JOURNAL OF MATERIALS SCIENCE: MATERIALS IN MEDICINE 9 (1998) 385–391

Formation of carbonate apatite on calcium phosphate coatings containing silver ions

M. SHIRKHANZADEH, M. AZADEGAN
Department of Materials and Metallurgical Engineering, Queen's University, Kingston, Ontario, Canada K7 3N6

The prerequisite for bioactive materials to bond to living bone is the formation of biologically equivalent carbonate apatite on their surfaces in the body. Calcium phosphate ceramic surfaces can be transformed to a biological apatite through a series of surface reactions including dissolution–precipitation and ion exchange. In the present work, apatite coatings with different crystallinity, compositions and crystal sizes, including a well-crystallized hydroxyapatite coating, were synthesized electrochemically and doped with silver ions in silver nitrate solution at room temperature. The formation of a new carbonate apatite on the surface of these coatings was investigated in an acellular simulated body fluid with ion concentrations comparable with those of human blood plasma, using scanning electron microscopy and Fourier transform–infrared spectroscopy. The results show that small quantities of silver ions incorporated into apatite coatings may have a strong stimulatory effect on the formation of carbonate apatite without adversely affecting the chemical stability of these coatings.

1. Introduction

The formation of a biologically equivalent carbonate apatite on the surface of bioactive materials such as bioglass, glass ceramics and calcium phosphate ceramics, is thought to be one of the events leading to bonding with bone [1–3]. There have been several explanations for the formation of biologically active apatite on bioactive materials. According to Hench [4], the combination of an alkaline interfacial pH resulting from the high content of soda in glasses and the surface SiOH groups, may be responsible for accumulation of calcium and phosphate from the body fluid and formation of carbonate apatite. In the case of glass ceramics, Kokubo *et al.* [5] have suggested that the calcium ions dissolved from these materials would increase the supersaturation degree of the surrounding body fluid, and the hydrated silica which forms on the surface of the glass ceramics provides the active sites for the nucleation of apatite. On the other hand, Li *et al.* [6] have shown that a carbonated apatite layer could also be formed on the surface of pure silica gel soaked in a simulated body fluid (SBF) solution containing Ca^{2+} and HPO_4^{2-} ions at a pH of 7.4. The concensus in all these works is that a gel–silica layer is essential for the nucleation and crystallization of carbonated apatite. According to Radin and Ducheyen, calcium phosphate ceramic surfaces can also be transformed to a biological apatite in simulated physiological solutions through a sequence of reactions including dissolution–precipitation and ion-exchange [3]. The behaviour of calcium phosphate ceramics was reported to be structure- and composition-dependent. The rate of carbonate apatite formation was found to be the highest for resorbable calcium-deficient hydroxyapatite (HA) and the lowest for the stoichiometric, well-crystallized HA. It has been suggested that while a more resorbable coating, such as calcium-deficient HA, may be advantageous for the immediate formation of carbonate apatite and bonding with bone, the long-term clinical stability and performance of such coatings may be inferior to more stable apatite coatings [7]. During our work on the *in vitro* evaluation of well-crystallized anti-microbial silver-containing HA coatings [8], we noticed that an extremely small concentration of silver ions present in these coatings could accelerate the formation of carbonate apatite in SBF solution, without adversely affecting the stability of the coatings. In view of its possible biological significance, we have investigated this effect in more detail.

2. Experimental procedure

To show the significance of silver ions in accelerating the formation of carbonate apatite, calcium phosphate coatings, with and without silver, were prepared. The coatings used, their designations, preparations and their silver contents, are summarized in Table I. API, a non-stoichiometric apatitic coating, was synthesized electrochemically on Ti6Al4V plates, according to the procedures described previously under galvanostatic conditions ($i = 0.65$ mA cm^{-2}) at 65 °C using an electrolyte containing 0.020 M $Ca(NO_3)_2$ and 0.012 M $NH_4H_2PO_4$ [9]. APII was synthesized by hydrothermal treatment of API for 4 h at 125 °C and APIII was prepared by hydrothermal treatment followed by heat treatment of API at 425 °C for 2 h. API–Ag, APII–Ag and APIII–Ag were prepared by treating corresponding apatite coatings in silver nitrate solution containing 50 p.p.m. Ag^+ ions (pH = 5.8) at room temperature for 1 h. The concentration of silver in the coatings was determined by atomic absorption spectroscopy. The formation of a biologically equivalent carbonate-containing apatite on the surface of the coatings was investigated by incubating the coatings in a simulated body fluid (SBF) solution. SBF solutions were prepared by dissolving reagent grade NaCl, $NaHCO_3 \cdot KCl$, $K_2HPO_4 \cdot 3H_2O$, $MgCl_2 \cdot 6H_2O$, $CaCl_2$ and Na_2SO_4 in distilled water. The ion concentrations of the SBF solution (Table II) were selected to be close to those in human blood plasma [6]. The SBF solution was buffered at pH 7.4 at 37 °C with *tris*-hydroxymethyl aminomethane (*tris*-$(CH_2OH)_3CNH_2$) and HCl. Coatings were incubated in triplicate in 400 ml SBF solution for 1 wk. Scanning electron microscopy (SEM) and Fourier transform–infrared (FT–IR) spectroscopy were employed to determine the surface morphological changes of the coatings and to investigate the mechanism of interactions between the coatings and the SBF solution.

