

Composites of bovine hydroxyapatite (BHA) and ZnO

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Received: 3 November 2007 / Accepted: 24 January 2008 / Published online: 10 February 2008
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Abstract Composites of calcinated bovine bone-derived hydroxyapatite (BHA), doped with 2.5, 5, and 10 wt.% ZnO were produced by sintering. Scanning electron microscopy (SEM) and X-ray diffraction analysis together with measurements of density, compressive strength, and Vickers microhardness were carried out in the sintered samples. The experimental results showed that the best mechanical properties were achieved in the samples with

5% addition of ZnO. The highest value of compression strength was achieved after sintering at 1200 °C (72 MPa) and of microhardness at 1300 °C (548 HV). Prolong heat treatment at 1300 °C results in vulnerable BHA–ZnO composites to over-firing effect.

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Introduction

Hydroxyapatite (HA) is one of the most popular, widely accepted, and clinically used biomaterial for skeletal and dental restorations and treatments. Biological HA features in several substitutions at the Ca^{2+} , PO_4^{3-} , and OH^- sites of its lattice, which also accommodates several trace elements of high importance in biological performance of HA after implantation [1–6]. Our team has addressed its recent systematic studies on biologically derived HA, either from bovine bones or human teeth, to reduce the high cost of synthetic HA and provide safe HA [7–13] from biological source [14–17]. Moreover, we have tried to face the second tough challenge of the brittleness of pure HA materials, which does not allow their direct use in heavy load-bearing biomedical applications [3, 18]. Accordingly, we have investigated the influence of incorporation of several phases in HA matrix on the properties of the resultant composite materials.

In this study, we investigate the influence of ZnO on mechanical properties and microstructure of HA, derived from bovine bones (BHA) [19]. ZnO is very popular in the daily dental practice, because it is the main substitute of some polycarboxylates [20], in temporary dental cement powders, or in the formula of dental impression materials. Zinc content normally ranges between 0.012 and 0.025 wt.% in human bone, which is relatively higher than

the Zn content in adult tissues and plasma. In vitro studies have shown that Zn has direct, specific proliferative effect on osteoblastic cells and a potent and selective inhibitory effect on osteoclastic bone resorption. Recent studies have also demonstrated a clinical relationship between osteoporosis and Zn deficiency in elderly subjects [19]. Many studies have been carried out on Zn-containing tri-calcium phosphate and apatite cements [21, 22]. The results have shown that Zn has a stimulating effect on osteoblastic cell proliferation and bone formation.

Materials and experimental procedure

The HA powder (BHA) was derived from calcinated bovine bone according to a method described in an earlier study [14]. In brief, freshly cut femurs were deproteinized with NaOH solution and after re-irrigation the samples were subjected to heat treatment at 850 °C for 4 h in air. Calcinated samples were properly ball-milled until fine BHA powder was obtained. This powder was mixed with 2.5, 5, and 10 wt.% ZnO (separately). The mixtures were planetary ball-milled in acetone media. The suspensions were dried and the powders were uniaxially pressed at 350 MPa to cylindrical pellets with diameter of 6 mm and height of 11 mm, according to British Standard for compression tests [23]. The pellets were sintered at 1000, 1100, 1200, and 1300 °C for 4 h in air.

Compressive tests were carried out with a universal testing machine (displacement 2 mm/min). Hardness tests were done with a Vickers microhardness testing unit (load 200 g). The density of the sintered samples was measured by Archimedes method by using mercury. The microstructure of the produced materials was observed by field emission scanning electron microscopy (FE-SEM, Hitachi S-4100, Japan, 25 kV acceleration voltage, beam current 10 μ A) under secondary electron mode, using carbon-coated samples. The crystalline phases of the bulk (not powder) samples were identified by X-ray diffraction analysis (XRD, Rigaku Geigerflex D/Mac, C Series, Cu K_{α} radiation, Japan). Copper K_{α} radiation ($\lambda = 1.5406$ nm) produced at 30 kV and 25 mA scanned the range of diffraction angles (2θ) between 20° and 60° with a 2θ -step of 0.02°/s. Identification of the phases was performed by comparing the experimental XRD patterns to standards compiled by the Joint Committee on Powder Diffraction Standards (JCPDS) using the cards 15-0876 for fluorapatite (whose XRD patterns are similar to HA), and 36-1451 for ZnO (TCP).

