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Biomimetic apatite formation on a CoCrMo alloy by using wollastonite, bioactive glass or hydroxyapatite

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A biomimetic method was used to promote a bioactive surface on a CoCrMo alloy (ASTM F75). To enhance the nucleation of apatite on the metallic substrate, wollastonite ceramics (W), bioactive glass (BG) or hydroxyapatite (HA) were used in the biomimetic method. Metallic samples were chemically treated and immersed for 7 days in SBF on a bed of bioactive material (W, BG or HA) followed by an immersion in 1.5SBF for 7 or 14 days without bioactive system.

A bonelike apatite layer was formed on the surface of all the samples tested. The samples treated with wollastonite showed a higher rate of apatite formation and the morphology of the layer was closer to that of the existing bioactive systems. A higher crystallinity of the apatite layer was also observed by using wollastonite. The pH of the SBF, the Ca/P ratio and the thickness of the layer on the samples treated with wollastonite and bioactive glass increased as increasing the immersion time. The thickness of the layer on the samples treated with hydroxyapatite also increased with time, but the pH of the SBF and the Ca/P ratio changed with no a defined trend.

1. Introduction

For a material to bond to living bone, the material has to form a biologically active bonelike apatite layer on its surface [1]. Those materials able to bond to bone, such as Bioglass® [2], A-W (Apatite-Wollastonite) glass ceramic [3] and pseudo-wollastonite ceramics [4], are known as bioactive. However, the bioactive systems cannot be used as bone implants for highly loaded applications due to their low fracture toughness. Therefore, there is an increasing amount of research in bioactive coatings on metallic substrates to promote a bond between the implant and bone. Several methods for coating hydroxyapatite (HA) on metals have been investigated. Among them, the plasma sprayed HA on metallic implants is already in clinical use but it has several disadvantages: the HA layer obtained is different in structure and composition to that of bone and it is not possible to add biological molecules [5] due to the high temperatures used in this technique.

On the other hand, by using a biomimetic method, that has widely been investigated [6–13], it is possible to form a spontaneous apatite layer on the surface of different substrates. The biomimetic method has several advantages: (a) it is a low temperature process, (b) it can be used to form an apatite layer on the surface of porous substrates or complex geometry implants and (c) it allows the incorporation of biological molecules [14].

During the biomimetic process, the surface of the metallic substrate can be modified to obtain effective sites for apatite to nucleate on the surface. Furthermore, it is necessary to provide calcium and phosphate ions. The impossibility of forming an apatite layer on cobalt base alloys, by using the biomimetic method, has been reported [6]. The samples were soaked in 10 M-NaOH aqueous solution at 60°C for 24 h, heated up to 600°C inside alumina containers and then, immersed in SBF for 1, 2 and 3 weeks [6]. On the other hand, an apatite layer has been observed on cobalt base alloys by using a 5 M-NaOH aqueous solution during the chemical treatment [15]. This treatment was performed for 24 h at 60°C. The alkali-treated samples were heated up to 400 or 600°C for 1 h. A homogeneous apatite layer was observed on the samples treated at 600°C after 21 days of immersion in SBF.

Additionally, the possibility of apatite formation on the surface of ceramics [10] and polymers [11–13]

after their immersion in simulated body fluid in contact with particles of bioactive glass, has been reported. The bioactive system promotes the apatite nucleation, not only on its own surface, but also on the treated substrate surface. According to this, other bioactive systems may show a similar behavior during the biomimetic process.

The main aim of this study was to increase the rate of apatite formation on cobalt base alloys. Thus, this work evaluates the effect of a bed of wollastonite, hydroxyapatite or bioactive glass during the first 7 days of immersion in simulated body fluid (SBF). The effect of a subsequent immersion in a more concentrated solution (1.5SBF) was also determined.

