Int. J. Adv. Eng. Pure Sci. 2021, 33(1): 116-121
DOI: 10.7240/jeps.771433

RESEARCH ARTICLE / ARAŞTIRMA MAKALESİ

Microstructure and Mechanical Properties of Composites of Bovine Derived Hydroxyapatite (BHA) Reinforced with MgF_2

MgF2 ile Güçlendirilmiş Sığır Kaynaklı Hidroksiapatit (BHA) Kompozitlerinin Mikroyapısı ve Mekanik Özellikleri

Mustafa ŞENGÖR [1]

[1] *Marmara Üniversitesi, Teknoloji Fakültesi Metalurji ve Malzeme Mühendisliği Bölümü, 34722, İstanbul, Türkiye*

Abstract
Composites of calcinated bovine bone derived hydroxyapatite (BHA) doped 1 and 2 wt% MgF_2 were prepared by a sintering process. Microstructure and crystallographic analyses along with measurements of density, compression strength, and microhardness were carried out in the produced samples. The experimental results indicated a beneficial effect of MgF_2 in the matrix of BHA reflected in the significant increase of compression strength and microhardness up to 143 MPa and 313 HV, respectively, achieved after sintering at 1300 ^{0}C for 2% MgF_2 addition. The presence of MgF_2 reduced the onset of sintering towards lower temperatures (i.e. 1100 ^{0}C) and increased the stability of hydroxyapatite towards transformation to TCP at 1300 ^{0}C. The influence of Mg^{2+} and F^- ions in the lattice of hydroxyapatite is discussed.
Keywords: Bovine derived hydroxyapatite; MgF_2; sintering; mechanical properties; microstructure.

Öz
Kalsine edilmiş sığır kemiğinden elde edilen hidroksiapatit (BHA) ile ağırlıkça% 1 ve ağırlıkça% 2 MgF_2 katkılı kompozitleri bir sinterleme işlemi ile hazırlandı. Üretilen numunelerde mikroyapı ve kristalografik analizler ile birlikte yoğunluk, basma mukavemeti ve mikrosertlik ölçümleri yapıldı. Deneysel sonuçlar, % 2 MgF_2 ilavesi ve 1300ºC'de sinterlemeden sonra elde edilen BHA matrisin, sıkıştırma mukavemeti ve mikro sertlikteki önemli artışa (sırasıyla 143 MPa ve 313 HV'ye kadar) neden olduğunu gösterdi. MgF2'nin varlığı, sinterleme başlangıcını daha düşük sıcaklıklara (~ 1100ºC) düşürdü ve hidroksiapatitin 1300ºC'de TCP'ye dönüşüme karşı stabilitesini arttırdı. Mg_2^+ ve F^- iyonlarının hidroksiapatit örgüsündeki etkisi tartışıldı.
Anahtar Kelimeler: Sığır hidroksiapatiti; MgF_2; sinterleme; mekanik özellikler; mikroyapı.

I. INTRODUCTION

The facts of life prolongation due to health care and the increase of sport and traffic accidents, in conjunction with the well-known intrinsic problems of autografts and allografts (e.g., availability, cost, viability, reproducibility), emerge the technology of biomaterials as to be of high social impact for the individuals, both elderly and young people, and the governments, especially the gross income of developed countries. In the case of hard-tissue prostheses and tissue engineering, hydroxyapatite (HA, $Ca_{10}(PO_4)_6(OH)_2$), that is the mineral of bones and teeth, is perhaps the most popular ceramic compound nowadays used in clinical applications, since it exhibits excellent biocompatibility and ability to establish direct bonding to natural bones. Comparing to synthetic HAs, biological apatites feature several substitutions at the Ca^{2+}, PO_4^{3-} and OH^- sites of HA-lattice [1].

Composite materials with key bio-ceramic inclusions are preferred in many clinical applications. Bone is a composite structure of our body based on crucial element of HA and calcium phosphates. Synthetic, artificial bone composite materials usually used a host polymer with calcium phosphates. Some of the problems that will arise are due to the compatibility issues, osseointegration problems and mechanical performances [2].

