

THE EFFECT OF SINTERING TEMPERATURE ON MICROSTRUCTURE OF GLASS-REINFORCED HYDROXYAPATITE BIOCOMPOSITES IMMERSSED IN SIMULATED BODY FLUID

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Hydroxyapatite is a ceramic material which is known as a highly bioactive and biocompatible material. It can be extracted from natural resources such as bovine bone. In the present study, hydroxyapatite composites with different percentage of sodalime glass were made and sintered at different temperatures. Finally, the specimens were evaluated with respect to bioactivity tests in simulated body fluid (SBF). Micrographs of scanning electron microscopy (SEM) showed that calcium phosphate precipitated on biocomposites surfaces. SEM images and XRD analysis showed that the composite with 5 % glass additive sintered at 1200°C has excellent capacity to form apatite layer on its surface.

INTRODUCTION

Biomaterials research has been advancing in recent years, particularly in the field of tissue engineering. The main purpose in tissue engineering is the stimulation of the body's own mechanisms to reconstruct diseased or damaged tissue to its original state and function [1-3]. The aging of the population involves certain problems that were not so generic some time ago, since fewer people reached those ages where incidence of such diseases is more evident. Osteoporosis is a fine example; this disease attacks the bone as a consequence of a major lack of osseous mass. Science and technique are looking for solutions to the problems derived from an aging population. The use of biomaterials can repair some of these tissues. Nowadays, it is possible to manufacture implants for any part of body, except for the brain. Obviously, different types of materials are in use depending on the tissue to be replaced [4]. Calcium phosphates are primarily used as bone and dental substitution in biomedical industry due to their biocompatibility, low density, chemical stability, their compositional and structural similarities to the mineral phase of bone and teeth [5-9]. Hydroxyapatite (HA_p), $[\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2]$, belongs to a group of calcium phosphate which is widely used in tissue engineering, bone replacement and biomedical applications because of its high bioactivity, biocompatibility and good osteoconduction [10-16]. Hydroxyapatite can be extracted from natural resources such as eggshells, corals and ostrich eggshells. It can also be produced from bovine bone by a sequence of thermal processes or by annealing and sintering, since hydroxyapatite is the main inorganic

constituent of natural bone. Two advantages of this process are the removal of all organic components of bone and also it prevents the possibility of transmission of dangerous disease. This process could be an economic method for producing hydroxyapatite to be used as a biomaterial in orthopedic and dental implants [17, 18]. Even if biocompatibility of HA_p is excellent, its bioactivity can be improved. One way to increase it would be to use the ability of the apatite to accept many substitution ions in its unit cell. In this case, an interesting way to improve the bioactivity of hydroxyapatite is the addition of silicone to the apatite structure [19].

Therefore, in this study hydroxyapatite was produced from bovine bone. Then it was combined with sodalime glass by powder metallurgy method. Eventually, their bioactivity evaluation was carried out in simulated body fluid (SBF). The aim of the present work was to investigate the effect of glass additive on the microstructural evaluation of hydroxyapatite based composites with respect to bioactivity test.

EXPERIMENTAL

Materials

For production of HA_p , in order to remove all fat, soft tissues and visible external components, the bone was boiled in boiling water for 3-4 hours. Then by using a gas torch and applying direct flame to the cleaned bone, organic components were burned. The product of this thermal process contained some char due to burning of organic components. To remove the remaining char, the black powder (bone ash) was placed in an air furnace

at 850°C temperature for 2.5 hours and eventually it was cooled inside the furnace. According to an earlier report [17], this temperature is an optimum temperature for extraction of HA_p powder. Sodalime glass powder also was prepared by milling. Its composition is given in Table 1.

The SBF solution was bought with the composition given in Table 2.

