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Composite bone substitute materials based on β-tricalcium phosphate and magnesium-containing sol–gel derived bioactive glass

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Abstract In the present study, bioceramic composites with improved mechanical and biological properties were synthesized by sintering mixtures of β-tricalcium phosphate and SiO_2–CaO–MgO–P_2O_5 sol–gel derived bioactive glass at 1000–1200°C. The physical, mechanical, structural and biological properties of the composites were evaluated by appropriate experiments such as microhardness, bending strength, XRD, SEM and MTT. The results showed that 1000 and 1100°C were not appropriate temperatures for sintering the composites and in contrast, the microhardness, bending strength and bulk density significantly increased by increasing in quantity of bioglass phase when the samples were sintered at 1200°C. No significant difference was found between the fracture toughness of the composites and pure β-tricalcium phosphate. β-tricalcium phosphate was structurally stable up to 1200°C and did not transform to its alpha form even in the presence of the bioglass phase but migration of magnesium cations from the glass composition into its lattice structure was found by right-shift in XRD patterns, especially when the composite contained higher amount of bioglass component. Calcium silicate was also crystallized in the composition of the composites, which was more detectable in higher sintering temperatures. The results of the MTT test showed that proliferation of human osteosarcoma cells on the composites was considerably better than that of pure β-TCP.

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1 Introduction

Replacement of extensive local bone loss is a significant clinical challenge [1, 2]. There are a variety of techniques available to the surgeon to manage this problem. The treatment can be done using graft materials such as autografts and allografts obtained from different sites in the patient or another donor, respectively [3]. Disadvantages of autogenous bone are procurement morbidity, long operative time and limited availability. The risk of diseases transmission is also the main drawback of the allografts. Thus, different artificial materials have been developed over the centuries to treat the bone defects [4]. Ideally, synthetic bone graft substitute should be biocompatible, osteoconductive, undergo remodeling and support new bone formation [5]. Because of its osteoconductivity and chemical similarity to that of bone mineral, hydroxyapatite (HA) has been widely used as bone substitute in orthopaedic surgeries [6]. Although poor mechanical properties of HA could be improved by different strategies such as densification with sintering aids [7, 8] or reinforcement with silicon carbide whisker [9], HA substitutes have a main drawback namely, poor solubility in physiological environments.

Thus, HA implants can not be replaced by newly formed tissues and failure may occur through refracture of HA itself or HA–host tissue interface [10]. β-Tricalcium phosphate (β-TCP) is another important calcium phosphate compound used as bone graft substitute. β-TCP has a higher resorption rate than HA and it is normally considered as a biodegradable material that allows bone growth and replacement. Unfortunately, β-TCP is also weak in mechanical strength and should be reinforced before implantation. In addition, β-TCP has a poor ability of inducing calcium phosphate formation both in vitro and in

vivo, so which it can not be chemically bonded to living tissues [11]. Few works have been done to improve the mechanical and biological properties of β-TCP. Composites of β-TCP and pyrophosphate glass with improved mechanical strength were previously reported by Yu et al. [12]. Sol–gel derived bioglasses are also appropriate candidate to be incorporated with β-TCP, because they exhibit high surface area as well as unique bioactivity.

Incorporation of a sol–gel derived magnesium-containing bioglass phase into β-TCP may provide strong materials with better biological properties than pure β-TCP. The results of various experiments performed in this study confirmed this suggestion.

