Enhanced performance of fluorine substituted hydroxyapatite composites for hard tissue engineering

HAE-WON KIM, YOON-JUNG NOH, YOUNG-HAG KOH, HYOUN-EE KIM*
School of Materials Science and Engineering, Seoul National University, Seoul 151-742, Korea
E-mail: kimhe@snu.ac.kr

Dense fluorine (F) substituted hydroxyapatite composites with yttria-doped zirconia (Y-TZP) and/or alumina (Al_2O_3) were successfully fabricated without applying pressure at 1400 °C for 3 h. The suppression of decomposition via the formation of a fluor-hydroxyapatite (FHA) solid solution allowed the sintered body to reach full density. Such fully densified FHA-composites exhibited improved mechanical properties, such as strength, toughness, and hardness, having values of more than ~ 2–4 times higher than those of pure HA or HA-composites. The proliferation behavior of osteoblast-like cells on the FHA-composites showed no cytotoxicity and comparable cell viability to that observed in pure HA for up to 10 days.

1. Introduction

Hydroxyapatite [HA, $Ca_{10}(PO_4)_6(OH)_2$] has attracted much attention for use as a substitute for bone and teeth due to its excellent biocompatibility to human tissues [1, 2]. Nevertheless, its intrinsic poor mechanical properties (strength, toughness, and hardness) have restricted wider applications in load-bearing implants [3]. Therefore, many attempts have been made to reinforce the HA by incorporating second phases, such as ceramic particles, whiskers, fibers, and metallic dispersoids. Among them, yttria-doped zirconia (Y-TZP) or alumina (Al_2O_3) has been introduced due to their excellent mechanical properties [4, 5]. However, the addition of those ceramic materials increases the decomposition rate of HA to tri-calcium phosphate [TCP (α- or β-)], resulting in a poor densification behavior and reduced mechanical properties [6, 7]. Hence, steps must be taken to produce fully densified HA–ZrO_2 or HA–Al_2O_3 composites without causing serious decomposition. It is known that fluorapatite [FA: $Ca_{10}(PO_4)_6F_2$] has a higher thermal stability than HA [8]. Therefore, it is expected that the introduction of FA will retard the decomposition of HA. In addition, the F itself retains advantages over other ions in that it protects teeth from dental caries, especially in the environment in the oral cavity as well as enhances mineralization and crystallization [9, 10]. However, only limited studies on the sintering behaviors and mechanical or biological properties of the FA or FA-composites have been done [11, 12].

In this study, fluor-hydroxyapatite [FHA: $Ca_{10}(PO_4)_6(OH,F)_2$] composites with yttria-doped ZrO_2 and/or Al_2O_3 were fabricated by pressureless sintering. The densification behavior and mechanical properties of the FHA-composites were investigated and correlated with their thermal stability by a comparison with HA-composites. Furthermore, in order to evaluate the cell viability, the human osteoblastic cellular responses to the composites were assessed.

2. Materials and methods

Initially, the FA powder was fabricated from a reaction between TCP (Aldrich, USA) and CaF_2 (Aldrich, USA) at 1000 °C for 3 h in air. The resulting FA was mixed with HA (Alfa, Aesar Co., USA) together with yttria-doped ZrO_2 (3Y-TZP, Tosoh Co., Japan) and/or Al_2O_3 (AKP-50, Tosoh Co., Japan) to fabricate the FHA–ZrO_2 and/or –Al_2O_3 composites. The molar ratio of FA to HA was 80%, and the volume ratio of yttria-doped ZrO_2 and Al_2O_3 to the FHA matrix was 40%. For a comparison, pure HA composites with yttria-doped ZrO_2 or Al_2O_3 were also fabricated. The designation and composition of the fabricated composites are shown in Table I. The resulting mixtures were ball-milled, oven-dried, and passed through a 70-mesh screen. After cold isostatic pressing (CIP) at 300 MPa, each sample was sintered without applying pressure at 1400 °C for 3 h in air. The specimens were machined into bar shapes with dimensions of ~ 3 × 4 × 25 mm and polished down to 1 μm, then subsequently beveled to minimize machining flaws. The apparent porosity was measured using the Archimedes method. The microstructure of the specimens was evaluated with scanning electron microscopy (SEM) and the phase composition after sintering was

*Author to whom all correspondence should be addressed.