Results and discussion

Table 1 presents the experimental results of densification (density), compressive strength, and Vickers microhardness

Table 1 Influence of sintering temperature and the amount (wt.%) of ZnO on density, compressive strength, and Vickers microhardness of the produced BHA–ZnO composites

<i>T</i> (°C)	2.5 wt.%	5 wt.%	10 wt.%
<i>d</i> (g/cm ³)			
1000	2.77 ± 0.060	2.86 ± 0.004	2.94 ± 0.002
1100	2.82 ± 0.002	2.17 ± 0.03	2.94 ± 0.002
1200	2.66 ± 0.036	2.76 ± 0.06	2.99 ± 0.058
1300	2.74 ± 0.004	2.82 ± 0.06	2.93 ± 0.069
σ (MPa)			
1000	21.00 ± 2.24	37.67 ± 10.41	28.36 ± 1.97
1100	39.45 ± 2.38	37.31 ± 2.38	27.99 ± 5.03
1200	52.24 ± 7.12	71.96 ± 10.90	53.22 ± 3.36
1300	35.41 ± 7.88	37.45 ± 12.60	32.99 ± 8.68
HV			
1000	49.02 ± 6.99	58.84 ± 5.57	67.87 ± 10.87
1100	94.39 ± 13.89	71.12 ± 28.43	95.13 ± 10.79
1200	208.20 ± 40.87	226.88 ± 11.30	203.10 ± 22.00
1300	302.82 ± 29.8	545.67 ± 45.70	338.10 ± 32.49

