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Microstructure and mechanical properties of sintered enamel hydroxyapatite

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Abstract

The influence of sintering temperature on the microstructure and the mechanical properties (i.e. compressive strength and microhardness) of samples of calcinated human enamel was investigated. The experimental results indicate a low densification regime after sintering at low temperatures (1000 °C, 1100 °C) and a high densification regime after sintering at higher temperatures (1200 °C, 1300 °C). With respect to earlier studies, high mechanical properties were achieved. The enhancement of mechanical properties was attributed to the presence of fluorine traces in enamel.

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Keywords: A. Sintering; C. Mechanical properties; Hydroxyapatite; Enamel; Fluorine

1. Introduction

The number of treated skeletal deficiencies steadily increases in a global scale. Effective ways for bone replacements and enhancement of bone formation [1] together with research directed to find ideal biomaterials for grafting purposes, which will feature biocompatibility and production simplicity and economy, are required. Hydroxyapatite (HA, $Ca_{10}(PO_4)_6(OH)_2$), the main mineral component of bones and teeth, is among the leading biomaterials satisfying these requirements [2]. Nevertheless, pure HA features low mechanical strength and fracture toughness, which limit their use in load-bearing applications [3]. Moreover, HA exhibits poor thermal stability. It decomposes to tricalcium phosphate (TCP, $Ca_3(PO_4)_2$) over firing at temperatures higher than 1200 °C [4]; undesirable dissolution of TCP rapidly takes place *in vivo*. F-substituted HA and fluoroapatite (FA) exhibit significantly higher thermal stability than HA even up to 1400 °C.

The HA bioceramics can be synthetic or natural. The former ones are reliable but their production is usually complicate and expensive. Natural bioceramics are economical, but they can potentially bear fatal diseases, such as human immunodeficiency virus (HIV), or bovine spongiform encephalopathy (BSE). Safety issues related to the preparation methods of bioceramics have been thoroughly discussed in some recent papers [5,6].

There are a lot of papers which encourage the use of HA derived from natural sources, such as human, animal, and corals, where thermal treatment or washing with diluted HC1 can prevent transmission of fatal diseases. Nevertheless, there is a significant lack of papers for encouraging the use of natural HA materials derived from human in calcinated form. Joshi et al. were the pioneers who have used calcinated human tooth HA for plasma spray [7]. The author has also performed studies on calcinated human enamel and dentine as candidate grafting materials and for plasma spray coatings [8–13].

This study addresses itself to dental enamel. Extracted human or animal teeth are natural sources of HA [2,8,14–16]. Calcinated tooth matter comprises 60 wt.% dentine and 40 wt.% enamel [8]. Enamel is the hardest biological substance of human body and comprises 92% HA (EHA), 6% water and 2% organic matter, while dentine composition is 47% HA (DHA), 30% organic matter and 23% water [17]. Enamel is actually a composite material. Its mineral phase primarily consists of calcium phosphate salts in the form of large hexagonal HA crystals that are carbonated and defective [18].

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Few early investigations focused in the understanding of basic properties of EHA with the aid of X-ray diffraction or thermal analyses, or in the determination of important features for plasma spraying, etc. [8,14,19–20]. Nevertheless, the use of a bioceramic in load bearing applications requires information about the fracture toughness, together with hardness (needed to evaluate the resistance to abrasion) and elastic modulus (to determine the degree of stress shielding) [21]. To the knowledge of the author, there are no articles of the influence of sintering on the properties of calcinated human enamel, which is the aim of the present study. The experimental results are discussed and compared with results from earlier similar studies.

2. Materials and experimental procedure

The EHA matter was obtained according to the instructions presented in earlier papers [8,14]. In brief, the processing was as follows. Extracted human teeth were collected from several different dental clinics. The teeth were kept in an antiseptic solution to avoid odour and possible contamination possibly caught during the preparation course. The teeth were cleaned with continuous water irrigation and then deproteinized in an alkali solution. After drying, calcination took place at 850 °C for 3 h. Enamel was very easily separated from dentine due to the significant difference of water content (as mentioned as on the first page).

The obtained enamel matter was ground and the powder was sieved. The collected fraction contained particles sized between 106 and 150 μm. The powders were kept for a couple of days in atmospheric conditions to uptake humidity. This trick aimed to skip the need for using lubricants or binders afterwards. Pellets of about 3.25 g each were prepared by uniaxial pressing of EHA powder at 350 MPa between hardened stainless steel dies, according to the British Standards for compression tests no. 7253. The reason of selecting the British Standards was because each sample requires less amount of powder than for the ASTM. Note that the brittle nature of HA makes difficult the preparation of large sized samples.