Fabrication of samples

Pure natural hydroxyapatite (NHA_p) and also mixtures containing NHA_p with 2.5 and 5 wt. % glass additives were wet mixed in an attrition mill using ethanol. The feed ratio was 1 g powder to 10 g ball (1 to 10 weight ratio). To increase green compacts strength, about 4 % PVA solution was added. The speed was fixed at 480 rpm and milling time was set at 2 hours. These mixed powders were pressed at 200 MPa to form disc-shaped samples in a 20 mm diameter die. Green compacts were sintered in an air furnace at different temperatures 900, 1100 and 1200°C. The heating rate was 5°C·minute⁻¹ for 2 hours and finally samples were cooled inside the furnace. The diameters of the samples after sintering are shown in Table 3:

Tests

Phase analysis was carried out by x-ray diffraction (XRD, advanced D8) using cu K α over the 2 θ rang of 5 – 100° (step size = 0.05 and step time = 1 s). Microstructural studies of specimens were performed by scanning electron microscopy (SEM, Cambridge S360). In-vitro studies were conducted by soaking samples in SBF at 37°C for 20 days using incubator [20]. Besides, the pH value and the volume of the SBF solution used for each sample were 7.40 and 63 ml respectively.

RESULTS AND DISCUSSION

HA_p powder characterisation

The X-ray diffraction analysis of NHA_p powder (input material) which was calcined at 850°C for 2.5 hours is shown in Figure 1. X^{pert} software showed that

Table 1. The composition of sodalime glass.

Oxide	SiO ₂	Na ₂ O	CaO	MgO	Al ₂ O ₃	K ₂ O
wt. %	63.8	20.7	8.7	4.1	1.3	1.6

Table 2. Composition of SBF and inorganic part of human blood plasma.

Ion	Na ⁺	K ⁺	Mg ²⁺	Ca ²⁺	Cl ⁻	HCO ₃ ⁻	HPO ₄ ²⁻	SO ₄ ²⁻
Plasma (mmol·l ⁻¹)	142.0	5.0	1.5	2.5	103.0	27	1.0	0.5
SBF (mmol·l ⁻¹)	142.0	5.0	1.5	2.5	147.8	4.2	1.0	0.5

the main phase is hydroxyapatite and there is not any secondary phase such as α - or β -TCP. In other words, there was no phase transformation to secondary phases. Therefore, according to these results and the previous work [17], this heat treatment temperature is suitable to produce HA_p (characterised material).

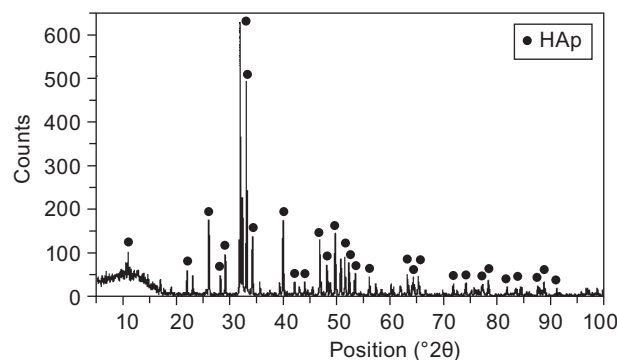


Figure 1. The XRD pattern of the NHA_p.

Microstructural examination

Before soaking

SEM images before soaking in simulated body fluid are illustrated in Figures 2-4:

These SEM micrographs are very similar to the SEM micrographs reported by the present authors previously [16]. However, the magnifications of the present micrographs are different from those reported before [16]. As SEM micrographs show there is no evidence based on calcium phosphate precipitates on granules in both pure and composite samples sintered at 900, 1100 and 1200°C without soaking. It can be said that SBF plays an essential role on precipitation.

It has been commonly accepted that the bioactivity of bioceramics relies on their ability to induce calcium phosphate formation generally and specifically hydroxyapatite in the physiological environment [21]. Thus, to emerge calcium phosphate precipitates samples have to be immersed in SBF.

Table 3. The diameters of the samples (mm) after sintering.