TABLE I Designation and composition of the fabricated materials

Designation	Materials	Composition		
		FA (relative to HA, mol %)	ZrO_2 (relative to HA + FA, vol %)	Al_2O_3 (relative to HA + FA, vol %)
HA	Pure HA	—	—	—
HA-Z	HA + ZrO_2	—	40	—
HA-A	HA + Al_2O_3	—	—	40
FHA-Z	FHA + ZrO_2	80	40	—
FHA-A	FHA + Al_2O_3	80	—	40
FHA-ZA	FHA + ZrO_2 + Al_2O_3	80	20	20

analyzed by X-ray diffraction (XRD). To observe the formation of the FHA solid solution, the lattice parameters were calculated from XRD using Si (99.999%) powder as an internal standard. The flexural strength and fracture toughness were determined by a four-point flexural configuration and indentation–strength (I–S) method, respectively [13]. The elastic modulus was measured by an ultrasonic pulse method and the hardness was measured using a Vickers indenter at a load of 1.96 N for 15 s. For each set of conditions, five specimens were tested. The *in vitro* proliferation test was performed as described previously [5]. In brief, the MG63 cells were placed on each of the polished samples and the Thermanox control with a cell density of 1.5×10^4 cells/cm^2, then cultured for up to 10 days in a 5% CO_2 humidified atmosphere in air at 37 °C. The used cell media was Dulbecco's modified Eagle's medium (DMEM, Life Technologies, Inc., MD) supplemented with 10% fetal bovine serum (FBS, Life Technologies, Inc., MD). After detaching them with a trypsin-EDTA solution and staining with trypan blue, the living cells were counted using a hemocytometer (Superior Co., Germany). Each test was performed in triplicate. The cell morphology was observed with SEM after fixation with 2.5% glutaraldehyde and dehydration with graded ethanol.

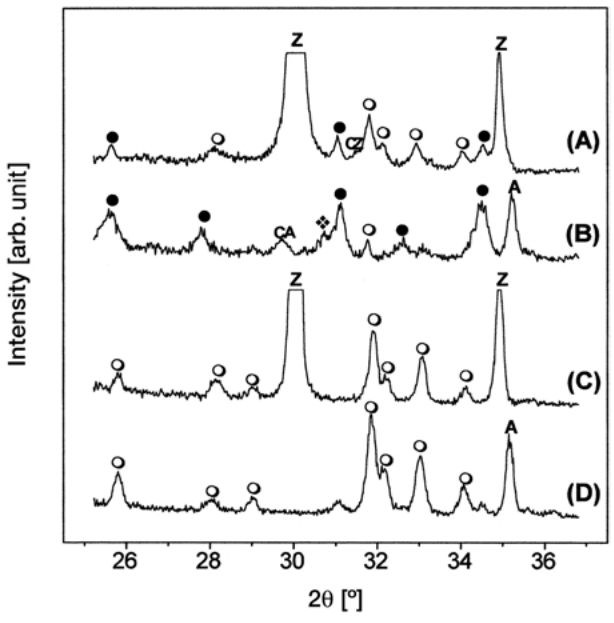

Figure 1 XRD patterns of the HA- and FHA-composites with ZrO_2 or Al_2O_3 after sintering at 1400 °C for 3 h; (A) HA-Z, (B) HA-A, (C) FHA-Z, and (D) FHA-A: (○; HA, ●; β-TCP, ❖; α-TCP, **Z**; ZrO_2, **A**; Al_2O_3, **CZ**; $CaZrO_3$, **CA**; $CaAl_2O_4$).

