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Influence of fluorapatite minor additions on behavior of hydroxyapatite ceramics

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Fluorinated hydroxyapatite is known to be less soluble by body fluids, resulting in enhanced resistance to biodegradation *in vivo* conditions, as compared to the pure hydroxyapatite ceramics. The present work was aimed at the investigation of the effect of minor additions of ultrafine fluorapatite (up to 10 wt %) on the sintering behavior and mechanical properties of hydroxyapatite ceramics. *In vitro* testing for the osteoblast-like cells viability and proliferation was performed with the samples of varying fluorapatite content. It was found that the fluorapatite addition hinders the sintering shrinkage and lowers the strength, but does not generally affect negatively the viability of the cells.

1. Introduction

Hydroxyapatite (HA) ceramics are of considerable interest for clinical applications due to their high biocompatibility with bone and teeth tissue. HA is the most stable calcium phosphate in an aqueous solution and that with the largest mechanical properties at a given porosity [1, 2]. The solubility of HA is believed to depend on the Ca/P ratio, crystallinity index, particle size, porosity and foreign ions such as carbonates, chlorine, fluorine, and magnesium ions, which are often present in HA as impurities or doping additives, these elements being present in natural HA of the human bone [1–3]. HA-base materials have found wide clinical applications as particulates, coatings, and dense or porous ceramics due to their ability to form strong bond with bone *in vivo* [2–6]. However, the bioresorption of HA, particularly resulting from the dissolution by extracellular fluids, affects negatively the fixation of implanted device into the bone or teeth tissue [7]. Fluorinated HA is known to be less soluble than pure HA [1, 7, 8]. For example, the estimation of the solubility product for HA and fluorine-substituted HA (FHA) gives 1.022×10^{-122} and 4.3×10^{-126}, respectively [7]. The data of the comparative *in vivo* testing of the HA, fluorapatite (FA) and FHA plasma-spray coatings in dogs jaws confirm the fluorine substitution for OH groups enhances significantly the resistance of apatite to resorption by body fluids [7]. Incorporation of fluorine increases the protection of teeth from caries, however, the high fluorine content can result in the fluorosis due to the CaF_2 and FA formation [9, 10]. A survey of the problem has been given by Jenkins [10].

FHA can be fabricated by the cyclic pH variation technique, e.g. using the reaction between solid HA and NaF solution [11, 12]. Solid-state synthesis based on the interaction between HA and ammonium fluoride at a high temperature, usually 1200 °C, has been described in Gineste *et al.* [7]. Another route is the solid state reaction of HA with CaF_2 [13]. However, there exists an apprehension that the rest of non-reacted fluoride could influence negatively the remineralization and promote to the formation of CaF_2-like deposits. Therefore, the further investigations of fluoride-containing HA-base materials are desirable which will be helpful in efforts to develop improved-efficacy anticaries compositions. One of such attempts was reported by Kurmaev *et al.* [14], who have studied the formation of FHA by the hydrolysis of tetracalcium phosphate, $Ca_4(PO_4)_2O$, in 0.1 mol/1 KH_2PO_4 solution in the presence of potassium fluoride, KF.

The present study was aimed at the investigation of an alternative way to introduce fluorine ions into HA-ceramics, namely through the direct sintering of HA-FA mixtures of varying FA content. Such a system might be

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applied to non-biodegradable (e.g. dental) implants. Influence of the FA additives on the sinterability, strength of sintered ceramics and osteoblast-like sarcoma cells behavior *in vitro* was evaluated.

2. Materials and methods

2.1. Materials

As the starting reagents, analytical grade $CaCl_2$, $(NH_4)_2HPO_4$, NH_4OH and NH_4F were used. The HA powder was fabricated by co-precipitation from aqueous solution according to the following reaction [15]:

$$10CaCl_2 + 6(NH_4)_2HPO_4 + 8NH_4OH \Rightarrow Ca_{10}(PO_4)_6(OH)_2 + 20NH_4Cl + 6H_2O$$

The reaction has previously been studied in details by the Tananaev's residual concentrations method [15].

