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CRYSTALLIZATION AND SINTERABILITY BEHAVIOR OF BIORESORBABLE CaO-P_2O_5-Na_2O-TiO_2 GLASS CERAMICS FOR BONE REGENERATION APPLICATION

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Abstract:*Some types of glass and glass ceramics have a great potential for making bone tissue engineering scaffolds, drug carrier and bone cements as they can bond to host bone, stimulate bone cells toward osteogenesis, and resorb at the same time as the bone is repaired. Calcium phosphate glass ceramics have very attractive properties that allow them to use in bone tissue engineering. Calcium phosphate glasses could be used for the fabrication of resorbable constructs, with controlled biodegradability. This work was investigated crystallization and sinterability of biodegradable glass ceramics in the CaO–P_2O_5–Na_2O–TiO_2 system using differential thermal analysis (DTA), X-ray diffraction (XRD) and scanning electron microscopy (SEM). Sinterability of the glasses also was investigated by measurement of sintering parameters. Different thermal treatments were applied to control the degree of devitrification of glasses. In the developed glass ceramics $Ca_2P_2O_7$ were the first phase to precipitate in the mother glass structure, followed by $Ca(PO_3)_2$ and TiP_2O_7 at elevated temperature. $Ca_2P_2O_7$ and $Ca(PO_3)_2$ seem to have a very positive effect in bone formation in vivo. It is therefore expected that glass ceramics understudy have good potential to be used for bone regeneration applications.*

Keywords: *Crystallization, Sinterability, Calcium Phosphate Glass, Bioresorbable.*

1. INTRODUCTION

Bone is usually in need of regeneration or substitution due to tumor removal, trauma, or age-related pathologies, such as osteoarthritis and osteoporosis. Two alternatives are possible for bone replacement: (i) transplantation or (ii) implantation. Transplants can be made by using living or nonliving tissues; at present, the commonly recognized gold standard in reconstructive bone surgery consists in the use of autografts that involve harvesting the patient’s own tissue from a donor site and transplanting it to the damaged region [1]. The most common strategy used to heal bone defects resulting from trauma or diseases is to fill the defect with autologous bone, typically from the iliac crest. Unfortunately, this technique has several drawbacks such as the need for a second surgery that prolongs the surgical operation and an increased risk of side-effects such as infections or pain [2,3]. Autologous bone causes no immunological problems but can be collected only in limited amount, and its harvesting can induce death of healthy tissue at the donor site; in addition, problems related to second site morbidity, mismatching in mechanical properties with respect to host bone, and tendency toward resorption may occur. A partial solution to these drawbacks is the use of allografts, i.e., the transplant of bone tissue from another living patient or from cadavers. However, allografts can cause disease transmission and carry the need for immunosuppressant drugs administration; furthermore, ethical and religious issues limit their use. Implantation involves the substitution of damaged tissues by using, in most cases, man-made biocompatible templates with proper 3D architecture able to promote tissue regeneration and/or remodeling. [1]. One alternative to bone grafts is the use of synthetic calcium phosphate substitutes [4]. These materials have generally a composition very close to that of the mineral part of bone and as a result present excellent biological properties such as biocompatibility, osteoconduction (ability to lead bone formation) and osteotransduction (replacement of the material by new bone) [5].

Phosphate-based glasses and glass ceramics offer great potential for usage as hard tissue implants and drug carriers due to their enhance bioactivity and similar chemical composition to the hard tissues.

And their chemical components, crystallization, structural characteristics, mechanical properties and even their dissolution rates can be governed by adjusting the compositions of the glasses and controlling crystallization to suit with their end applications [6-11].

Kyun Park et al in their study investigated the in vitro biocompatibility of calcium metaphosphate (CMP) with human bone marrow stromal cells (HBMSCs) and its effect on osteoblastic differentiation. Powder and disk forms of CMP do not exert a cytotoxic effect on the HBMSCs undergoing osteoblastic differentiation. In addition, the HBMSCs adhere to the surface of the CMP disk as successfully as to the culture plate or hydroxyapatite (HA) disk. The HBMSCs adhered to either the HA or CMP disk display an undistinguishable actin arrangement and cellular phenotypes, indicating that the CMP does not disrupt normal cellular responses. An analysis of the differentiation of the HBMSCs cultured on culture plate, the HA and the CMP disk shows that three matrices are capable of supporting osteoblastic differentiation of the HBMSCs as accessed by alkaline phosphatase (ALP) staining. Further molecular analysis of osteoblastic differentiation of HBMSCs reveals that the CMP disk has a better ability than the HA disk to induce an expression of osteoblast-related genes, including ALP, osteoprotegerin (OPG), a decoy receptor for RANK ligand, and osteopontin (OPN), a non-collagenous bone matrix protein. Researches demonstrate that, in addition to favorable biocompatibility, the CMP can stimulate osteoblastic differentiation of the HBMSCs in vitro [12,13,14].

