

## Reinforcement of sheep-bone derived hydroxyapatite with bioactive glass

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Productions, mechanical and microstructural analyses of bioglass/sheep hydroxyapatite (SHA) composites are introduced. Bioglass were added to calcinated SHA with various concentrations 5 and 10 wt. %. Powder mixture compacted with pressure (350 MPa) in steel moulds to obtain cylinder samples. As the next step, samples sintered in air at various temperatures (1000, 1100, 1200, 1300 °C for 4 hrs). Compression strength test, density and the Vickers microhardness (HV) measurements were carried out and microstructural characterizations were performed by scanning electron microscopy (SEM) and X-ray diffraction analysis (XRD) in order to determine optimum sintering temperature and mechanical properties. At last average hardness value  $1612 \pm 80$  HV, average density of  $2.65 \pm 0.31$  g/cm<sup>3</sup>, and compressive strength value of  $\sigma_{avr} = 99.14$  MPa were obtained by sintering at 1300 °C with the addition of 5 wt. % bioglass in SHA. Results show potential of brittle biomaterial but resistant to plastic strain.

**Key words:** Composites, Sheep bone, Hydroxyapatite (HA), Bioglass, Bioceramic.

### Introduction

Anticipated cost of hip, vertebral and wrist fractures treatments increased to \$10 billion per year in US and the number of operations including bone grafts surge around 4,000,000 per annum in worldwide due to natural aging process in years and increase in life expectancy comes with advances in medicine and technology. It is envisaged that in 27 of EU countries, life expectancy will increase and population around 65 years will be rise from 17% to 30% between 2010 and 2060. In addition to that population around 80 years will rise over 5% to 12% in same period [1]. With this statistics, demand in the biomaterial market also be expected to increase due to rising numbers of injuries ended with bone defects and bone fractures which are mostly effecting aged people.

As a standard treatment of these injuries, materials called allografts and autografts (defined as golden standards) are used to heal defects. However, these materials would not be a perfect choice from time to time due to problems like donor site scarceness, rejection by the immune system, insufficient resorption and transfer of the pathogens [1]. Due to these negativity, biomaterial market demands started to change. Therefore, aim of the studies done by researchers headed to composite materials which are consisting of two separate material or compound. The reason why these materials starting to become

crucial instrument in biomaterial market is because they are capable of showing good biocompatibility and mechanical features which are unreachable with usage of materials itself.

One of the mostly used and widely accepted biomaterial in skeletal and dental surgery practises is hydroxyapatite (HA). It has good biological features which allow conversion of some trace elements on its lattice and it has capability to form strong bond with the related tissue [2, 3]. Despite all, mechanical properties of pure HA are not good for using to produce synthetic bone structure particularly in wet environment. For this reason, they can only used in applications which don't need any excessive loadings [4]. However their composites with ceramics and metallic materials show promising mechanical properties, therefore they can be used to fill bone defects [5]. But choosing of production method of HA should be done intelligently. It can be produced chemically from natural sources but some prions can survive at the end of the production process. Another method of manufacturing HA is using of chemicals which is resulted with synthetic HA. But this method will be time consuming and expensive due to multistage steps [6]. Here, HA derived from sheep bones obtained by calcination method which is more time saving and economical than the chemical methods.

Bioglasses are another material used as biomaterial because of their high bioactivity both in vitro and in vivo, they are capable of forming valiant bonds on surface of contact of glass-bone structure. However, they have low fracture toughness, this characteristic delimitate the suitable applications for them. But they have higher fracture toughness and bioactivity than HA

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[5]. Due to that it's expected that composite of bioglass/HA will be more bioactive and resistant than just HA.

In this study, to improve mechanical properties of sheep derived hydroxyapatite (SHA), bioglass/SHA composites prepared by using different sintering temperatures and different weight percentage of 45S5<sup>®</sup> bioglass. In order to determine effect of bioglass, microstructural and mechanical properties of composites were investigated.

## Materials and Methods

### Materials

Method used to obtain SHA powders in this study explained before in the study of Oktar *et al.* [7] Briefly, the heads of fresh femurs obtained from fresh sheep bones were cut off. Remaining parts of the bones were deproteinized in the presence of NaOH and cleaned with the tap water. After the washing, bones were dried and calcinated at 850 °C for 4 hrs in air. Then, to get fine powder, calcinated parts crashed, ball milled and sieved through 100 µm sieve.