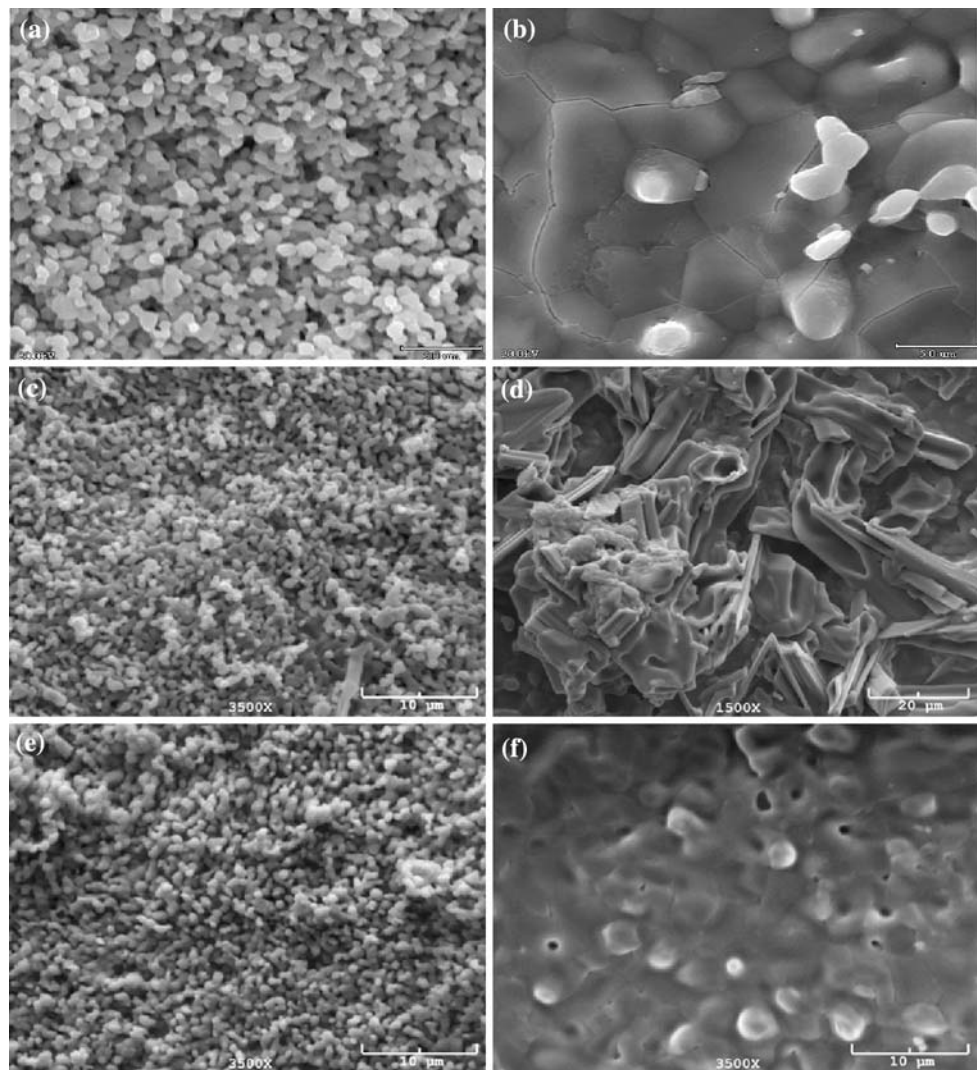
of the BHA–ZnO samples sintered at different temperatures. For comparison purposes, the relevant values of pure BHA samples, produced with the same procedure and reported in earlier studies [15, 24, 25], are presented in Table 2. It is clearly seen that sintering occurs at temperatures between 1100 °C and 1200 °C, where significant increase of condensation and mechanical properties is observed, while the presence of ZnO does not shift sintering temperature. This conclusion is also confirmed with the SEM images of Fig. 1, where poor sintering is suggested at 1000 °C, while microstructures of well-sintered bodies are observed in the samples heat treated at 1300 °C. The high density of ZnO (5.6 g/cm³, as compared with BHA) does not allow us to draw out firm conclusions about sintering ability from the density values of Table 1.

The comparison of the values of Tables 1 and 2 indicates that ZnO did not increase the compressive strength of BHA but only the hardness. The highest values of compressive strength were observed after sintering at 1200 °C for all ZnO additions, but among them, the best was that one with 5% ZnO. Apparently, 2.5% ZnO seems to be a small addition to BHA, whereas 10% is too high. The microstructure of BHA–5% ZnO in Fig. 1d, which comprised well-developed crystals (likely ZnO) dispersed in the BHA matrix, perhaps provides the high compressive strength to materials. The X-ray diffractograms at that temperature (1300 °C), shown in Fig. 2, indicate well-crystallized materials, where no crystalline phase, resulted from reaction between BHA and ZnO, was registered.

Furthermore, the presence of ZnO narrows the sintering temperature range because the resultant BHA–ZnO

Table 2 Mean values of density and mechanical properties of BHA samples sintered at different temperatures obtained from earlier similar studies [15, 24, 25]

T (°C)	[15]			[24, 25]		
	d (g/cm ³)	σ (MPa)	HV	d (g/cm ³)	σ (MPa)	HV
1000	1.98 ± 0.01	12 ± 2	42 ± 2	2.46 ± 0.04	48.17 ± 20.02	85.37 ± 9.43
1100	2.59 ± 0.01	23 ± 3	92 ± 4	2.34 ± 0.04	22.16 ± 5.09	74.20 ± 19.80
1200	2.62 ± 0.01	67 ± 7	138 ± 3	2.59 ± 0.04	75.20 ± 18.30	148.50 ± 10.5
1300	2.72 ± 0.01	62 ± 11	145 ± 3	2.48 ± 0.08	65.01 ± 41.57	130.68 ± 17.9