As far as the application of HA in biomedicine is concerned, the brittle nature of HA limits its use only in coatings or low-load bearing applications. Accordingly, the reinforcement of HA matrix will expand the applications of this highly osseointegrating and conductive material. Reinforcing bioinert ceramics like Al_2O_3, TiO_2, ZrO_2 and MgF_2 to increase mechanical performances of HA [3]. In the present study, the influence of MgF_2-doping (1-2 wt%) on reinforcing sintered HA materials were investigated, where HA was derived from calcined bovine bone (BHA). The reinforcing effect of the dopant is assumed to be a result of the developed microstructure and the

Corresponding Author: Mustafa ŞENGÖR, Tel: +90 (216) 777 3954, e-posta: mustafa.sengor@marmara.edu.tr
Submitted: 19.07.2020, **Revised:** 29.10.2020, **Accepted:** 29.10.2020

crystalline phases formed after sintering. Magnesium is a body-friendly material with an essential role in the human body [4], for instance at reducing risks of cardiovascular diseases, promoting catalytic reactions, controlling biological functions, and in the mineralization of calcined tissues. Approximately 60 to 70% of total body's Mg-content is in the skeleton, as substitution ion in HA lattice. Approximately 0.38 wt% of cattle bone is Mg [4]. Mg^{2+} ions seemingly affect the conductivity of osteoclasts and osteoblasts [5]. Traces of Mg in natural bone affect cell adhesion and bone formation [6]. Hence, several compounds of Mg have been considered in biomedicine, such as bredigite ($Ca_7MgSi_4O_{16}$, considered as similarly bioactive to $CaO\text{-}SiO_2$), forsterite (Mg_2SiO_4), and enstatite ($MgSiO_3$, considered as machinable biomaterial) [7].

With respect to HA composites, the presence of Mg in HA is related to apatite crystallization and stability, such as the thermal conversion to β-tricalcium phosphate (ß-TCP, $Ca_3(PO_4)_2$). Lim and Kim *et al.* doped HA with 1% MgO [8], MgF_2 and CaF_2 [9] and found that substitution of F^- for OH^- caused an enhancing effect on cell culture tests. The same occurred in the case of MgF_2 comparing to CaF_2. From the same study, using HA-20% Al_2O_3 doped with 5% MgF_2, HA-dissociation was entirely inhibited below 1400°C. The effect of fluorine was also verified by Evis *et al.*, in terms of increasing chemical stability, thermal stability and hardness, who produced HA-composites by doping with partially stabilized zirconia and MgF_2 or MgO [10]. The presence of MgF_2 in HA matrix favors the reduction of porosity of sintered bodies and the formation of secondary phases (i.e., α-TCP) after sintering at 1400°C [11].

II. MATERIALS AND EXPERIMENTAL PROCEDURE

The powder of bovine derived hydroxyapatite, BHA, was produced according to the method described in earlier studies [12-17]. In brief, freshly cut femurs were deproteinized with 4 N NaOH solution (48 h). After re-irrigation, the samples were subjected to heat treatment at 850 ^{0}C for 4 h in open air to securely eliminate any risk of fatal disease transmission [18]. The calcinated BHA bulk pieces were properly ball-milled until fine (<10 μm) BHA powder was obtained.

The BHA fine powder was mixed with 1 and 2 wt% MgF_2 powder (Sigma Aldrich, 99.9% purity, 3-6 mm particle size). The powder mixture was well homogenized by planetary ball-milling in acetone media using a zirconium oxide (yttrium stabilized) jar and balls (Retsch PM 100 SM 100, Haan, Germany). The resultant powders were well dried. Cylindrical pellets were prepared by uni-axially pressing at 350 MPa according to British Standard BS 7253. The green pellets were sintered at 1000, 1100, 1200, and 1300 ^{0}C for 4 h in an open air furnace (Nabertherm HTC 3 /14, Lilienthal, Germany) with heating and cooling rates of 5 K/min.