3. Results and discussion

3.1. Characterization of the coatings prior to the incubation tests in SBF

Fig. 1 shows scanning electron micrographs of the apatite coatings without silver (API, APII, APIII). The corresponding FT–IR spectra of these coatings are shown in Fig. 2. It is seen that (API) consists of an interlocking network of non-uniform plate-like crystals in the range of 4–6 μm (Fig. 1a). The FT–IR spectrum of this coating (Fig. 2a) shows, in addition to bands associated with hydroxyapatite (1100–1032, 962, 632, 3570 cm^{-1}) [10], bands attributed to octacalcium phosphate (OCP) (525 and 900–865, 1200 and 1280 cm^{-1}) very close to those reported for pure OCP [11]. The appearance of both OCP and HA

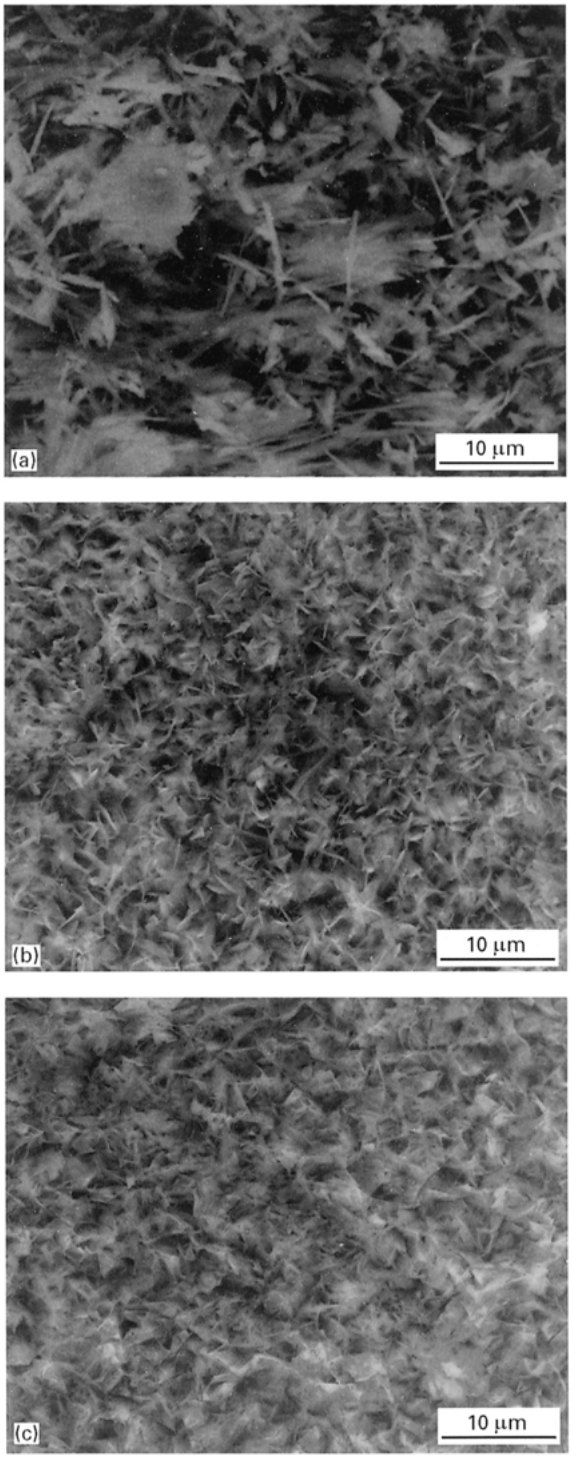

Figure 1 Scanning electron micrographs of (a) API, (b) APII and (c) APIII prior to incubation in SBF solution.

TABLE I Apatite coatings used in the study

Coating name	Synthesis and treatment	Silver content (%)
API	As-prepared at 65 °C	0
APII	API hydrothermally treated for 4 h	0
APIII	API hydrothermally treated for 4 h followed by heat treatment at 425 °C for 2 h	0
API–Ag	API doped with Ag in $AgNO_3$ solution for 1 h	0.85
APII–Ag	APII doped with Ag in $AgNO_3$ solution for 1 h	0.25
APIII–Ag	APIII doped with Ag in $AgNO_3$ solution for 1 h	0.21

TABLE II Ion concentrations of SBF and human blood plasma [6]

	Ion concentration (mM)							
	Na^+	K^+	Ca^{2+}	Mg^{2+}	Cl^-	HCO_3^-	HPO_4^{2-}	SO_4^{2-}
SBF	142.0	5.0	2.5	1.5	147.8	4.2	1.0	0.5
Human plasma	142.0	5.0	2.5	1.5	103.0	27.0	1.0	0.5

