

Surface modifications of glass-reinforced hydroxyapatite composites

J.D. Santos^{*†}, Lakhan J. Jha[†] and F.J. Monteiro^{*†}

^{*}Department of Metallurgical Engineering, FEUP, University of Porto, Rua dos Bragas 4099-Porto Codex, Portugal;

[†]INEB-National Institute for Biomedical Engineering, Praça do Coronel Pacheco 1, 4000-Porto, Portugal

Surface modifications of glass-reinforced hydroxyapatite composites immersed in a simulated physiological solution were studied using X-ray photoelectron spectroscopy, scanning electron microscopy and energy dispersive X-ray spectroscopy. In the first stages of the apatite formation process, calcium and silicon ions were leached out from the surface of the composites. After 12 days of immersion, apatite crystals were detected on the surface indicating bioactive behaviour. Sodium ions attributed to sodium sulphate were also found in the apatite layer. Bound water on the surface also increased with immersion time.

Keywords: Composite materials, hydroxyapatite, bioactive glass, surface modification

Received 21 March 1994; accepted 22 September 1994

Recent work has shown that hydroxyapatite (HA) could be reinforced by adding a glassy phase to its sintering process¹. Chemical similarity between the glassy phase and HA seems to be an essential requirement for the reinforcement mechanism to occur². Small quantities of phosphate-based glasses may be easily added to HA powders enhancing the densification process. Not only are the mechanical properties of these composites found to be higher than those usually reported for dense HA^{3–6} but the glass addition also acts as a means of incorporating ions commonly found in bone tissues, such as Na⁺, K⁺, etc.⁷.

The rate of bone bonding to implant and the strength and stability of the bond vary with the composition and microstructure of the bioactive material^{8,9}. The bonding mechanism generally involves a bioactive acellular layer rich in calcium phosphate, mucopolysaccharides and glycoproteins. The bioactive layer provides an acceptable environment for collagen and bone mineral deposition¹⁰. It is considered that calcium phosphate layers formed on the surfaces of bioactive materials implanted in the body play an essential role in forming the chemical bond of the bioactive material to the living bone. It is possible to evaluate the bioactivity of a material by examining the precipitation and growth of the calcium phosphate, resulting in the formation of a calcium phosphate layer on its surface after immersion in appropriate physiological solutions^{11–13}.

In the present work a series of newly developed HA glass composites have been tested in order to evaluate

eventual changes in surface chemistry occurring through immersion in synthetic plasma solution. This was done by using X-ray photoelectron spectroscopy (XPS), scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDS).

MATERIALS AND METHODS

Two phosphate-based glasses (P1 and P2) and one silica-based glass (S1) were used to reinforce HA. The phosphate-based glasses were produced from reagent grade chemicals heated at 1300°C for 1 h in a platinum crucible. The silica-based glass was obtained from Bioglass Research Center, University of Florida, USA. Chemical compositions of the glasses are given in Table 1. In order to obtain a homogeneous mixture, glass and HA powders were wet milled and mixed in a ball mill pot using methanol. The amount of glass added to HA was 2.5 wt%. The method used to fabricate the composites is described fully elsewhere^{1,2,14}.

Samples were machined to the dimension of 10 × 10 × 1 mm³ and ground to 200 μm. The specimens were ultrasonically washed in acetone, rinsed in double distilled water and dried. Duplicate samples of each type were immersed in separated polyethylene bottles containing 50 cm³ synthetic plasma solution buffered at a pH of 7.3 with aminomethanetrimethanol-HCl. Samples were immersed for 1, 2, 6 and 12 d at 37°C without stirring. The composition of synthetic plasma solution is given in Table 2. Immediately after immersion, samples were gently rinsed in double distilled water and dried at room temperature.

Correspondence to Professor J.D. Santos.