The compressive strength of the sintered samples was measured in a universal testing machine (Instron-8511, Norwood, MA 02062-2643, USA, displacement 2 mm/min). Their Vickers microhardness was measured in a Shimadzu testing unit (Shimadzu Inc., HMV-2, Japan, load 200 g). Density was measured by Archimedes method by employing a pycnometer (ULTRAPYC 1200e, Quantachrome, Boynton Beach, Florida 33426, USA). The presenting results were obtained from 10 different samples. The microstructure of the samples was observed in a field emission scanning electron microscope (FE-SEM, Hitachi S-4100, Japan) under secondary electron mode (Figure1). The crystalline phases were identified by X-ray diffraction analysis (XRD, Rigaku Geigerflex D/Mac, C Series, Japan, Cu K_a radiation 1.5406 nm produced at 30 kV and 25 mA, 2θ step 0.02 ^{0}/s).

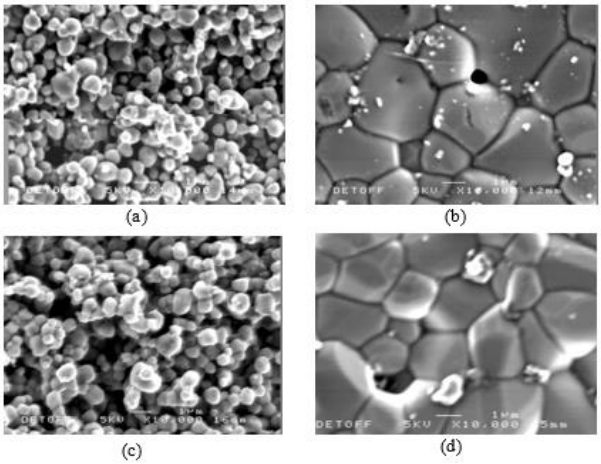

Figure 1. Microstructure of BHA-MgF_2 composites sintered at different temperatures: (a) BHA-1 wt% MgF_2 1000 ^{0}C, (b) BHA-1 wt% MgF_2 1300 ^{0}C, (c) BHA-2 wt% MgF_2 1000 ^{0}C, (d) BHA- 2 wt% MgF_2 1300 ^{0}C.

III. RESULTS AND DISCUSSION

Table 1 presents the experimental results (and their standard deviation) of density (d), compressive strength (σ), and Vickers microhardness (HV) of the produced BHA-MgF_2 composites sintered at different temperatures. Tables 2 and 3 were prepared with results reported in earlier studies for similarly produced samples of BHA doped with CaF_2 (0.5 and 1.0 wt%) [19], and pure BHA [20]. Table 3 in conjunction with the results of earlier studies [21] indicates 1200 and 1300 ^{0}C as the optimum sintering temperatures with respect to the maximum strength of pure BHA-materials obtained. The results of compressive strength of Table 1 indicate that doping of BHA with MgF_2 shifts sintering onset towards lower temperatures. This effect is more pronounced in the case of lower (1 wt%) amount of MgF_2. This result has higher significance since the samples have still low densification degree at

1100 ^{0}C, which means that there are porosity and little formation of glassy phase (due to eutectic melting) that closes the pores and increases microhardness. Therefore, if moderate compressive strength is required in a specific application, then MgF_2-doping can result in a suitably strong scaffold of small porosity (i.e., low densification) that maintains all the features of calcinated BHA before the occurrence of the strong effect of extensive sintering.

Table 1. Influence of MgF_2-content (1 and 2 wt%) on density (d), compression strength (σ) and Vickers micro-hardness (HV) of BHA-MgF_2 composites sintered at different temperatures (the standard deviation of density was <0.001 g/cm^3).

T (ºC)	d (g/cm^3)		σ (MPa)		HV	
	1 wt%	2 wt%	1 wt%	2 wt%	1 wt%	2 wt%
1000	2.181	2.156	35.5 ± 2.1	29.4 ± 6.6	115 ± 94	139 ± 7
1100	2.281	2.165	83.5 ± 8.6	45.9 ± 7.0	168 ± 15	155 ± 13
1200	2.611	2.446	109.0 ± 3.1	63.5 ± 15.6	289 ± 14	231 ± 14
1300	2.976	2.884	125.0 ± 4.5	143.0 ±27.48	291 ± 14	313 ± 22