The pressed pellets were sintered in an open atmospheric furnace (Heraus, Germany) at four different temperatures, i.e. 1000, 1100, 1200, and 1300 °C, for 4 h. The heating rate was 4 °C/min. To avoid chipping effect due to thermal stresses, the samples were let to cool down naturally to room temperature inside the furnace.

The crystalline structure of the sintered samples was determined by X-ray diffraction analysis (XRD) using a Rigaku Rint-100 X-ray diffractometer. The outer surfaces of the samples were observed by scanning electron microscopy (SEM, Jeol 5410). The density of the samples was measured by the classical Archimedes method (i.e. immersion in water). The presenting results are the average (and the standard deviations) from 10 different samples. The Vickers microhardness (HV) of the samples was measured on polished surfaces with a Shimadzu microhardness testing machine, with a load of 200 g and 20 s indentation period. The presenting results are the average (and the standard deviations) from 10 different samples, where five indentations were done at each sample. The compression strength tests were done with a universal testing machine DVT (Devotrans Inc., Istanbul, Turkey). The crosshead speed was 2 mm/min. The presenting results are the average (and the standard deviations) from 10 samples.

3. Results

From the diffractograms of Fig. 1 it is concluded that the samples were highly crystallized after sintering at all the tested temperatures between 1000 and 1300 °C. Careful fitting of the patterns of standard HA (card 84-1998) and FA (card 84-1997) to these diffractograms suggests that both phases should be present. However, due to the small shift of the peaks between these two phases, they cannot be easily distinguished one from the other. Evidence of formation of calcium phosphate (card 21-839) was also registered in all spectra.

The increase of temperature caused a slight decay of the intensity of the diffractograms (Fig. 1). This decrease of crystallinity agrees fairly well with the SEM images of the samples sintered at the four tested temperatures (Fig. 2). These

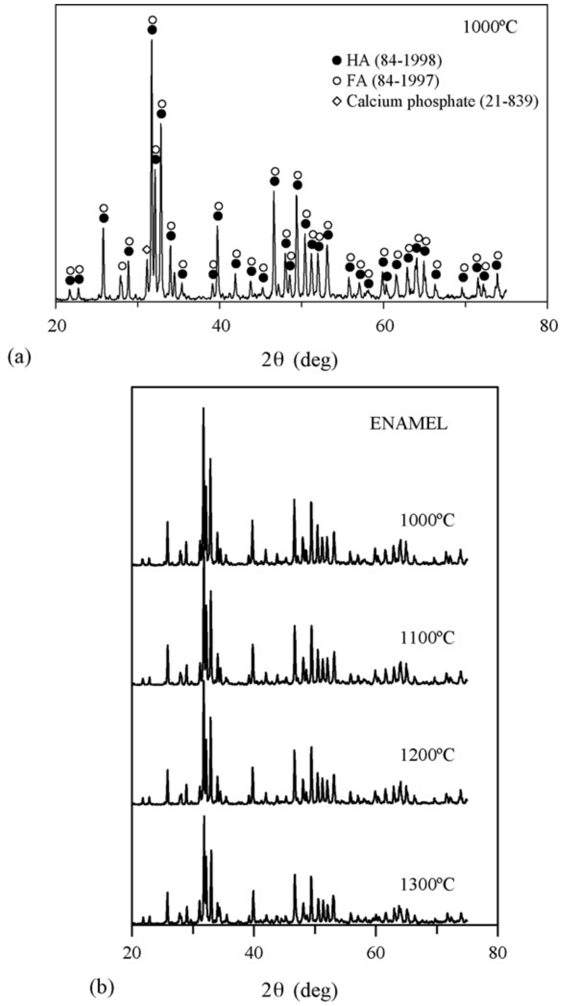

Fig. 1. X-ray diffractogram of EHA samples sintered at 1000 °C with the identification of the phases (a), and overview of all the diffractograms obtained from the EHA samples sintered at different temperatures (b). (The intensities have not been normalized).

