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In vitro calcium phosphate formation on SiO_2–Na_2O–CaO–P_2O_5 glass reinforced hydroxyapatite composite: a study by XPS analysis

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In-vitro apatite layer formation on the surface of a newly developed glass reinforced hydroxyapatite composite was characterized using X-ray photoelectron spectroscopy (XPS) and scanning electron microscopy (SEM) equipped with energy dispersive X-ray analysis (EDXA). After 1 week soaking in an acellular simulated body fluid the composite surface was entirely covered by a Ca, P-layer, suggesting a bioactive behaviour. XPS binding energy results revealed that this surface layer was a carbonated and non-stoichiometric apatite with Ca/P ratio of 1.3. This apatite layer was composed of very fine needle-like crystallites. Comparative studies on a commercially available hydroxyapatite showed that a similar apatite layer was also formed on its surface.

1. Introduction

A wide range of dental and orthopaedic applications require ceramic materials ranging from nondegradable to entirely degradable ones [1–4]. Therefore, there is a great need to fabricate composites with controlled biodegradation, especially for bone grafting, filling or substitution [5–8]. Various attempts have been made to associate the advantageous properties of hydroxyapatite and bioactive glasses [9–12]. While pure crystalline hydroxyapatite is a fairly inert material in aqueous solutions, bioactive glasses exhibit controlled ion release depending on their chemical composition [13–15]. A suitable combination of both may provide composites with different degrees of biodegradation. In earlier work [16–18], Santos *et al.* demonstrated that Bioglass® could be added to hydroxyapatite by a simple liquid phase sintering process. This association may lead to hydroxyapatite composites with variable biodegradation, as different glass percentages could be easily added to hydroxyapatite. The Bioglass® incorporated into hydroxyapatite has been considered to promote a rapid new bone formation *in vivo* [19, 20] and therefore, its presence in the microstructure of the composites may play an important role in their biological behaviour for many biomedical applications, particularly in the repair of periodontal osseous defects and maxillofacial surgery.

Bioactive materials bond to living bone through a collagen-free apatite layer which is formed on their surface, although its thickness and crystal size seem to be dependent on the bioactive material [21]. This Ca, P-layer can also be reproduced *in vitro* using simulated physiological solutions [22, 23] and the test may then be used as a criterion to assess *in vivo* bioactivity.

Earlier work [24] showed that strong surface dissolution occurs for Bioglass®–hydroxyapatite composite in the initial stage of the apatite formation process, probably with leaching of Ca^{2+} and Si^{4+} from the sample surface. The mechanism of apatite nucleation was discussed in terms of being induced by surface dissolution due to immersion in the simulated physiological solution.

In this work slight changes in the physiological solution were introduced, particularly in terms of its Ca^{2+} and HPO_4^{-2} ionic concentration in order to obtain a faster response in the Ca, P-layer nucleation and growth, since these two ions strongly influence the apatite nucleation process [25]. Longer immersion times, up to 4 weeks, were also used to increase the thickness of the apatite layers. Having thicker surface layers, more reliable surface analysis could be performed reducing the interference from the substrate.

The chemical composition of the apatite layer formed on the surface of a Bioglass®–hydroxyapatite composite was determined using X-ray photoelectron spectroscopy (XPS) analysis. Comparative studies with sintered hydroxyapatite samples were also conducted. The use of XPS allows one to determine

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precisely the chemical composition of the surface apatite layers.

2. Materials and methods

Commercial hydroxyapatite supplied by Plasmâ Biotal, Tideswell, UK was sintered at 1300 °C for 1 h at 4 °C/min heating rate. X-ray diffraction (XRD) analysis performed after sintering showed that the mineral composition of the samples fully corresponded to hydroxyapatite without any other mineral phases. Bioglass®–hydroxyapatite composite samples were also prepared by a liquid phase sintering process, using a 2.5 wt % addition of Bioglass® to hydroxyapatite. Bioglass® had the following chemical composition, in mol%: 46.1 SiO_2, 24.4 Na_2O, 26.9 CaO and 2.6 P_2O_5. The composite preparation is fully described elsewhere [17]. The microstructure of this composite was composed of a mixture of hydroxyapatite, tricalcium phosphate and calcium phosphate silicate [16].

Samples described above were machined to $10 \times 10 \times 1$ mm and ground to 200 μm. After being ultrasonically washed in acetone, rinsed in double destilled water and dried, samples were soaked in an acellular physiological solution, whose chemical composition is presented in Table I. The solution was buffered at a pH = 7.3 with trimethanol aminomethane-HCl. Duplicate samples of each type were immersed for 1, 2, 3 and 4 weeks at 37 ± 0.5 °C without stirring.