However, Table 1 shows that sintering successfully takes place at 1200 and 1300 ^{0}C. The highest values of the measured magnitudes were obtained at 1300 ^{0}C. This means that the glassy phase formed, due to the presence of MgF_2 and the phenomenon of sintering itself, did not jeopardize the properties of the resultant composites, whereas the properties of pure BHA samples (Table 3) slightly decay at 1300 ^{0}C (than 1200 ^{0}C). The presence of MgF_2 should favor the formation of strong glassy phase at 1300 ^{0}C since the highest values of compression strength and hardness were achieved in the case of 2 wt% MgF_2 than 1%. The slightly lower values of density in the samples with 2 wt% than 1 wt% may not represent poorer densification, but they are due to the lower density of MgF_2 than BHA (so, the higher amount of MgF_2 added, the lower density of the resultant composite – densification results of BHA doped with the heavy CaF_2, shown in Table 2, support this consideration).

Table 2. Influence of CaF_2-content (0.5% and 1.0%) on density (d) and compression strength (σ) of BHA-CaF_2 composites sintered at different temperatures reported in an earlier study [19].

T (ºC)	d (g/cm^3)		σ (MPa)	
	0.5%	1.0%	0.5%	1.0%
1000	2.893 ± 0.01	3.117 ± 0.01	31.8 ± 9.1	15.4 ± 4.4
1100	3.002 ± 0.01	3.074 ± 0.03	42.7 ± 7.4	16.7 ± 3.9
1200	3.152 ± 0.03	3.134 ± 0.05	48.3 ± 10.2	28.2 ± 13.3
1300	3.026 ± 0.01	3.183 ± 0.01	35.5 ± 14.5	36.0 ± 4.6

In general, doping with MgF_2 caused a considerable increase in compression strength and hardness of BHA. The low density of MgF_2 and the small amount of MgF_2 needed to achieve high mechanical properties are two extra advantages since the final implant material will be both strong and with low weight. The superior effect of MgF_2 is clear by comparing the results of Table 1 with the poor mechanical properties of the BHA samples doped with CaF_2, assuming the similar chemical nature of the two alkaline earth ions of Mg and Ca.

Table 3. Mechanical properties (compression strength, σ and Vickers micro-hardness, HV) of BHA samples sintered at different temperatures reported in earlier studies [20].

T (ºC)	BHA		BHA	
	σ (MPa)	HV	σ (MPa)	HV
1000	12 ± 2	42 ± 2	48.2 ± 20.0	85.4 ± 9.4
1100	23 ± 3	92 ± 4	22.2 ± 5.1	74.2 ± 19.8
1200	67 ± 7	138 ± 3	75.2 ± 18.3	148.5 ± 10.5
1300	62 ± 11	145 ± 3	65.0 ± 41.6	130.7 ± 17.9

From the literature, BHA-MgO composites sintered at 1300 ^{0}C have reached compression strength of 121.85 and 98.23 MPa [6] and microhardness of 305 and 218 HV [8] for 5 and 10 % MgO, respectively. Evis *et al.* found that HA composites doped with 5 % MgF_2 have higher hardness than those with 5 % MgO [11]. They postulated that the fluorine ions of MgF_2 diffuse into the lattice of HA and result in increased thermal stability and mechanical properties of the sintered materials. Similar high values have been reported for BHA-TiO_2 composites (at 1300 °C 105 MPa and 204 HV for 5% TiO_2 and 247 HV for 10% TiO_2, and at 1200 °C 104 MPa for 10% TiO_2) [22]. However, the mechanism of reinforcement should be rather governed by the high chemical affinity of Ti to oxygen than the diffusion of the cation of titanium into HA lattice. Strong materials were fabricated when BHA was doped with inert glass (105 MPa for 5% glass at 1300 ^{0}C and 133 MPa for 10% glass at 1200 ^{0}C) [23]. Erkmen *et al.* have achieved 100 MPa of compression strength in tooth-enamel derived-HA doped with partially stabilized zirconia [24].