2. Materials and methods

The bioactive glass (BG) was prepared from a mixture of reagent grade chemicals of SiO_2, Na_2O, CaO and P_2O_5 (Sigma-Aldrich). The aim was to obtain a glass with a composition close to that of Bioglass® 45S5. The melting of the oxides mixture was performed at 1360°C in an alumina-zirconia crucible. The glass was crushed with a laboratory planetary-type agate ball mill. The hydroxyapatite (HA) was obtained by a precipitation method [16] using reagent grade chemicals of $(NH_4)_2HPO_4$ and $Ca(NO_3)_2 4H_2O$ and the wollastonite (W) was used as provided (Gosa, SA).

Chemical analyses of the BG, HA and W were performed by using atomic absorption spectroscopy and wet way methods. Particle size distribution analyses were also performed by using laser diffraction (Coulter).

To promote nucleation and growth of apatite crystals, two different simulated body fluid solutions were used, one with an ionic concentration nearly equal to that of human blood plasma (SBF) and other with a concentration of 1.5 times of that of the SBF (1.5SBF). Both solutions were prepared according to the procedure described by Kokubo [17]. Appropriate amounts of reagent grade chemicals of sodium chloride (NaCl), sodium hydrogen carbonate ($NaHCO_3$), potassium chloride (KCl), dipotassium hydrogen phosphate ($K_2HPO_4 \cdot 3H_2O$), magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$), calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$), sodium sulfate (Na_2SO_4) and tris-hydroxymethyl aminomethane ($(CH_2OH)_3CNH_2$) were dissolved into de-ionised water and buffered to pH 7.25 at 37°C with hydrochloric acid.

Bars of the CoCrMo alloy (ASTM F75) were machined to obtain cylindrical samples of 1.27 cm diameter × 1 cm height. The specimens were ground with silicon carbide papers ranging from 120 to 800 grit.

The polished samples were soaked in 5 M NaOH aqueous solution for 24 h at 60°C. Then, the samples were washed with de-ionised water and dried at 40°C for 24 h. The first stage of the immersion procedure was performed as follows: (a) 1.5 g of the corresponding bioactive material (BG, HA or W) was placed on the bottom of a cleaned glass, (b) 50 ml of SBF, previously heated at 37°C, was poured carefully into the glass avoiding the suspension of bioactive particles, (c) the alkali-treated sample was placed on the bed of bioactive powder, (d) each glass was placed in an incubator previously set at 37°C. The samples were kept under these conditions for 7 days. In a second stage, samples were taken out from the glass and immersed in 50 ml of 1.5SBF for 7 or 14 days at 37°C. When immersing the samples for 14 days, the 1.5SBF was renewed after 7 days. No bioactive material was present in this second immersion stage. The pH of the simulated body fluids was measured at different intervals.

The surface of the substrates was analyzed by scanning electron microscopy (SEM), energy dispersive X-ray analysis (EDX) and X-ray diffraction (XRD). By using EDX, the Ca/P ratio of the compound formed on the surface was evaluated. Reagent grade chemical of hydroxyapatite (Sigma-Aldrich) was used for the calibration.

3. Results

Table I shows the chemical analysis of the bioactive glass obtained. This composition differs slightly from that of the Bioglass® 45S5. However, as stated in the literature [1] the composition of the glass obtained in this work is within the specified range for bioactive glasses. The chemical analysis of the wollastonite used and that of the hydroxyapatite obtained by a precipitation technique are shown in Tables II and III, respectively. For the HA, the Ca and P contents are slightly different to the theoretical values (Table III). According to the literature [16], by using this precipitation method, apart from hydroxyapatite, a minor quantity of $CaHPO_4$ is also obtained. The mean diameters of the wollastonite, bioactive glass and hydroxyapatite particles were 11, 15 and 27 μm, respectively.