Surface morphology was examined using a Jeol scanning electron microscope equipped with energy dispersive X-ray analysis (EDXA). A VG ESCALAB X-ray photoelectron spectroscope was used for quantitative determination of the elements present on the samples surface before and after immersion. The instrument was calibrated against Au 4f7/2 spectrum at 84.0 eV binding energy, as reported previously [24]. Spectra were referenced to the C 1s peak of adventitious carbon fixed at 285.0 eV.

3. Results

Fig. 1a, b shows the surface morphology of Bioglass®–hydroxyapatite composite before and after 1 week soaking in simulated physiological solution. Scratches from the grinding are clearly observed before immersion, whereas after immersion the material surface is entirely covered by a layer composed of large spherulites, due to surface reaction with the solution. This layer seemed to be weak as it tended to fracture in some areas during scanning electron microscopy (SEM) analysis by electron bombardment of the incident beam. A similar layer was also detected for longer soaking periods. At higher magnification, very small elongated crystallites could be observed, as may be seen in Fig. 1c. Equivalent findings were also observed for commercial sintered hydroxyapatite, as represented in Fig. 2a, b. Energy dispersive spectroscopy analysis (EDXA) indicated that, in all cases, these layers were composed of Ca and P.

XPS analysis of the elements present on the surface of sintered hydroxyapatite and Bioglass®–hydroxyapatite composite were recorded after 1 and

TABLE I Chemical composition of acellular simulated body fluid

Chemical	Composition (g/dm^3)
NaCl	8.00
$CaCl_2.2H_2O$	0.19
$MgSO_4$	0.10
KCl	0.40
KH_2PO_4	0.06
$NaHCO_3$	0.35
Na_2HPO_4	0.05

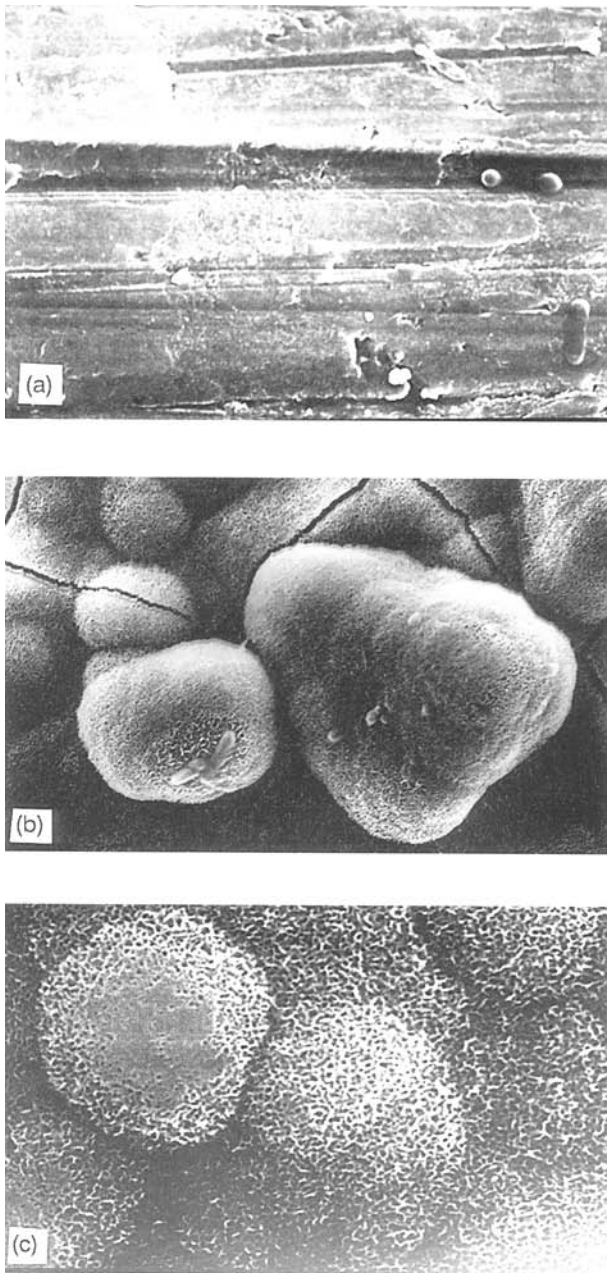

Figure 1 SEM micrograph of the surface of Bioglass®–hydroxyapatite composite (a) before immersion in the simulated physiological solution (4000 ×). Grinding scratches are clearly observed. After immersion for (b) 1 week (c) 4 weeks the material surface is entirely covered by a Ca, P-layer which could be resolved into very small crystallites.