2 Preparation and characterization of starting materials

The bioactive glass used in this study was selected from the system $CaO–SiO_2–P_2O_5–MgO$ with 64% SiO_2, 26% CaO, 5% P_2O_5 and 5% MgO in molar percentage [13]. The precursors used for the preparation of the bioglass were tetraethylorthosilicate (TEOS), triethylphosphate (TEP), calcium nitrate tetrahydrate and magnesium nitrate hexahydrate, all of them were purchased from Merck Company. The solution of the gel was prepared by the stoichiometric amount of precursors and the gel was obtained according to the method described previously [14]. Briefly, TEOS was introduced into 0.1 M nitric acid solution and stirred for 1 h at room temperature for acid hydrolysis. TEP, calcium nitrate and magnesium nitrate were then added to the TEOS solution in sequence allowing 45 min for each reagent to react completely. After the final addition, the mixture was stirred for 1 h for completion of the hydrolysis reaction. The solution was poured into a Teflon container, kept sealed at 25°C for 6 days (until the gel was formed), dried at 70°C for 3 days and then at 120°C for 2 days, and finally, heated at 700°C for 5 h to eliminate the residual nitrate and organic substances.

$CaCO_3$ and $CaHPO_4$ reagents (both from Merck, Germany) were used for synthesizing β-TCP through solid-state reaction. They were mixed together in Ca/P molar ratio of 1:5 and homogenized in ethanol medium in a ball mill for 6 h. Then, the powder was dried at 100°C for 12 h and calcined at 900°C for 3 h. The surface area of both β-TCP and bioglass were recorded by the Brunauer, Emmel and Teller (BET) method (Micrometrics, Gimini).Thermal behavior of the dried bioglass powder (before stabilizing) were evaluated in air atmosphere up to 1200°C using a simultaneous differential thermal analysis (DTA) and thermogravimetry analysis (TGA) technique (PL-STA 1600, England).

3 Preparation and characterization of composites

β-TCP was mixed with the sol–gel derived bioglass (G) in various β-TCP to bioglass weight ratios, i.e. 90TCP/10G, 75TCP/25G and 60TCP/40G, and homogenized in a dried ball mill for 2 h. Disc-shaped specimens (5 mm in diameter and 3 mm in height) were formed in an press apparatus, where the mixed powder was uniaxially compacted within a mold at a pressure of 200 MPa. In this paper the vocabularies β-TCP and TCP are synonyms. Finally, the samples were heated in an electrical furnace at 1000–1200°C for 3 h.

Phase compositions of the synthesized composites were evaluated using X-ray diffraction analysis (Philips PW 3710) with Cu-Kα radiation operated at 40 kV, 30 mA and scan rate of 0.02 2θ/s. The XRD patterns were checked by X'Pert HighScore software, version 1.0d, 2003 (PANalytical B.V., Almelo, the Netherlands).

The shrinkage behavior of the specimens was recorded, in the term of volume contraction (ΔV), using the following formula:

$$\Delta V\% = \frac{V_0 - V_S}{V_0} \times 100 \quad (1)$$

where V_0 and V_S are volume of the specimen before and after the firing process, respectively.

The total porosity (P_t) of the samples was also determined in accordance with density method using to the following formula [15]:

$$P_t = 100\left(1 - \frac{D_b}{D_t}\right) \quad (2)$$

where D_b and D_t are bulk density and theoretical density of the composites, respectively. D_b was calculated from the following equation:

$$D_b = \frac{m}{v} \quad (3)$$

where m is the mass of the specimen and v is its volume. However, powder density (D_p) was used instead of D_t, because it was difficult to measure the theoretical density of β-TCP/G composites. To measure D_p, the sintered composite specimen was ground in an agate mortar and passed through a 500-mesh sieve and then transferred into the sample holder of a gas picnometer device (Micrometrics, Accupy C1330) for the analysis.

Three-point bending strength (BS) was performed on cuboid-shaped specimens, with finally nominal dimensions of 5:1:1 in length: width: thickness. The specimens had been shaped in a uniaxial press device at a pressure of 200 MPa and sintered at 1000–1200°C for 3 h. A universal testing device (Instron, 1196) with crosshead speed of 1 mm/min was used and BS was calculated using the following formula:

$$BS = \frac{3Pl}{2bd^2} \quad (4)$$

where P is the load at the time of the fracture and b, d and l were width, thickness and length of the specimen, respectively.