Fig. 1 shows the change of pH of the SBF with time for each bioactive material used. The pH of the SBF increased with time during the first days of immersion when bioactive glass (BG) and wollastonite ceramics (W) were in contact with the metallic substrate during the immersion in SBF. This increase may indicate that an ionic exchange between the bioactive material (BG or W) and the SBF is taking place. As observed, the

TABLE I Chemical analysis of the bioactive glass obtained (wt%)

	SiO_2	CaO	Na_2O	P_2O_5
Bioactive glass	43.86	23.99	26.01	6.12
Bioglass® 45S5	45	24.5	24.5	6

TABLE II Chemical analysis of the wollastonite used (wt%)

	SiO_2	CaO	Fe_2O_3	Al_2O_3	Na_2O	MgO	K_2O
Wollastonite	49.06	47.70	0.26	0.47	0.26	2.06	0.25

TABLE III Chemical analysis of the HA obtained and the theoretical values (wt%)

	Ca	P
HA	40.26	17.63
Theoretical values for HA	39.68	18.45

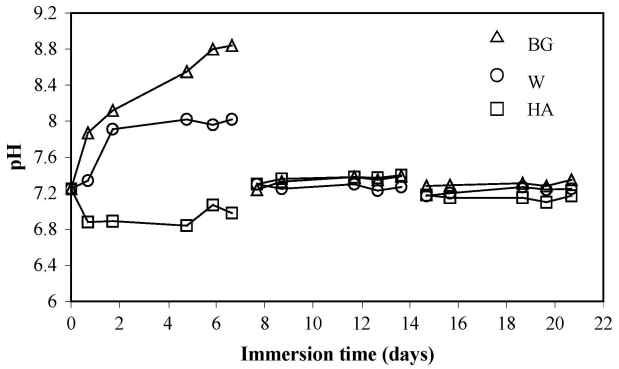

Figure 1 Change of pH of the SBF with time after the immersion of samples treated bioactive glass (BG), wollastonite (W) and hydroxyapatite (HA).

increase in pH values is higher for the solutions corresponding to the samples treated with the bioactive glass. This can be explained taking into account the reported in the literature: when a bioactive glass is immersed in SBF, Ca^{2+} and Na^{+} exchange with H^{+} [18], while by testing wollastonite, the exchange occurs only between Ca^{2+} and H^{+} [19]. By using W or BG, the decrease in pH after 7 days is due to the change of the sample to a glass containing 1.5SBF without bioactive material present.

By using HA a different trend was observed. The pH of the SBF decreases slightly during the first 5 days of immersion. This may be due to the partial dissolution of HA that occurs when this material is soaked in SBF leading to the decrease of pH values [20]. After 5 days of immersion the pH of this solution increased. However, this increment is lower than that observed by using W or BG. During the immersion of the samples in 1.5SBF (longer immersion times) there is no a substantial change of pH in all the cases.

Figs 2 to 4 show the surface and the corresponding EDX elemental spectrum of the samples treated with W, BG and HA, respectively. After 7 days of immersion in SBF with a bed of W, BG or HA, some particles and