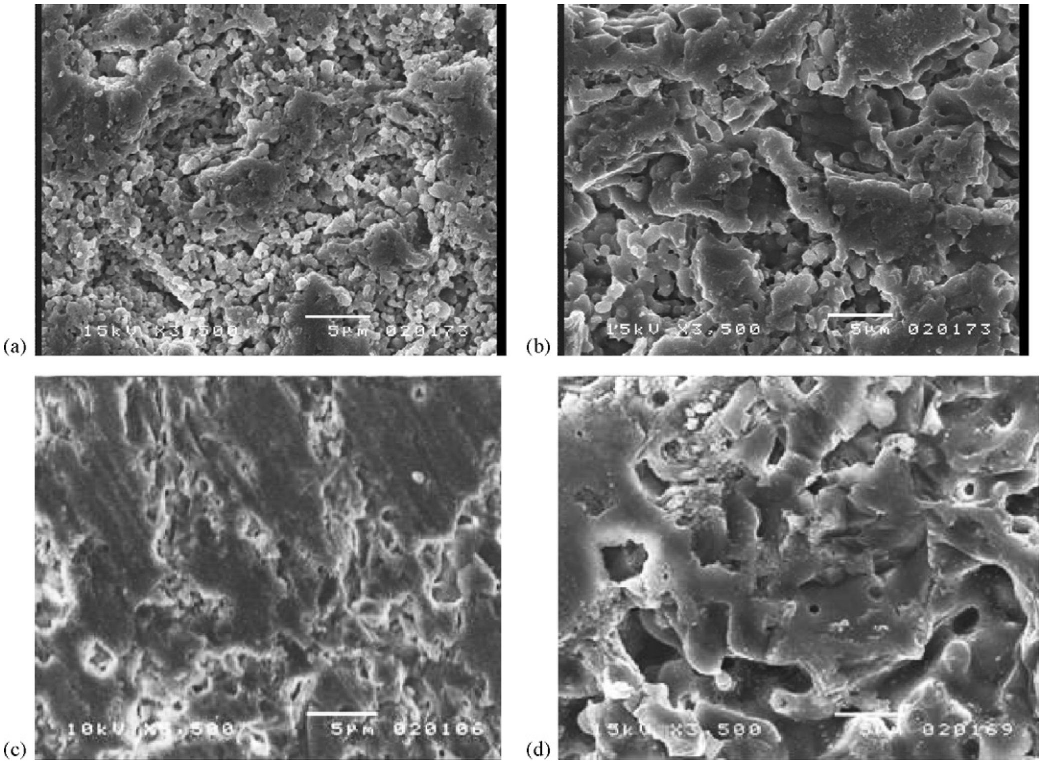

Fig. 2. Microstructure of the EHA samples sintered at (a) 1000 °C, (b) 1100 °C, (c) 1200 °C, and (d) 1300 °C.

images show that the densification was perceptibly improved from 1000 °C (Fig. 2a) to 1100 °C (Fig. 2b). However, a regime of high densification was actually achieved after sintering at the highest tested temperatures, i.e. at 1200 °C (Fig. 2c) and at 1300 °C (Fig. 2d). These differences in densification are reflected fairly well in a quantitative manner at the values of density, measured as 2.003 (±0.070) g/cm^3 for the samples sintered at 1.000 °C, 2.161 (±0.086) g/cm^3 at 1100 °C, 2.661 (±0.120) g/cm^3 at 1200 °C, and 2.622 (±0.093) g/cm^3 at 1300 °C.

Table 1 summarizes the results of the mechanical tests, specifically bending strength and Vickers microhardness. The values of bending strength clearly follow the above mentioned general trend of the low densification regime obtained after sintering at the lower tested temperatures (i.e. 1000 and 1100 °C) and the high densification regime for the samples sintered at the higher tested temperatures (1200 and 1300 °C). This is not, however, the general trend of the values of microhardness, where the maximum value was the one obtained at the lowest temperature (i.e. 1000 °C), then the hardness decays for the samples sintered at 1100 and 1200 °C, and finally increases after sintering at 1300 °C.