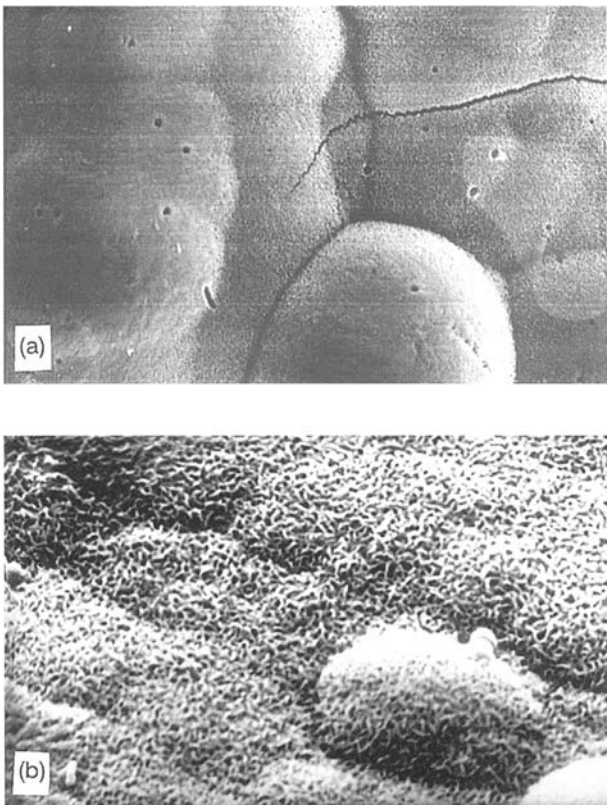

Figure 2 A Ca, P-layer observed on the surface of a commercially available hydroxyapatite after (a) 1 week and (b) 4 weeks (4000 ×).

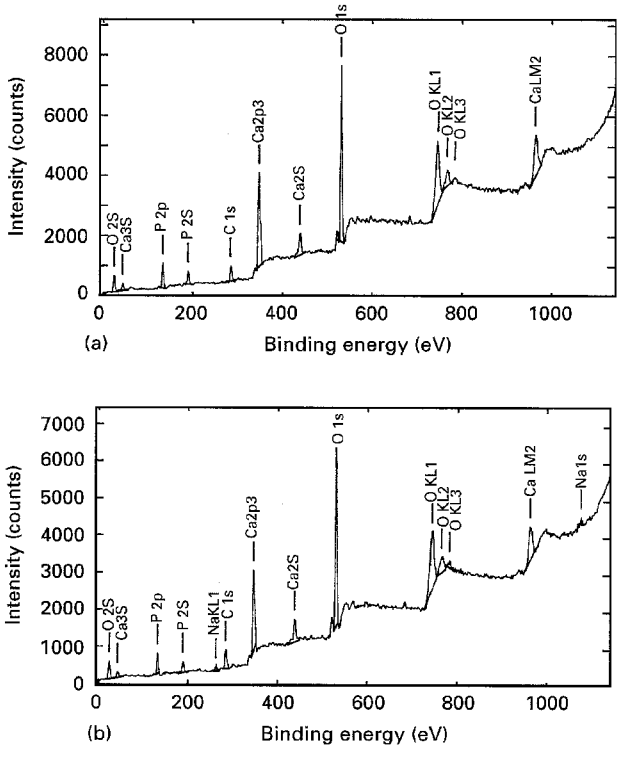

Figure 3 XPS spectra of hydroxyapatite soaked for (a) 1 week and (b) 4 weeks.

4 weeks immersion, as shown in Figs 3 and 4, respectively. Ca 2p3, P 2p, O1s and C1s level spectra were identified for all test conditions. An additional peak of Na 1s was also found, particularly after 4 weeks immersion. For samples immersed for 1 week, the Na 1s peak could not be clearly detected. The peak position