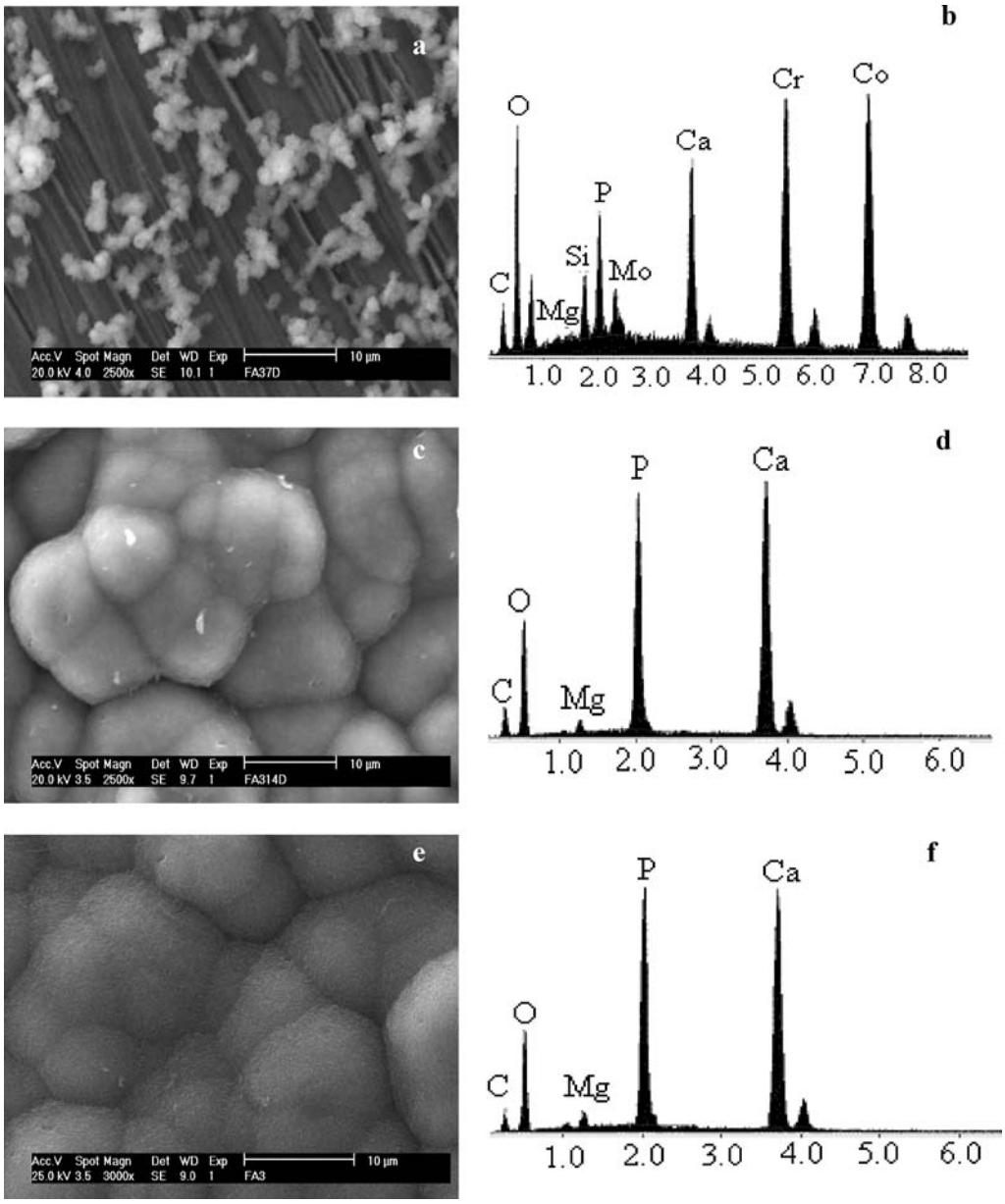

Figure 2 Samples treated with wollastonite: (a, b) after 7 days of immersion in SBF with wollastonite, (c, d) after 7 days in SBF with wollastonite +7 days in 1.5SBF, (e, f) after 7 days in SBF with wollastonite +14 days in 1.5SBF.