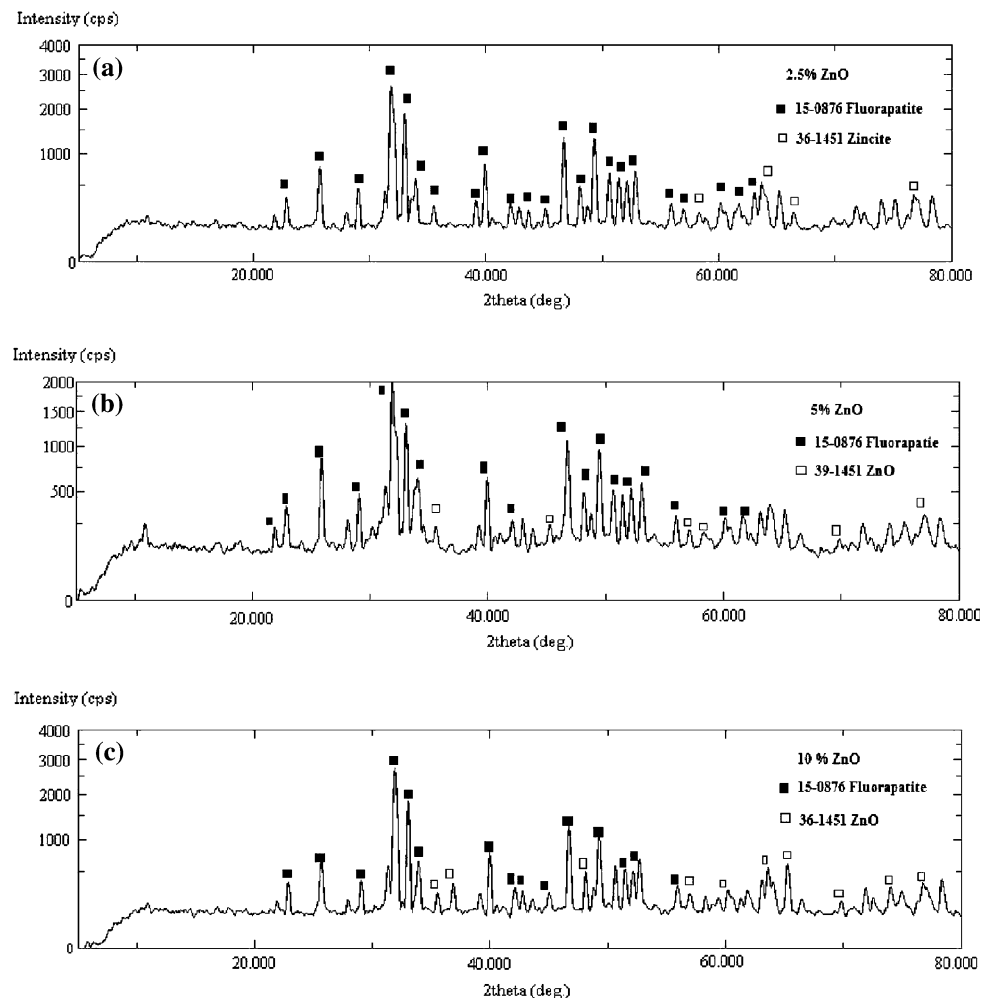
Fig. 1 Typical microstructures of the BHA–ZnO produced composites. (a) 2.5 wt.% ZnO, 1000 °C; (b) 2.5 wt.% ZnO, 1300 °C; (c) 5 wt.% ZnO, 1000 °C; (d) 5 wt.% ZnO, 1300 °C; (e) 10 wt.% ZnO, 1000 °C; (f) 10 wt.% ZnO, 1300 °C

materials are vulnerable to over-firing effect at 1300 °C, indicated by the dramatic decrease of compressive strength values at that high temperature. At 1300 °C, more glass phase (than at 1200 °C) is expected to exist, which should advance sintering by closing porosity. Hence, higher values of hardness were measured at 1300 °C (Table 1), which actually resemble microhardness values of glasses but not HA (Table 2). Nevertheless, the resultant material seems to

be intrinsically (presumably by its nature, itself) vulnerable to over-firing, which means that secondary porosity (Fig. 1f) together with microcracks (Fig. 1b, perhaps developed during cooling due to the glassy phase) resulted in the dramatic decay of compressive strength of the samples sintered at 1300 °C.