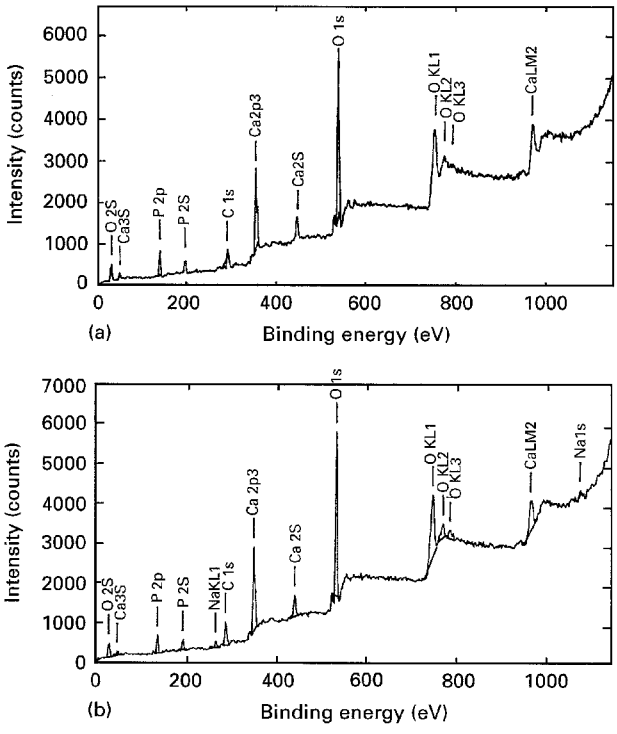

Figure 4 XPS spectra of Bioglass®–hydroxyapatite composite immersed for (a) 1 week and (b) 4 weeks.

TABLE II Binding energy of the elements detected on the surface

Sample	Immersion time (weeks)	Binding energy (eV)				
		O	P	Ca	C	Na
Hydroxyapatite	1	531.3	133.2	347.1	284.5	–
	4	531.5	133.1	347.0	284.5	1071.0
HA/Bioglass®	1	531.4	133.4	347.5	284.5	–
	4	531.3	133.1	347.1	284.6	1071.2

TABLE III Relative concentration of the elements detected on the surface

Sample	Immersion time (weeks)	Relative concentration (at %)					
		O	P	Ca	C	Na	Ca/P
Hydroxyapatite	1	51.0	14.7	19.1	15.1	–	1.3
	4	49.3	13.9	17.9	17.6	1.2	1.3
HA/Bioglass®	1	48.4	14.6	18.7	18.2	–	1.3
	4	47.6	11.8	15.7	22.8	1.9	1.3

of elements detected on the surface and their relative concentration are presented in Tables II and III, respectively.

Quantitative elemental analysis for sintered hydroxyapatite revealed that the Ca, P-layer had a Ca/P ratio of approximately 1.3, which remained practically unchanged after immersion for 4 weeks. Silicon was never detected on the surface of Bioglass®–hydroxyapatite composite. This effect was due to coverage of the composite surface by the Ca, P-layer.

Since the identified C 1s peak was very broad and strong for both hydroxyapatite and Bioglass®–hydroxyapatite composite after immersion for 1 and

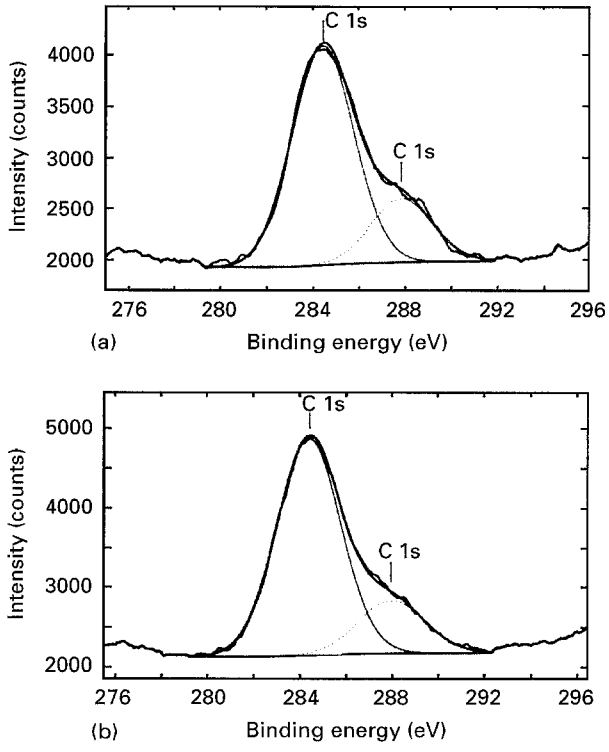

Figure 5 The deconvolution of C 1s revealed that it was composed of two peaks, one at 284.5 ± 0.1 eV and another at approximately 287.8 ± 0.1 eV for both (a) sintered hydroxyapatite and (b) Bioglass®–hydroxyapatite composite.