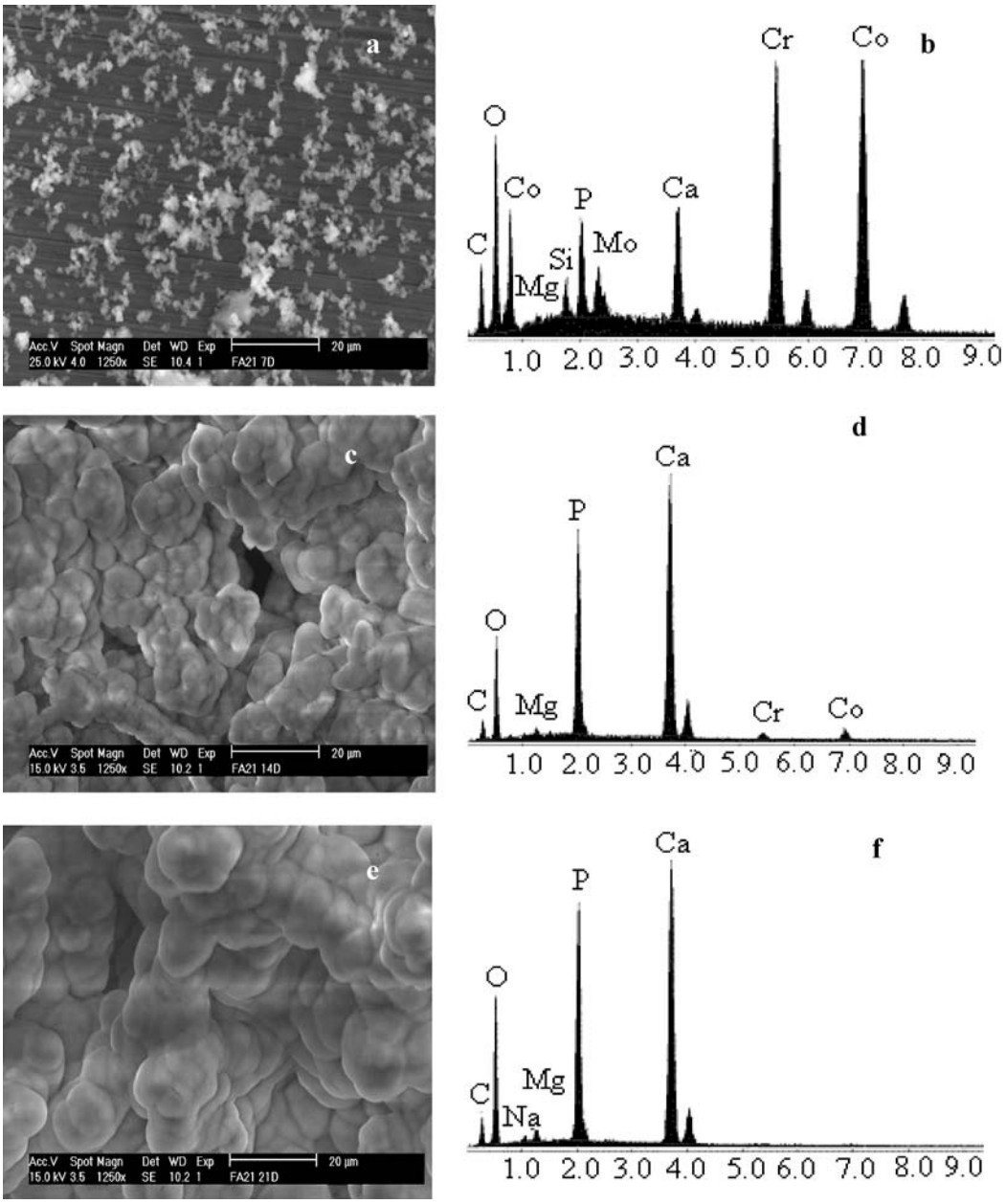

Figure 3 Samples treated with bioactive glass: (a, b) after 7 days of immersion in SBF with bioactive glass, (c, d) after 7 days in SBF with bioactive glass +7 days in 1.5SBF, (e, f) after 7 days in SBF with bioactive glass +14 days in 1.5SBF.

agglomerates were observed (Figs 2a–b, 3a–b and 4a–b). The EDX analysis shows the presence of Ca, P, Si and the alloying elements. After 14 days immersion (7 days in SBF with W, BG or HA+7 days in 1.5SBF without bioactive material) a homogeneous layer is observed (Figs 2c–d, 3c–d, 4c–d). The peaks corresponding to Ca and P increased in intensity and the alloying elements are not longer detected on the samples treated with W and HA and, on that treated with BG, the intensity of the peaks corresponding to the alloying elements decreased considerably. This indicates that the thickness of the ceramic layer increases with time of immersion.

After 21 days (7 days in SBF with W, BG or HA + 14 days in 1.5SBF) a more homogeneous layer is observed in all the cases (Figs 2e–f, 3e–f, 4e–f). Additionally, the agglomerates for the samples treated with wollastonite (Fig. 2e) are less angular than that of the agglomerates shown in Fig. 2c and this morphology is similar to that observed on the existing bioactive systems.