Table 1 Chemical composition of the glasses (mol%)

	P ₂ O ₅	CaO	Na ₂ O	Al ₂ O ₃	SiO ₂
P1	45.5	54.5	—	—	—
P2	62.9	10.1	10.1	16.9	—
S1	2.6	26.9	24.4	—	46.1

Table 2 Composition of the simulated physiological solution¹²

Chemical	Composition (g dm ⁻³)
NaCl	8.04
KCl	0.22
CaCl ₂ ·2H ₂ O	0.37
NaHCO ₃	0.35
K ₂ HPO ₄ ·3H ₂ O	0.30
Na ₂ SO ₄ ·10H ₂ O	0.16
MgCl ₂ ·6H ₂ O	0.30
Aminomethanetrimethanol	6.05
HCl, 37.5%	3.68 (cm ³ dm ⁻³)

Surface analysis

A JEOL scanning microscope JSM 35C, along with EDS (Voyager, Norman Instruments, UK), was used to examine surface morphology. VG ESCALAB 200A was used for quantitative analysis of elements present on the sample surfaces. An Mg K α X-ray source was used during analysis at a pass energy of 1253 eV and current of 5 mA. A 5.3×10^{-7} Nm⁻² vacuum in the specimen chamber was achieved. Detailed higher energy resolution scans over pertinent kinetic energy regions were recorded to provide more accurate quantitative and chemical bonding information. The instrument was calibrated against the Au 4f_{7/2} spectrum at 84.0 eV binding energy. Spectra were referenced to the C 1s peak of adventitious carbon fixed at 285.0 eV. A minimum of two samples was analysed by XPS and only reproducible data are reported in the present study.

RESULTS

The microstructure of P1 HA-phosphate glass composite was composed of an HA matrix and tricalcium phosphate (TCP) phases, β -TCP and α -TCP, in a proportion of 50%, 20% and 30% respectively, as reported previously². The P2 sample had a phase content of 40% HA, 15% β -TCP and 45% α -TCP. In the HA-silica-based glass composite, calcium phosphate silicate was detected using X-ray diffraction analysis^{2,14}, besides apatitic structures.

SEM analysis revealed that after 6 d immersion in the physiological solution the P1 sample was almost covered by a layer formed on its surface, as shown in Figure 1. EDS analysis showed that this layer was composed of Ca and P crystals with a Ca/P ratio = 1.65, which corresponds to a structure of apatite. Some dissolution also seems to have occurred in some areas of the surface. Similar behaviour was also detected for the P2 sample. Ca and P crystals were analysed by EDS along with some surface dissolution after 2 d

immersion, as shown in Figure 2. More intense apatite formation occurred on its surface after 12 d immersion.

Significant surface dissolution occurred for the HA-silica-based glass composite after 6 d immersion, without any apatite formation, as may be observed in Figure 3. This result indicated that only surface dissolution was active in the initial stages and more ions were leached out from the composite surface than from HA-phosphate-based glass composites. However, after 12 d immersion its surface was also covered by an apatite structure, as for the HA-phosphate-based glass composites.

For qualitative evaluation of the elements present on the surface, wide scan spectra of P1, P2 and S1 were recorded. Changes in the elemental composition of P2 and S1 samples with respect to immersion times were compared with a control, as shown in Figures 4 and 5. Besides the most intense peaks of the constituent elements (P 2p, Ca 2p and O 1s for P1 and P2, and P 2p, Ca 2p, Si 2p and O 1s for S1), a strong peak for C 1s indicates the presence of a large amount of carbon on the surface. This could be derived partly from the solution and partly from unavoidable carbon contamination. Sodium was never detected on P2 and S1 control samples; however, it was found on the surface after immersion for 6 d in the synthetic plasma