3. Results and discussion

Fig. 1(A)–(D) show the XRD patterns of the HA- or FHA-composites after sintering at 1400 °C for 3 h. In both HA-composites with yttria-doped ZrO_2 and Al_2O_3 (Fig. 1(A) and (B), respectively), large amounts of β-TCP together with $CaZrO_3$ or $CaAl_2O_4$ were observed. This showed that a significant degree of decomposition occurred, as illustrated in the following reactions (1) and (2):

$$Ca_{10}(PO_4)_6(OH)_2\ (s) + ZrO_2\ (s) = 3Ca_3(PO_4)_2\ (s) + CaZrO_3\ (s) + H_2O\ (g) \quad (1)$$

$$Ca_{10}(PO_4)_6(OH)_2\ (s) + Al_2O_3\ (s) = 3Ca_3(PO_4)_2\ (s) + CaAl_2O_4\ (s) + H_2O\ (g) \quad (2)$$

Even though pure HA is known to decompose to β-TCP [$Ca_3(PO_4)_2$] at ~ 1300–1350 °C, the presence of other oxides (yttria-doped ZrO_2 or Al_2O_3) accelerates the decomposition of HA by capturing Ca ions, resulting in the formation of reaction products ($CaZrO_3$ or $CaAl_2O_4$) along with β-TCP [6, 7]. Such a decomposition process has been the main drawback in the fabrication of dense HA-composites with other ceramics. In contrast, only slight amounts of β-TCP were observed in the FHA-composites (Fig. 1(C) and (D)). Moreover, the $CaZrO_3$/$CaAl_2O_4$ were barely detected. These XRD patterns confirm that the employing of FHA into the yttria-doped ZrO_2 or Al_2O_3 effectively suppressed the above decomposition process.

In Fig. 2, the degree of decomposition to β-TCP was analyzed by calculating the relative peak intensity of β-TCP to HA from the XRD patterns [14]. The amounts of β-TCP that formed in the HA-composites (HA-Z and HA-A) were sharply reduced in the FHA-composites (FHA-Z and FHA-A). This suppression of decomposition was attributed to the formation of a FHA solid solution, which retained a high chemical and thermal stability at the sintering temperatures. The formation of the FHA solid solution was further confirmed by the lattice parameters. There was significant decrease in the *a*-axis from 9.407 Å for HA down to 9.372 Å for FHA, and similar values in the *c*-axis (~ 6.88 Å) for both cases were observed. It has been recognized that the incorporation of F into the HA lattice contracts the *a*-axis with little change in the *c*-axis [15, 16].

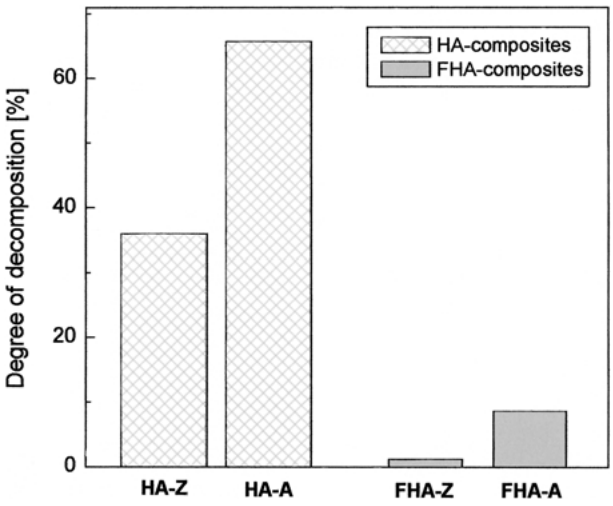

Figure 2 Degrees of decomposition of the HA- and FHA-composites with ZrO_2 or Al_2O_3 after sintering at 1400 °C for 3 h.