The Ca/P ratios of the compounds formed on the samples immersed in simulated body fluids at different immersion times are shown in Table IV. These values indicate that the compound formed on the metallic

TABLE IV Ca/P ratios of the compounds formed on the substrates

Immersion time	W	BG	HA
7 days in SBF	1.21 ± 0.051	1.19 ± 0.04	1.21 ± 0.039
14 days (7 days in SBF + 7 days in 1.5SBF)	1.29 ± 0.058	1.36 ± 0.051	1.46 ± 0.042
21 days (7 days in SBF + 14 days in 1.5SBF)	1.49 ± 0.052	1.45 ± 0.048	1.39 ± 0.044

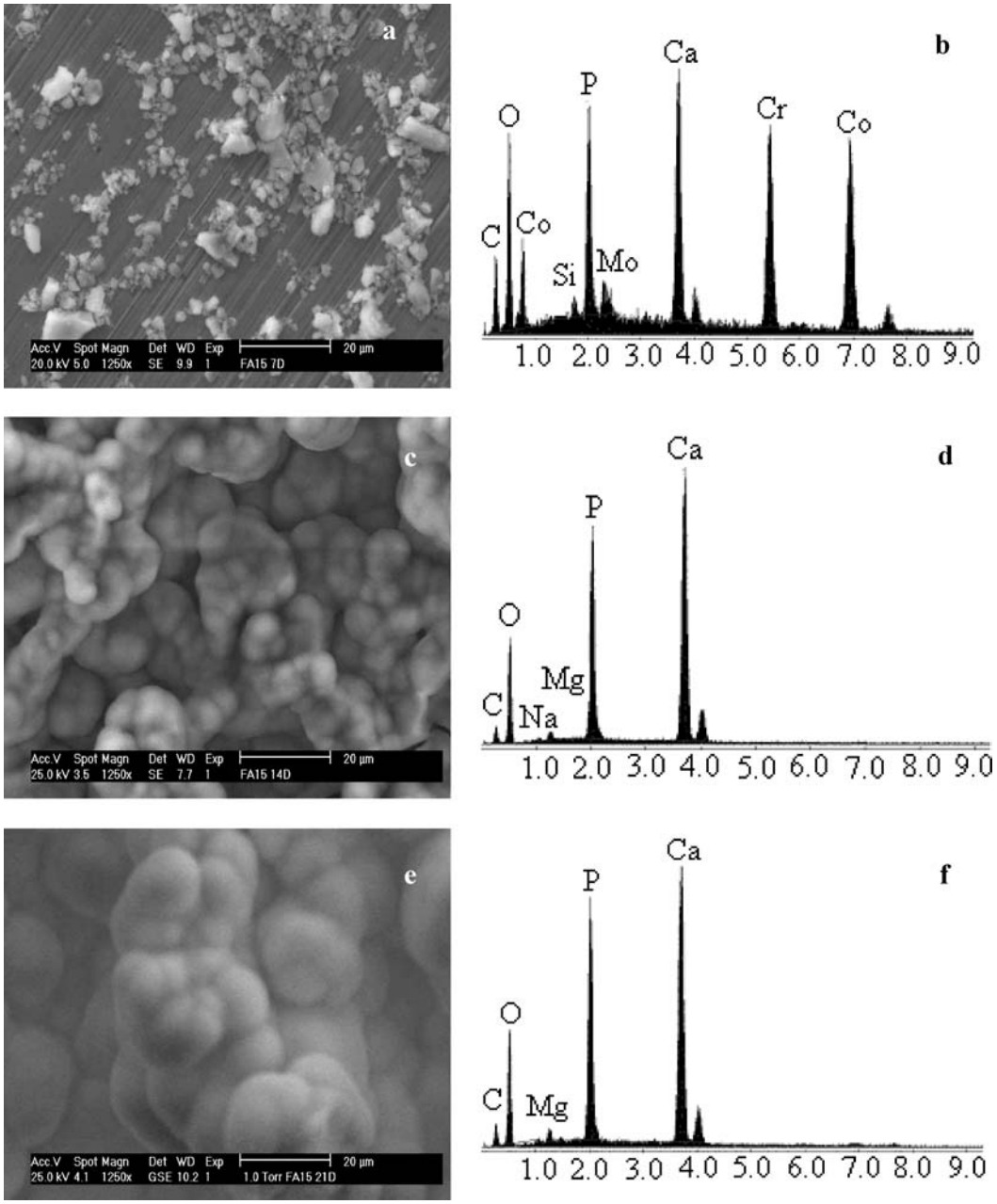

Figure 4 Samples treated with hydroxyapatite: (a, b) after 7 days of immersion in SBF with hydroxyapatite, (c, d) after 7 days in SBF with hydroxyapatite +7 days in 1.5SBF, (e, f) after 7 days in SBF with hydroxyapatite +14 days in 1.5SBF.

samples corresponds to apatite (1.2–1.66). The Ca/P ratio for HA is 1.67. As observed, the Ca/P ratio of the samples treated with W and BG increases as the immersion time is increased. However, by using HA, a different behavior was observed. The Ca/P ratio increases from 7 to 14 days of immersion, but decreases slightly from 14 to 21 days. This may be an indicative that, when HA is immersed in SBF, both dissolution and precipitation occurs and Ca^{2+}, HPO_2^{2-} and $PO_4^{3-}$are continuously released [21]. This release has an effect on the pH of the solution and the pH is an important parameter on the Ca/P ratio of the apatite formed.

Fig. 5 shows the XRD patterns of the samples treated with W, BG and HA after different times of immersion. After 7 days in SBF, by using wollastonite, peaks corresponding to the alloying elements and peaks of lower intensity, corresponding to hydroxyapatite (HA), can be observed. On the other hand, after 7 days of immersion of the samples treated with bioactive glass or hydroxyapatite, no peaks corresponding to HA were detected. This may indicate that the ceramic particles analyzed by SEM and EDX (Figs 3a–b and 4a–b) are not thick enough, thus, they can not be detected by XRD. After 14 days of immersion of the samples treated with W, BG or HA (7 days in SBF with W, BG or HA + 7 days in 1.5SBF) the peaks detected correspond to HA and those corresponding to alloying elements are no longer detected.

After 21 days of immersion (7 days in SBF with W, BG or HA + 14 days in 1.5SBF) the intensity of the peaks corresponding to HA has increased considerably in all the cases. This is in agreement with the SEM and EDX results, the thickness of the apatite layer increases by increasing the time of immersion. After 21 days, the peaks intensity is higher for the materials treated with wollastonite. This shows that,