4 weeks, it was deconvoluted using a Gaussian curve-fitting process. Fig. 5a and b show the C1s peaks for hydroxyapatite and Bioglass®–hydroxyapatite composite, respectively, after immersion for 4 weeks.

The deconvoluted peaks revealed that the C1s peak was composed of two peaks: one at 284.5 ± 0.1 eV and another at approximately 287.8 ± 0.1 eV.

4. Discussion

Several authors have reported that the Ca, P formation on a material's surface in simulated acellular body fluids is a decisive indicator of their bioactivity, since bioactive materials bond to bone *in vivo* through similar surface layer [21, 26]. Several techniques have also been used to characterize these Ca, P-layers, particularly thin-film XRD and Fourier transform infra-red (FTIR) analysis, on an extensive range of materials [22, 23, 27]. In this work, the authors have compared the Ca, P formation on the surface of a commonly accepted bioactive material, sintered hydroxyapatite, to that of a newly developed composite, using a surface analysis technique capable of characterizing the surface chemistry of the Ca, P-layer and quantitatively determining its chemical composition.

SEM analysis revealed that the Ca, P-layer formed on the surface of both sintered hydroxyapatite and the Bioglass®–hydroxyapatite composite was composed of randomly oriented crystallites with a needle-like shape. Similar findings were also observed by other authors for sintered hydroxyapatite [22, 28].

The addition of Bioglass® to hydroxyapatite has an important effect on the rapid formation of the Ca, P-layer, as the time required for nucleation to start was much shorter for the composite than for sintered hydroxyapatite, as reported previously [24]. This behaviour was attributed to the presence of a highly soluble tricalcium phosphate phase, β-TCP, formed from the reaction between Bioglass® and the hydroxyapatite matrix during liquid phase sintering process, which induces a much faster Ca, P formation than hydroxyapatite [22]. However, these XPS results have shown that the chemical characteristics of this layer did not seem to be influenced by the glass reinforcement, as the peak positions of all detected elements that composed the Ca, P-layer and their relative atomic proportions were practically the same for both materials, as presented in Tables II and III.

The Ca, P-layer formed on a specimen's surface is composed of spherulites with very fine crystallites, suggesting a high Ca, P nucleation rate. Some spherulites were formed directly on the surface of other growing spherulites (Figs 1b and 2b) or at their interface (Fig. 2a). This type of occurrence suggests that the front of a growing layer is also a preferential nucleation site for other spherulites.

The reference for XPS binding energy data was the adventitious C 1s peak at 285.0 eV. The peak position of all the detected elements were corrected with respect to this C 1s level. The binding energies determined for P2p, Ca2p3 and O1s obtained at, respectively, 133.1 eV, 347.1 eV and 531.3 eV undoubtedly corresponded to calcium phosphate (apatite), $Ca_{10}(PO_4)_6(OH)_2$, formation on the surface, as determined by Landis *et al.* [29]. However, this apatite is nonstoichiometric as its Ca/P ratio is much lower than the Ca/P = 1.67 stoichiometric value. Although using a different material, Andersson *et al.* [30] have also reported calcium-deficient apatite layers with Ca/P ratios of 1 and 1.3 on the surface of bioactive glass. Silicon was never detected on the surface of Bioglass®–hydroxyapatite, indicating that the whole surface was covered by the Ca and P rich layer which agreed with SEM observations.

The deconvoluted C1s peaks at 284.6 eV and 287.8 eV, respectively, corresponded to carbon contamination and carbonate group, CO_3^{-2}, on the surface layer. This is in agreement with the reference standard binding energy for CO_3^{-2}, as determined by Verhoeven *et al.* [31]. Therefore, it may be concluded that the surface layer is a carbonated apatite. Other authors have reported the presence of carbonated apatite layers on biomaterials surface after immersion in physiological solutions, although using different analysis techniques, namely infrared spectroscopy [20, 32].

Na 1s was detected at 1071.1 ± 0.1 eV and its concentration on the materials surface tended to increase with immersion time. Both occurrences indicate the existence of some Na_2SO_4 [33] in the surface layer which is in agreement with earlier results [24].

5. Conclusions

A Ca, P surface layer was formed on the surface of a newly developed Bioglass®–hydroxyapatite

composite after immersion for 1 week in acellular simulated body fluid. The use of XPS analysis allowed precise determination of its chemical composition, which revealed that this layer was a non-stoichiometric apatite, with a Ca/P ratio of 1.3, containing carbonate ions, CO_3^{-2}, in its structure.

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