4. Discussion

4.1. Comparison with earlier studies

Results of compression strength and microhardness of similarly produced samples of dentine derived HA (DHA) are summarized in Table 2 [2]. Comparison of the values of Tables 1 and 2 reveals similar general trends as mentioned above. However, there is a slight but a constant superiority of EHA than DHA. The high HV values obtained at the lowest sintering temperatures (1000 °C) suggest that sintering does not extensively occur at this low temperature for DHA. Hence, the samples maintain the hardness of the initial raw material, while the influence of sintering on microhardness is still negligible. The very low values of bending strength support this conclusion. Moreover, the microhardness of tooth enamel has been experimentally measured as −300 HV

Table 1
Compressive strength (σ, in MPa) and Vickers microhardness (in HV) of samples of EHA sintered at different temperatures

T (°C)	σ	HV
1000	27.60 ± 2.15	325.8 ± 15.5
1100	38.81 ± 3.01	252.9 ± 10.3
1200	54.32 ± 8.90	238.8 ± 11.1
1300	61.27 ± 15.02	286.6 ± 14.4

Table 2
Compressive strength (σ, in MPa) and Vickers microhardness (in HV) of samples of DHA sintered at different temperatures [2]

T (°C)	σ	HV
1000	9.83 ± 3.37	258 ± 12.9
1100	14.48 ± 4.58	163.1 ± 8.1
1200	56.30 ± 11.05	229.8 ± 11.5
1300	56.77 ± 18.63	251.9 ± 12.6

Table 3
Compressive strength (σ, in MPa) and Vickers microhardness (in HV) of DHA composites doped with 5 and 10 wt.% Bioglass® 45S5, sintered at different temperatures [15]

T (°C)	5 wt.% Bioglass®		10 wt.% Bioglass®	
	σ	HV	σ	HV
1200	62.23 ± 20.29	472 ± 66	83.03 ± 33.95	383 ± 38
1300	68.81 ± 9.72	365 ± 60	43.38 ± 5.14	234 ± 32

Table 4
Compressive strength (σ, in MPa) and Vickers microhardness (in HV) of DHA composites doped with 5 and 10 wt.% metallic Ti, sintered at different temperatures [16]

T (°C)	5 wt.% Ti		10 wt.% Ti	
	σ	HV	σ	HV
1200	16.96 ± 8.197	80 ± 12	11.31 ± 3.702	91 ± 12
1300	17.56 ± 2.55	149 ± 19.7	32.1 ± 2.64	204 ± 42.2

[2], which fits very well to the value reported for EHA at 1000 °C in Table 1.

The importance of the presence of glassy phase (Fig. 2c and d) on the mechanical properties becomes more evident by comparing the experimental results of the present study (Table 1) with results of sintered composites of DHA doped with 5 wt.% and 10 wt.% bioglass® “45S5” (Table 3) [15]. The presence of the glass clearly favoured densification, and hence mechanical properties were enhanced. Nevertheless, excess of glass content can cause some (probably) overfiring effects at elevated sintering temperatures. This is evident in the dramatic decrease of mechanical properties in the case of using 10% glass after sintering at 1300 °C (Table 3). It should be also noticed that glasses have usually high chemical affinity with HA [22–25].

With regards to the importance of chemical affinity between the dissimilar phases in the structure of composite materials, one can see that the composites of DHA with metallic Ti resulted in considerably weaker materials (Table 4) [16]. The weakening is evidently due to the significant difference of the chemical nature between HA and Ti [26], the high chemical reactivity between Ti and HA that results in reaction products of probably brittle nature, and the mismatch of thermal expansion coefficients [23].

Composites of DHA have been also prepared by doping (with 5 and 10 wt.%) with two (Table 5, 45.5% P_2O_5–54.5% CaO) and three oxides (Table 6, 45% P_2O_5–28% CaO–27% Na_2O) [25]. Comparison of these results with Tables 1 and 2 as well as Table 3 indicates that the nature of the oxides greatly influence the interfacial interactions. Moreover, the presence of many oxides increases the possibility of spontaneous formation of crystalline phases (during heating or during cooling) that make the resulting composite more fragile [23]. Therefore, the intrinsic capability of the system to form an optimum amount of glassy phase during sintering (as evidently achieved in the present study, Fig. 2), seems to be a necessary condition to anticipate high mechanical properties by favouring sintering and by suppressing formation of undesirable reaction products and devitrification.