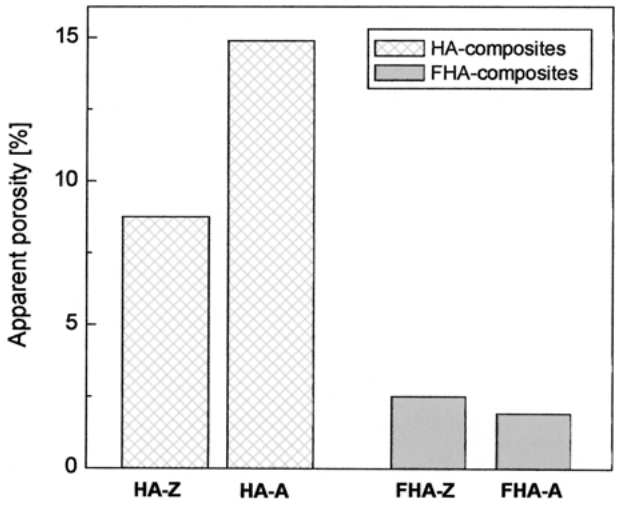

Figure 3 Apparent porosities of the HA- and FHA-composites with ZrO_2 or Al_2O_3 after sintering at 1400 °C for 3 h.

The reduced decomposition rate influenced the densification behavior of the composites significantly, as shown in Fig. 3. A large degree of apparent porosity (~ 10–15%) was observed in the HA-composites (HA-Z and HA-A). In contrast, almost full densification occurred in the FHA-composites (FHA-Z and FHA-A) with only a small degree of porosity (~ 2%).

The surface microstructures of the composites after sintering at 1400 °C for 3 h are shown in Fig. 4(a)–(d), reflecting the porosity result. In the HA-composites with yttria-doped ZrO_2 and Al_2O_3 (Fig. 4(a) and (b), respectively), lots of pores were observed throughout the surface, indicating the occurrence of poor densification of the HA-composites. This high porosity was attributed to the considerable degree of decomposition. The pronounced decomposition, which had already occurred prior to the sintering process, is known to be detrimental to the densification of ceramic materials. However, the pores observed in the HA-composites almost completely disappeared in the FHA-composites with yttria-doped ZrO_2 and Al_2O_3 (Fig. 4(c) and (d), respectively). Moreover, each reinforcing agent (yttria-doped ZrO_2 or Al_2O_3) was finely and uniformly dispersed within the FHA matrix.

As a result of the improved sinterability and well-developed microstructure, superior mechanical properties were obtained in the FHA-composites when compared to the HA-composites. The four-point flexural strengths and fracture toughness of the composites are shown in Figs. 5 and 6, respectively. Both the mechanical properties behaved in a similar manner. In the HA-composites (HA-Z and HA-A), relatively low strength (~ 70–100 MPa from Fig. 5) and toughness (~ 1 $MPam^{1/2}$ from Fig. 6) were observed. Even though