In this study, crystallization and sinterability of bioresorbable CaO-P_2O_5-Na_2O-TiO_2 glass ceramics system were investigated.

2. EXPERIMENTAL PROCEDURE

2. 1. Material and Methods

For the elaboration of the glass ceramics, calcium phosphate glass particles with the following weight composition, 65.9%P_2O_5 –26.04%CaO–3.88%Na_2O–4.18%TiO_2 were used. For the preparation of the glasses, β-tri calcium phosphate, P_2O_5, Na_2CO_3 and TiO_2 (all of them prepare from Merck, Germany) were used as the basic reagents. The reactants were weighted, homogeneously mixed and melted in a alumina crucible at 1380 °C for 1 h in air. The mixture was casted on a steel rotary roller to obtain frit. Frits were pulverized in an agate planetary mill and sifted (mesh No. 140). DTA analysis of the glass particles was performed to obtain the values of the glass transition temperature (T_g) and the crystallization temperature (T_c) in order to know the most adequate temperature range for the different thermal treatments. Both, T_g and T_c were measured in a differential thermal analyzer at a heating rate of 10 °C/min in air atmosphere (Mettler Toledo, SDTA 851 E, Malek Ashtar Tehran University). The analysis was conducted up to 900 °C.

Glass powder (<106 μm) was mixed homogeneously, with 0.5 wt % PVA and were placed into a stainless steel cylindrical die and then uniaxially pressed at 426.85 Kg/cm^2 to form a green compact tablet (with 15 mm diameter and 5 mm height). In order to understand that what phases crystallized in the temperature of crystallization peaks in DTA, 2 samples were placed in electrical furnace and heated with heating rate of 10 °C/min in air atmosphere to temperature of first peak of DTA, and for 10 minutes remained in this temperature. Then samples removed from furnace and investigated with XRD. The second sample heat treated to second temperature with the same rate above and remained in this temperature for 10 min and removed quickly from furnace. This sample also evaluated by X-ray powder diffraction. Finally, considering the DTA graph, green compacts obtained by the pressing process, were fired at different temperatures. For any temperature, 5 green compact were prepared and fired by heating rate of 10 °C/min. The duration time for heat treatment was 2 h and then the samples were free cooling down inside the furnace. Sinterability parameters such as liner shrinkage, liquid absorption, bulk density and open pore percentage were measured for each of 5 samples in all temperatures and average of them for each temperature was calculated. The bulk density of the sintered glass-ceramics was determined by the Archimedes method. In this method, petroleum with low density (1.47 gr/cm^3) was employed. Because the sintered glass ceramics a little dissolved in cold water after 24 h.

2. 2. Characterizations

The porosity and morphology of the optimum samples were analyzed by scanning electron microscopy (SEM, Vega Tescan, Iran University of Science and Technology).

The devitrification or formation of new crystalline phases was evaluated by X-ray powder diffraction (XRD). XRD measurements were performed in a Bragg–Brentano θ/2θ diffractometer with CuKα radiation (Philips-PW1800). The starting and the final 2θ angles were 10° and 70°, respectively. The step size was 0.04° for 2θ.