The fracture toughness (K_{IC}) of the samples was determined according to the method described previously [16]. Briefly, Vickers indenter (MVK-C11 Akashi, Japan) prints an effect on the center of the mirror polished surface in ambient air. Crack length was recorded using the indenter microscope and to minimize the slow crack growth the measurement was carried out 30 s after the indentation.

The fracture toughness (K_{IC}) was calculated according to the equation developed by Lawn and Fuller [16], which relates the indentation load (P) and the size of the median cracks (c):

$$K_{IC} = 0.0726\left(\frac{P}{c^{1.5}}\right) \quad (5)$$

The Vickers hardness was also measured on the polished surfaces of the samples at indentation load of 200 gr using a MVK-H21 (Akashi, Japan) hardness tester.

For morphological analysis, the surfaces of the specimens were coated with a thin layer of gold and the microstructures were examined using a scanning electron microscope (SEM, Streoscan S360, Cambridge Ltd.).

To study the effect of incorporation of G phase into β-TCP on the viability and proliferation of the osteoblastic cells, the preliminary in vitro tests were carried out using the G-292 human osteosarcoma cells (NCBI C 116 national cell bank of Iran).

G-292 cells were cultured in tissue culture polystyrene (PS) flasks (Falcon, USA) at 37°C under 5% CO_2 atmosphere in Dulbecco's modified Eagle's medium (DMEM) with L-glutamine, supplemented 10% fetal bovine serum (FBS) and antibioticantimycotic (100 units penicillin G sodium, 100 mg streptomycin sulfate, and 0.25 mg amphotericin B in saline) and harvested after the treatment with 0.05% trypsin-EDTA. The sterilized specimens (β-TCP and composites) were mounted in test wells and the cells were seeded at 3×10^4 cell/ml. The cell culture on the samples was carried out in humidified incubator at 37°C with 5% CO_2 and 95% air for 3, 5 and 7 days. The cell proliferation in contact with the samples was determined by using water soluble enzyme substrate 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT; Sigma), which is converted to blue water insoluble product formazan accumulated in the cytoplasm of viable cells. In brief, 100 µl of MTT solution (5 mg/ml) was freshly added to culture dish containing 2 ml of fresh medium and incubated at 37°C and 5% CO_2 for 4 h. The intracellular formazan was solubilized using 2 ml of lysing buffer containing 45% dimethyl formamide and 10% sodium dodecyl sulfate (SDS; Sigma). The absorbance of formazan produced was measured at 590 nm with a spectrophotometer (ICN, Switzerland). Cell number was determined using a linear correlation between absorbance and G-292 cell concentration.

Data were processed using Microsoft Excel 2003 software and the results were produced as mean ± standard deviation of at least four experiments. Significance between the mean values was calculated using standard software program (SPSS GmbH, Munich, Germany) and the $P \leq 0.05$ was considered significant.

4 Results and discussion

Sol–gel-derived bioglasses are important class of bioceramic materials with excellent bioactivity and wide uses in clinical orthopaedy. These materials exhibits high surface area that is characteristics of the sol–gel derived powders. Thus, these materials may accelerate sintering phenomenon (formation of a ceramic bond between the particles in an elevated temperature) when they are added or admixed to other bioceramics such as calcium phosphates. The specific surface area of the bioglass powder synthesized in this study was 212.33 m^2/g, a value much higher than that of β-TCP i.e. 2.58 m^2/g.

SiO_2–CaO–MgO–P_2O_5 ternary system was used in this study, because of its superior bioactivity and stimulating effect on human osteoblastic cell proliferation and differentiation, as described in the previous works [13, 17].