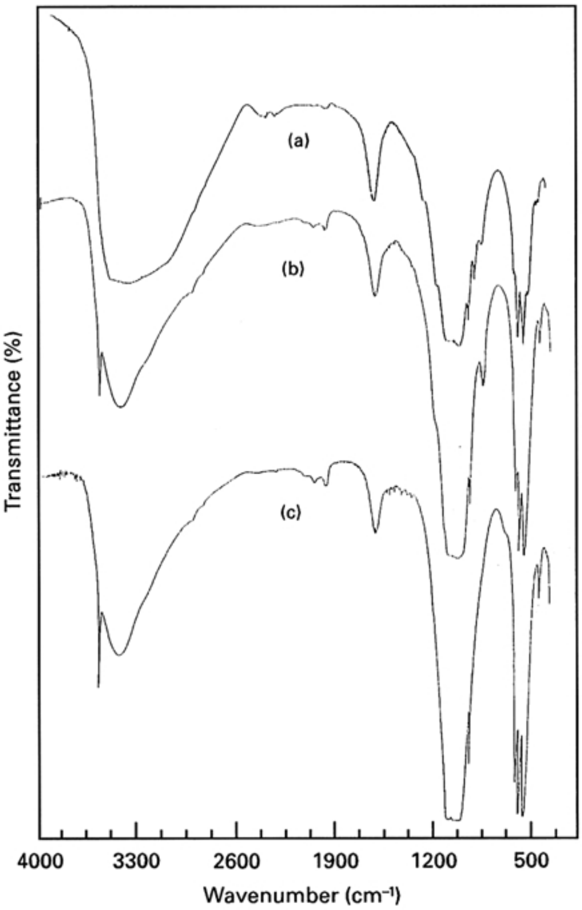

Figure 2 FT–IR spectra of (a) API, (b) APII and (c) APIII prior to incubation in SBF solution.

bands in the FT–IR spectrum of the API may signify the presence of OCP–apatite interlayering similar to bone apatite. Upon hydrothermal treatment or hydrothermal treatment followed by the heat-treatment process in air at 425 °C, API was transformed to APII and APIII, respectively, characterized by more uniform crystals in the range of 2–3 μm (Fig. 1b and c). FT–IR spectra of APII and APIII (Fig. 2b and c) clearly show that the contribution of OCP phase in these samples has decreased as a result of hydrothermal and heat-treatment processes. In particular, it is seen that the infrared spectrum of APIII is generally similar to those reported for stoichiometric HA of high purity [10]. The bands at 1100–1032, 962, 601 and 565 cm^{-1}, derived from PO_4 modes of HA are very sharp, indicating that the coating consists of well-crystallized HA crystals. The transformation of API to APII and APIII may involve chemical changes which take place, especially during the steam treatment resulting in improved crystallinity of the coatings. These chemical changes may involve the crystal interior, crystal surface, or both, in some hydrolysis or other dissolution and recrystallization process.

Fig. 3 shows scanning electron micrographs of the apatite coatings, containing silver (API–Ag, APII–Ag and APIII–Ag). The corresponding FT–IR spectra of these samples are shown in Fig. 4. As shown in Fig. 3a, API–Ag is amorphous-like (as seen by SEM) and may consist of extremely small crystals exhibiting both OCP and HA bands (Fig. 4a) similar to API. The amorphous-like structure of the API–Ag sample may be due to the rapid dissolution and recrystallization of API in silver nitrate solution and inhibition of the crystal growth process by silver ions. The incorporation of silver in this coating may occur both through dissolution–precipitation and ion-exchange mechanisms. Thus, it is possible that phosphate ions released from the coating as a result of hydrolysis may react with silver ions to form silver phosphate precipitates on this coating. This may explain the relatively high silver content (0.85% by weight) observed in API–Ag