3. RESULTS AND DISCUSSION

Fig. 1 shows the X ray diffraction of glass powder. XRD pattern shows an amorphous pattern and no crystalline phase exist. Fig. 2 shows the DTA diagram of glass powder. The two exothermic signals at 672 °C and 747 °C are ascribed to the crystallization from glass matrix,

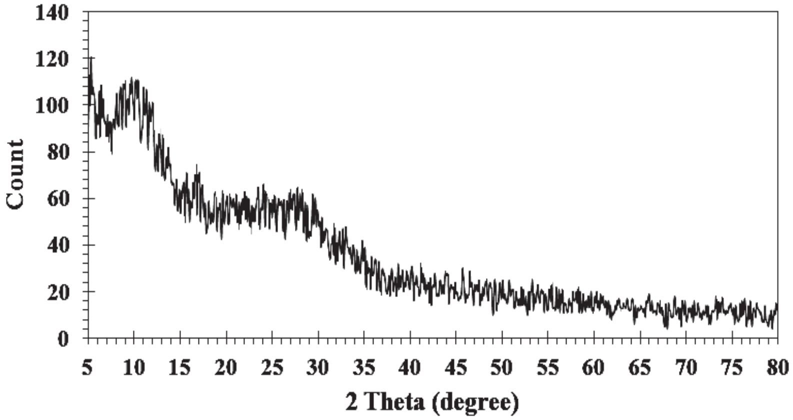

Fig. 1. X ray diffraction of CaO-P_2O_5-Na_2O-TiO_2 glass powder

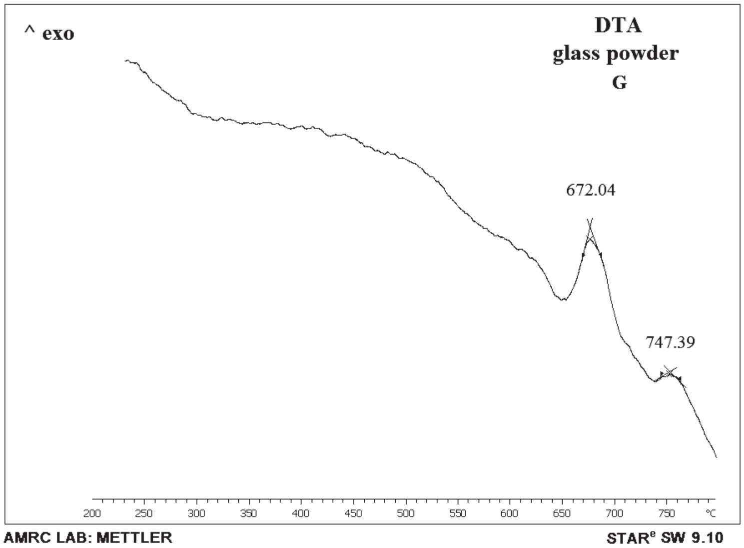

Fig. 2. DTA trace of CaO-P_2O_5-Na_2O-TiO_2 glass powder

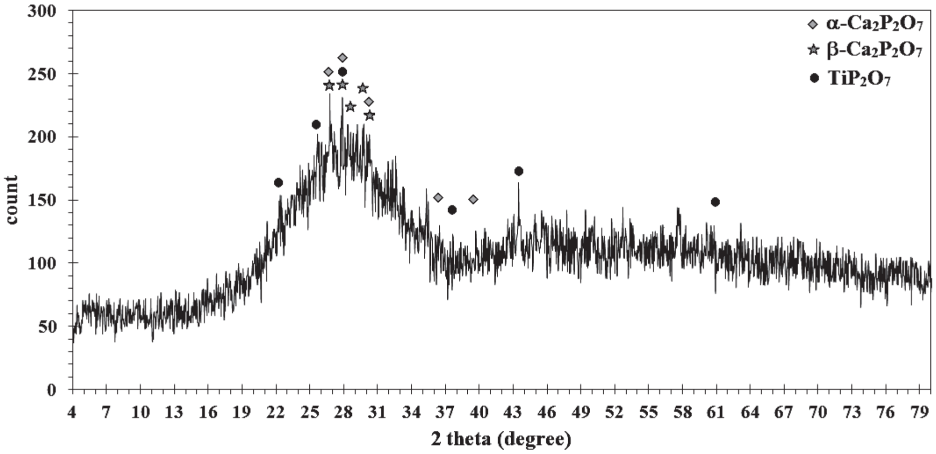

Fig. 3. XRD pattern of $CaO-P_2O_5-Na_2O-TiO_2$ glass ceramic heat treated at 672 °C for 10 minutes