Bioglass used in this study also known as 45S5 has composition of 45 wt. % SiO<sub>2</sub>, 6 wt. % P<sub>2</sub>O<sub>5</sub>, 24,5 wt. % Na<sub>2</sub>O, 24,5 wt. % CaO. Compositions were installed in platinum crucible and heated to 1330 °C for 4 hrs. Afterwards, in order to get granular form, melted composition poured into water. Then, they were milled in a porcelain ball until getting powder type particle [5].

### Preparation of SHA-bioglass composites

SHA powders obtained from calcination were subsequently mixed with 5 and 10 wt. % of 45S5 bioglass content separately. Firstly, SHA and bioglass powders were blended together and wet milled for 3 hrs with a ball grinder (Retsch S100, Haan, Germany). Each mixture kept in drying-oven to get rid of excessive ethanol. Then, dried powder mixture compacted in steel moulds with hydraulic vertical press in order to obtain cylindrical samples according to British Standard 7253. Briefly powders pressed between steel dies under the 350 MPa pressure. After the compaction samples were sintered at 1000, 1100, 1200 and 1300 °C for 4 hrs (Nabertherm HT 16/1, Lilienthal, Germany).

### SHA-bioglass composite characterization

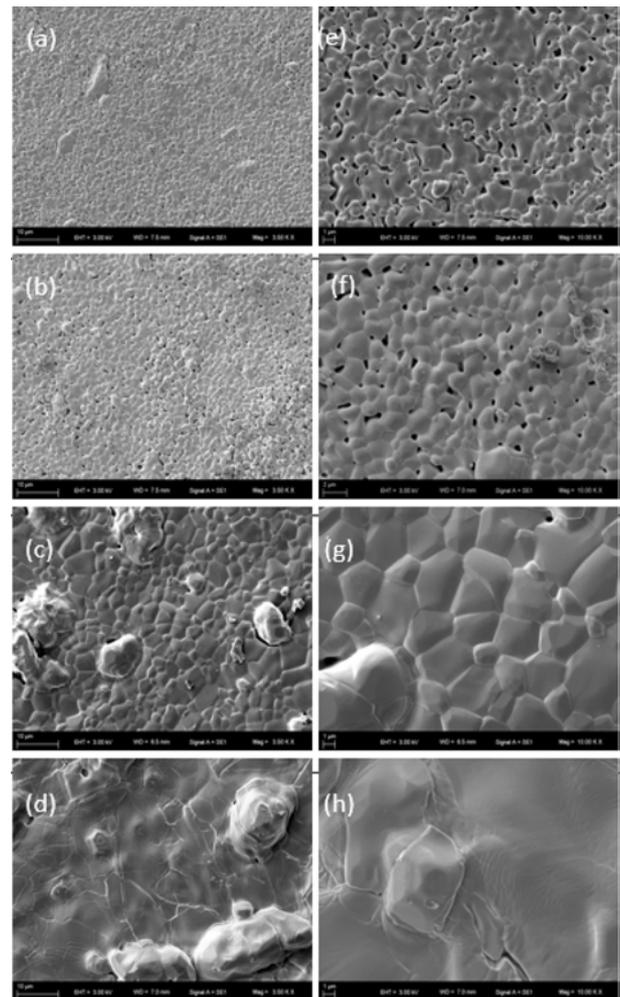
The microhardness measurements (Table 1) carried out with 300 g load applied with 20s of dwell time (Shimadzu HMV-2, Kyoto, Japan), compression test (Table 1) carried out with a 2 mm/min loading rate (Devotrans Universal Testing Machine, Istanbul, Turkey) and density of sintered samples (Table 1) were measured using an Archimedes method in order to determine optimum sintering temperature. Scanning electron microscopy (Evo LS10 SEM) and X-ray diffraction (XRD, Bruker<sup>™</sup>D8 Advanced Series Powder Diffractometer with CuKα (λ = 1.5406 Å)) analysis method were used to characterize microstructure and

phases.