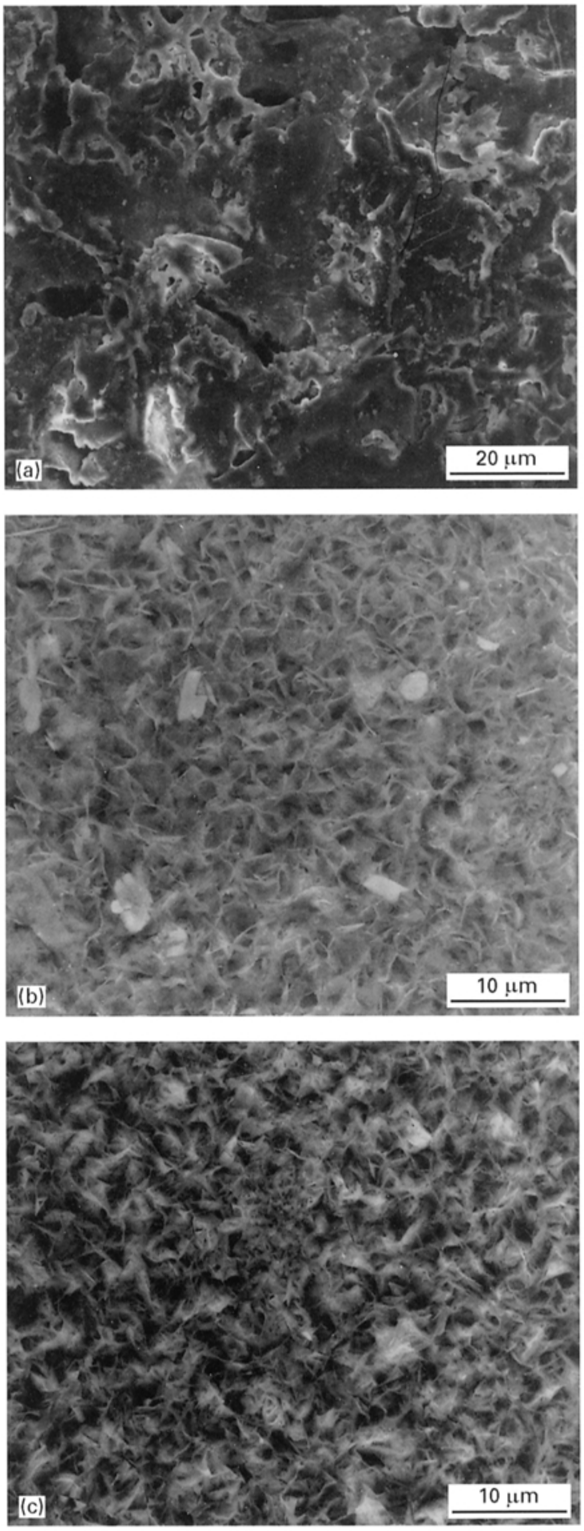

Figure 3 Scanning electron micrographs of (a) API–Ag, (b) APII–Ag and (c) APIII–Ag prior to incubation in SBF solution.

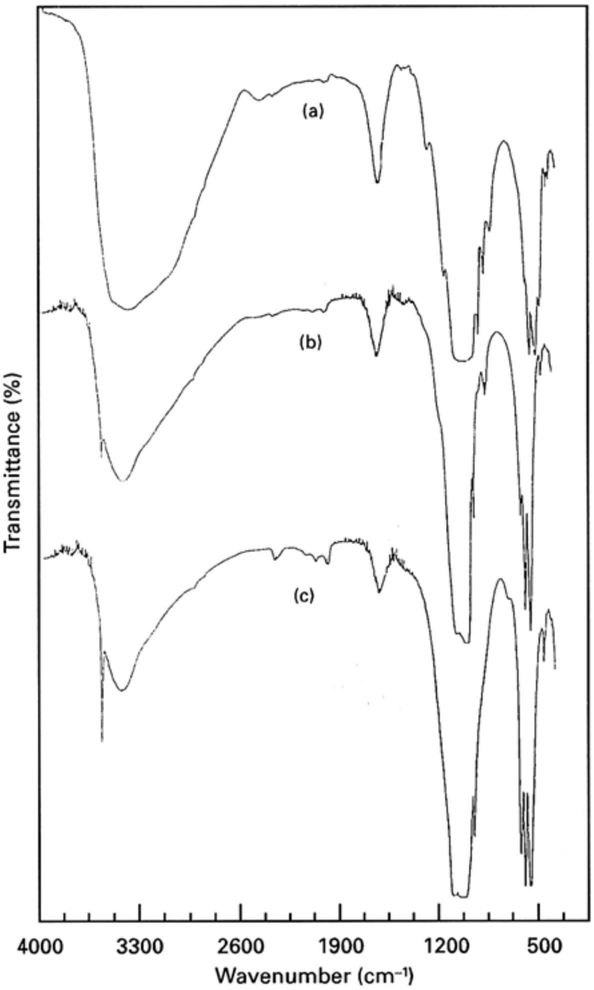

Figure 4 FT–IR spectra of (a) API–Ag, (b) APII–Ag, and (c) APIII–Ag prior to incubation in SBF solution.

coating. In contrast to API, the microstructure of both APII and APIII remained unchanged in silver nitrate solution (Fig. 3b and c). The FT–IR spectra of these samples were also unaltered (Fig. 4b and c). Despite this, a significant amount of Ag^+ ions were incorporated into these samples as reported in Table I. Thus, the uptake of silver ions by these coatings appears to occur predominantly through an ion-exchange mechanism. The hydration layer associated with the apatite crystals is believed to play an important role in this ion-exchange process. It is seen in Fig. 4 that, in all cases, the FT–IR spectra of apatite coatings exhibit a small peak at $\sim 1630\ cm^{-1}$ and a broad band in the range of $3000–3600\ cm^{-1}$ indicative of "bound water" or a hydration layer associated with apatite crystals. It is known that the hydration layer contains a high concentration of calcium and phosphate ions [12]. Neuman and Neuman [13] have shown that ions in the hydration layer are readily exchangeable with isoions in bulk solution. These results have also been confirmed by Pak and Bartter [14] and Avnimetech [15]. Suzuki *et al.* [16] have also demonstrated that cations such as Ca^{2+} and Pb^{2+} and Zn^{2+} in aqueous solutions can be removed by synthetic HA through an ion-exchange reaction between ions in solution and Ca^{2+} ions of the apatite. It is envisaged that Ag^+ ions may be incorporated into apatite crystals by a similar ion-exchange mechanism.