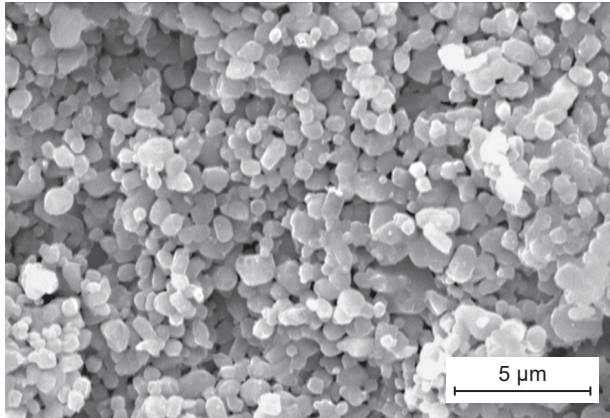
wt. %	Temperature (°C)		
	900	1100	1200
0	19.89	19.51	18.00
2.5	19.90	19.77	18.56
5	19.96	19.87	18.96

After soaking

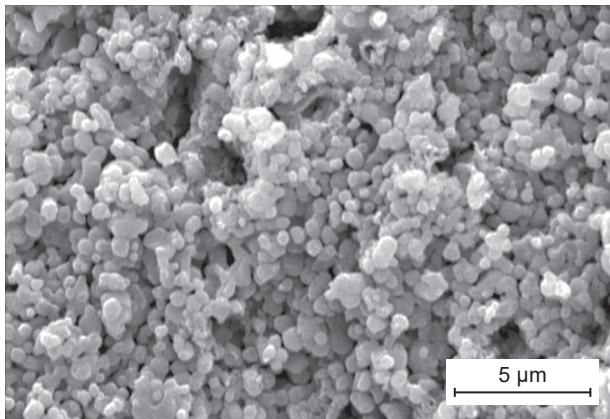
SEM micrographs of soaked specimens in SBF are given in Figures 5-7:

Based on SEM micrographs Ca-P precipitation appeared on all bioceramics with various morphology and amount after immersion in SBF. Calcium phosphate precipitation prefer to form on the surfaces with sharp curvature. This behaviour can be attributed to high levels

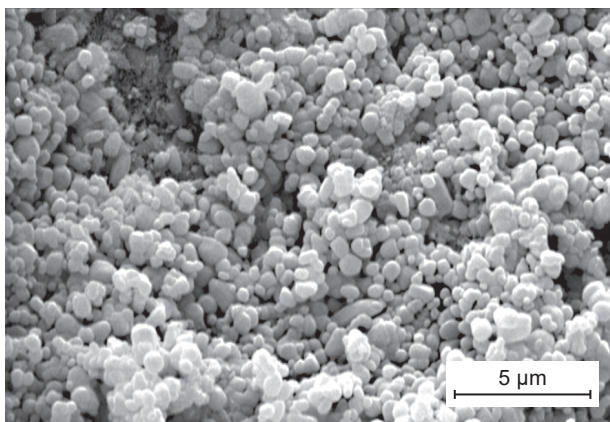
of energy in these areas. The flake-like morphology in Figure 5 expresses the first stage of Ca-P precipitation. These precipitates gradually are connected together to form spherical structures as shown in Figure 6 and 7. R. Xin et al. [22] have reported that each calcium phosphate granule consists of a large number of tiny flake-like crystals. Diana Horkavcova et al. [18] have expressed that the surface of bovine hydroxyapatite (HA-B) covered with new phase made of nanocrystalline plates of