Table 5
Compressive strength (σ, in MPa) and Vickers microhardness (in HV) of DHA composites doped with 5 and 10 wt.% mixture of 45.5%P_2O_5 + 54.5%CaO, sintered at different temperatures [25]

T (°C)	5 wt.% P_2O_5–CaO		10 wt.% P_2O_5–CaO	
	σ	HV	σ	HV
1200	64 ± 27	250 ± 19	67 ± 17	285 ± 19
1300	39 ± 9	238 ± 26	53 ± 11	279 ± 12

Table 6
Compressive strength (σ, in MPa) and Vickers microhardness (in HV) of DHA composites doped with 5 and 10 wt.% mixture of 45 wt.%P_2O_5 + 28 wt.%-CaO + 27 wt.%Na_2O, sintered at different temperatures [25]

T (°C)	5 wt.% P_2O_5–CaO–Na_2O		10 wt.% P_2O_5–CaO–Na_2O	
	σ	HV	σ	HV
1200	36 ± 11	222 ± 23	40 ± 21	259 ± 15
1300	55 ± 14	358 ± 18	39 ± 12	301 ± 8

4.2. The role of F

The X-ray analysis (Fig. 1) suggested that HA and FA should be simultaneously present in the investigated sintered samples. Literature survey reveals that the F-content in enamel ranges between 0.01 and 0.07 wt.% (Table 7) [14,27–32]. Some researchers describe enamel as fluorohydroxyapatite obtained due to reaction of F ions within enamel apatites [19]. The changes of HA crystallographic structure in enamel due to ion substitutions (among them F-substitution), occurring in oral cavity, have been described by several investigators [33–35]. The mineral phase of apatite in tooth enamel contains 0.04–0.07 wt.% F [27]. There is a descending concentration gradient of F from the enamel surface toward the inner layers [36]. EHA is not directly equivalent to FA, but it behaves exactly like FA. Hence, enamel can be named as F-rich HA.

Table 7
F-content (wt.%) in several tissues

Tissue	F (wt.%)	References
Human enamel	0.021	[14]
Human enamel	0.04–0.07	[27]
Human enamel	0.01	[28]
Human bone	0.03	[28]
Human enamel	0.1	[29]
Human enamel with F rinse	0.61	[30]
Human enamel without F rinse	0.04	[30]
Human enamel with F rinse (before study)	0.54–0.65	[31]
Human enamel without F rinse (control group) (before study)	0.34–0.45	[31]
Human enamel with F rinse (after study)	0.50–0.71	[31]
Human enamel without F rinse (control group) (after study)	0.27–0.35	[31]
Human enamel	0.01	[32]

The grain boundaries in human enamel is a result of a very complex regime that various impurities (K, Na, Mg, Sr, etc.) are involved [35]. Reformulations, may occur due to these impurities, can considerably change the mechanical behaviour of the resulting materials. Accordingly, the positive impact of F-content in the mechanical properties of natural materials is often assigned to specific changes of crystallite dimensions and orientations, higher mineral contents in the bone, or modifications of bone architecture. This should be also the case in the present study for the sintered samples of EHA and the enhanced mechanical properties achieved (Table 1).

In the recent years, F-containing inorganic materials have been increasingly investigated as potential biomaterials for stimulatory purposes in hard tissues or for maintaining the stability of materials during processing [36–41,24]. However, there is an open debate about the positive (e.g. water fluoridation, treatment for osteoporosis, prevention of dental caries) and the negative impact (e.g. possible increase of bone fracture, bone health of females, skeletal fluorosis) of F in human health [42–48], but further discussion is beyond the scope of the present paper.

4.3. Potential

The investigated materials (EHA) have already successfully passed *in vitro* tests with cell cultures. Valerio et al. have reported that EHA performs as good as the commercial HA [49]. This fact in conjunction with the results of the present study indicate EHA as interesting biomaterial for general grafting purposes in pure form but also modified in combination with other reinforcing materials, such as in coatings produced according to the ASTM C-633-01 method. The practical application of such coatings has been shown earlier [50]. Regarding the performance achieved, EHA has been used to coat femoral implants by plasma spraying. X-ray radiography revealed no evidence of any inflammatory reaction [51].

Accordingly, the studied EHA material can be qualified for further experimentation in animal tests and eventually in clinical studies. Nevertheless, the particular nature of the raw materials certainly puts plausible reservations with regards to economic aspects and the availability of large amounts of human teeth. It is really hard to get large amounts of EHA or DHA from teeth. Those who are not aware in dentistry practice would propose that large amounts of EHA could be obtained from bovine teeth. Nevertheless, it is really more difficult to extract a tooth even from a dead cow than from a human. Moreover, current (unpublished) studies of the author on bovine teeth have indicated that the inorganic content of bovine teeth is much lower than human teeth. In particular, the crown/root mass ratio is 1/3 for human tooth and 1/5 for cow tooth (note that the crown predominantly consists of enamel.