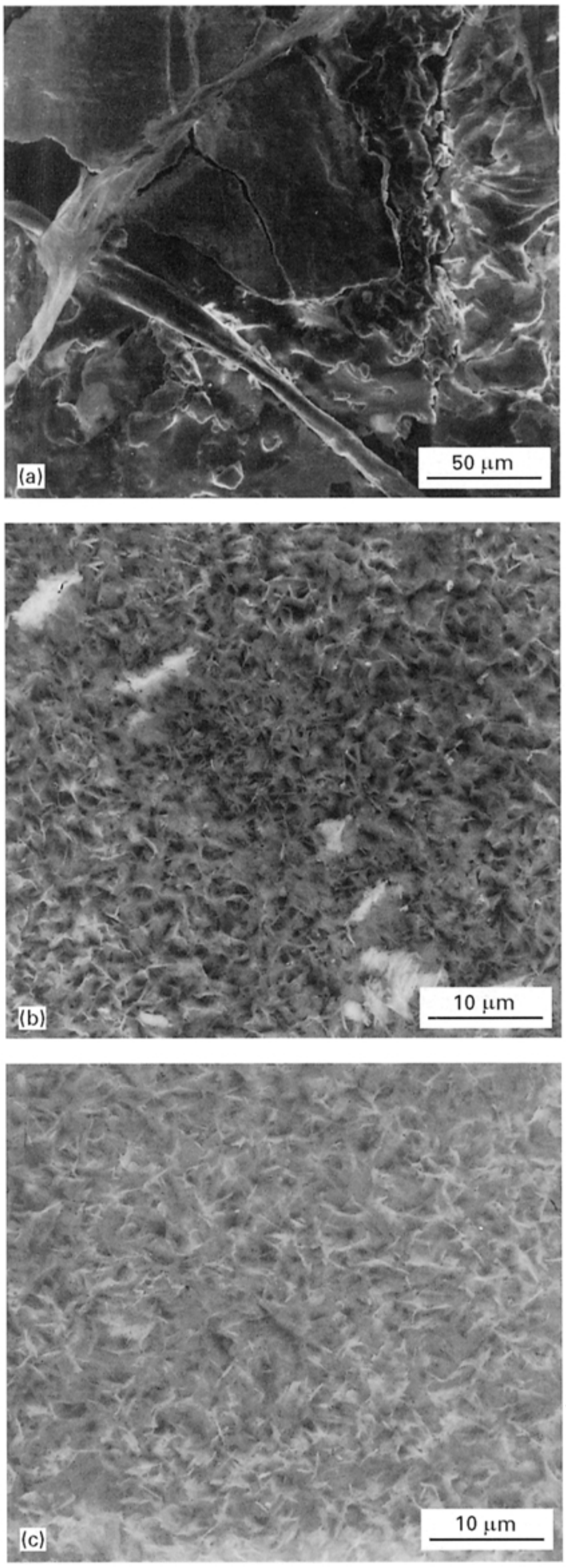

Figure 5 Scanning electron micrographs of (a) API, (b) APII and (c) APIII after 7 d incubation in SBF solution.

3.2. Characterization of coatings after incubation in SBF solution

Scanning electron micrographs of the apatite coatings without and with silver after 7 d incubation in SBF are shown in Figs 5 and 6, respectively. The corresponding FT–IR results are also presented in Figs 7 and 8. API exhibited considerable morphological and chemical changes as a result of the extensive hydrolytic transformations (Figs 5a and 7a). The presence of