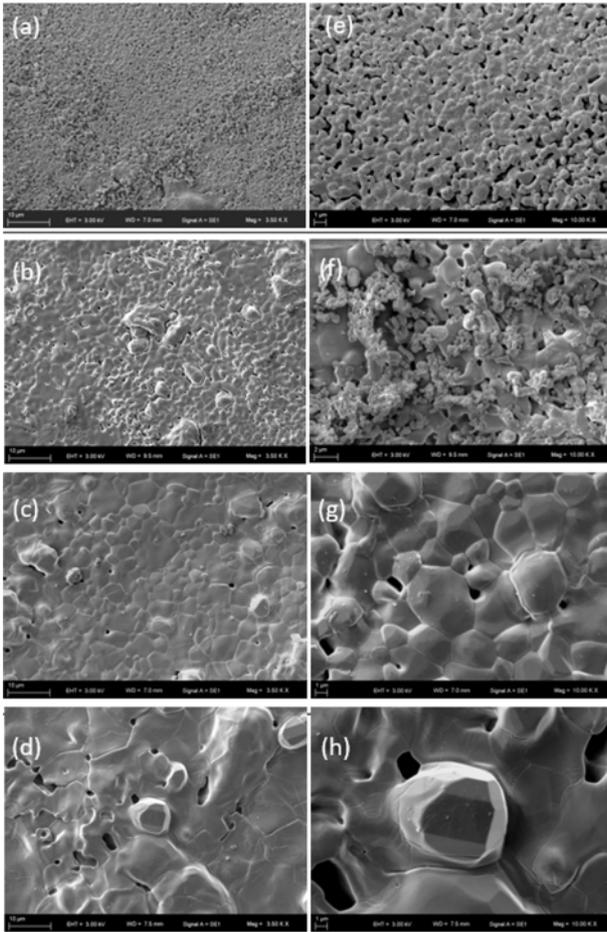
## Results and Discussion

Micro structure of 5 and 10 wt. % bioglass-SHA composites were given in Fig. 1 and Fig. 2 respectively. It is clearly visible that at 1200 and 1300 °C, glassy phase developed with the association with SHA structure. In addition to that, porosity occurred in all of the samples. Table 1 shows the experimental results of Vickers microhardness (HV), compression strength and density ( $\sigma$ ) of the samples sintered at different temperatures. Experimental results suggest that sintering at higher temperature makes the composite more improved in terms of mechanical properties.

Density of the bioglass-SHA composites were inverse proportional with the reinforcement content and directly proportional with the increasing sintering temperature. As it seems, with the rising temperature, 5 wt. % 1000 °C composite density increased from 2.15 g/cm<sup>3</sup> up to 2.65 g/cm<sup>3</sup> but it's not the same thing for increasing



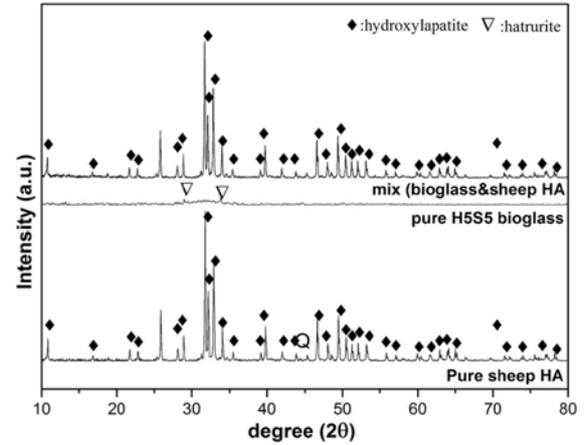
**Fig. 1.** SEM images of SHA composites with the addition of 5 wt.% bioglass sintered at 1000 °C (a,e), 1100 °C (b,f), 1200 °C (c,g), and 1300 °C (d,h) for 4 hrs in air.



**Fig. 2.** SEM images of BHA composites with the addition of 10 wt.% bioglass sintered at 1000 °C (a,e), 1100 °C (b,f), 1200 °C (c,g), and 1300 °C (d,h) for 4 hrs in air.

reinforcement content. Same sample density decreased to  $2.13 \text{ g/cm}^3$  from  $2.15 \text{ g/cm}^3$  with the 10 wt. % bioglass addition. This is caused by sintering behavior of SHA and extensive glassy phase occurred at higher temperatures, which wets the particles and seals the pores and made the material denser.

Furthermore, microhardness values of the bioglass-SHA composites increased both with the sintering temperature and reinforcement content. As seen in Table 1, increasing the sintering temperature of 5 wt.% bioglass composite from 1000 °C to 1300 °C causes an increment of the hardness of composite from 165,33