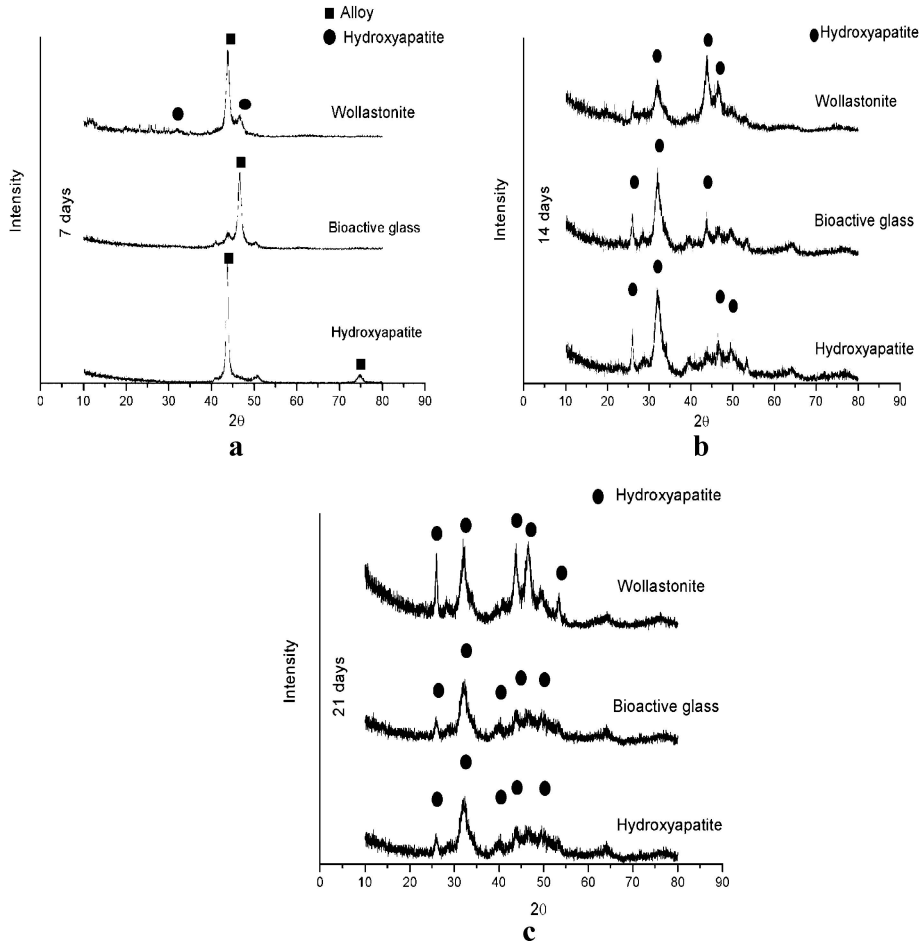

Figure 5 XRD patterns: (a) after 7 days in SBF with bioactive material, (b) after 7 days in SBF with bioactive material + 7 days in 1.5SBF, (c) after 7 days in SBF with bioactive material + 14 days in 1.5SBF.

by using wollastonite, the apatite layer formed on the samples has a higher crystallinity and more defined apatite crystals.

Among the three bioactive systems used, the wollastonite powder increases the rate of bonelike apatite formation on the CoCrMo alloy by this biomimetic method. A thin HA layer can be detected by XRD after 7 days of immersion in SBF with wollastonite, while, by using bioactive glass or hydroxyapatite, the apatite layer was detected after 14 days of immersion (7 days of immersion in SBF with bioactive material +7 days of immersion in 1.5SBF). This is may be due to the higher content of Si in wollastonite than in bioactive glass. According to the literature [4, 22], the silicon dissolved from the existing bioactive systems plays an important role in forming the apatite layer. The amount of Si^{4+} adsorbed on the surface acts as a nucleating agent [9]. As observed from the EDX spectra (Figs 2b, 3b and 4b) the intensity of the peak corresponding to Si, after 7 days of immersion in SBF, is higher for the samples treated with W than that of the samples treated with BG or HA. Additionally, the apatite layer formation on the substrates depends strongly on the ion concentration in the vicinity of the surface of the substrates, where the degree of supersaturation of SBF to apatite increases with increasing Ca^{2+} dissolved from the W, BG or HA. After longer immersion times, a thicker ceramic layer, consisting of Ca, P and O, is formed in all the cases and the silicon can not longer be detected by EDX.

4. Conclusions

A bonelike apatite layer was formed on samples of CoCrMo alloy (ASTM F75) by using a biomimetic method that includes the use of a bed of wollastonite, bioactive glass or hydroxyapatite. As analyzed by SEM and EDX, all the samples formed apatite crystals on their surfaces after 7 days of immersion in SBF and an homogeneous apatite layer after longer immersion times in 1.5SBF. However, by using XRD, hydroxyapatite was detected only on the samples treated with wollastonite after 7 days of immersion. No apatite was detected on the samples treated with hydroxyapatite or bioactive glass after this short period of immersion. This indicates that the rate of apatite formation increases by using wollastonite. Furthermore, after 21 days, the morphology of the apatite layer that was closer to that formed on the existing bioactive systems was observed on the samples treated with wollastonite.

Acknowledgments

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