Therefore, the main aim of this study is to show that the presence of F traces in HA, like in the sintered natural EHA studied in the present work, which should be related to the enhancement of the mechanical properties achieved, can be considered as a guide line for further studies on developing biomaterials with superior mechanical properties. More specifically, experiments with very small additions of F should be carried out to allow a comparison with earlier studies [37–39]. However, one should be careful at defining the level of F-traces because there is a big difference between the range of 0.01–0.07 and 0.75–3.77 wt.%, for instance. This is because pure FA usually develops neutral interactions with the neighbouring biological vicinity.

5. Conclusions

The study of the influence of sintering temperature on the microstructure and the mechanical properties of samples of calcinated human enamel, obtained from extracted human teeth, revealed that a low densification regime is reached after sintering at low temperatures (1000 °C, 1100 °C) and a high densification regime is reached after sintering at higher temperatures (1200 °C, 1300 °C).

With respect to earlier studies, high mechanical properties were achieved. These results, in conjunction with good results obtained from earlier studies on plasma spray coatings and *in vitro* biological tests with cell cultures, qualify the investigated materials of EHA for further experimentation in animal tests and clinical studies.

The enhancement of mechanical properties should be related to the presence of fluorine traces in enamel. Therefore, experiments with even smaller amounts of fluorine in HA must be carried out. It is expected that materials with superior mechanical properties may be achieved.

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References

[1] B. Ben-Nissan, A. Milev, R. Vago, Morphology of sol-gel derived nano-coated coralline hydroxyapatite, Biomaterials 25 (2004) 4971–4975.

[2] G. Goller, F.N. Oktar, Sintering effects on mechanical properties of biologically derived dentine hydroxyapatite, Mater. Lett. 56 (2002) 142–147.

[3] H. Guo, K.A. Khor, Y.C. Boey, X. Miao, Laminated and functionally graded hydroxyapatite/yttria stabilized tetragonal zirconia composites fabricated by spark plasma sintering, Biomaterials 24 (2003) 667–675.

[4] E.Z. Erkmen, The effect of heat treatment on the morphology of D-Gun sprayed hydroxyapatite coatings, J. Biomed. Mater. Res. (Appl. Biomater.) 48 (1999) 861–868.

[5] L.S. Ozyegin, F.N. Oktar, G. Goller, E.S. Kayali, T. Yazici, Plasma-sprayed bovine hydroxyapatite coatings, Mater. Lett. 58 (2004) 2605–2609.

[6] G. Goller, F.N. Oktar, L.S. Ozyegin, E.S. Kayali, E. Demirkesen, Plasma-sprayed human bone-derived hydroxyapatite coatings: effective and reliable, Mater. Lett. 58 (2004) 2599–2604.

[7] S.V. Joshi, M.P. Srivastava, A. Pal, S. Pal, Plasma spraying of biologically derived HA on implantable materials, J. Sci.: Mater. Med. 4 (1993) 251–255.

[8] F.N. Oktar, Ph.D. Thesis, Characterization of tooth processed tooth hydroxyapatite and bioglass for potential applications in dentistry (Supervisor S. Altintas) Bogazici University, Istanbul, Turkey (1999).

[9] F.N. Oktar, H. Sayrak, S. Ozsoy, N. Heybeli, A histological study of human derived tooth-hydroxyapatite (THA), Key Eng. Mater. 309–311 (2006) 27–30.

[10] F.N. Oktar, S. Ozsoy, H.T. Turoglu, S. Altmtas, An in vitro comparative study of bioglass and biologically derived hydroxyapatite with bone scintigraphy (BS) method, Key Eng. Mater. 309–311 (2006) 163–167.

[11] F.N. Oktar, L.E. Zdrentu, S. Petrescu, P. Valerio, L.S. Ozyegin, I. Peker, A complementary biocompatibility evaluation of plasma sprayed dentine (DHA) and enamel derived hydroxyapatite (EHA) using fibroblasts, Key Eng. Mater. 309–311 (2006) 619–622.

[12] F.N. Oktar, F. Ozgen, S. Altintas, Shear stress studies of plasma-sprayed teeth derived hydroxyapatite on titanium, Key Eng. Mater. 309-311 (2006) 627–630.

[13] F.N. Oktar, S. Ozsoy, S. Altintas, A practical method for clinical veterinary use: use of dentine hydroxyapatite (DHA) as a graft material, Key Eng. Mater. 309–311 (2006) 1387–1390.