Thermal analysis was performed on the synthesized bioglass to find out the major phenomena occur during sintering. Figure 1 shows thermal behavior of the synthesized gel powder. The first endothermic phenomenon accompanied with a weight loss of 40% is doublet, one of

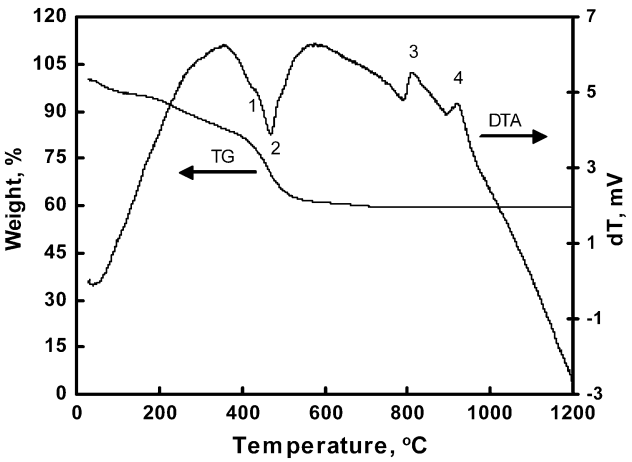

Fig. 1 DTA and TG curves of dried bioglass gel

them appeared at 450°C (1) is attributed to condensation of silanol groups and another one found at 500°C (2) is due to removal of nitrate groups. The exothermic peak observed at 810°C (3) is assigned to glass transition phenomenon in which change occurs in the glass structure. The second exothermic peak (4) at 900°C is corresponded to crystallization of the glass phase to calcium silicates.

The results showed that incorporation of the bioglass phase into the β-TCP considerably influenced its structural, mechanical and biological properties.

β-TCP was structurally stable up to 1200°C and did not transform to its alpha form even in the presence of the bioglass phase. Figure 2 shows the XRD patterns of pure β-TCP and TCP/G composites heated at 1100°C (Fig. 2a) and 1200°C (Fig. 2b). β-TCP was the main phase in all specimens (in both pure β-TCP and composites). In other words, the XRD patterns revealed that the addition of the bioglass phase to β-TCP did not influence the phase composition or form of β-TCP. In the XRD patterns of 75TCP/25G and 60TCP/40G, the peaks observed at $2\theta = 27.6°$ and 31.9° were assigned to $CaSiO_3$, an osteointegrative and potential bioactive material [18] (PDF No. 00-001-0720), crystallized within the bioglass phase. These peaks were not easily observed in the patterns of 90TCP/10G. It suggests that the presence of this crystalline phase could further improve the mechanical strength of the composites.

Migration of Mg ions from the glass phase into the β-TCP lattice structure is suggestible when the composites are sintered at 1200°C. This phenomenon is more tangible when higher amount of bioglass phase is used. This suggestion is simply confirmed by the right-shift in XRD pattern of 60TCP/40G composite, indicating formation of a Mg-substituted β-TCP phase (such as $Ca_{2.859}Mg_{0.411}(PO_4)_2$ solid solution according to the card No. 01-087-1582 in the X'Pert HighScore database).

It was found from the results that both firing temperature and the quantity of the bioglass phase are important factors that influence the physical and mechanical properties of the composites. As shown in Fig. 3, these parameters had an increasing effect on volume contraction of specimens, so which the maximum contraction occurred in 60TCP/40G specimen sintered at 1200°C.

Bulk density of specimens was also improved using higher firing temperature; meanwhile for those specimens sintered at 1200°C, the bulk density values of composites were significantly higher than that of pure β-TCP (Fig. 4a). Adverse results were obtained for total porosity and the values of composite formulations (Fig. 4b) were significantly lower than the values of pure TCP. This suggests that it was related to elimination of open pores at the surfaces of the β-TCP/G composites. There was no significant difference between the bulk density/total porosity of specimens sintered at 1100°C and those sintered at 1000°C.