The synthesis product was calcined at 900 °C, resulting in fine HA powder of the following characteristics: Ca/P ratio 1.67; chemical composition (wet chemical analysis), in wt %: Ca^{2+} 36.00, PO_4^{3-} 51.23, H_2O 12.87; BET specific area as determined by nitrogen gas adsorption (a Quantachrome Autosorb) is about 4.5 sq m/g; median powder agglomerate size (a Coulter Counter) is about 2.06 μm; density is 3.16 g/cm^3. No minor phase was detected by X-ray diffractometry (XRD, a DRON-3 and a Rigaku Denki diffractometers) in the calcined powder. Crystallinity index of the powder is 0.76.

The FA was prepared by precipitation from aqueous solution according to the reaction:

$$10CaCl_2 + 6(NH_4)_2HPO_4 + 2NH_4F + 6NH_4OH \Rightarrow Ca_{10}(PO_4)_6F_2 + 20NH_4Cl + 6H_2O$$

The characteristics of the synthesis product after calcination at 900 °C were as follows: Ca/P = 1.67; Ca/F = 5.0 according to wet chemical analysis; BET specific area is 13.5 sq m/g; particles size is less than 1.0 μm; density is 3.18 g/cm^3. No minor phase was detected by XRD. The powder is poorly crystallized, according to the XRD and small-angle X-ray scattering data. The synthesis product is of following chemical composition (wt %): Ca^{2+} 39.7, PO_4^{3-} 56.6, F^- 3.8, the theoretical composition of FA being (wt %): Ca^{2+} 39.54, PO_4^{3-} 56.50, F^- 3.96. The absence of an OH^- band at 3570 cm^{-1} in IR spectrum confirms the synthesis product is FA.

The mixtures of HA with FA were prepared in a ball mill using an ethanol media and corundum balls. The batches containing 0, 1, 2 and 10 wt % FA were uniaxially pressed under the pressure from 80 to 200 MPa, followed by sintering in an air furnace at 1150, 1200, 1250, 1350 and 1400 °C for 2 h. The samples were discs of about 14 mm diameter and 1.5 mm thickness, or rectangular bars of $3 \times 4 \times 35$ mm^3 dimension.

2.2. Methods

The samples were characterized for open pores content (Archimedean method): open pores size distribution (mercury porometry, a Quntachrome Autopore); linear sintering shrinkage (dilatometry, an Adhamel Lomargy Dilatometer); specific area (a BET method which is based on the measurements of the quantity of nitrogen gas adsorbed onto or desorbed from a solid surface at some equilibrium vapor pressure, a Quantochrome Autosorb equipment); phases content (XRD, DRON 3 diffractometer, CuK$_\alpha$ radiation); three-point bending strength (UTS-100 Testmachine, an error of load measurement was kept at ± 1%); Vickers microhardness (a PMT-3 hardness tester, indentation load 0.49 N, the imprint diagonal measurement error was about 10%); scanning electron microscopy (SEM, a Philips XL 20 microscope coupled with energy dispersion X-ray analyzer, EDX), IR spectrometry (a Specord 75 spectrometer), vertical scanning interferometry (a WYKO microscope), and laser mass-spectrometry (a EMAL-2 analyzer).

To characterize the crystallinity index, an innovative ''four peaks'' technique was developed. This XRD method is accounted for four main peaks of the diffraction pattern: (211), (112), (300) and (202). The crystallinity index, I_c, was evaluated as the ratio

$$I_c = [I(211) + I(112) + I(300) + I(202)]_s / [I(211) + I(112) + I(300) + I(202)]_r$$

where I is the peak intensity; subscripts s and r denote sample and reference, respectively. To measure the XRD patterns, a common X-ray diffractometry (radiation wavelength $\lambda = 1.54$ Å) and diffraction of synchrotronic radiation (ESRF Grenoble, $\lambda = 0.32$ Å, 37 KeV) were employed. The crystallinity index of a reference sample was evaluated according to the procedure of AFNOR S94068 standard using an alpha-alumina powder (RM 300) as the sample of 100% crystallinity.