The results of microstructure observations, shown in Fig. 1, and crystallographic analysis, shown in Fig. 2, agree with the above conclusions for the BHA-MgF_2 composites. The SEM images of Figs. 1a and 1c suggest a poor sintering regime at 1000 ^{0}C, where the initial submicron powder particles of BHA are loosely connected one to the other. Sintering is complete at 1300^{o}C (Figs. 1b and 1d). The corresponding X-ray diffractograms predominantly registered the patterns of apatite. Although the X-ray peaks of HA and fluorapatite (FA) are almost superimposed, the presence of fluorine (due to MgF_2) in FA may be suggested due to the high mechanical properties of Table 1 (comparing to pure BHA of Table 2), since it is well known that fluorine reinforces HA. Peaks of isokite ($CaMgPO_4F$) can also be suggested. It is worthy noted that the produced materials exhibited remarkable thermal stability at 1300 ^{0}C since the peaks of apatite maintain their intensity. No peaks of TCP were registered at all, although the SEM images suggest intensive sintering. This stability was already indicated in the results of Table 1. The low amount of MgF_2 does not cause any perceptible difference between the samples having a different amount of MgF_2 in either SEM-micrographs or X-ray diffractograms.

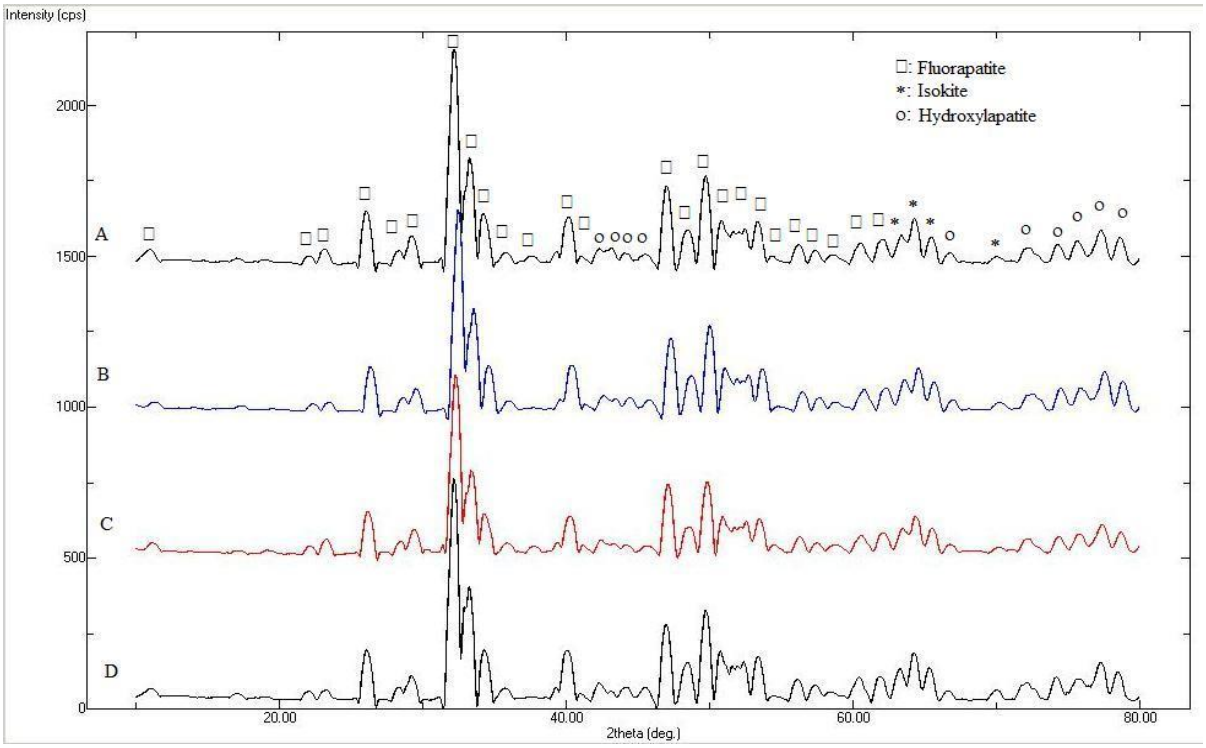

Figure 2. X-ray diffractograms of BHA-MgF_2 composites sintered at different temperatures: (A) BHA-1 wt% MgF_2 1000 ^{0}C, (B) BHA-1 wt% MgF_2 1300 ^{0}C, (C) BHA-2 wt% MgF_2 1000 ^{0}C, (D) BHA-2 wt% MgF_2 1300 ^{0}C. (The peaks have been identified with the ICDD cards: HA 84-1998; FA 15- 0876; Isokite ($CaMgPO_4F$) 7-0406).