**Fig. 3.** XRD patterns of pure SHA, pure bioglass, and mix of bioglass /SHA.

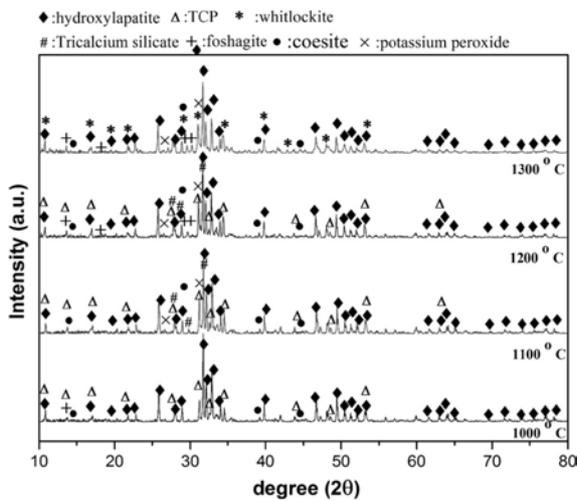
HV to 1612 HV. Similar increasing in the difference can also be visible for 10 wt% bioglass composite. Increasing the sintering temperature from 1000 °C to 1300 °C causes an increment in hardness values of composite from 488 HV to 1310,66 HV. The microhardness of pure hydroxyapatite derived from sheep bones was investigated in a previous study and it was found 189 HV which is notably lower than in the present study [8]. This improvement might be due to the finer grain size of SHA and bioglass. Also, it shows that bioglass supports SHA with its higher fracture toughness.

In addition to that, alteration of compressive strength for both reinforcement content and sintering temperatures is given in Table 1. Increasing the reinforcement content has a negative impact on compressive strength while increasing sintering temperatures has the opposite effect. At 1000 °C, 5 wt% bioglass composite has a 60,47 MPa compressive strength while 10 wt% bioglass composite has a 33.26 MPa compressive strength. Previous studies of Gunduz *et al.* demonstrate that the existence of a relatively high glassy phase at delicate concentrations can lead to reduced compression values when compared to lower percentages of reinforcement material [9].

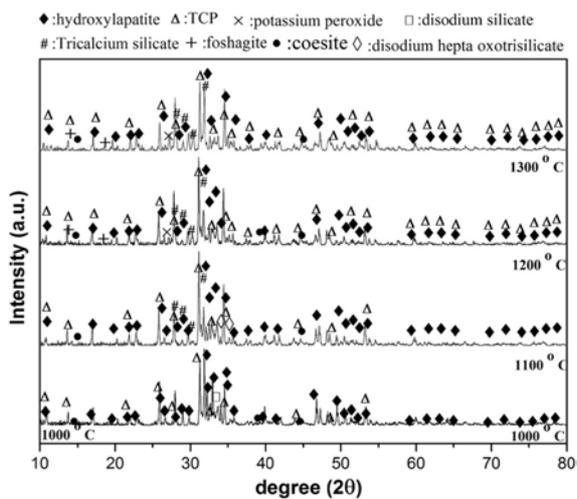
Fig. 1 and Fig. 2 show scanning electron micrographs of various bioglass-SHA samples sintered at various temperatures and prepared with 5 wt% and 10 wt% bioglass addition. Common microstructure formations were generally observed for all samples. Small

**Table 1.** Mechanical properties of composites sintered at different temperatures (1000 °C, 1100 °C, 1200 °C, and 1300 °C).

T (°C)	d (g/cm <sup>3</sup> )		σ (MPa)		Hardness (HV)	
	5 wt%	10 wt%	5 wt%	10 wt%	5 wt%	10 wt%
1000	2.15 ± 0.24	2.13 ± 0.2	60.47 ± 9.3	33.26 ± 4.2	165.33 ± 15	488 ± 43.5
1100	2.36 ± 0.81	2.35 ± 0.19	72.26 ± 34.8	38.70 ± 8.6	374 ± 87.22	612.33 ± 44.4
1200	2.61 ± 0.16	2.45 ± 0.29	78.21 ± 51.1	52.44 ± 7.1	1272.66 ± 74	774.33 ± 64.4
1300	2.65 ± 0.31	2.51 ± 0.03	99.14 ± 65.7	63.14 ± 9.7	1612 ± 80	1310.66 ± 164.6



**Fig. 4.** XRD patterns of SHA composites doped with 5 wt. % bioglass and sintered at 1000, 1100, 1200 and 1300 °C.



**Fig. 5.** XRD patterns of SHA composites doped with 10 wt. % bioglass and sintered at 1000, 1100, 1200 and 1300 °C.

particles which are connected poorly detected at lower sintering temperatures while perfect grain growth occurred at high temperature sintered samples and it can clearly visible both at 1200 °C and 1300 °C. This grain growth covered up the porosities and made the composite more dense. Low porosity rate makes possible to use this composite in orthopedics. For both bioglass addition it can be seen bioglass particles mixed with SHA but after 1200 °C, glass phase started to occur. As with the growing temperature and concentration of bioglass, grain growth and grain boundaries were much more evident than lower temperatures.

The XRD analysis of the pure materials given in Fig. 3, diffractograms of the composites with 5 and 10 wt.