The surface roughness of the sintered ceramics was evaluated by vertical scanning interferometry method (a WYCO microscope). The following parameters were computed: R_a (roughness average), R_q (root mean square roughness), R_z (average maximum amplitude) and R_t (maximum amplitude of the profile).

In vitro studies for MG-63 osteoblast-like cells viability and proliferation were performed. The cells were isolated from a human osteosarcoma and cultured in 25 cm^3 culture flask containing Dulbecco minimum essential media (DMEM) supplemented with 10% human serum, L-glutamine, penicillin–streptomycin and fungizone. The ceramic samples were discs of 14 mm diameter and 1.5 mm thickness. The cells were also seeded onto polystyrene surfaces (tissue culture plastic) to serve as controls. Characterization of osteoblast behavior on the different surfaces was made after 1, 4, 10, 24 and 48 h. Both microscopic and biochemical methods were used, such as SEM, environmental SEM (a special type of SEM that operates at moderate vacuum and in a humid atmosphere, enabling surface studies of biological tissues) and detection of initial cells attachment, proliferation and viability by measurement of mitochondrial enzyme activity (MTT assay). Approximately 15 000 cells were seeded onto each substratum. The cells were counted using a hemacytometer. Non-attached cells were removed by rinsing. Living cells were detected by reducing the

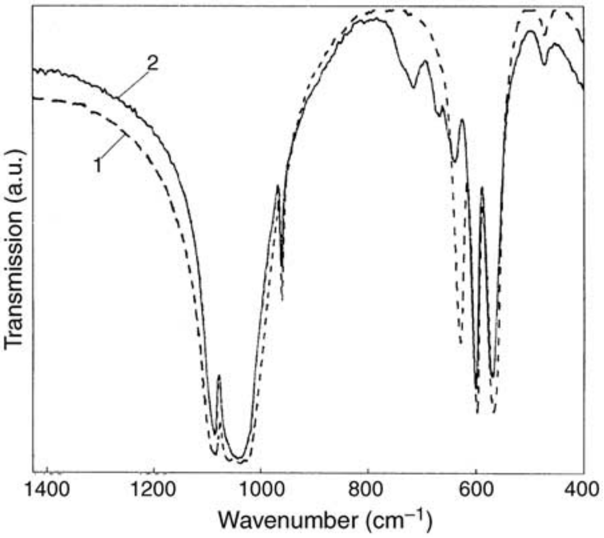

Figure 1 IR spectra of initial mechanical mixture HA–10 wt %FA (curve 1) and the sintered at 1200 °C sample of the same composition (curve 2).

tetrazolin salt to intensively colored formazan derivatives followed by absorbance measurements at $\lambda = 652$ nm.

3. Results and discussion

XRD diffraction patterns of calcined powders correspond to the standard ASTM data. No additional phases were revealed. The IR spectra of the initial powders were typical for the HA and FA, respectively. There is only one O–H stretching band at 3573 cm^{-1} for pure HA sample, while the band at 3540 cm^{-1} was present for FA [13]. Besides, the spectra in the 500–800 cm^{-1} range contains two strong bands at 601 and 571 cm^{-1} due to the ν_4 mode of the PO_4 tetrahedra in both the compounds, and the band at 631 cm^{-1} assigned to the Ca_3–OH librational mode for the HA only. The position and intensity of this band depends strongly on the introduction of F^- into the linear $[OH]^-$ chains. At the substitution of fluorine for OH^- groups of about 10%, the position of this band corresponds to 637 cm^{-1}, being the characteristic of the FHA spectrum.

Shown in Fig. 1 is the IR spectra from the mechanical mixture of 90%HA with 10%FA initial powders (curve 1) and from the sample of the same composition sintered at 1200 °C for 1 h in ambient air atmosphere (curve 2). The difference in the region 600 to 800 cm^{-1} is obvious. The solid solution HA–FA formation can be concluded from these data.

Table I gives the data of the laser mass spectrometry analysis of the initial powders (HA and 90%HA–10%FA mixture) and the ceramics of the same compositions sintered at 1200 °C in air atmosphere. It can be seen that the sintering does not practically influence the composition, e.g. indicating the fluorine does not evaporate from the powder during sintering. Some minor fluorine content in the HA powder and ceramics is probably due to the contamination from the furnace refractory lining.