Figures 5 and 6 show the fracture toughness and microhardness values of the samples, respectively. All values

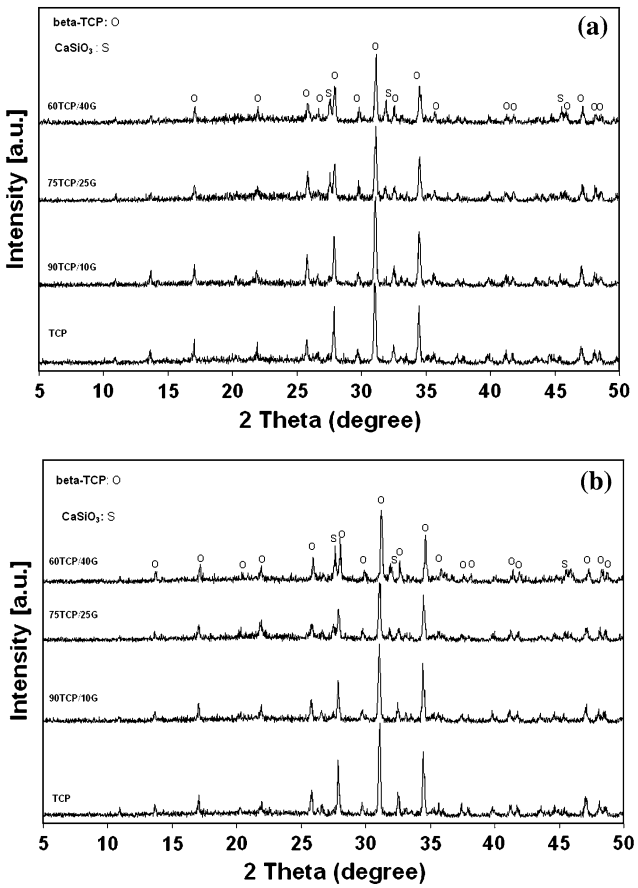

Fig. 2 XRD patterns of TCP and TCP/G composites sintered at 1100°C (**a**) and 1200°C (**b**)