a) pure HA_p

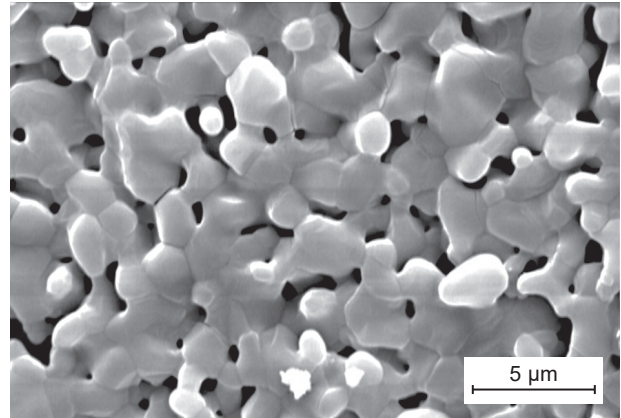


b) 2.5 %glass

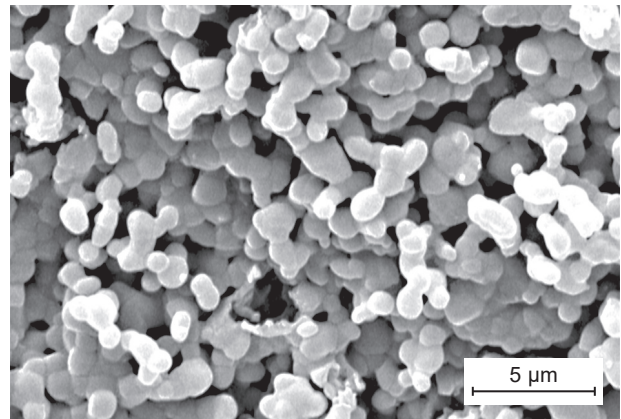


c) 5 % glass

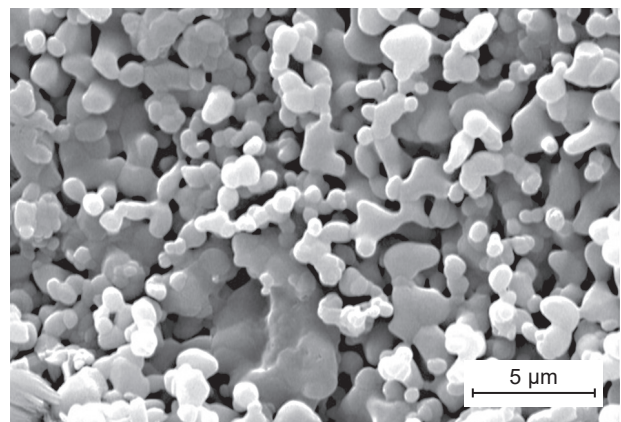
Figure 2. SEM micrographs of specimens before soaking in SBF with sintering temperature of 900 °C: a) pure HA_p, b) 2.5 % glass, c) 5 % glass.



a) pure HA_p



b) 2.5 %glass



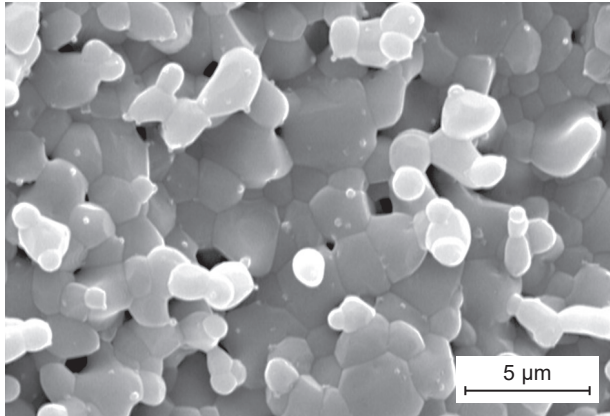
c) 5 % glass

Figure 3. SEM micrographs of specimens before soaking in SBF with sintering temperature of 1100°C: a) pure HA_p, b) 2.5 % glass, c) 5 % glass.

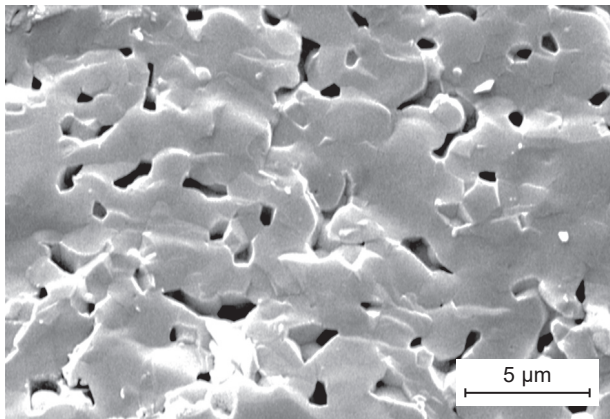
hydroxyapatite during the first 14 days of immersion in SBF. Similarly they found that the nanocrystals gradually formed a continuous layer [18]. Once the crystals are formed, they can grow spontaneously by consuming calcium and phosphate ions from the surrounding fluid [8].