TABLE I The results of laser mass spectrometry analysis of initial powders and sintered at 1200 °C ceramics

Material		Content, wt %				
		Ca	P	F	O	Si
HA	Powder	39.52	18.25	0.03	41.63	0.16
	Ceramics	39.82	18.25	0.05	41.57	0.11
90%HA–10%FA	Powder	39.62	18.81	0.61	40.32	0.22
	Ceramics	39.91	18.26	0.65	40.86	0.11

Given in Table II is the crystallinity index of sintered at 1200 °C ceramic samples evaluated with four peaks method, the index value equals to 1.0 being accepted for the ceramics sintered of the initial HA powder, as a reference. The FA addition decreases slightly the index, the lowest value was found for the ceramics containing 10 wt % FA. Therefore, the FA either was not crystallized fully at 1200 °C, or does induce distortion in the HA lattice resulting in the broadening of the peaks of the diffraction pattern.

Generally, an increase in the pressing pressure up to 150 MPa enhances monotonously the sintering shrinkage, however further rise of the pressure over 150 MPa can result in an overpressing effect and the lateral cracks formation in the green body, probably due to the fine FA additives, a phenomenon that is well known in the powders compaction practice. The addition of FA decreases the linear shrinkage at a certain sintering temperature, as it can be seen in Fig. 2, as an example. Fig. 3 shows the effect of sintering temperature on the linear shrinkage of the samples, the green bodies being compacted by applying of uniaxial pressure of 150 MPa. The addition of FA is seen to reduce the sinterability of the composite mixtures, in spite of the FA powder was of smaller particle size, as compared to the HA initial powder. In particular, the BET specific area of the sintered at 1200 °C samples increases from 0.3 up to 0.8 sq.m/g with an increase of the FA content from 0 to 10 wt %, indicating an enlargement in porosity.

FA additions are seen to hinder the sintering of the HA–FA mixtures. XRD revealed the samples with 2 wt % FA addition sintered at 1400 °C contain beta tricalcium phosphate (TCP), and these with 10 wt % FA contain both alpha and beta TCP in total amount about 7 wt %, the β-TCP content being of about 3 wt %. Due to the low percentage of TCP, only semi-quantitative estimations were made. Because both the synthetic FA and HA do not contain the impurities of Mg and other elements, which

TABLE II Crystallinity index of sintered ceramics

Material	Intensity	FHWM	Intensity	FHWM	Intensity	FHWM	Intensity	FHWM	Crystallinity index
	Peak (211)		Peak (112)		Peak (300)		Peak (202)		
Reference	935.1	0.2478	467.7	0.2376	573.9	0.260	212.3	0.2526	1
HA–1%FA	892.0	0.2403	451.7	0.2255	560.6	0.247	211.3	0.2550	0.936
HA–2%FA	946.1	0.2268	453.6	0.2177	611.6	0.233	230.5	0.2305	0.937
HA–10%FA	961.1	0.2179	467.1	0.2077	593.1	0.226	244.9	0.2234	0.912

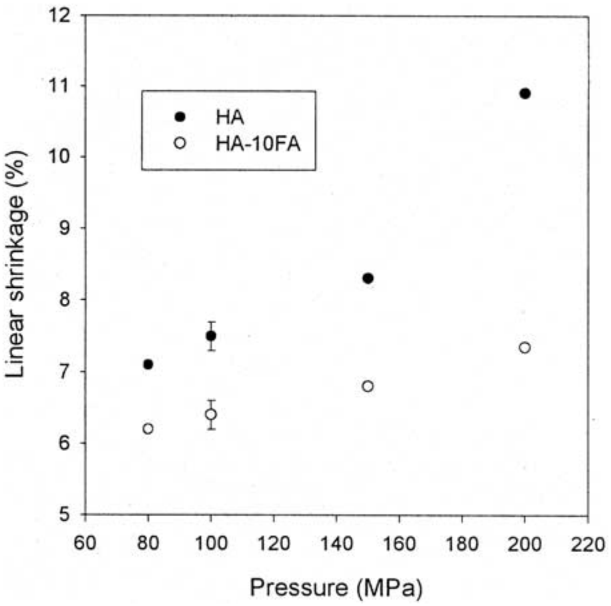

Figure 2 Linear shrinkage vs. compacting pressure for ceramics sintered at 1200 °C.