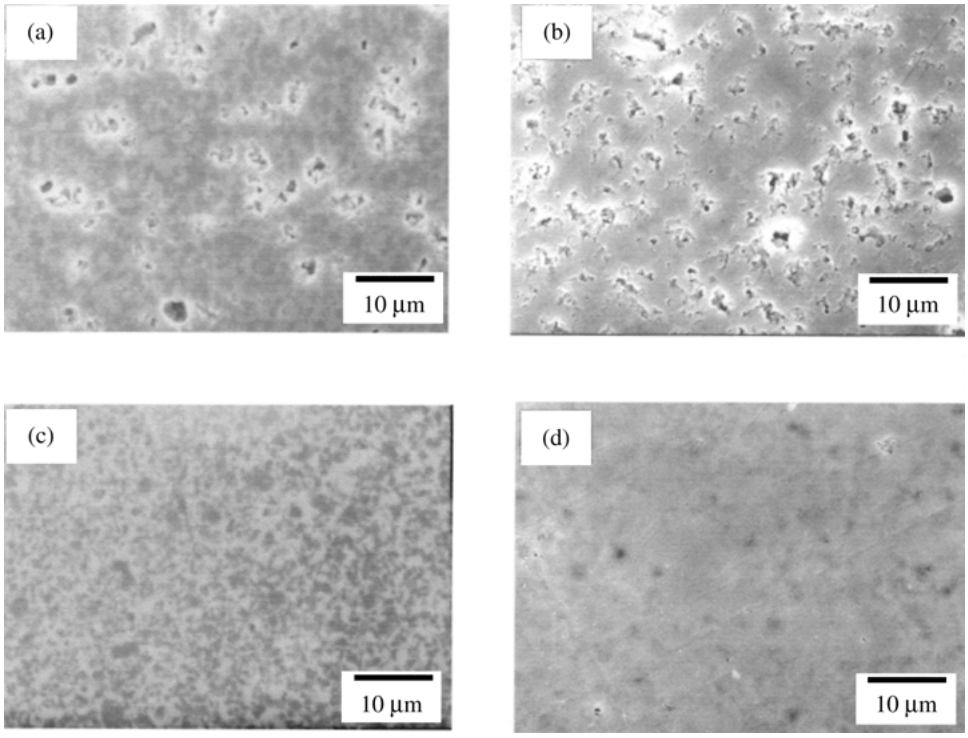

Figure 4 SEM of the HA- and FHA-composites with ZrO_2 or Al_2O_3 after sintering at 1400 °C for 3 h; (a) HA-Z, (b) HA-A, (c) FHA-Z, and (d) FHA-A.

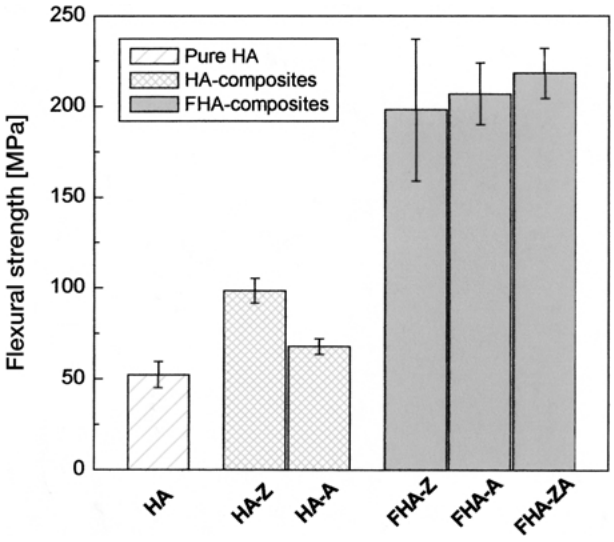

Figure 5 Flexural Strengths of the HA- and FHA-composites with ZrO_2 or/and Al_2O_3 after sintering at 1400 °C for 3 h. Data on the pure-HA is for comparison, in which the sintering condition was 1300 °C for 1 h.

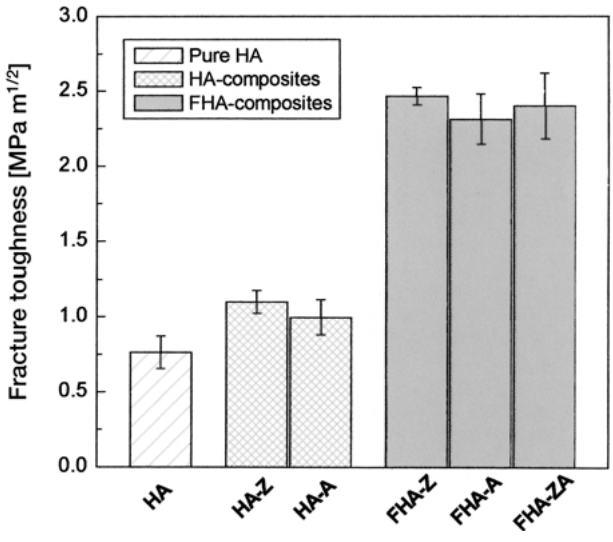

Figure 6 Fracture toughness of the HA- and FHA-composites with ZrO_2 or/and Al_2O_3 after sintering at 1400 °C for 3 h. Data on the pure-HA is included for comparison, in which the sintering condition was 1300 °C for 1 h.