In addition, SEM images examined by Radu Alexandru Roşu et al. [8] showed the germination of the biological hydroxyapatite after the immersion in SBF for

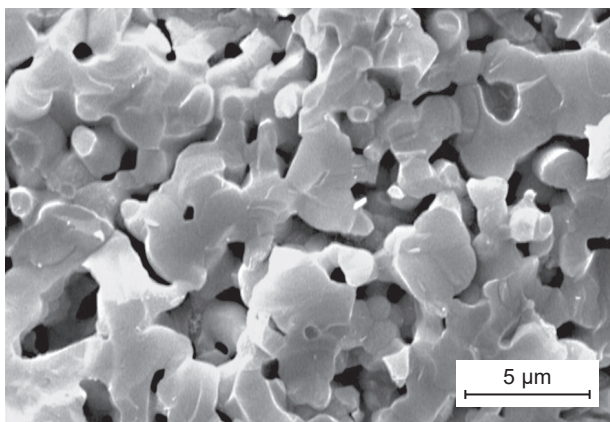
21 days. They found that an essential condition required for coated implants with bioactive materials, to obtain their contact with the living tissue, is the formation on their surface of an apatite layer which is similar to bone. M. U. Hashmi et al. [9] assessed the effect of sintering temperature on in-vitro behaviour of bioactive glass-ceramics by immersion the samples in SBF for thirty days at 37°C; hence, EDS results confirmed the bioactivity of all samples by showing the presence of carbon