[14] F.N. Oktar, K. Kesenci, E. Piskin, Characterization of processed tooth hydroxyapatite for potential biomedical implant applications, Int. J. Artif. Cells, Blood Substitu. Immob. Biotech. 27 (1999) 367–379.

[15] G. Goller, H. Demirkiran, F.N. Oktar, E. Demirkesen, Processing and characterization of bioglass reinforced hydroxyapatite composites, Ceram. Int. 29 (2003) 721–724.

[16] G. Goller, F.N. Oktar, D. Toykan, E.S. Kayali, The improvement of titanium reinforced hydroxyapatite for biomedical applications, Key Eng. Mater. 240–242 (2003) 619–622.

[17] A.A. Caputo, Standlee F P., Biomechanics in Clinical Dentistry, Quintessence Pub, Co. Inc., Chicago, London, Berlin, Sao Paulo, Tokyo, Hong-Kong, 1987.

[18] J.L. Cuy, A.B. Mann, K.J. Livi, M.F. Teaford, T.P. Weihs, Nanoindentation mapping of the mechanical properties of human molar tooth enamel, Arch. Oral Biol. 47 (2002) 281–291.

[19] L. Lagocka, K. Jakubowska, K. Opalko, D. Chlubek, Content of florides soluble and insoluble in potassium hydroxide in superficial layers of enamel in adults regularly using fluoride-supplemented toothpaste, in: XXIIIrd ISFR Conference, Vol. 33, No. 3, Szczecin, June 11-14 Poland, 2000.

[20] E. Lugscheider, M. Knepper, K.A. Gross, Spray drying of apatite powders for thermal spraying applications, in: Proceedings International Thermal Spray Conference & Exhibition, Orlando, Florida, USA, (1992), pp. 459–464.

[21] S. Agathopoulos, A.U. Tulyaganov, P.A.A.P. Marques, M.C. Ferro, M.H.V. Fernandes, R.N. Correia, The fluorapatite-anorthite system in biomedicine, Biomaterials 24 (2003) 1317–1331.

[22] S. Agathopoulos, P. Nikolopoulos, A. Salomoni, I.A. Stamenkovic, Preparation and properties of binary oxide bioceramics, J. Mater. Sci.:Mater. Med. 7 (1996) 629–636.

[23] I.A. Key, S. Pina, S. Agathopoulos, D.U. Tulyaganov, J.M.F. Ferreira, Interfacial interactions between liquid new biocompatible model glasses and solid metallic and ceramic substrates used in biomedicine, Key Eng. Mater. 284-286 (2005) 835–838.

[24] S. Agathopoulos, D.U. Tulyaganov, P. Valerio, J.M.F. Ferreira, A new model formulation of the SiO_2–Al_2O_3–B_2O_3–MgO–CaO–Na_2O–F glass–ceramics, Biomaterials 26 (2005) 2255–2264.

[25] F.N. Oktar, G. Goller, Sintering effects on mechanical properties of glass-reinforced hydroxyapatite composites, Ceram. Int. 28 (2002) 617–621.

[26] J.V. Naidich, The wettability of solids by liquid metals, Prog. Surf. Membr. Sci. 14 (1981) 353–484.

[27] R. Murugan, T.S. Kumar, K.P. Rao, Fluorinated bovine hydroxyapatite: preparation and characterization, Mater. Lett. 57 (2002) 429–433.

[28] E.A. Monroe, Biomaterials–Teeth, Course Notes, State University of New York, College of Ceramics at Alfred University, Alfred, NY, USA, 1987 (June 30).

[29] R.Z. LeGeros, Calcium Phosphates in Oral Biology and Medicine (Monographs in Oral Science), S. KargerPub., 1991. p. 145.

[30] A.R. TenCate, Oral Histology, 4th ed., Mosby Pub, 1997.

[31] B.R. Schemehorn, J.C. Orban, G.D. Wood, G.M. Fischer, A.E. Winston, Remineralization by fluoride enhanced with calcium and phosphate ingredients, J. Clin. Dent. 10 (1999) 13–16.

[32] A. Ravaglioli, A. Krajewski, Bioceramics, Materials, Properties, Applications, Chapman & Hall, London, New York, Tokyo, Melbourne and Madras, 1992, p. 193.

[33] D. Waszkiel, L. Lagocka, K. Jakubowska, K. Opalko, I. Nocen, D. Chlubek, Content of fluoride and magnesium in superficial layer of human enamel with erosion - an in vivo study, in: XXIIIrd ISFR Conference, Vol. 33, No. 3, Szczecin, June 11-14 Poland, 2000.