In our earlier studies [15, 26], we have presented SEM images of sintered BHA samples, similar to the one

Fig. 2 X-ray diffractograms of BHA–ZnO composites sintered at 1300 °C. (a) 2.5 wt.% ZnO, (b) 5 wt.% ZnO, and (c) 10 wt.% ZnO



prepared in the present study. Comparison of those images with the SEM images of the Fig. 1b, d, and f would suggest that the BHA–ZnO composites apparently exhibit better densification behaviour over sintering than in BHA. Therefore, it is suggested that shorter dwelling of BHA–ZnO samples at sintering temperatures may result in stronger samples. In any case, the fact is that ZnO is currently a heavily investigated oxide in biology [21, 22, 27–33], and therefore it features high potential in biomedicine, as briefly outlined in the Introduction. Hence, the investigation of incorporation of ZnO in BHA matrix, which, as produced in this study, anticipates low production cost and high safety regarding elimination of any risk of transmitting fatal diseases [7–13, 16,], must continue in a systematic and thorough way.

Conclusions

ZnO attracts great interest in biology research because it is postulated that it can influence and control many cells' biological expressions, related to the general health of

humans or healing processes. Therefore, the incorporation of ZnO in the matrix of HA materials emerges as a realistic challenge in the field of biomaterials. The experimental results of this study showed that the optimum addition of ZnO in the matrix of biologically derived HA, which is 5 wt.% ZnO, negligibly affects the compressive strength but increases the microhardness of the resultant materials, compared to pure BHA. It is clear, however, that addition of ZnO narrows the sintering temperature range because the produced BHA–ZnO composite materials are more vulnerable to over-firing effect at 1300°C. Hence, it is suggested that shorter dwelling of samples at sintering temperatures may result in stronger BHA–ZnO composite materials.

Acknowledgements This study was carried out with the financial support of the Turkish Republic Government Planning Organization in the framework of the Project “Manufacturing and Characterization of Electro-Conductive Bioceramics” with project number 2003 K120810. The authors also acknowledge the technical support of assistant O. Meydanoglu, associate professor G. Goller, professor E.S. Kayali from Metallurgical and Materials Engineering Department of Istanbul Technical University, and L.S. Ozyegin from Dental Technical School of Health Related Professions, Istanbul, Turkey, for

BHA production. The work of S. Agathopoulos in this paper has been in the framework of the Project ENTER 04EP26, co-financed by E.U.-European Social Fund (75%) and the Greek Ministry of Development-GSRT (25%). O. Gunduz also acknowledges the support of Prof. M. Edirisinghe from University College London where he is spending a sabbatical year.