the values for the composites were slightly higher than those of pure HA, these values did not represent the reinforcing effects of yttria-doped ZrO_2 and Al_2O_3, and were insufficient for the applications in the load-bearing implants. These low strength values were related to those pores driven by the incomplete densification since the large pore acted as a main fracture origin. The fracture toughness was also low since the toughness of ceramics is directly correlated with strength. However, in the FHA-composites (FHA-Z, FHA-A, and FHA-ZA), both mechanical properties were much higher (over $\sim$ 2–3 times) than those of the HA-composites, with the values of $\sim$ 220 MPa in strength and $\sim$ 2.5 MPam$^{1/2}$ in toughness. There were no significant differences in the values between the composite with ZrO_2 and Al_2O_3 reinforcements. These significant increases in strength and toughness were presumably due to the improvement in densification, and the consequent reduction in porosity. Moreover, the refinement of the microstructure with the addition of yttria-doped ZrO_2 and Al_2O_3 also contributed to the improvement. As a result, these FHA-composites reinforced with yttria-doped ZrO_2 and/or Al_2O_3 are potentially very useful for hard tissue engineering.

Along with the strength and toughness, other mechanical parameters, such as the hardness and elastic modulus of the fabricated materials are summarized in Table II. Usually, higher hardness implies superior performance in a wear-involving environment. Moreover, the elastic modulus of an implant, which is similar to that of the host hard tissue, is preferable in view of the stress distribution. As shown in Table II, the FHA-composites with yttria-doped ZrO_2 and Al_2O_3 retain the higher hardness ($\sim$ 9–10 GPa) and elastic modulus ($\sim$ 150–180 GPa) than the HA-composites do ($\sim$ 5 GPa in elastic modulus and $\sim$ 90 GPa in hardness). The considerable increases in both hardness and elastic modulus are directly related with the reduction in the porosity, which is known as the Duckworth–Knudsen exponential model. This model is commonly used to characterize the mechanical behaviors of ceramics [17, 18]. In particular, the elastic modulus values of the FHA-composite with Al_2O_3 are superior to those with yttria-doped ZrO_2, due to the intrinsic properties of reinforcement, i.e. the higher elastic modulus of pure Al_2O_3 than yttria-doped ZrO_2. When compared to pure HA, the improvements in hardness in the FHA-composites are promising, whereas, the increased elastic modulus casts a question of the stress concentration on the implants and consequent failure. Therefore, the composite with yttria-doped ZrO_2 seemed to be a better choice than that with Al_2O_3 from the elastic modulus point of view. However, a real biological environment is quite complicated and the situation under load is different from the testing conditions in many factors such as the loading state (cyclic/static, shear/tensile/compressive), the surrounding fluid (wet/dry), and the loading period. Hence, a decisive conclusion requires further study.

TABLE II Hardness and elastic modulus of the fabricated materials after sintering at 1400 °C for 3 h; for pure HA, the sintering condition was 1300 °C for 1 h

Materials	Hardness (GPa)	Elastic modulus (GPa)
HA	5.53 $\pm$ 0.22	117.2
HA-Z	4.93 $\pm$ 0.32	88.9
HA-A	5.43 $\pm$ 0.50	92.6
FHA-Z	9.68 $\pm$ 0.84	143.5
FHA-A	9.88 $\pm$ 1.01	181.9
FHA-ZA	10.2 $\pm$ 0.90	158.0

The cell viability of the fabricated FHA-composites was evaluated by an *in vitro* culture method. Fig. 7(a)–(d) show the MG63 cells that proliferated on the FHA-composites and pure HA after culturing for 3 days. At low magnification, large numbers of cells were well adhered and uniformly spread on the FHA composites with yttria-doped ZrO_2 (Fig. 7(a)), which was manifested by the distribution of dark nuclei throughout the surface. At higher magnification, a rather apparent morphology of the MG63 cells with round nuclei and surrounding