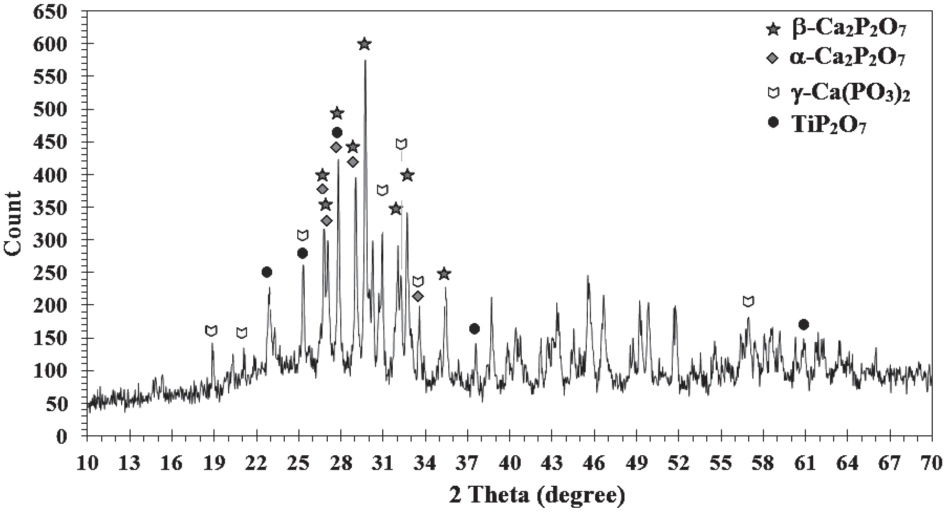

Fig. 4. XRD pattern of $CaO-P_2O_5-Na_2O-TiO_2$ glass ceramic heat treated at 747 °C for 10 minutes

which are confirmed by XRD patterns. XRD patterns for the samples heat-treated at 672 °C and 747 °C for 10 minutes represented in Fig. 3 and Fig. 4, respectively. From Fig. 3 it can be inferred that in 672°C, α-$Ca_2P_2O_7$ (JCPDS file No. 09-0345) and β-$Ca_2P_2O_7$ (JCPDS file No. 09-0346) were the major phases and TiP_2O_7 (JCPDS file No. 38-1468) was the minor phase that crystallized from glass matrix. Large amount of glass phase exist in this sample that was indicated with X-ray pattern.

As seen in Fig. 4, at 747 °C, the glass phase was reduced and γ-$Ca(PO_3)_2$ (JCPDS file No. 50-0584) was crystallized within glass matrix so that β-$Ca_2P_2O_7$ (JCPDS file No. 09-0346) was the major phase in this temperature.

XRD patterns of the samples heat-treated at different temperatures for 2 hr are shown in Fig. 5, where the structural evolution of the phases is clearly presented.

Samples heat treated at 592 °C was ratherly amorphous. After heat treatment of the samples at 632 °C, amorphous phase was partially disappeared and α-$Ca_2P_2O_7$ (JCPDS file No. 09-0345) and β-$Ca_2P_2O_7$ (JCPDS file No. 09-0346) phases were precipitated in the samples. By