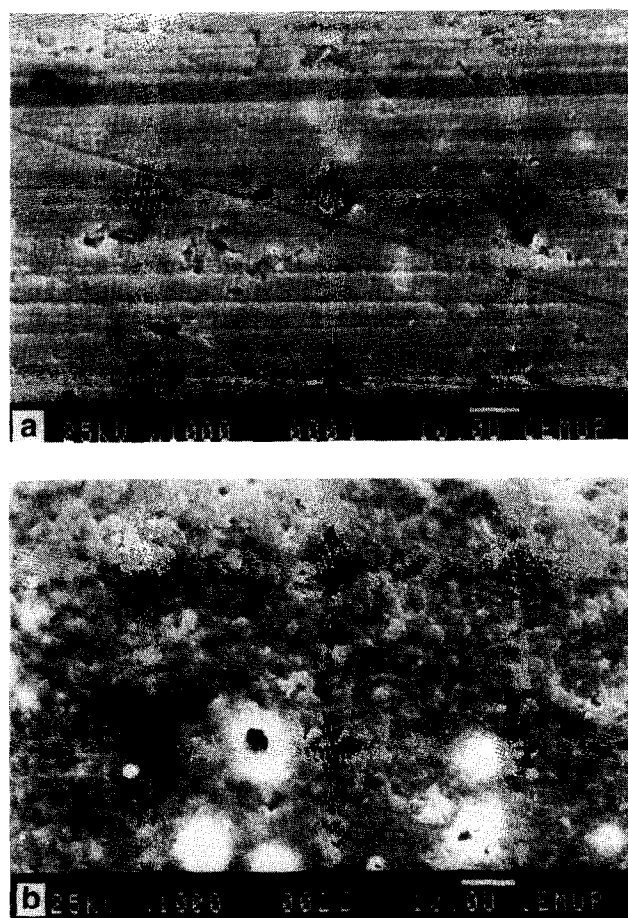


Figure 1 Scanning electron micrographs of P1 sample **a**, before and **b**, after 6 d immersion, showing that the surface was covered by an apatite layer. Scratches of the grinding process are not seen. EDS analysis revealed that this layer had a Ca/P ratio = 1.65.

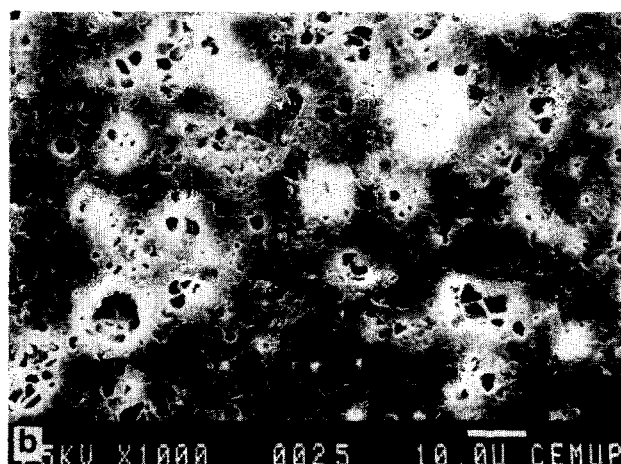
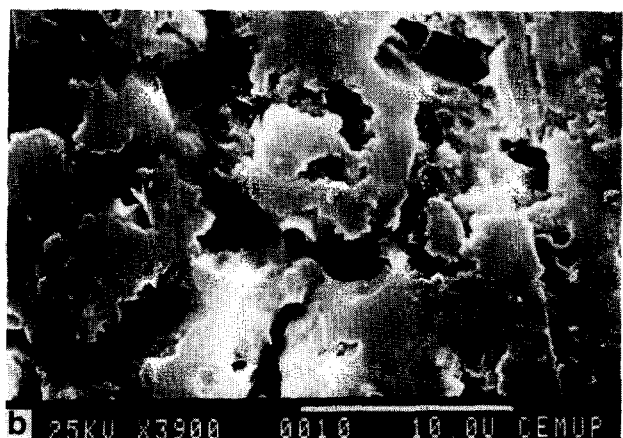
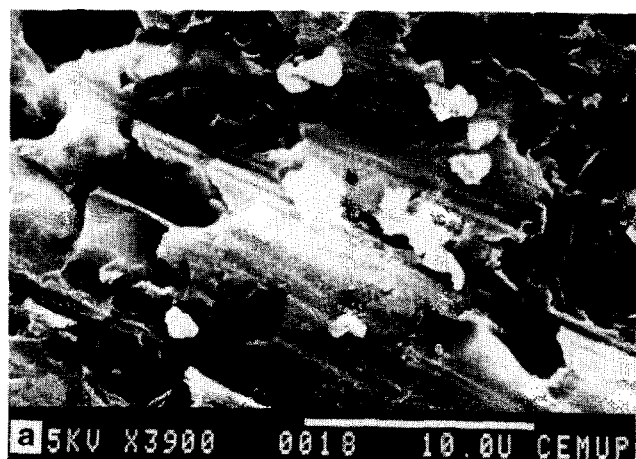