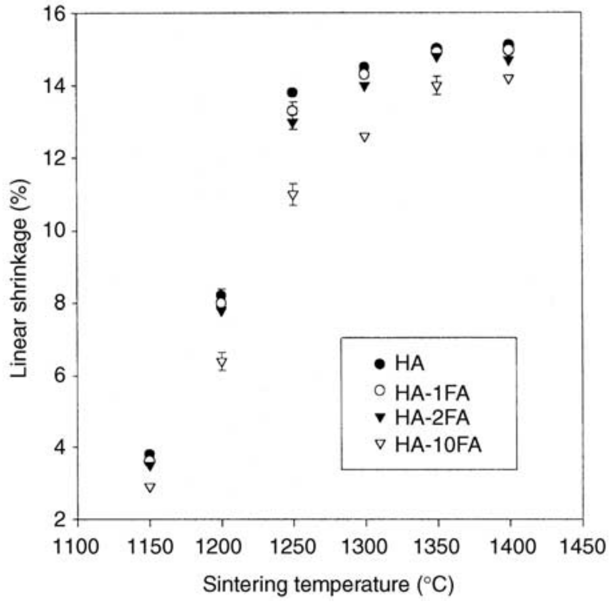

Figure 3 Linear shrinkage vs. sintering temperature for samples which were compacted at a pressure 150 MPa.

can destabilize the apatite structure of HA at high temperatures favoring its thermal conversion to TCP [15], this phenomenon can be related to the influence of FA itself on the decomposition of HA during the heat treatment. Density of sintered HA samples reaches the theoretical one only at sintering temperature of 1350 °C and above.

Residual porosity affects the mechanical properties. Fig. 4 shows bending strength *versus* sintering temperature plot for the HA–FA samples, which have been compacted at the pressing pressure of 150 MPa. The strength is falling down with the rise in both the FA percentage and pores content, the latter dependence being in accordance to an exponential law. Fracture toughness decreases from 0.77 down to 0.69 MPa m$^{1/2}$ (measured by indentation method using the common relation between the length of semi-circular crack and the indentation load [18]), and the microhardness of the samples decreases from 1.87 down to 1.63 GPa with an increase of FA content from 0 to 10 wt %. The significant difference in strength for the samples of varying FA content is rather surprising, just because the pore content does not differ so much, especially for the samples which have been sintered at relatively high temperature, e.g. 1350–1400 °C. The effect could probably be explained considering the destabilizing action of the FA on the phase composition of ceramics. It can be supposed that the residual stresses, resulting from the phase decomposition induced by FA at the sintering, lead to the microcracking and, therefore, to the weakening of the ceramics.

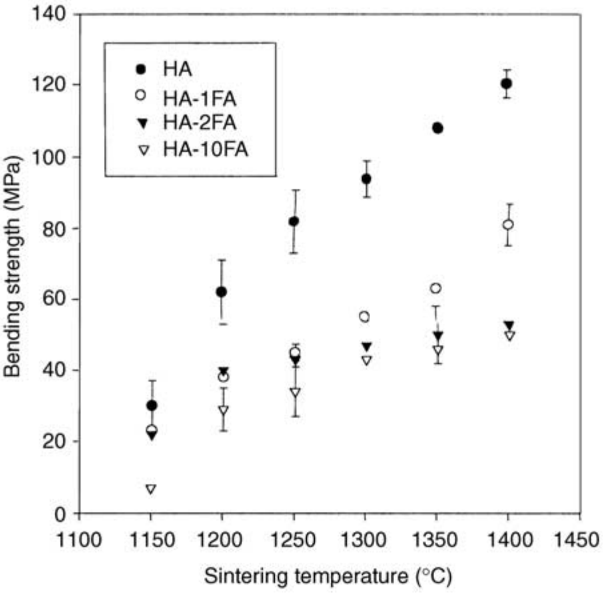

Figure 4 Bending strength vs. sintering temperature for the samples which were compacted at a pressure of 150 MPa.