% bioglass addition which were sintered at 1000, 1100, 1200 and 1300 °C, shown in Fig. 4 and Fig. 5. Results of XRD analysis indicates that, pure bioglass contains the hatrurite phase which has  $\text{Ca}_3\text{SiO}_5$  chemical formula. Similar phase was found in the study carried out by Ashuri *et al.*  $\text{Ca}_2\text{SiO}_4$  phase found in sol-gel derived bioactive glass which is very similar to hatrurite [10]. XRD peaks of composites showing that, main peaks are similar and contains hydroxyapatite (HA) and partial decomposition of HA to tricalcium phosphate (TCP), beside of that only in 5 wt. % bioglass addition with 1300 °C sintering temperature has whitlockite ( $(3\text{CaO}\cdot\text{P}_2\text{O}_5)$ ) phase which is another form of calcium phosphate like TCP. Whitlockite phase found in previous studies at higher temperature sintering of bovine bones [11]. Weaker peaks showing variety of phases like foshagite ( $\text{Ca}_4\text{Si}_3\text{O}_9(\text{OH})_2$ ), tricalcium silicate ( $\text{Ca}_3\text{SiO}_5$ ), coesite ( $\text{SiO}_2$ ) which were occurred as minor phases during sintering. There is no information for foshagite in biomaterial literature. Tricalcium silicate has another name called alite, which is formulated as  $3\text{CaO}\cdot\text{SiO}_2$  and it contains different oxides besides CaO and  $\text{SiO}_2$ . It has different polymorphic states depending on temperature, below 1100 °C it has monoclinic crystal formation and above 1100 °C it has rhombohedral crystal formation [12]. Other weak phase called foshagite has  $\text{Ca}_4\text{Si}_3\text{O}_9(\text{OH})_2$  chemical formula and monoclinic crystal structure. It has brittle character which can reinforce composite mechanical properties [13]. With presence of heat, minor phases transform to each other and major phases which have similar compounds in their structure.

## Conclusions

The characterization and preparation of bioglass- SHA composites have 5 wt% and 10 wt% bioglass addition were successfully achieved. The samples which are sintered at 1000, 1100, 1200 and 1300 °C showed that it has potential to used as biomaterial composite. Compression strength and the microhardness values could show versatility depending on concentration of reinforcement bioglass content and sintering temperature. Optimal conditions for production identified based on obtained results.

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## References

1. O. Gunduz, C. Gode, Z. Ahmad, H. Gokce, M. Yetmez, C. Kalkandelen, Y.M. Sahin, F.N. Oktar, *J. Mech. Behav. Biomed. Mater.* 35 (2014) 70-76.
2. O. Gunduz, *J. Austral. Ceram. Soc.* 50[2] (2014) 52-58.
3. F. N. Oktar, G. Goller, *Ceram. Int.* 28[6] (2002) 617-621.
4. S. Salman, O. Gunduz, S. Yilmaz, M.L. Ovecoglu, R.L. Snyder, S. Agathopoulos, F.N. Oktar, *Ceram. Int.* 35[7] (2009) 2965-2971.
5. G. Goller, H. Demirkiran, F.N. Oktar, E. *Ceram. Int.* 29[6] (2003) 721-724.
6. G. Goller, F.N. Oktar, L.S. Ozyegin, E.S. Kayali, E. Demirkesen, *Mater. Lett.* 58[21] (2004) 2599-2604.
7. F.N. Oktar, S. Agathopoulos, L.S. Ozyegin, O. Gunduz, N. Demirkol, Y. Bozkurt, S. Salman, *J. Mater. Sci.: Mater. in Med.* 18[11] (2007) 2137-2143.
8. N. Demirkol, F. N. Oktar, E. S. Kayali, *Acta Phys. Polonica.* 121[1] (2012) 274.
9. O. Gunduz, Z. Ahmad, N. Ekren, S. Agathopoulos, S. Salman, F. N. Oktar, *J. Thermoplas. Comp. Mater.* 22[4] (2009) 407-419.
10. M. Ashuri, F. Moztafzadeh, N. Nezafati, A.A. Hamedani, M. Tahriri, *Mater. Sci. Eng. C.* 32[8] (2012) 2330-2339.
11. A. Dobrádi, M. Enisz-Bódogh, K. Kovács, I. Balczár, *Ceram. Int.* 41[3] (2015) 4874-4881.
12. J.P. Bournazel, Y. Malier, M. Moranville Regourd, in *Proceedings of the RILEM International Conference, Arles, September (1996).*
13. J.W. Anthony, R.A. Bideaux, K.W. Bladh, M.C. Nichols, *Handbook of Mineralogy, Vol. IV, Mineral Data Publishing (2000).*