivity and mechanical properties of diopside nano-bioceramic synthesized by a facile method using eggshell as raw material. Materials Science and Engineering: C, 71, 604-610.
7) Khandan, A., & Ozada, N. (2017). Bredigite-Magnetite (Ca7MgSi4O16-Fe3O4) nanoparticles: A study on their magnetic properties. Journal of Alloys and Compounds, 726, 729-736.
8) Khandan, A., Jazayeri, H., Fahmy, M. D., & Razavi, M. (2017). Hydrogels: Types, structure, properties, and applications. Biomat Tiss Eng, 4(27), 143-69.
9) Sharafabadi, A. K., Abdellahi, M., Kazemi, A., Khandan, A., & Ozada, N. (2017). A novel and economical route for synthesizing akermanite (Ca2MgSi2O7) nano-bioceramic. Materials Science and Engineering: C, 71, 1072-1078.
10) Khandan, A., Abdellahi, M., Ozada, N., & Ghayour, H. (2016). Study of the bioactivity, wettability and hardness behaviour of the bovine hydroxyapatite-diopside bio-nanocomposite coating. Journal of the Taiwan Institute of Chemical Engineers, 60, 538-546.
11) Shayan, A., Abdellahi, M., Shahmohammadian, F., Jabbarzare, S., Khandan, A., & Ghayour, H. (2017). Mechanochemically aided sintering process for the synthesis of barium ferrite: Effect of aluminum substitution on microstructure, magnetic properties and microwave absorption. Journal of Alloys and Compounds, 708, 538-546
12) Heydary, H. A., Karamian, E., Poorazizi, E., Khandan, A., & Heydaripour, J. (2015). A novel nano-fiber of Iranian gum tragacanth-polyvinyl alcohol/nanoclay composite for wound healing applications. Procedia Materials Science, 11, 176-182.
13) Karamian, E., Khandan, A., Kalantar Motamedi, M. R., & Mirmohammadi, H. (2014). Surface characteristics and bioactivity of a novel natural HA/zircon nanocomposite coated on dental implants. BioMed research international, 2014.
14) Jabbarzare, S., Abdellahi, M., Ghayour, H., Arpanahi, A., & Khandan, A. (2017). A study on the synthesis and magnetic properties of the cerium ferrite ceramic. Journal of Alloys and Compounds, 694, 800-807.
15) Razavi, M., & Khandan, A. (2017). Safety, regulatory issues, long-term biotoxicity, and the processing environment. In Nanobiomaterials Science, Development and Evaluation (pp. 261-279). Woodhead Publishing.
16) Khandan, A., Ozada, N., & Karamian, E. (2015). Novel microstructure mechanical activated nano composites for tissue engineering applications. J Bioeng Biomed Sci, 5(1), 1.
17) Ghayour, H., Abdellahi, M., Bahmanpour, M., & Khandan, A. (2016). Simulation of dielectric behavior in RFeO $$ _ {3} $$3 orthoferrite ceramics (R= rare earth metals). Journal of Computational Electronics, 15(4), 1275-1283.
18) Saeedi, M., Abdellahi, M., Rahimi, A., & Khandan, A. (2016). Preparation and characterization of nanocrystalline barium ferrite ceramic. Functional Materials Letters, 9(05), 1650068.
19) Khandan, A., Karamian, E., Faghih, M., & Bataille, A. (2014). Formation of AlN Nano Particles Precipitated in St-14 Low Carbon Steel by Micro and Nanoscopic Observations. Journal of Iron and Steel Research International, 21(9), 886-890.
20) Karamian, E. B., Motamedi, M. R., Mirmohammadi, K., Soltani, P. A., & Khandan, A. M. (2014). Correlation between crystallographic parameters and biodegradation rate of natural hydroxyapatite in physiological solutions. Indian J Sci Res, 4(3), 092-9.
21) Khandan, A., & Esmaeili, S. (2019). Fabrication of polycaprolactone and polylactic acid shapeless scaffolds via fused deposition modelling technology. Journal of Advanced Materials and Processing, 7(4), 16-29.
22) Ravi, G. R., & Subramanyam, R. V. (2012). Calcium hydroxide-induced resorption of deciduous teeth: A possible explanation. Dental Hypotheses, 3(3), 90.
23) Anttonen, V., Tanner, T., Kämppi, A., Päkkilä, J., Tjäderhane, L., & Patinen, P. (2012). A methodological pilot study on oral health of young, healthy males. Dental Hypotheses, 3(3), 106.
24) Gupta, R., Thakur, N., Thakur, S., Gupta, B., & Gupta, M. (2013). Talon cusp: a case report with management guidelines for practicing dentists. Dental Hypotheses, 4(2), 67.
25) Kjaer, I. (2013). External root resorption: Different etiologies explained from the composition of the human root-close periodontal membrane. Dental Hypotheses, 4(3), 75.
26) Motamedi, M. R. K., Behzadi, A., Khodadad, N., Zadeh, A. K., & Nilchian, F. (2014). Oral health and quality of life in children: a cross-sectional study. Dental Hypotheses, 5(2), 53.