In this study, naturally derived apatite (and not synthetic chemically produced HA) was intentionally selected, considering it as an economic, endlessly available, and designed by the nature herself material. Indeed, HA materials produced via powder processing route, where HA was obtained from bovine bone, has been proven that have great potential for bone substitute owing to their excellent biocompatible and osteoconductive properties [25]. Animal bones [26], teeth [27] as well as hydrothermally transformed

aragonite skeletons of sea creatures [28] are abundant sources of naturally derived apatite. However, all the necessary safety precautions for eliminating all possible risks of transmitting incurable and fatal diseases from the animal-donors, such as human immunodeficiency virus (HIV), or bovine spongiform encephalopathy (BSE), should be taken [29]. Calcination method, as applied in this present study, makes the transmission of such prions impossible since no protein can survive at a high temperature of 850 °C for several hours [22].

The good results of the present study, in terms of the considerable increase of mechanical properties of BHA matrix by doping with only 1 and 2% MgF_2, indicate the produced composites for further consideration and experimentation in biomedicine, either in bulk or in coating (e.g., plasma-spray) form. These good results should be attributed to the influence of both the cation Mg^{2+} and the anion F^- in apatite lattice. However, to shed light on the precise influence of these elements in apatite lattice (such as by Rietvelt analysis [30], that is beyond the scope of the present article), HA of high purity must be used, since biological apatites feature several substitutions in their lattice.

IV. CONCLUSION

The results of this study showed that MgF_2 could successfully dope the matrix of bovine derived hydroxypatite (BHA) since doping with only 1 and 2 wt% MgF_2 significantly increased the mechanical properties of BHA. The highest values of compression strength were achieved after sintering at 1300 °C: 143 MPa for 2 wt% MgF_2 and 125 MPa for 1 wt% MgF_2 addition. At the same sintering temperature, the highest HV values were obtained as 291 HV for 1% MgF_2 and 313 HV for 2% MgF_2. The effect of reinforcement should be attributed to the influence of both the cation Mg^{2+} and the anion F^- in apatite lattice. Moreover, the experimental results suggest that the presence of MgF_2 seemingly favors the shift of the onset of sintering towards lower temperatures (i.e. towards 1100 ^{0}C) and increases the thermal stability of HA towards transformation to TCP at 1300 ^{0}C.

ACKNOWLEDGEMENTS

I wish to very special thank Prof. Faik Nuzhet Oktar and Oguzhan Gunduz for supporting and also helping in this research.

REFERENCES

[1] Oktar, F.N., Yetmez, M., Agathopoulos, S., Lopez Goerne, T.M., Goller, G., Peker, I. and et al., (2006). Bond-coating in plasma-sprayed calcium-phosphate coatings. *J Mater Sci: Mater in Med*, 17, 1161-71.

[2] Moukbil, Y., Isindag, B., Gayir, V., Ozbek, B., Haskoylu, M. E., Oner, E. T., Oktar, F. N., Ikram, F., Sengor, M., & Gunduz, O. (2020). 3D printed bioactive composite scaffolds for bone tissue engineering. *Bioprinting*, 17, e00064.

[3] Ibrahim, M., Labaki, M., Giraudon, J.-M., & Lamonier, J.-F. (2020). Hydroxyapatite, a multifunctional material for air, water and soil pollution control: A review. *Journal of Hazardous Materials*, 383, 121139.

[4] Tiyyagura, H. R., Puliyalil, H., Filipič, G., Kumar, K. C., Pottathara, Y. B., Rudolf, R., Fuchs-Godec, R., Mohan, M. K., & Cvelbar, U. (2020). Corrosion studies of plasma modified magnesium alloy in simulated body fluid (SBF) solutions. *Surface and Coatings Technology*, 385, 125434.

[5] Yamasaki, Y., Yoshida, Y., Okazaki, M., Shimazu, A., Kubo, T., Akagawa, Y., & Uchida, T. (2003). Action of FGMgCO3Ap-collagen composite in promoting bone formation. *Biomaterials*, 24(27), 4913—4920.