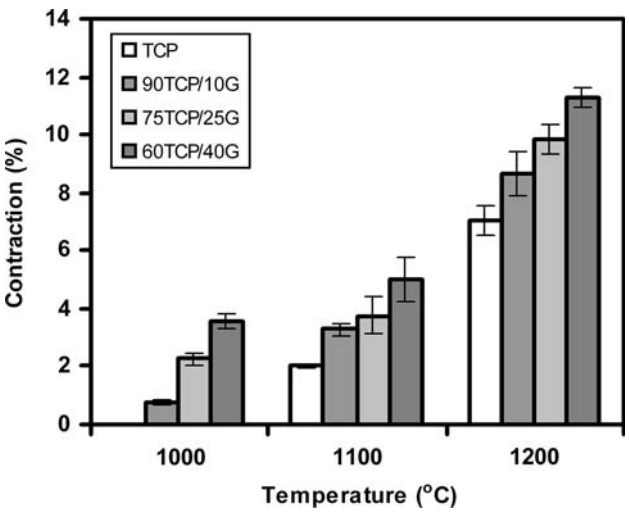

Fig. 3 Shrinkage of TCP and TCP/G composites at different sintering temperatures

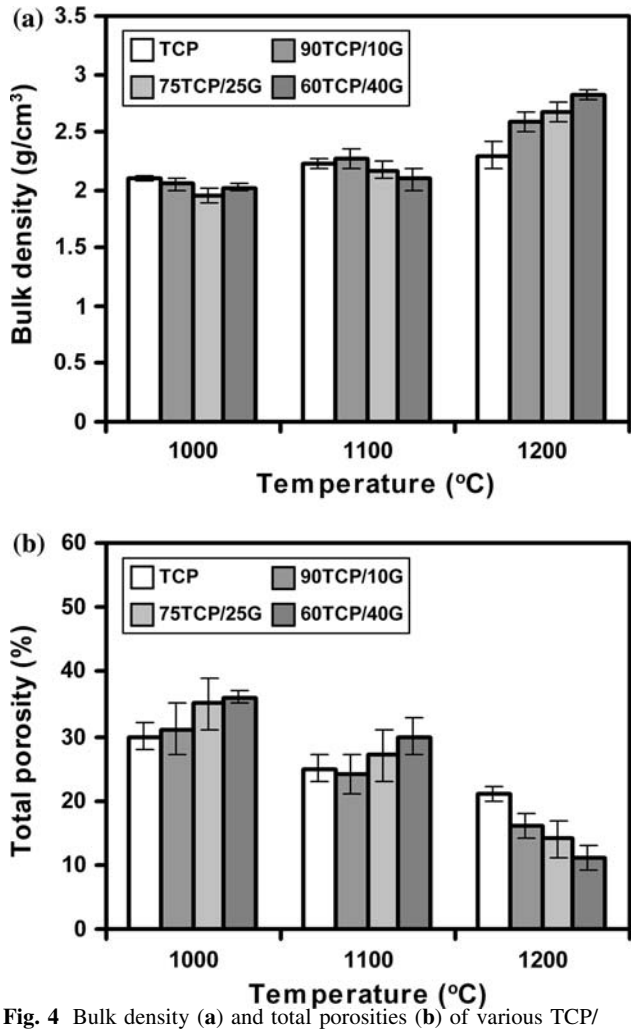

Fig. 4 Bulk density (**a**) and total porosities (**b**) of various TCP/bioglass composites sintered at different temperatures

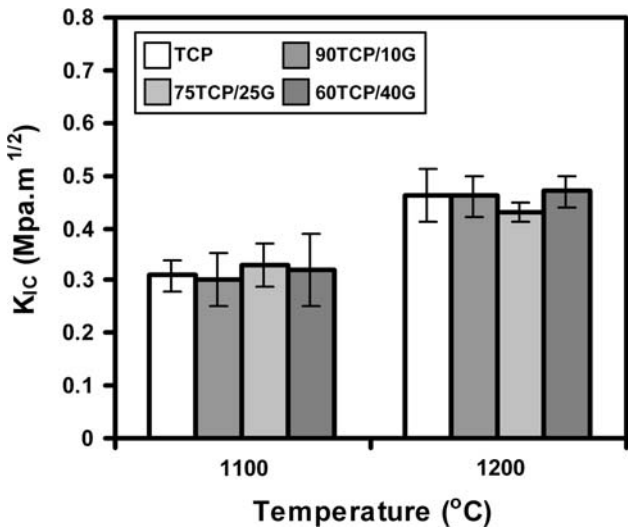

Fig. 5 The change in fracture toughness of TCP and composites by sintering at different temperatures

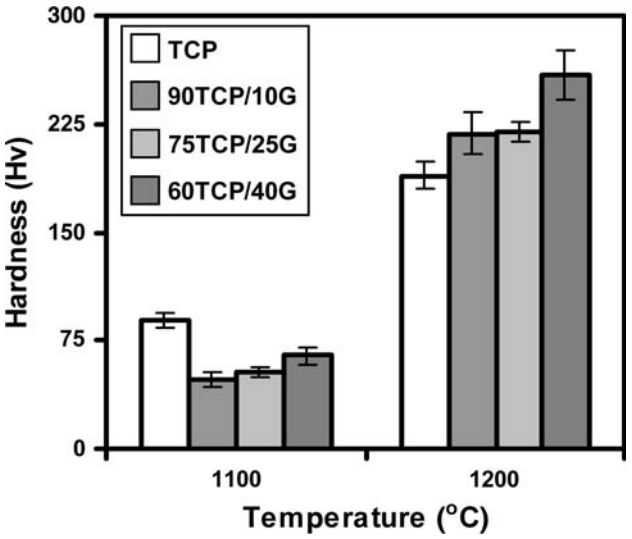

Fig. 6 Microhardness of TCP and TCP/G composites sintered at different temperatures