a) pure HA_p

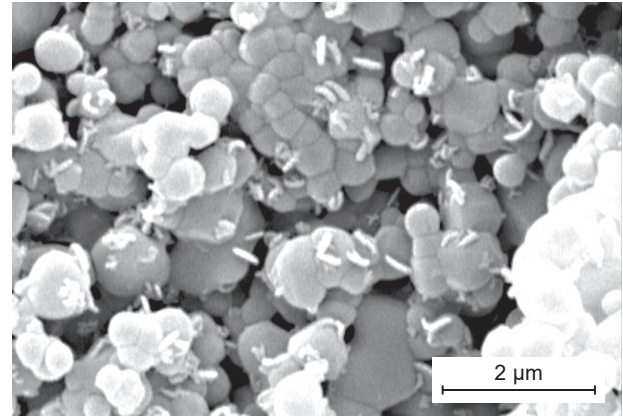


b) 2.5 %glass

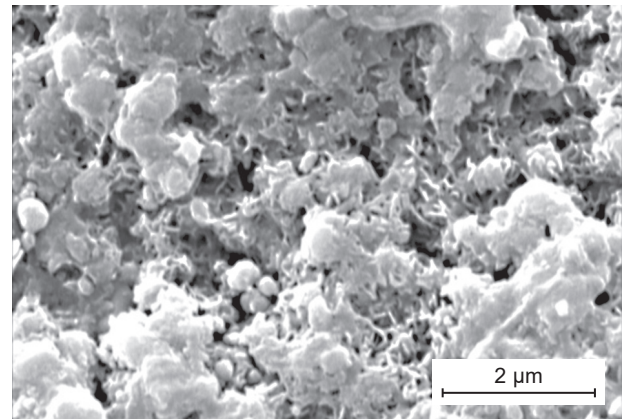


c) 5 % glass

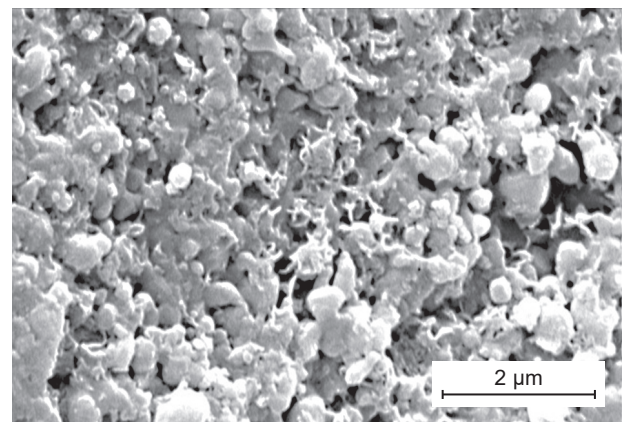
Figure 4. SEM micrographs of specimens before soaking in SBF with sintering temperature of 1200°C: a) pure HA_p, b) 2.5 % glass, c) 5 % glass.



a) pure HA_p



b) 2.5 %glass



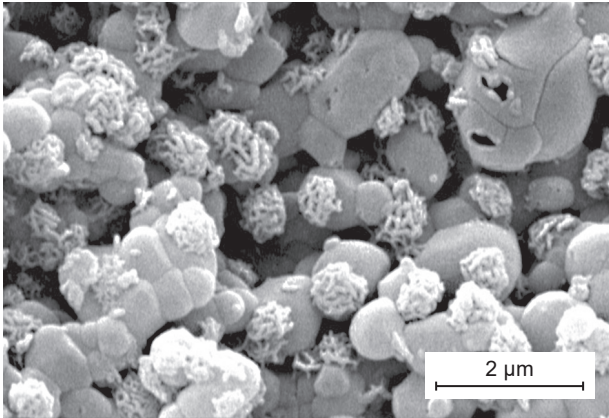
c) 5 % glass

Figure 5. SEM micrographs of soaked specimens with sintering temperature of 900°C: a) pure HA_p, b) 2.5 % glass, c) 5 % glass.

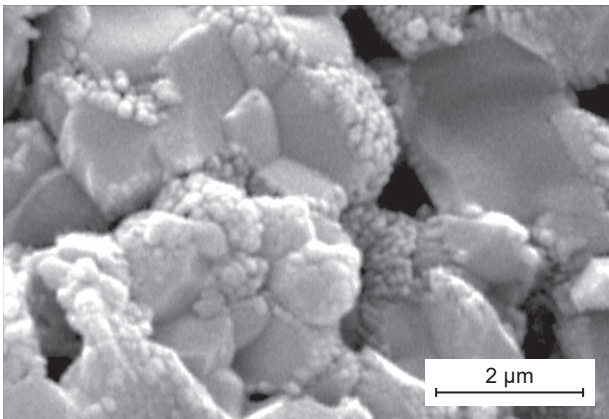
along with Ca and P corresponding to the formation of hydroxycarbonate apatite in physiological solution.

Hydroxyapatite formation does not occur on every type of bioceramics and it is less likely to occur on the surfaces of HA_p samples [22]. Because of sintering temperature there is not any secondary phase of β-TCP in Figure 5 and hydroxyapatite is the leading phase of specimens. Therefore, it can be said that the precipitates are not hydroxyapatite.

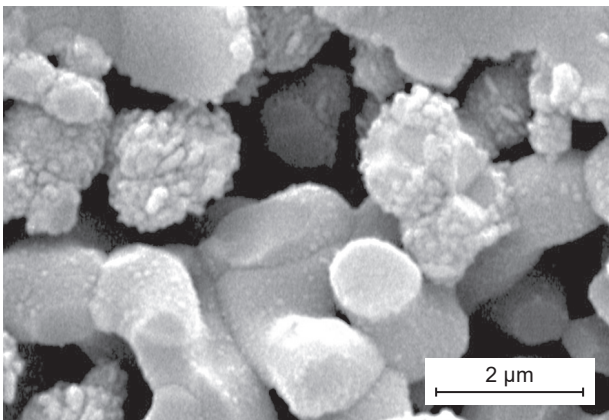
Moreover, hydroxyapatite is not the only phase of calcium phosphate which may be formed under physiological conditions. Other calcium phosphate phases such as octa calcium phosphate (OCP) and dicalcium phosphate dehydrate (DCPD) may form on the bioactive ceramic surfaces. OCP and DCPD are considered as precursors for HA_p formation. Theoretical analysis showed that the OCP nucleation could be much faster than HA_p in physiological environments [22].