27) Narayanan, N., & Thangavelu, L. (2015). Salvia officinalis in dentistry. Dental Hypotheses, 6(1), 27.
28) Khandan, A., Karamian, E., & Bonakdarchian, M. (2014). Mechanochemical synthesis evaluation of nanocrystalline bone-derived bioceramic powder using for bone tissue engineering. Dental Hypotheses, 5(4), 155.
29) Shavarani, S. M., Nejad, M. G., Rismanchian, F., & Izbirak, G. (2018). Application of hierarchical facility location problem for optimization of a drone delivery system: a case study of Amazon prime air in the city of San Francisco. The International Journal of Advanced Manufacturing Technology, 95(9), 3141-3153.
30) Shirani, K., Sheikhbahaei, E., Torkpour, Z., Nejad, M. G., Moghadas, B. K., Ghasemi, M., ... & Khandan, A. (2020). A narrative review of COVID-19: the new pandemic disease. Iranian Journal of Medical Sciences, 45(4), 233.
31) Ghadirinejad, N., Nejad, M. G., & Alsaadi, N. (2021). A fuzzy logic model and a neuro-fuzzy system development on supercritical CO2 regeneration of Ni/Al2O3 catalysts. Journal of CO2 Utilization, 54, 101706.
32) Ghasemi, M., Nejad, M. G., & Aghaei, I. (2021). Knowledge management orientation and operational performance relationship in medical tourism (overview of the model performance in the COVID-19 pandemic

and post-pandemic era). Health Services Management Research, 34(4), 208-222.
33) Ghasemi, M., Nejad, M. G., & Bagzibagli, K. (2017). Knowledge management orientation: an innovative perspective to hospital management. Iranian journal of public health, 46(12), 1639.
34) Mosallaeipour, S., Nejad, M. G., Shavarani, S. M., & Nazerian, R. (2018). Mobile robot scheduling for cycle time optimization in flow-shop cells, a case study. Production Engineering, 12(1), 83-94.
35) Nejad, M. G., Güden, H., & Vizvári, B. (2019). Time minimization in flexible robotic cells considering intermediate input buffers: a comparative study of three well-known problems. International Journal of Computer Integrated Manufacturing, 32(8), 809-819.
36) Ghasemi, M., Nejad, M. G., Alsaadi, N., Abdel-Jaber, M. T., Yajid, A., Shukri, M., & Habib, M. (2022). Performance measurment and lead-time reduction in epc project-based organizations: a mathematical modeling approach. Mathematical Problems in Engineering, 2022.
37) Golabi, M., & Nejad, M. G. (2022). Intelligent and fuzzy UAV transportation applications in aviation 4.0. In Intelligent and Fuzzy Techniques in Aviation 4.0 (pp. 431-458). Springer, Cham.
38) Nejad, M. G., & Kashan, A. H. (2019). An effective grouping evolution strategy algorithm enhanced with heuristic methods for assembly line balancing problem. Journal of Advanced Manufacturing Systems, 18(03), 487-509.
39) Davani, P. P., Kloub, A. W. M., & Ghadiri Nejad, M. (2020). Optimizing the first type of U-shaped assembly line balancing problems. Annals of Optimization Theory and Practice, 3(4), 65-82.
40) Saeedi, M. R., Morovvati, M. R., & Mollaei-Dariani, B. (2020). Experimental and numerical investigation of impact resistance of aluminum–copper cladded sheets using an energy-based damage model. Journal of the Brazilian Society of Mechanical Sciences and Engineering, 42(6), 1-24.
41) Kardan-Halvaei, M., Morovvati, M. R., & Mollaei-Dariani, B. (2020). Crystal plasticity finite element simulation and experimental investigation of the micro-upsetting process of OFHC copper. Journal of Micromechanics and Microengineering, 30(7), 075005.
42) Fazlollahi, M., Morovvati, M. R., & Mollaei Dariani, B. (2019). Theoretical, numerical and experimental investigation of hydro-mechanical deep drawing of steel/polymer/steel sandwich sheets. Proceedings of the Institution of Mechanical Engineers, Part B: Journal of Engineering Manufacture, 233(5), 1529-1546.
43) Saeedi, M. R., Morovvati, M. R., & Alizadeh-Vaghasloo, Y. (2018). Experimental and numerical study of mode-I and mixed-mode fracture of ductile U-notched functionally graded materials. International Journal of Mechanical Sciences, 144, 324-340.
44) Morovvati, M. R., & Mollaei-Dariani, B. (2018). The formability investigation of CNT-reinforced aluminum nano-composite sheets manufactured by accumulative roll bonding. The International Journal of Advanced Manufacturing Technology, 95(9), 3523-3533.
45) Morovvati, M. R., & Dariani, B. M. (2017). The effect of annealing on the formability of aluminum 1200 after accumulative roll bonding. Journal of Manufacturing Processes, 30, 241-254.