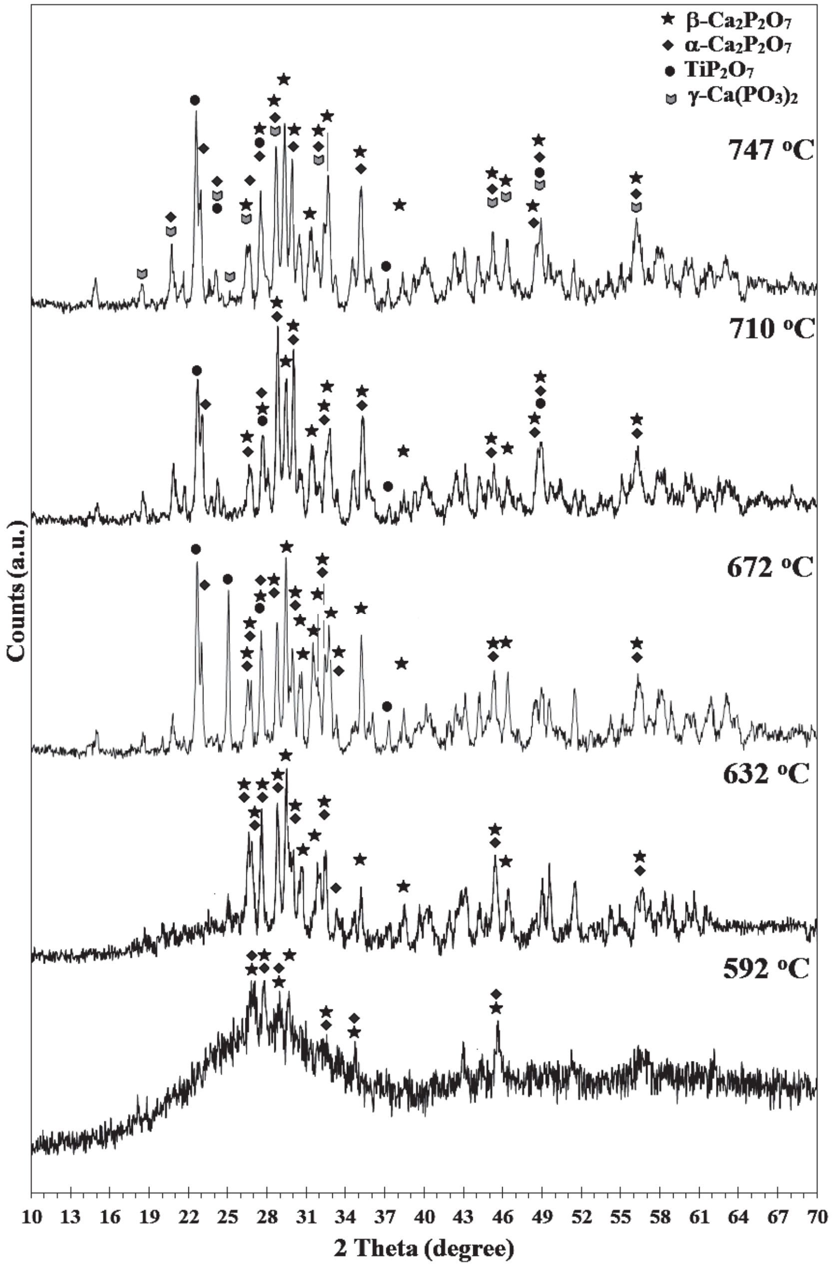

Fig. 5. XRD patterns of the samples heat-treated at different temperatures for 2hr

increasing of temperature to 672 °C beside α and β-$Ca_2P_2O_7$, formation of TiP_2O_7 (JCPDS file No. 38-1468), was happen and more increasing temperature up to 747 °C, resulted in formation of γ-$Ca(PO_3)_2$ (JCPDS file No. 50-0584).

Figure 6 depicts the changes of average bulk density of the samples heat treated between 592 °C and 747 °C. Furthermore, variation of liner shrinkage, liquid absorption and open porosity of these samples have been depicted in Fig 7, Fig. 8 and Fig. 9, respectively. According to Figs. 6-9, the best temperature for sintering of the

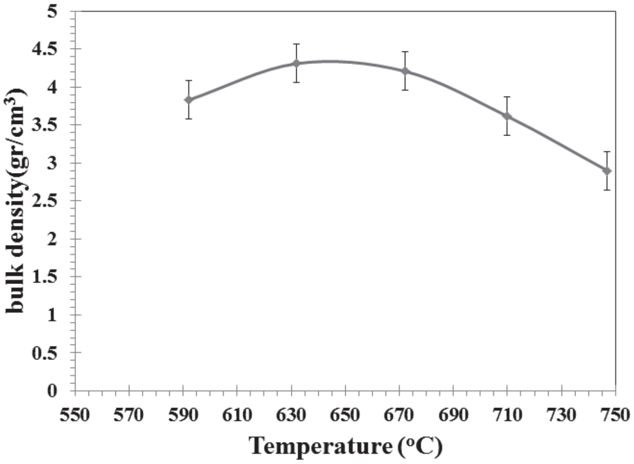

Fig. 6. Changes of bulk density versus temperature of the samples heat-treated at different temperatures for 2hr