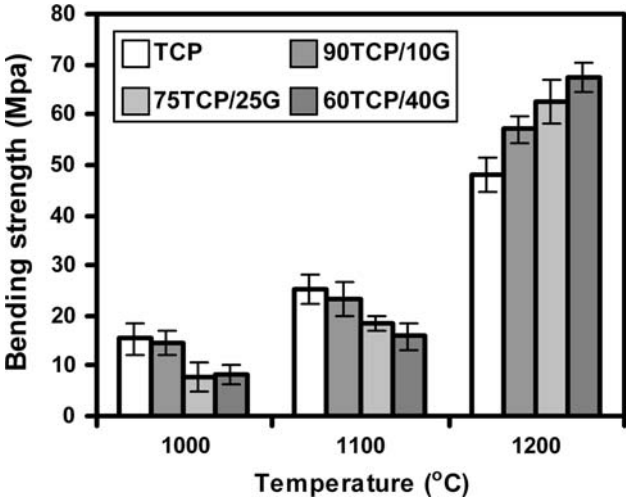

Fig. 7 Bending strength of TCP and TCP/G composites sintered at different temperatures

increased with increasing sintering temperature from 1100 to 1200°C. The use of bioglass phase did not significantly change the resistance of the materials to crack propagation, i.e. fracture toughness, indicating the brittle nature of the composites like to pure β-TCP. However, microhardness values of the TCP/G composites sintered at 1200°C were significantly higher than that of TCP and higher microhardness value was obtained using higher amount of bioglass phase.

The BS of all samples increased with increasing the firing temperature (Fig. 7). When the specimens were heated at 1000 or 1100°C, the BS decreased by incorporation of bioglass phase into TCP and further decrease in BS value was observed when higher amount of bioglass

phase was used. In contrast, for those samples sintered at 1200°C, the BS values of composite specimens were significantly ($P \leq 0.05$) higher than that of pure TCP and the maximum value belonged to 60TCP/40G specimen. These results suggest that the bioglass particles do not bond to TCP particles thoroughly at 1000 or 1100°C but decrease homogeneity (uniformity) of the composite microstructure, leading to decrease in mechanical strength. It is clear that the higher amount of bioglass phase deteriorate this situation. In contrast, sintering the samples at 1200°C favors formation of ceramic bonds between the β-TCP particles and bioglass phase through a viscous flow mechanism and interparticulate diffusion, leading to improve the bending strength.

The maximal mechanical properties of the β-TCP/G composites obtained in the present study may differ with those of other literatures [12]. Discrepancies can rise form the compositional variety of the glasses, which may influence their fluidity at elevated temperature, as well as differences in other processing parameters such as particle size of staring materials, pressing condition (uniaxial or isostatic), amount of pressure applied to the samples when forming discs/cuboids, and finally firing program (firing time, rate and temperature).

Typical surface micrographs of TCP and TCP/G composite (75TCP/25G) sintered at 1200°C have been illustrated in Fig. 8. Different morphologies were observed for different compositions. The microstructure of TCP (Fig. 8a) consisted of micropores and well grown grains resulted from the sintering process. A nearly homogenous distribution of β-TCP particles embedded within a crystallized bioglass phase was observed in the surface micrograph of TCP/G (Fig. 8b), which was indicative of a well accomplished sintering phenomenon in the presence of liquid phase.

The in vitro biological properties of the composite were not the goal of this study and are under investigation using different cell lines and experiments. However, the preliminary MTT test confirmed the better proliferation of G-292 cells in contact with TCP/G composites compared to pure TCP or control (polystyrene). The results are shown in Fig. 9. Both TCP and TCP/G composites increased the number of the cells, in a time-dependent manner. The number of the cells proliferated in contact with the composites were significantly higher than that of pure TCP. It is notable that the presence of Mg ions in the composition of the TCP/G materials and thus, in the composition of the culture medium can prefer the biological properties of the composites compared to pure β-TCP. It has been thoroughly found that magnesium ions can influence on both cell functions and bone formation metabolisms [19, 20]. Furthermore, it has been found that Mg is an important ion in the qualitative changes of bone matrix, determining