[34] E.K. Mahoney, R. Rohanizadeh, F.S.M. Ismail, N.M. Kilpatrick, M.V. Swain, Mechanical properties and microstructure of hypomineralised enamel of permanent teeth, Biomaterials 25 (2004) 5091–15100.

[35] Q. Song, C. Wang, S. Wen, Effects of doping on crystal and grain boundary in human-enamel, Mater. Sci. Eng. A 297 (2000) 272–280.

[36] M. Okazaki, M. Taira, J. Takashi, H. Tohda, T. Yanagisawa, Heterogeneous fluoridated hydroxyapatites partially substituted with fluoride ions, Bio-Med. Mater. Eng. 7 (1997) 141–148.

[37] K.A. Gross, L.M. Rodriguez- Lorenzo, Sintered hydroxyfluorapatites. Part I: sintering ability of precipitated solid solution powders, Biomaterials 25 (2004) 1375–1385.

[38] K.A. Gross, Rodriguez-LorenzoF L.M., Sintered hydroxyfluorapatites. Part II: mechanical properties of solid solutions determined by micro-indentation, Biomaterials 25 (2004) 1385–1394.

[39] K.A. Gross, L.M.K.A. Bhadang, Sintered hydroxyfluorapatites. Part HI: Sintering and resultant mechanical properties of sintered blends of hydroxyapatite and fluorapatite, Biomaterials 25 (2004) 1395–1405.

[40] S. Agathopoulos, D.U. Tulyaganov, P.A.A.P. Marques, M.C. Ferro, M.H.V. Fernandes, R.N. Correia, The Fluorapatite-anorthite system in biomedicine, Biomaterials 24 (7) (2003) 1317–1331.

[41] S. Agathopoulos, D.U. Tulyaganov, J.M.G. Ventura, S. Kannan, M.A. Karakassides, J.M.F. Ferreira, Formation of hydroxyapatite onto glasses from the CaO–MgO–SiO_2 system with B_2O_3, Na_2O, CaF_2 and P_2O_5 additives, Biomaterials 27 (2006) 1832–1840.

[42] B. Oral, D. Ozbasar, The effect of sodium monofluorophosphate therapy on lipid and lipoprotein metabolism in postmenopausal women, Euro. J. Obst. Gyn. Rep. Bio. 107 (2003) 180–184.

[43] S. Hillier, C. Cooper, S. Kellingray, G. Russell, H. Hughes, D. Coggon, Fluoride in drinking water and risk of hip fracture in the UK: a case–control study, Lancet 355 (2000) 265–269.

[44] R. Lehmann, M. Wapniarz, B. Hofmann, B. Pieper, I. Haubitz, B. Allolio, Drinking water fluoridation: Bone mineral density and hip fracture incidence, Bone 22 (1998) 273–278.

[45] M.R. Karagas, J.A. Baron, J.A. Barrett, Si. Jacobsen, Patterns of fracture among the United States elderly: geographic and fluoride effects, Ann. Epidemiol. 6 (1996) 209–216.

[46] J. Cunha-Cruz, P. Nadanovsky, Dental fluorosis increases caries risk, J. Evid. Bas. Dent. Prac. 5 (2005) 170–171.

[47] P.T.C. Harrison, Fluoride in water: a UK perspective, J. Fluor. Chem. 126 (2005) 1448–1456.

[48] N. Guafiabens, J. Farrerons, L. Perez-Edo, A. Monegal, A. Renau, J. Carbonell, M. Roca, M. Torra, M. Pavesi, Cyclical etidronate versus sodium fluoride in established postmenopausal osteoporosis: a randomized 3 year trial, Bone 27 (2000) 123–128.

[49] P. Valerio, F.N. Oktar, L.S. Ozyegin, G. Goller, A.M. Goes, M.F. Leite, Biocompatibility evaluation of dentine, enamel and bone derived hydroxyapatite, Key Eng. Mater. 254–256 (2004) 837–840.

[50] F.N. Oktar, G. Goller, M. Yetmez, D. Toykan, Effects of bond-coatings on plasma sprayed calciumphosphate coatings, Key Eng. Mater. 240–242 (2003) 315–318.

[51] F.N. Oktar, S. Ozsoy, L.S. Ozyegin, A radiological follow-up study of plasma-sprayed fluorapatite (FA) coatings, Key Eng. Mater. 240–242 (2003) 327–329.