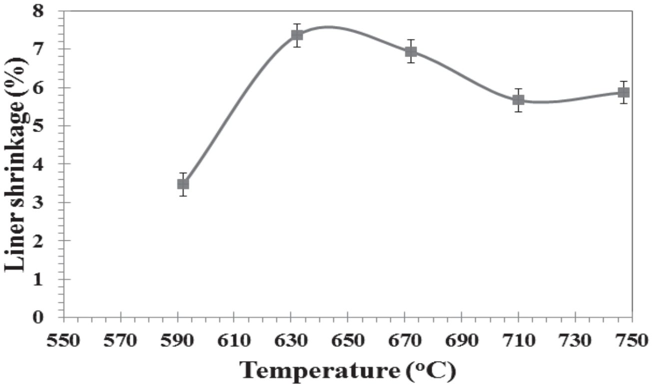

Fig. 7. Changes of liner shrinkage versus temperature of the samples heat-treated at different temperatures for 2hr

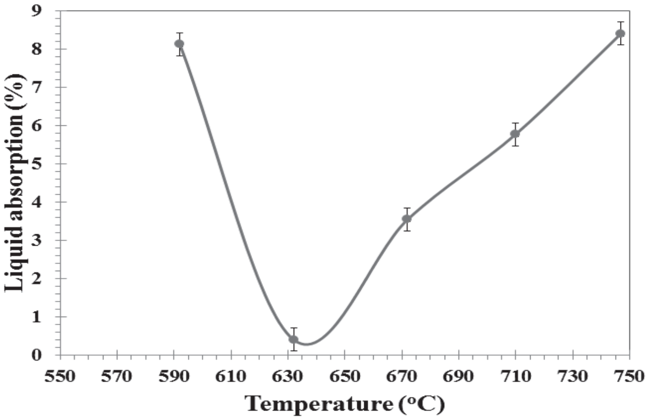

Fig. 8. Changes of liquid absorption versus temperature of the samples heat-treated at different temperatures for 2hr

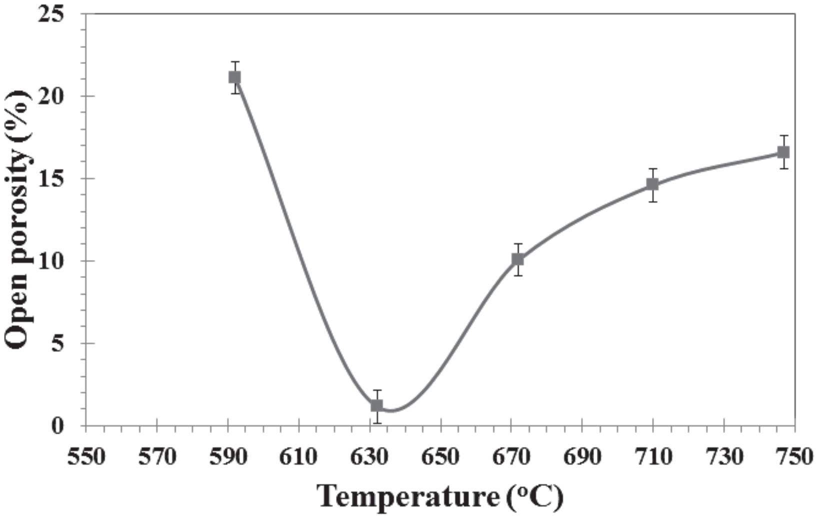

Fig. 9. Changes of open porosity versus temperature of the samples heat-treated at different temperatures for 2hr

compacted calcium phosphate glass powder is approximately 632 °C. At this temperature the resulted specimen contains α and β-$Ca_2P_2O_7$ and shows maximum of liner shrinkage and bulk density and minimum of open porosity and liquid absorption. At elevated temperatures (> 670 °C) volume crystallization was occurred and samples could not be sintered enough; therefore, the properties corrupted and porous structure obtained that has not enough strength. Pyrocalcium phosphates (α and β-$Ca_2P_2O_7$) have a positive effect in point of bone formation view and are biodegradable phases that can be used in scaffold fabrication for regeneration application in bone tissue engineering. Recently, calcium metaphosphate (CMP) ceramic has been proposed as a new bone substitute material because of its biodegradable property and osteoconductivity [15,16]. The in vitro and in vivo biocompatibility of CMP with an animal model was examined in previous studies [12]. The chemical composition of several biomaterials has developed toward a composition that resembles the natural bone matrix as much as possible. The best known of these so called chemical anisotropic biomaterials are calcium phosphate biomaterials such as hydroxyapatite and tricalcium phosphates. Other calcium phosphates include the calcium pyrophosphates [$Ca_2P_2O_7$] and other oxide compounds [XCaO. P_2O_5] [17]. β-$Ca_2P_2O_7$ crystal belongs to a tetragonal, space group $P4_1$ [18]. The ab plane is spread by a three-dimensional sheet consisting of P_2O_7–Ca infinite chains based on the chelate-like rings, giving a matted effect when viewed down c-axis [19]. The crystal structure of β-$Ca_2P_2O_7$ having the matted sheets is believed to be related closely to the cleavability. The easy machinability in the glass-ceramic may imply that β-$Ca_2P_2O_7$ crystal has cleavability. The machinability is suggested to result from the interlocking plate-like microstructure of β-$Ca_2P_2O_7$ crystals dispersed in the glassy matrix phase [20]. Hence, the best temperature for the crystallization of calcium phosphate glass powder also is 632 °C.