References

- Oktar FN (2006) *Mater Lett* 60:2207
- Oktar FN, Yetmez M, Agathopoulos S, Lopez Goerne TM, Goller G, Peker I, Ferreira JMF (2006) *J Mater Sci Mater Med* 17:1161
- Goller G, Oktar FN (2002) *Mater Lett* 56:142
- Oktar FN (2007) *Ceram Int* 33:1309
- Kurkcu M, Benlidayi ME, Ozsoy S, Ozyegin LS, Oktar FN, Kurtoglu C (2008) *J Mater Sci Mater Med* 19:59
- Erkmen EZ, Genc Y, Oktar FN (2007) *J Am Ceram Soc* 90:2285
- Goller G, Oktar FN, Ozyegin LS, Kayali ES, Demirkesen E (2004) *Mater Lett* 58:2599
- http://www.oie.int/eng/info/en_statesb.htm. Bovine spongiform encephalopathy (updated: 01/08/2006)
- Mad cow disease\BSE and vCJD Instant Expert - life - 04 September 2006 - Print - New Scientist.htm
- http://www.oie.int/eng/info/en_esbmonde.htm. Last update: 27 July 2007 (fr)
- http://www.oie.int/eng/info/en_esbcarte.htm. Geographical Distribution of Countries that reported BSE Confirmed Cases since 1989. Last update: 04 January 2007
- http://www.europa.eu.int/comm/food/fs/sc/ssc/outcome_en.html. Final Report on the assessment of the Geographical BSE-risk of Turkey 27 June 2002
- Opinion of the Scientific Steering Committee on the Geographical Risk Of Bovine Spongiform Encephalopathy (GBR) in Turkey Adopted by the SSC on 27 June 2002
- Oktar FN, Kesenci K, Piskin E (1999) *Artif Cell Blood Substit Immobil Biotechnol* 27:367
- Goller G, Oktar FN, Agathopoulos S, Tulyaganov DU, Ferreira JMF, Kayali ES, Peker I (2006) *J Sol-Gel Sci Technol* 37:111
- Ozyegin LS, Oktar FN, Goller G, Kayali ES, Yazici T (2004) *Mater Lett* 58:2605
- Carvalho AL, Faria PEP, Grisi MFM, Souza SLS, Taba MJ Jr, Palioto DB, Novaes AB Jr, Fraga AF, Ozyegin LS, Oktar FN, Salata LA (2007) *J Oral Implantol* 33:267
- Oktar FN, Goller G (2002) *Ceram Int* 28:617
- Bandyopadhyay A, Withey EA, Moore J, Bose S (2007) *Mater Sci Eng C* 27:14
- Bertenshaw BW, Combe EC (1972) *J Dent* 1:65
- Otsuka M, Marunaka S, Matsuda Y, Ito A, Layrolle P, Naito H, Ichinose N (2000) *J Biomed Mater Res* 52:819
- Otsuka M, Ohshita Y, Marunaka S, Matsuda Y, Ito A, Ichinose N, Otsuka K, William I, Higuchi WI (2004) *J Biomed Mater Res Part A* 69A:552
- British Standard for compression tests (No: 7253), BS EN. ISO 7253:2001, Test methods. Determination of modulus of elasticity in compression. www.bsiglobal.com/upload/Standards%20&%20Publications/Membership/UpdateStandards/October06.pdf
- Goren S, Gokbayrak H, Altıntaş S (2004) *Key Eng Mater* 264–268:1949
- Gokbayrak H (1996) Production of hydroxyapatite ceramics. M.Sc. Thesis, Bogazici University, Istanbul
- Oktar FN, Aydin H, Goller G, Agathopoulos S, Rocha G, Senaroglu B, Kayali S (2006) *Key Eng Mater* 309–311:45
- Kamitakahara M, Ohtsuki C, Inada H, Tanihara M, Miyazaki T (2006) *Acta Biomater* 2:467
- Du RL, Chang J, Ni SY, Zhai WY, Wang JY (2006) *J Biomater Appl* 20:341
- Miaoa S, Wenga W, Chengb K, Dua P, Shena G, Hana G, Zhang S (2005) *Surf Coat Technol* 198:223
- Wang Z, Xue D, Chen X, Lu B, Ratajczak H (2005) *J Non-Cryst Solids* 351:1675
- Balamurugan A, Balossier G, Kannan S, Michel J, Rebelo AHS, Ferreira JMF (2007) *Acta Biomater* 3:255
- Ito M (1991) *Biomaterials* 12:41
- Vélez D, Arita IH, García-Garduño MV, Castaño VM (1994) *Mater Lett* 19:309