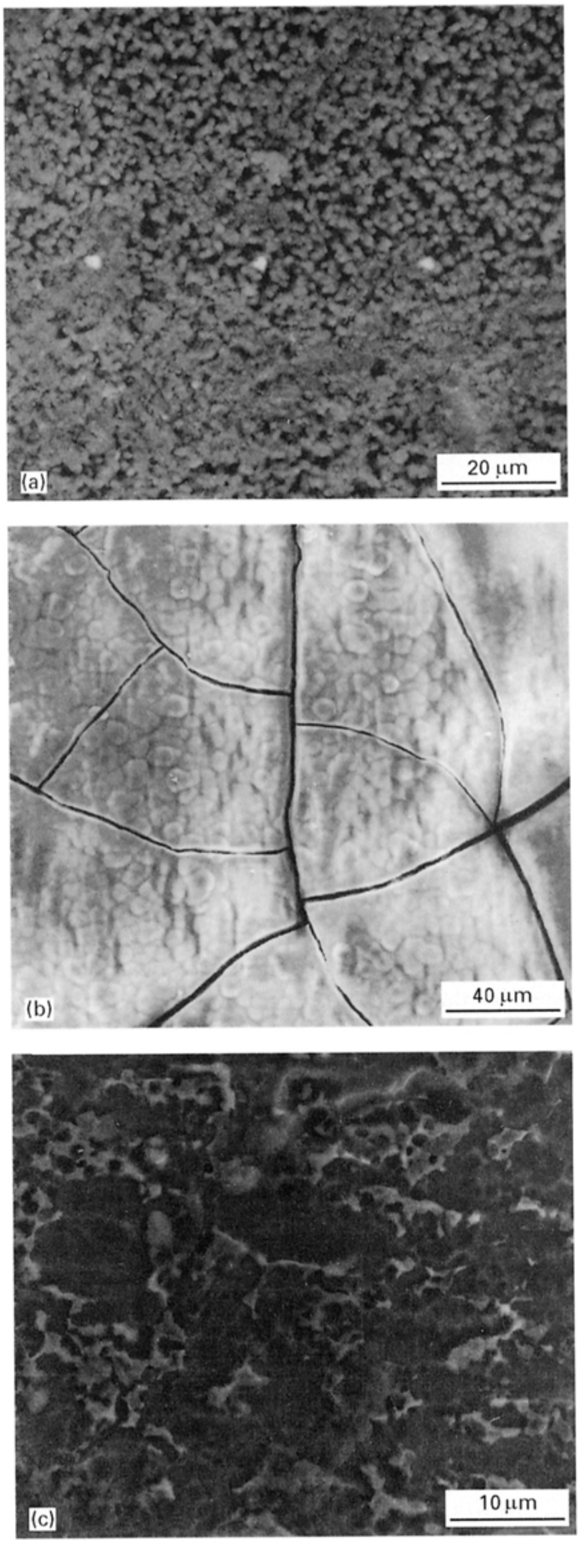

Figure 6 Scanning electron micrographs of (a) API–Ag, (b) APII–Ag and (c) APIII–Ag after 7 d incubation in SBF solution.

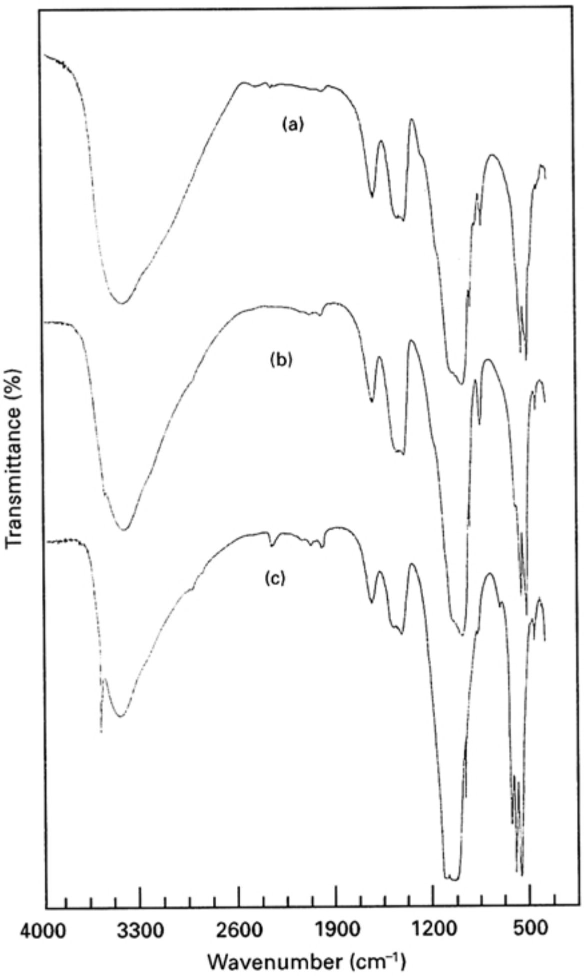

Figure 7 FT–IR spectra of (a) API, (b) APII and (c) APIII after 7 d incubation in SBF solution.

Mg^{2+} ions in the SBF solution may have acted as a crystal-growth inhibitor resulting in the formation of an amorphous-like hydrolytic product. The FT–IR spectrum of API after incubation in SBF solution (Fig. 7a) shows, in addition to phosphate bands, the principal bands due to carbonate ions in type B sites (i.e. at 872, 1420 and 1460 cm^{-1}), indicating the formation of a carbonate apatite. It is also seen from Fig. 7a that the intensities of the phosphate bands associated with hydroxyapatite have increased as a result of the progressive hydrolysis of API in SBF solution. At the same time, the intensities of the OCP bands have considerably decreased. The formation of the new carbonate apatite on API was also confirmed by the weight gain of approximately 55% (Table III) after 7 d incubation in the SBF solution. Because of the extensive morphological changes, it is likely that the entire coating has transformed to a carbonate apatite phase in this case. In contrast to API, the morphology of the APII and APIII did not change appreciably in SBF solution (Fig. 5b and c, respectively) and relatively less weight gain was observed for these samples. It is also seen that the phosphate and OH bands associated with HA in these samples have remained unchanged (Fig. 7b and c). Nevertheless, the FT–IR results confirmed the presence of a considerable amount of carbonate ions in these coatings. Thus the incorporation of carbonate ions in these coatings may largely occur by an ion-exchange mechanism whereby CO_3^{2-} ions in the SBF solution partially replace phosphate and probably OH^- ions present in the apatite crystals.