Fig. 5 shows the SEM micrographs of the samples surface to be tested *in vitro*. The sintering conditions were selected in the range from 1180 to 1250 °C to match the microstructure of the samples, which must be generally independent on the FA content to get the reasonable results of the comparative *in vitro* testing. Mercury porometry measurements revealed that two pore size populations exist in the ceramics. The former corresponds to the submicropores of 0.4 to 1.0 μm effective diameter (this population occupies about 40–60% of the total pores volume), the latter being the pores of 3–10 μm size. These pores were revealed by SEM observations (Fig. 5), and there is practically no difference between the pores size and distribution in the samples of different FA content. It can be supposed that large-size pores are interconnected by slit-like channels in the microstructure. The latter are critical for mercury penetration into the porous body during the pore size distribution measurements by a mercury porometry method [16]. The total open pores content in the ceramics was in the range from 25 to 28 vol %.

The initial surface roughness of the *in vitro* tested samples is given in Table III. The roughness parameters of the samples are almost identical. This can also be seen on the SEM micrographs of the surfaces.

Shown in Fig. 6 is the amount of viable cells on the four groups of materials under study and on the control. It is clear that the number of viable cells increases with

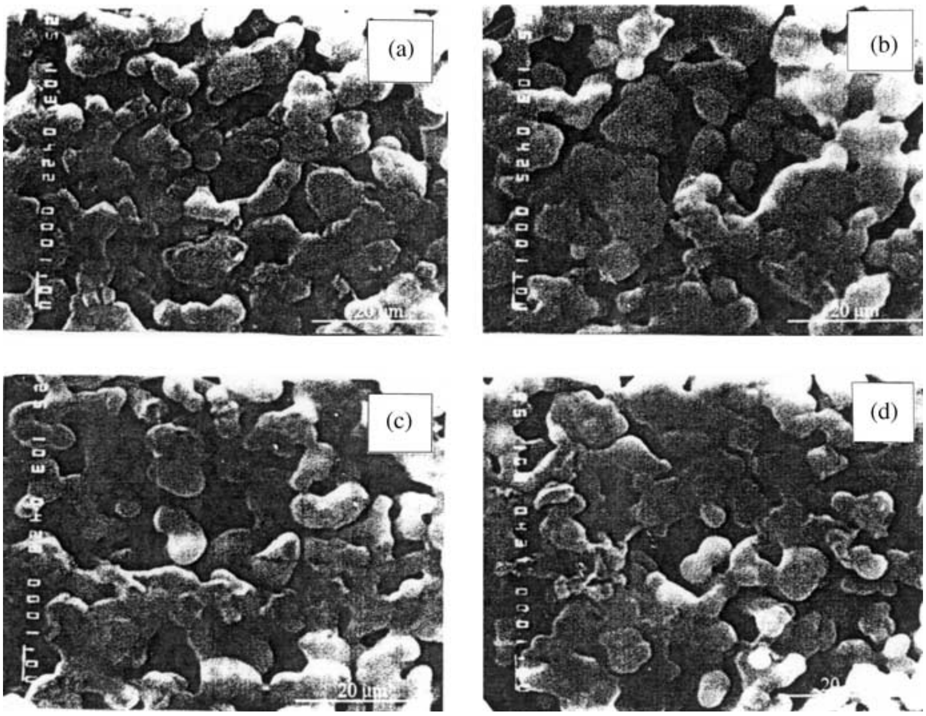

Figure 5 SEM micrographs of the surfaces of the samples: HA (A); HA–1%FA (B); HA–2%FA (C) and HA–10%FA (D).