To investigate the microstructural of the best samples, selected from sintering their behavior, SEM was conducted on the samples heat treated at 632°C. Fig. 10 (A-C), which are consisted of dense structure and in which grains are immersed in the residual glass matrix.

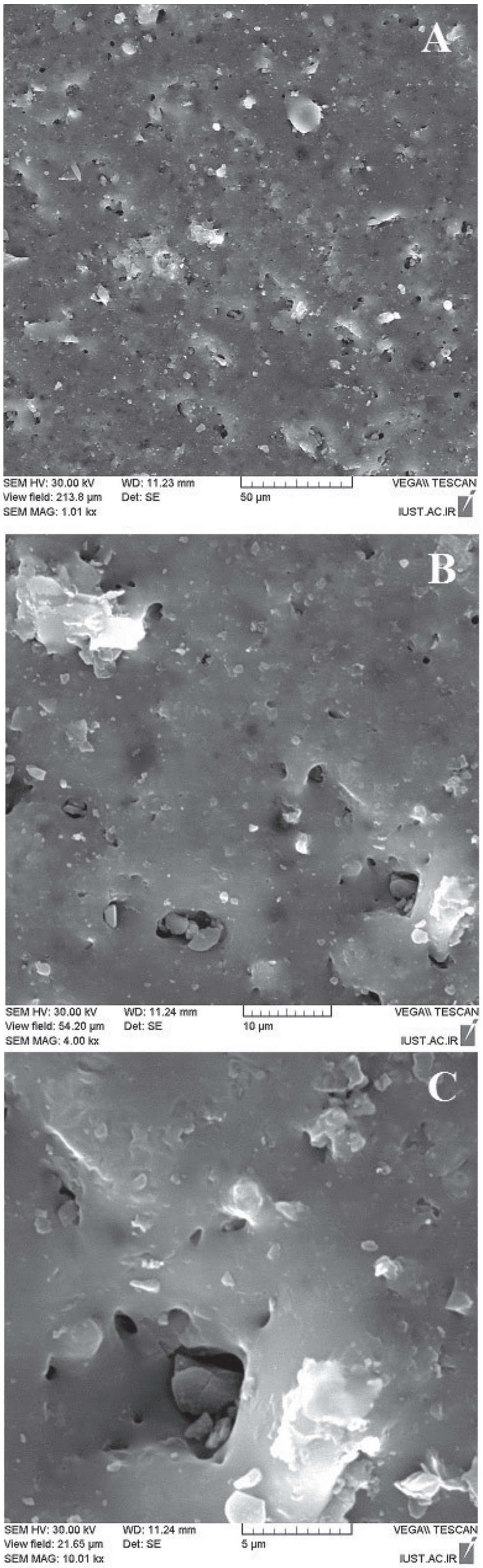

Fig. 10. SEM images of samples heat treated in 632 °C for 2 hr in different magnification: (A) 1000X, (B) 4000 X, (C) 10000 X

4. CONCLUSIONS

Sinterability and crystallization behavior of bioresorbable $CaO-P_2O_5-Na_2O-TiO_2$ glass ceramics was investigated the best sintering and crystallization temperature was 632 C. In this temperature calcium pyrophosphate was crystallized from glass matrix and the sintering parameter such as bulk density, liner shrinkage, open porosity and liquid absorption were optimum. Therefore, this glass ceramic can be used in a scaffold or dense forms or as a powder at bone regeneration application.

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