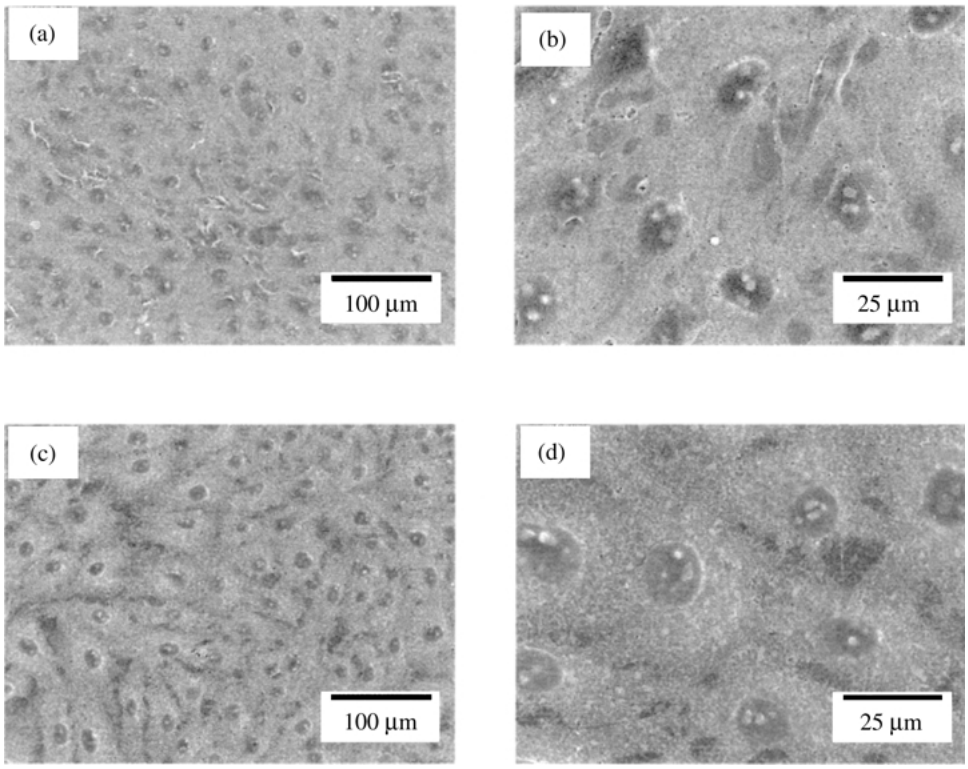

Figure 7 Morphologies of the proliferated MG63 cells on the fabricated materials after culture for 3 days ((a) FHA-Z at low magnification, (b) FHA-Z at high magnification, (c) HA at low magnification, and (d) HA at high magnification).

cytosols in intimate contact each other were observed (Fig. 7(b)). Such cell behavior on the FHA composites with yttria-doped ZrO_2 was very similar to that on the pure HA (Fig. 7(c) and (d)), which confirmed the comparable cellular response with no indication of cytotoxicity. The cells that proliferated on the FHA–Al_2O_3 composites were also found to behave in a similar manner (not shown here).

The proliferated cells on the FHA-composites along with on the Thermanox and pure HA were quantified in Fig. 8, after culture periods of 3–10 days. For all the test materials, the number of cells increased considerably with increasing culture period. Even though no significant difference was found among the test samples at day 3, the cell numbers on the pure HA and FHA-composites were higher than that of the Thermanox control after culturing for 7 days and the trends were more pronounced at day 10. However, little differences were observed between pure HA and FHA-composites. Based on the morphology and the numbers of the proliferated cells, it is clear that the FHA-composites have comparable cell viability to pure HA. However, for a deeper understanding of the biological performance of the FHA-composites, further study including not only the *in vitro* properties of the proliferated cells, such as cell differentiation and mineralization, but also the *in vivo* behaviors of bone ingrowth and bone/implant interfacial characteristics are needed.

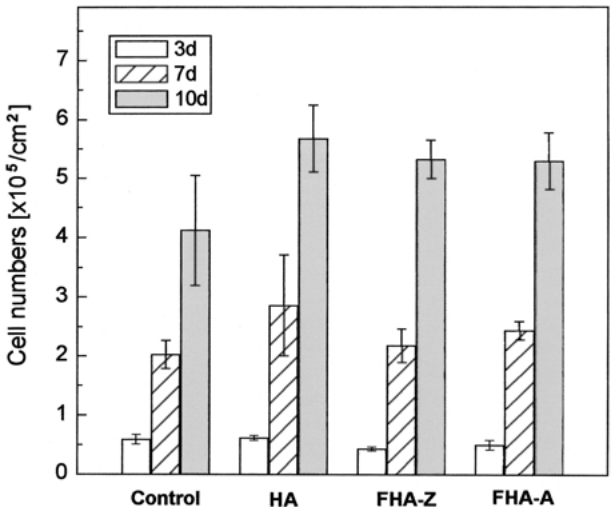

Figure 8 Proliferation rates of the MG63 cells on the fabricated materials. Thermanox cover slip is used as a control. Data on the pure HA is included for comparison.