Figure 2 a, Apatite crystals (indicated by arrows) on the surface of P2 sample after 2 d immersion. b, Higher apatite formation occurred after 12 d immersion.

solution. Detailed spectra of each detected element were recorded in order to evaluate the chemical state and the surface chemical composition. The quantitative data of the detected elements revealed depletion of Ca from all specimen surfaces after immersion in the solution as compared with that of control samples. However, the atomic percentage of Ca increased significantly for samples immersed for 12 d. The variation in the quantity of Ca and P was more intense in the case of S1 than for P1 and P2.

DISCUSSION

It has been reported in the literature that apatite formation on HA involves a partial dissolution of its surface in the initial stage of the process, followed by secondary nucleation and/or reprecipitation^{15,16}. These results seem to have been confirmed by the present SEM results as the surface degradation process occurred in concurrence with the apatite formation process. Kodri *et al.*¹⁵ compared the behaviour of pure sintered HA to that of mixtures of HA and TCP materials after 1 wk immersion period in a phosphate buffer solution. While no surface modifications were detected for HA, the biphasic calcium phosphate ceramics formed apatite crystals covering their whole surface.

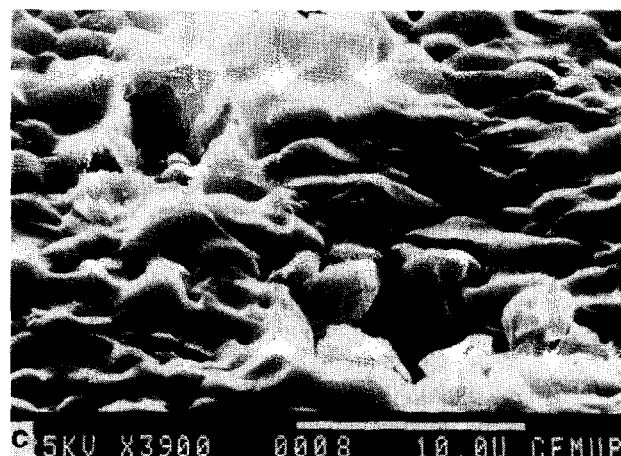


Figure 3 Very intense surface degradation occurred after 6 d immersion for sample S1, when comparing a with b. After 12 d the surface was almost covered by an apatite layer, c.

These differences were attributed to the higher solubility of the TCP phase compared with HA. Identical behaviour has been reported by Hyakuna *et al.*¹¹.

A much more intense surface dissolution occurred for the HA-silica-based glass composite in the initial stages of the apatite formation process. Kokubo *et al.*¹³ proposed that not only calcium but also silicon is dissolved from the surface of bioactive glass-ceramic which may explain the degree of dissolution observed.