The behaviour of silver-containing apatite coatings in SBF solution was found to be very different from those without silver. A much higher weight gain was observed for API–Ag (Table III) in comparison with API after 7 d incubation in the SBF solution. The scanning electron micrograph of API–Ag after incubation in the SBF solution shows, in addition to extensive dissolution and restructuring of the coating,

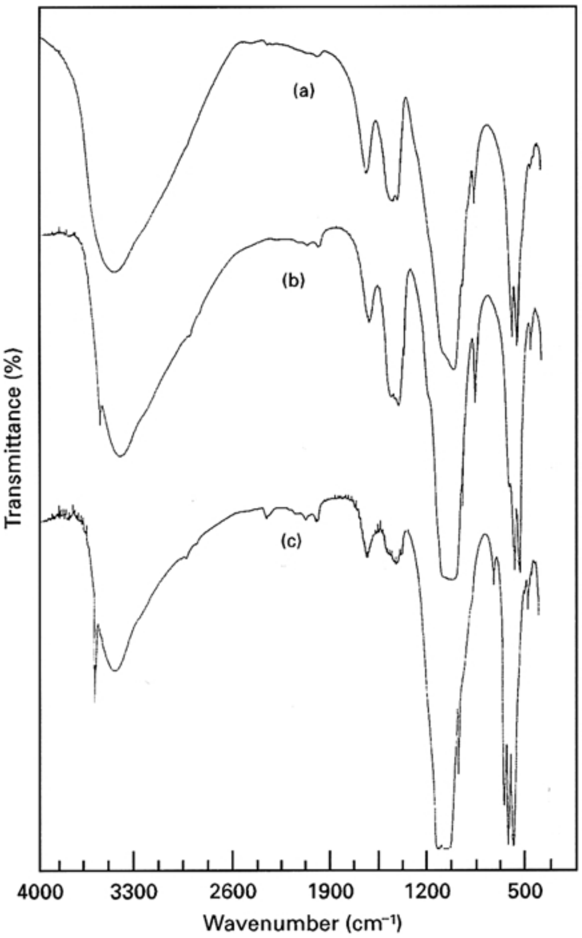

Figure 8 FT–IR spectra of (a) API–Ag, (b) APII–Ag and (c) APIII–Ag after 7 d incubation in SBF solution.

TABLE III Weight gain of the apatite coatings incubated in SBF solution for 7 d

	Coating					
	API	APII	APIII	API–Ag	APII–Ag	APIII–Ag
Weight gain	55%	30%	15%	170%	100%	40%

formation of a new and distinct carbonated apatite phase in the form of tiny spherulites on the surface of the coating. Relatively large weight gains were also observed for APII–Ag and APIII–Ag in SBF solution. Distinct bone-like carbonated apatite films were also formed on these coatings although no significant dissolution and restructuring of the original coatings took place in these cases. This can be seen particularly in Fig. 6c where the shadows of the original crystals of the HA coating are apparent beneath the newly formed carbonated apatite layer. The above results clearly show that small amounts of silver present in the apatite coatings have a significant activating effect upon the formation of new bone-like apatite in the SBF solution. Others have reported a relationship between heavy metals and formation of calcium phosphate minerals in solutions. For example, Fleisch *et al.* [7] have found that concentrations of Pb^{2+} as low as 5×10^{-2} M lower the ion product for spontaneous precipitation of a calcium phosphate mineral from solution, and Hidaka *et al.* [18] have recently found that indium ions eluted from dental casting alloys may stimulate the formation of oral calcium phosphate precipitates, counteracting the inhibition by saliva-derived phosphoproteins. The exact mechanism by which silver activates the formation of carbonated apatite in the present work is not known. It is possible that silver ions present in the hydration layer of apatite crystals may bind phosphate ions and form nanoclusters of silver phosphate uniformly dispersed on the apatite crystals, which may act as the nucleation sites for the precipitation of carbonate apatite. Alternatively, Ag^+ ions present in the apatite crystals may be released in SBF and lower the minimum ion product $[Ca] \times [P]$ necessary for the formation of carbonated apatite. Thus the strong stimulatory effect of silver may be related to the high stability of Ag^+ ions which, in turn, is influenced by the short- and medium-range atomic/ionic environment of the silver in the apatite. Other materials such as clay have been used in the past to support silver in the form of Ag^+ ions as an active antimicrobial agent. However, it has been reported that Ag^+ ions incorporated into clay can be easily reduced to silver metal particles, leading to a lowering of antimicrobial activity and to changing of its colour from ochre to black [19]. Keller-Besrest *et al.* [20] have investigated the unstable nature of the Ag^+ ions in clay using EXAFS technique. Triangular silver clusters were identified, which supports the possible coexistence of two silver species, Ag^0 and Ag^+, related to the observed unstable behaviour of Ag^+ ions in clay. According to these authors, the formation of metallic silver clusters results from the interaction of Ag^+ ions with clay which acts like an electron donor. In the present work, all apatite coatings doped with silver were white in colour and did not darken with time in the SBF solution. Thus, it appears that unlike silver in clay, Ag^+ ions incorporated in the apatite coatings may not be prone to reduction. The mechanism by which Ag^+ ions remain active in the apatite is not known at present. Further structural studies are required to determine precisely the localization of silver in the apatite coatings and explain the mechanisms responsible for its long-term activity.