4. Conclusions

Fully dense FHA-composites with 40% yttria-doped ZrO_2 or/and Al_2O_3 were fabricated by pressureless sintering. These composites exhibited improved thermal stability at high temperatures without phase decomposition. The obtained FHA-composites exhibited excellent mechanical properties, such as flexural strength, fracture toughness, and hardness, with values of $\sim$2–3 times higher than those of either pure HA or HA-composites. The highest values obtained in the FHA-composites were $\sim$220 MPa in strength, $\sim$2.5 MPam$^{1/2}$ in fracture toughness, and $\sim$10 GPa in hardness. These fabricated FHA-composites did not display any cytotoxicity and showed a similar cell proliferation behavior to pure HA.

Acknowledgment

This work was supported by a grant of the Korea Health 21 R&D Project, Ministry of Health and Welfare, Republic of Korea (02-PJ3-PG6-EV11-0002).

References

1. L. L. HENCH, *J. Am. Ceram. Soc.* **74** (1991) 1487.
2. R. Z. LEGEROS, *Prog. Cryst. Grow. Char.* **4** (1981) 1.
3. K. DE GROOT, C. DE PUTTER, P. SMITT and A. DRIESSEN, *Sci. Ceram.* **11** (1981) 433.
4. E. CHAMPION, S. GAUTIER and D. BERNACHE, *J. Mater. Sci. Mater. Med.* **7** (1996) 125.
5. H.-W. KIM, Y.-J. NOH, Y.-H. KOH, H.-E. KIM and H.-M. KIM, *Biomaterials* **22** (2002) 4113.
6. J. LI, H. LIAO and L. HERMANSSON, *ibid.* **17** (1996) 1787.
7. J. HUAXIA and P. M. MARQUIS, *J. Mater. Sci.* **28** (1993) 1941.
8. A. KRAJEWSKI, A. RAVAGLIOLI, N. ROVERI, A. BIGI and E. FORESI, *ibid.* **25** (1990) 3203.
9. E. C. MORENO, M. KRESAK and R. T. ZAHRADNIK, *Nature* **247** (1974) 64.
10. R. Z. LEGEROS, L. M. SILVERSTONE, G. DACULSI and L. M. KEREBEL, *J. Dent. Res.* **62** (1985) 138.
11. F. B. AYED, J. BOUAZIZ and K. BOUZOUITA, *J. Eur. Ceram. Soc.* **20** (2000) 1069.
12. W. RAJARAO and R. F. BOEHEM, *J. Dent.* **53** (1974) 1351.
13. P. CHANTILKUL, G. R. ANSTIS, B. R. LAWN and D. B. MARSHALL, *J. Am. Ceram. Soc.* **64** (1981) 539.
14. X. YANG and Z. WANG, *J. Mater. Chem.* **8** (1998) 2233.
15. L. J. JHA, S. M. BEST, J. C. KNOWLES, I. REHMAN, J. D. SANTOS and W. BONFIELD, *J. Mater. Sci. Mater. Med.* **8** (1997) 185.
16. M. J. LARSEN and S. J. JENSEN, *Archs. Oral Biol.* **34** (1989) 969.
17. R. W. RICE, in ''Treatise on Materials Science and Technology'', Vol. 11, edited by R. K. MacCrone (Academy, New York, 1977) p. 199.
18. M. A. LOPES, F. J. MONTEIRO and J. D. SANTOS, *J. Biomed. Mater. Res.* **48** (1999) 734.

Received 9 July 2002
and accepted 4 February 2003