[6] Cai, Y., Zhang, S., Zeng, X., Wang, Y., Qian, M., & Weng, W. (2009). Improvement of bioactivity with magnesium and fluorine ions incorporated hydroxyapatite coatings via sol–gel deposition on Ti6Al4V alloys. *Thin Solid Films*, 517(17), 5347–5351.

[7] Jadidi, A., & Salahinejad, E. (2020). Mechanical strength and biocompatibility of bredigite (Ca7MgSi4O16) tissue-engineering scaffolds modified by aliphatic polyester coatings. *Ceramics International*, 46(10, Part B), 16439–16446.

[8] Oktar, F. N., Agathopoulos, S., Ozyegin, L. S., Gunduz, O., Demirkol, N., Bozkurt, Y., & Salman, S. (2007). Mechanical properties of bovine hydroxyapatite (BHA) composites doped with SiO2, MgO, Al2O3, and ZrO2. *Journal of Materials Science: Materials in Medicine*, 18(11), 2137–2143.

[9] Kim, S.-J., Bang, H.-G., Song, J.-H., & Park, S.-Y. (2009). Effect of fluoride additive on the mechanical properties of hydroxyapatite/alumina composites. *Ceramics International*, 35(4), 1647–1650.

[10] Evis, Z., Usta, M., & Kutbay, I. (2009). Improvement in sinterability and phase stability of hydroxyapatite and partially stabilized zirconia composites. *Journal of the European Ceramic Society*, 29(4), 621–628.

[11] Evis, Z., Usta, M., & Kutbay, I. (2008). Hydroxyapatite and zirconia composites: Effect of MgO and MgF2 on the stability of phases and sinterability. *Materials Chemistry and Physics*, 110(1), 68–75.

[12] Oktar, F. N., K, K., & E, P. (1999). Characterization of Processed Tooth Hydroxyapatite for Potential Biomedical Implant Applications. Artificial Cells, Blood Substitutes, and *Biotechnology*, 27(4), 367–379.

[13] Carvalho, A. L., Faria, P. E. P., Grisi, M. F. M., Souza, S. L. S., Taba Jr, M., Palioto, D. B., Novaes Jr, A. B., Fraga, A. F., Ozyegin, L. S.,

Oktar, F. N., & Salata, L. A. (2007). Effects of Granule Size on the Osteoconductivity of Bovine and Synthetic Hydroxyapatite: A Histologic and Histometric Study in Dogs. *Journal of Oral Implantology*, 33(5), 267–276.

[14] Gunduz, O., Daglilar, S., Salman, S., Ekren, N., Agathopoulos, S., & Oktar, F. N. (2008). Effect of Yttria-doping on Mechanical Properties of Bovine Hydroxyapatite (BHA). *Journal of Composite Materials*, 42(13), 1281–1287.

[15] Komur, B., Lohse, T., Can, H. M., Khalilova, G., Geçimli, Z. N., Aydoğdu, M. O., Kalkandelen, C., Stan, G. E., Sahin, Y. M., Sengil, A. Z., Suleymanoglu, M., Kuruca, S. E., Oktar, F. N., Salman, S., Ekren, N., Ficai, A., & Gunduz, O. (2016). Fabrication of naturel pumice/hydroxyapatite composite for biomedical engineering. *BioMedical Engineering OnLine*, 15(1), 81.

[16] Salman, S., Gunduz, O., Yilmaz, S., Öveçoğlu, M. L., Snyder, R. L., Agathopoulos, S., & Oktar, F. N. (2009). Sintering effect on mechanical properties of composites of natural hydroxyapatites and titanium. *Ceramics International*, 35(7), 2965–2971.

[17] Gunduz, O., Erkan, E. M., Daglilar, S., Salman, S., Agathopoulos, S., & Oktar, F. N. (2008). Composites of bovine hydroxyapatite (BHA) and ZnO. *Journal of Materials Science*, 43(8), 2536–2540.

[18] Ozyegin, L. S., Oktar, F. N., Goller, G., Kayali, E. S., & Yazici, T. (2004). Plasma-sprayed bovine hydroxyapatite coatings. *Materials Letters*, 58(21), 2605–2609.