4. Conclusion

The results in this work have shown that small concentrations of silver ions incorporated into apatite coatings may have a profound effect on the formation of new carbonated apatite in SBF solution. Silver ions may, therefore, enhance the bone-bonding properties of these coatings *in vivo*, The strong stimulatory effect of silver present in the well-crystallized HA coating is particularly important because the formation of carbonate apatite on this coating may be initiated without compromising its long-term integrity. Other metal ions such as Fe^{2+}, Zn^{2+}, and In^{3+} may also show a stimulatory effect when incorporated into apatite coatings. However, silver ions are known to have strong bactericidal effects. Thus, the potential bone-bonding properties of silver-containing apatite coatings combined with the potent antibiotic effects of silver ions may provide an effective means for eradicating the bacteria from the bone/implant interface.

Acknowledgement

The authors thank the Natural Sciences and Engineering Research Council of Canada (NSERC) for the financial support provided for this research project.

References

1. L. L. HENCH, *Ann. NY Acad. Sci.* **523** (1988) 54.
2. T. KOKUBO, *J. Non-Cryst. Solids* **120** (1990) 138.
3. S. R. RADIN and P. DUCHEYRIE, *J. Biomed. Mater. Res.* **27** (1993) 35.
4. L. L. HENCH and J. WILSON, in "Silicon Biochemistry", edited by D. Evered, CIBA Foundation Symposium 121 (Wiley, New York, 1986) p. 231–46.
5. T. KOKUBO, H. KUSHITANI, C. OHTSUKI, S. SAKKA and T. YAMAMURO, *J. Mater. Sci. Mater. Med.* **3** (1992) 79.
6. P. LI, C. OHTSUKI, T. KOKUBO, K. NAKANISHI and N. SOGA, *J. Am. Ceram. Soc.* **75** (1992) 2094.
7. S. H. MAXIAN, J. P. ZAWADSKY and M. G. DUNN, *J. Biomed. Mater. Res.* **27** (1993) 111.
8. M. SHIRKHANZADEH, M. AZADEGAN and G. Q. LIU, *Mater. Lett.* **24** (1995) 7.
9. M. SHIRKHANZADEH, *Nano-Struct. Mater.* **4** (1994) 677.
10. J. ARENDS, J. CHRISTOFFERSEN, M. R. CHRISTOFFERSEN, H. ECKERT, B. O. FOWLER, J. C. HEUGHEBAERT, G. H. NANCOLLAS, J. P. YESINOWAKI and S. J. ZOWAKI, *J. Crystal. Growth* **84** (1987) 515.
11. B. O. FOWLER, E. C. MORENO and W. E. BROWN, *Arch. Oral. Biol.* **11** (1966) 477.
12. W. G. ROBERTSON and D. B. MORGAN, *Biochim. Biophys. Acta* **230** (1971) 495.
13. W. F. NEUMAN and M. W. NEUMAN, "The Chemical Dynamics of Bone Mineral" (University of Chicago Press, Chicago, IL, 1958).
14. C. Y. C. PAK and F. C. BARTTER, *Biochim. Biophys. Acta* **141** (1967) 401.
15. Y. AVNIMETECH, *Israel J. Chem.* **6** (1968) 375.
16. T. SUZUKI, T. HATSUSHIKA and Y. HAYAKAWA, *J. Chem. Soc. Farad. Trans.* **77** (1981) 1059.
17. H. FLEISCH, S. BISAZ and R. RUSSELL, *Proc. Soc. Exp. Biol. Med.* **118** (1965) 882.
18. S. HIDAKA, Y. OKAMATO, K. ABE and K. MIYAZAKI, *J. Biomed. Mater. Res.* **31** (1996) 11.
19. A. OYA, T. BANSE, F. OHASHI and S. OTANI, *Appl. Clay Sci.* **6** (1991) 135.
20. F. KELLER-BESREST, S. BENAZETH and Ch. SOULEAU, *Mater. Lett.* **24** (1995) 17.

Received 14 April
and accepted 25 September 1997