a) pure HA_p

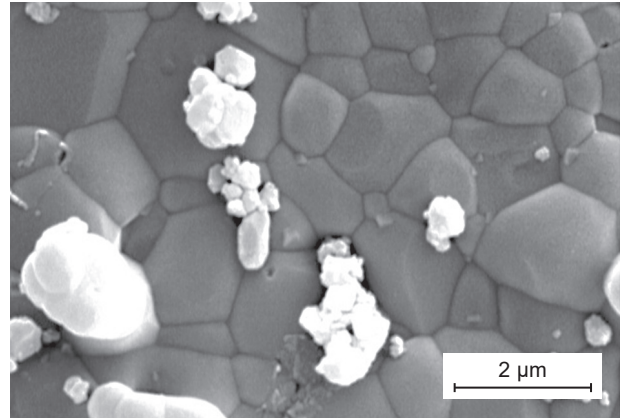


b) 2.5 % glass

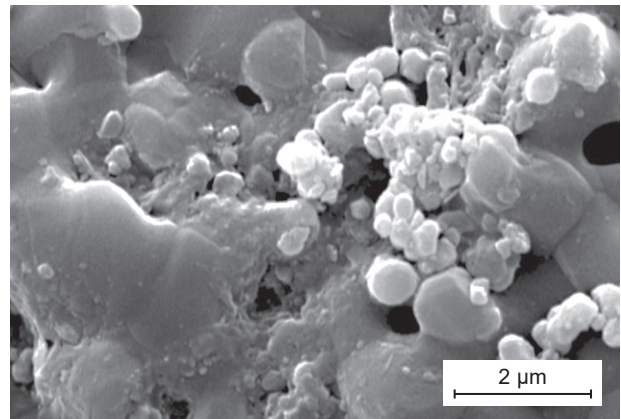


c) 5 % glass

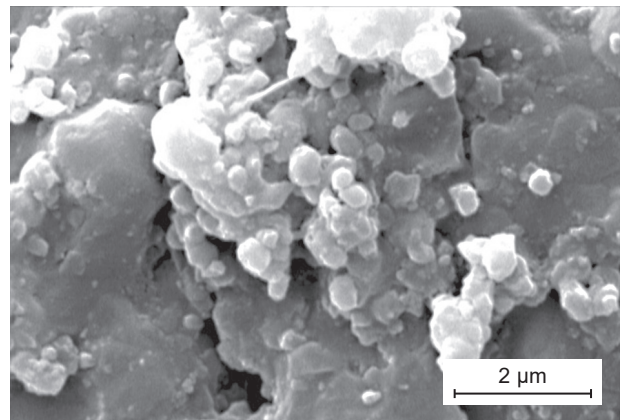
Figure 6. SEM micrographs of soaked specimens with sintering temperature of 1100°C: a) pure HA_p, b) 2.5 %glass, c) 5 % glass.



a) pure HA_p



b) 2.5 % glass



c) 5 % glass

Figure 7. SEM micrographs of soaked specimens with sintering temperature of 1200°C: a) pure HA_p, b) 2.5 % glass, c) 5 % glass.

Therefore, the precipitate in Figure 5 can be a precursor constituent of hydroxyapatite.

The ability of precipitation formation was increased with sintering temperature and glass additions by comparison of pure and glass reinforced specimens. Based on Figures 6 and 7 (b, c) there is a dense layer of calcium phosphate precipitation on the surface of specimens. Due to phase transformation some hydroxyapatite was converted into β -TCP above 1100°C temperature and finally biphasic calcium phosphate (BCP) appeared. As shown in Figures 6 and 7 biphasic bioceramics (HA_p/TCP) have high capacity to form ball-like Ca-P precipitation. R. Ravarian et al. [19] showed formation of apatite with ball-like morphology on the surface of their specimens.