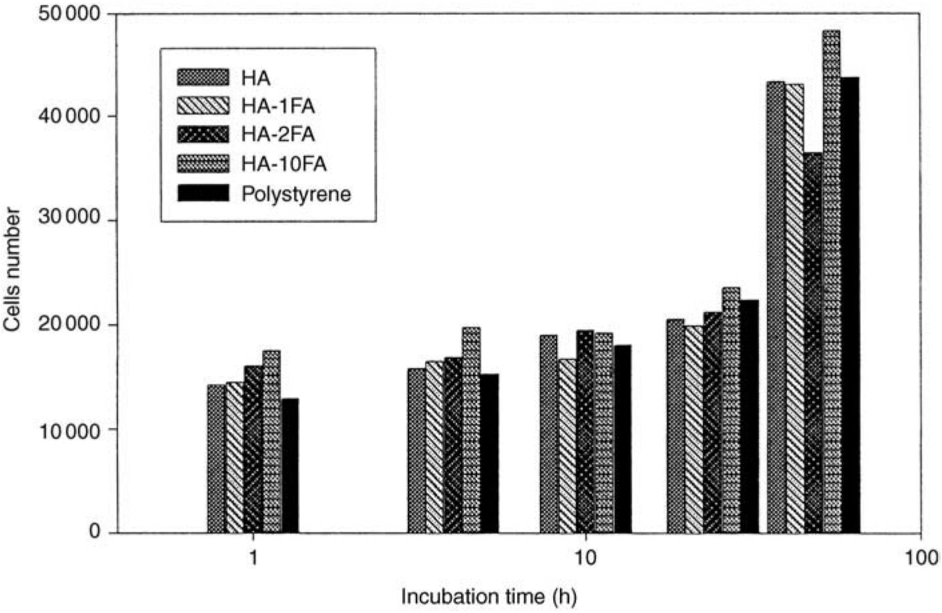

Figure 6 Comparison of amount of viable cells between the four groups of ceramic samples (including the control) cultured for 1, 4, 10, 24 and 48 h.

TABLE III Roughness parameters (in µm)

Material	R_a	R_q	R_z	R_t
HA	0.45 ± 0.07	0.56 ± 0.08	8.81 ± 0.90	10.61 ± 1.33
HA–1%FA	0.61 ± 0.20	0.83 ± 0.22	10.09 ± 1.39	12.45 ± 2.14
HA–2%FA	0.51 ± 0.17	0.72 ± 0.23	9.47 ± 1.62	11.77 ± 2.19
HA–10%FA	0.57 ± 0.12	0.72 ± 0.10	9.07 ± 0.81	10.88 ± 1.17

incubation time for all materials. One can also conclude that the difference in cells proliferation is small between all materials. The lowest proliferation rate on HA–2%FA surface can be explained by the pores size on these samples are smaller than for any of other materials. No pores are big enough to fit a whole cell but the pseudopodia might very well find their way into the pores. If the pores are large enough for two pseudopodia, the cells could still communicate, but if there is only room for one, the cells cannot send each other ‘‘signals’’ as efficiently. The latter could lead to a decrease in proliferation.

Thus, the addition of the synthetic, fine-grained FA promotes the decomposition of HA at high temperature, hindering the sintering densification and decreasing the mechanical properties, in spite of the solid-solution of FA in HA is formed. The FA additions up to 10 wt % do not affect negatively the viability of osteoblast-like cells on the HA-base ceramics. This study has naturally to be continued with freshly isolated human osteoblasts to see if they show receptors and release factors such as alkaline phosphate and collagen in the same way as they do it in the human environment and if they mineralize and differentiate into osteocytes.

4. Conclusions

The study of the effect of fluorapatite minor additions on sintreability, mechanical properties and *in vitro* behavior of HA-base ceramics was performed. The following conclusions can be drawn from the results.

1. HA forms solid solution with 0–10 wt % FA at the sintering temperature 1200 °C and above.
2. FA hinders the sintering shrinkage, promotes decomposition of HA and lowers the mechanical properties of sintered ceramics.
3. FA additives in the range up to 10 wt % do not influence negatively the viability behavior of the osteoblast-like human cells onto ceramics of 25–28% open pores content.

Acknowledgments

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