46) Morovvati, M. R., Lalehpour, A., & Esmaeilzare, A. (2016). Effect of nano/micro B4C and SiC particles on fracture properties of aluminum 7075 particulate composites under chevron-notch plane strain fracture toughness test. Materials Research Express, 3(12), 125026.
47) Fatemi, A., Morovvati, M. R., & Biglari, F. R. (2013). The effect of tube material, microstructure, and heat treatment on process responses of tube hydroforming without axial force. The International Journal of Advanced Manufacturing Technology, 68(1), 263-276.
48) Pourmoghadam, M. N., Esfahani, R. S., Morovvati, M. R., & Rizi, B. N. (2013). Bifurcation analysis of plastic wrinkling formation for anisotropic laminated sheets (AA2024–Polyamide–AA2024). Computational materials science, 77, 35-43.
49) Morovvati, M. R., Fatemi, A., & Sadighi, M. (2011). Experimental and finite element investigation on wrinkling of circular single layer and two-layer sheet metals in deep drawing process. The International Journal of Advanced Manufacturing Technology, 54(1), 113-121.
50) Morovvati, M. R., Mollaei-Dariani, B., & Haddadzadeh, M. (2010). Initial blank optimization in multilayer deep drawing process using GONNS. Journal of manufacturing science and engineering, 132(6).
51) Ghomi, F., Daliri, M., Godarzi, V., & Hemati, M. (2021). A novel investigation on the characterization of bioactive glass cement and chitosan-gelatin membrane for jawbone tissue engineering. Journal of Nanoanalysis, 8(4), 292-301.
52) Anarestani, S. S., Morovvati, M. R., & Vaghasloo, Y. A. (2015). Influence of anisotropy and lubrication on wrinkling of circular plates using bifurcation theory. International Journal of Material Forming, 8(3), 439-454.
53) Monfared, R. M., Ayatollahi, M. R., & Isfahani, R. B. (2018). Synergistic effects of hybrid MWCNT/nanosilica on the tensile and tribological properties of woven carbon fabric epoxy composites. Theoretical and Applied Fracture Mechanics, 96, 272-284.
54) Kamarian, S., Bodaghi, M., Isfahani, R. B., & Song, J. I. (2021). Thermal buckling analysis of sandwich plates with soft core and CNT-Reinforced composite face sheets. Journal of Sandwich Structures & Materials, 23(8), 3606-3644.
55) Kamarian, S., Bodaghi, M., Isfahani, R. B., & Song, J. I. (2022). A comparison between the effects of shape memory alloys and carbon nanotubes on the thermal buckling of laminated composite beams. Mechanics Based Design of Structures and Machines, 50(7), 2250-2273.
56) Barbaz-I, R. (2014). Experimental determining of the elastic modulus and strength of composites reinforced with two nanoparticles (Doctoral dissertation, Doctoral dissertation, MSc Thesis, School of Mechanical Engineering Iran University of Science and Technology, Tehran, Iran).
57) Lucchini, R., Carnelli, D., Gastaldi, D., Shahgholi, M., Contro, R., & Vena, P. (2012). A damage model to simulate nanoindentation tests of lamellar bone at multiple penetration depth. In 6th European Congress on Computational Methods in Applied Sciences and Engineering, ECCOMAS 2012 (pp. 5919-5924).
58) Mahjoory, M., Shahgholi, M., & Karimipour, A. (2022). The effects of initial temperature and pressure on the mechanical properties of reinforced calcium phosphate cement with magnesium nanoparticles: A molecular dynamics approach. International Communications in

Heat and Mass Transfer, 135, 106067.
59) Talebi, M., Abbasi-Rad, S., Malekzadeh, M., Shahgholi, M., Ardakani, A. A., Foudeh, K., & Rad, H. S. (2021). Cortical bone mechanical assessment via free water relaxometry at 3 T. Journal of Magnetic Resonance Imaging, 54(6), 1744-1751.
60) Shahgholi, M., Oliviero, S., Baino, F., Vitale-Brovarone, C., Gastaldi, D., & Vena, P. (2016). Mechanical characterization of glass-ceramic scaffolds at multiple characteristic lengths through nanoindentation. Journal of the European Ceramic Society, 36(9), 2403-2409.
61) Fada, R., Farhadi Babadi, N., Azimi, R., Karimian, M., & Shahgholi, M. (2021). Mechanical properties improvement and bone regeneration of calcium phosphate bone cement, Polymethyl methacrylate and glass ionomer. Journal of Nanoanalysis, 8(1), 60-79.
62) Malekipour, M., Norouzi, Z., & Shahlaei, S. (2019). Effect of remineralizing agents on tooth color after home bleaching. Frontiers in dentistry, 16(3), 158.
63) Malekipour, M. R., Shirani, F., Taromi, Z., & Shahnazari, S. (2017). Comparison of color stability of two resin composites in blood area. Dental Hypotheses, 8(3), 65.
64) Malekipour, M. R., Shirani, F., & Ebrahimi, M. (2016). The effect of washing water temperature on resin-dentin micro-shear bond strength. Dental Research Journal, 13(2), 174.