[19] Ozyegin, L. S., Oktar, F. N., Agathopoulos, S., Salman, S., Bozkurt, Y., & Eruslu, N. (2007). Improvement of Microstructure of Bovine Hydroxyapatite (BHA) by Doping with Calcium Fluoride. *Bioceramics* 19, 330, 43–46.

[20] Goller, G., Oktar, F. N., Agathopoulos, S., Tulyaganov, D. U., Ferreira, J. M. F., Kayali, E. S., & Peker, I. (2006). Effect of sintering temperature on mechanical and microstructural properties of bovine hydroxyapatite (BHA). *Journal of Sol-Gel Science and Technology*, 37(2), 111–115.

[21] Gören, Ş., Gökbayrak, H., & Altintaş, S. (2004). Production of hydroxylapatite from animal bone. *Key Engineering Materials*, 264–268, Issue III, pp. 1949–1952.

[22] Oktar, F. N. (2006). Hydroxyapatite–TiO2 composites. *Materials Letters*, 60(17), 2207–2210.

[23] Salman, S., Oktar, F. N., Gunduz, O., Agathopoulos, S., Öveçoğlu, M. L., & Kayali, E. S. (2007). Sintering Effect on Mechanical Properties of Composites Made of Bovine Hydroxyapatite (BHA) and Commercial Inert Glass (CIG). *Key Engineering Materials*, 330–332, 189–192.

[24] Erkmen, Z. E., Genç, Y., & Oktar, F. N. (2007). Microstructural and mechanical properties of hydroxyapatite-zirconia composites. *Journal of the American Ceramic Society*, 90(9), 2885–2892.

[25] Göller, G., & Oktar, F. N. (2002). Sintering effects on mechanical properties of biologically derived dentine hydroxyapatite. *Materials Letters*, 56(3), 142–147.

[26] Mucalo, M. R. (2015). 14 - *Animal-bone derived hydroxyapatite in biomedical applications*. In M. Mucalo (Ed.), Hydroxyapatite (Hap) for Biomedical Applications (pp. 307–342). Woodhead Publishing.

[27] Kürkcü, M., Benlidayı, M. E., Özsoy, S., Özyeğin, L. S., Oktar, F. N., & Kurtoğlu, C. (2008). Histomorphometric evaluation of implants coated with enamel or dentine derived fluoride-substituted apatite. *Journal of Materials Science: Materials in Medicine*, 19(1), 59–65.

[28] Rocha, J., Lemos, A. F., Sanjeevi, K., Agathopoulos, S., & Ferreira, J. (2005). Hydroxyapatite Scaffolds Hydrothermally Grown from Aragonitic Cuttlefish Bones. *Journal of Materials Chemistry*, 15.

[29] Anderson, I., Mucalo, M., Johnson, G., & Lorier, M. A. (2000). The processing and characterization of animal-derived bone to yield materials with biomedical applications. Part III. *Journal of Materials Science. Materials in Medicine*, 11, 743–749.

[30] Kannan, S., Goetz-Neunhoeffer, F., Neubauer, J., & Ferreira, J. M. F. (2008). Ionic substitutions in biphasic hydroxyapatite and $β$-tricalcium phosphate mixtures: Structural analysis by rietveld refinement. *Journal of the American Ceramic Society*, 91(1), 1–12.

CAPTIONS OF FIGURES

Fig. 1. Microstructure of BHA-MgF_2 composites sintered at different temperatures: (a) BHA-1 wt% MgF_2 1000 ^{0}C, (b) BHA-1 wt% MgF_2 1300 ^{0}C, (c) BHA-2 wt% MgF_2 1000 ^{0}C, (d) BHA- 2 wt% MgF_2 1300 ^{0}C.

Fig. 2. X-ray diffractograms of BHA-MgF_2 composites sintered at different temperatures: (A) BHA-1 wt% MgF_2 1000 ^{0}C, (B) BHA-1 wt% MgF_2 1300 ^{0}C, (C) BHA-2 wt% MgF_2 1000 ^{0}C, (D) BHA-2 wt% MgF_2 1300 ^{0}C. (The peaks have been identified with the ICDD cards: HA 84-1998; FA 15- 0876; Isokite ($CaMgPO_4F$) 7-0406).