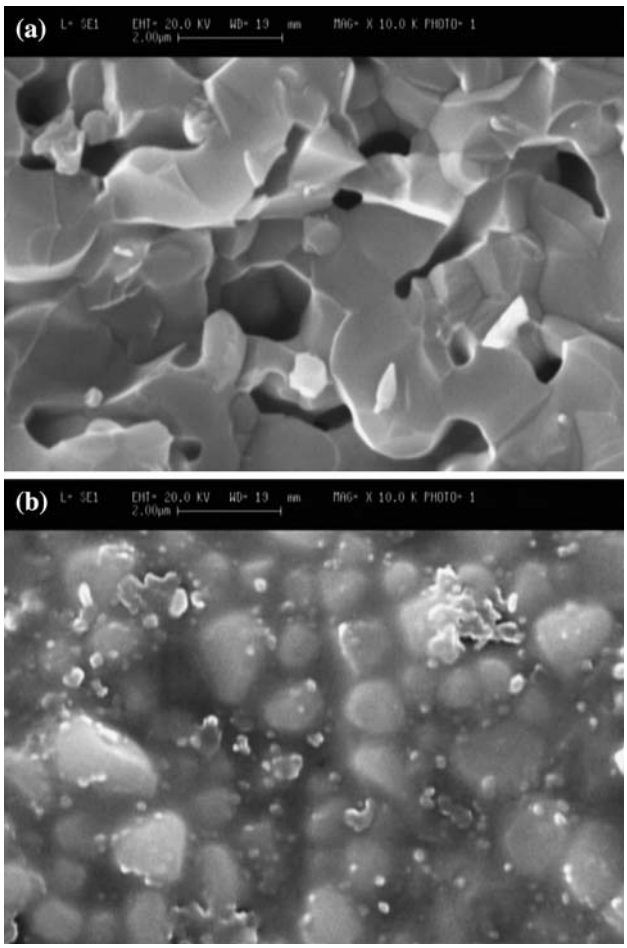

Fig. 8 Surface micrograph of β-TCP (**a**) and 75TCP/25G (**b**) sintered at 1200°C

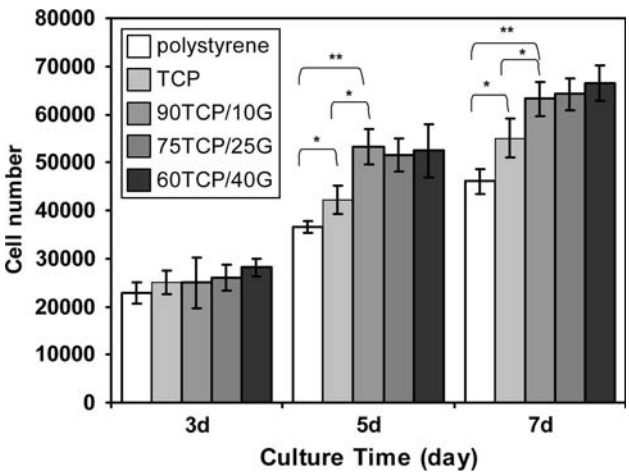

Fig. 9 Proliferation of the osteosarcoma cells grown in culture medium in contact with TCP or TCP/G composites (* $P < 0.05$, ** $P < 0.005$)

fragility of bones. Mg depletion adversely affects all stages of skeletal metabolism; causing cessation of bone growth, decrease of osteoblastic and osteoclastic activities and

osteopenia [21]. Moreover, the role of Si ions which can be released from the composite material into the culture medium should not be ignored. These ions can play an important role in cellular response. It has been documented in other studies that Si ions enhanced proliferation of osteoblastic cells and increased synthesis of collagen [22, 23].

5 Conclusions

It was concluded from the present study that incorporation of the sol–gel derived SiO_2–CaO–MgO–P_2O_5 bioglass powder into β-TCP produced biocomposites with improved mechanical strength and biological responses to osteoblastic cells compared to pure β-TCP. The mechanical strength of the composites was influenced by the quantity of the bioglass phase as well as firing temperature. Addition of the bioglass phase to β-TCP did not significantly change its brittleness. The results of this study may be used to indicate which compositions and processing parameters can provide better materials for the hard tissue replacement.

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