The glass has a major effect on the structure of the HA_p. It is highly reactive at high temperatures and forces major chemical changes associated with the hydroxyl site. When HAp was used with glass, a silicated hydroxyapatite may have been produced which is much more bioactive than hydroxyapatite [19]. Based on Figure 7 (b, c) glass-reinforced composites sintered at 1200°C have good ability to form calcium phosphate precipitation. This is related to silicate hydroxyapatite existence with increasing sintering temperature and the glassy phase. Moreover, the ball-like morphology of calcium phosphate precipitates is obvious in Figure 7 showing more tendency to precipitate. Kaili Lin et al. [21] have showed that the ball-like particles consisted a fine structure form on surfaces after one day soaking in SBF.

Substitution of silicate ions with phosphate ions at ultra-trace levels will enhance the bioactivity of HAp through either their effect on surface chemistry or controlled local bioavailable Si release [19]. Some other reports [23] have shown the improved bioactivity and dissolution rate of HAp in the presence of silicon.

The weights of the samples before and after immersion in SBF solution are given in Table 4:

Table 4. The weights of the specimens (g) before and after soaking in SBF.

wt. %	Temperature (°C)					
	900		1100		1200	
	before	after	before	after	before	after
0	1.97	1.98	1.92	1.94	1.90	1.92
2.5	1.94	1.95	1.91	1.93	1.87	1.91
5	1.89	1.90	1.87	1.89	1.85	1.90

According to the results shown in Table 4 both pure and glass-reinforced composites had a weight increase after immersion in SBF solution due to apatite formation. It can be said that the mass of specimens increased with glass addition particularly at higher temperatures. For

instance, samples with 2.5 % and 5 % of glass addition at 1200°C had 2.14 and 2.70 percentage of mass increase respectively. Therefore, composites with 5 % glass additive sintered at 1200°C has shown the best result with respect to bioactivity studies; similarly, microstructural observations after immersion in SBF expressed that the amount of calcium phosphate precipitations was higher compared to other specimens; as a result, they are more biocompatible than other samples. XRD analysis was carried out to confirm the SEM observations because of HAp precipitation. Figure 8a, b give information on the XRD patterns of composites with 5 % glass additive sintered at 1100 and 1200°C temperatures respectively after immersion in SBF. XRD patterns show that the main phase was HAp synthesised (HAp, syn.) after 20 days immersion in SBF for both sintering temperatures. As the patterns illustrated both specimens have good capability for the synthesis of HAp on their surfaces, resulting from the existence of biphasic bioceramics (HA_p/TCP) at mentioned temperatures, however, the intensity of synthesised HAp (HAp, syn.) peaks are more pronounced regarding the sintering temperature of 1200°C.

The formation of HAp precipitations rises considerably with increasing sintering temperature and formation of biphasic bioceramics (HA_p/TCP) [21].

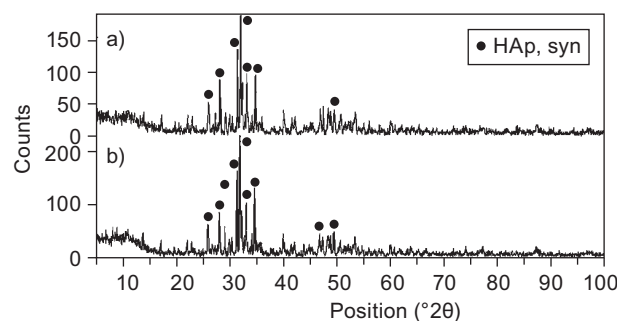


Figure 8. XRD patterns of 5 % glass-reinforced composite at: a) 1100°C, b) 1200°C after immersion in SBF.

CONCLUSION

Calcium phosphate precipitates formed on all specimen surfaces with different morphology. However, the ability of formation was increased by sintering temperature and glass additions. This behaviour originates from the substitution of silicon ions in HA_p structure and production of silicate hydroxyapatite at higher temperatures. XRD analysis and SEM images showed that the composite with 5% glass additive sintered at 1200°C has excellent ability to form apatite layer on its surface.

Acknowledgment

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