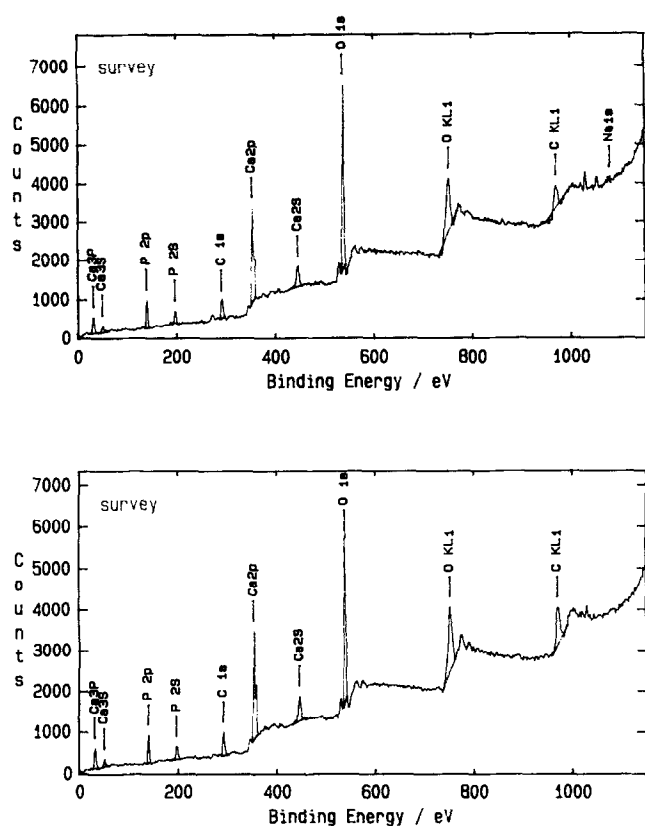


Figure 4 X-ray photoelectron spectroscopy of the P2 control sample before (lower half) and after 6 d immersion (upper half). Sodium was detected after immersion.

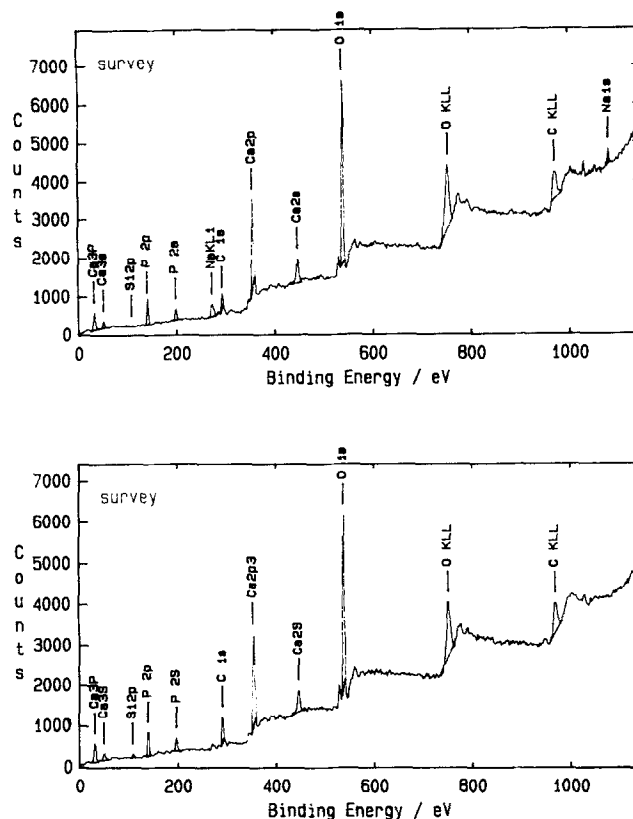


Figure 5 X-ray photoelectron spectra indicated that after 6 d immersion (upper half) Si was leached out from the composite control surface (lower half) and adsorption of Na occurred.

However, after 12 d immersion apatite formation also occurred on the surface of the HA-silica-based glass composite, indicating bioactive behaviour.

The peak positions of O 1s at 531.4 eV, P 2p at 133.0 eV and Ca 2p³ at 347.6 eV did not change with respect to immersion time compared with the P1 control. This indicates that the surface chemistry of the composite was unchanged during immersion. Calcium, phosphorus and oxygen detected at the above-mentioned peak positions correspond to an apatite structure, more precisely HA¹⁷. However, the Ca/P ratio decreased with increasing immersion time. Probably this decrease in Ca/P ratio was due to surface degradation of the composite in the course of time.

O 1s, P 2p, C 1s and Ca 2p³ peaks were found at 531.5, 133.1, 285.0 and 347.8 eV respectively, for the P2 control. The peak positions did not change significantly with respect to immersion time. However, an additional peak of Na 1s at 1072.5 eV was detected on the surface after 6 d immersion. The Ca/P ratio decreased on the surface of the P2 composite after immersion for 6 d, but increased for the specimen immersed for 12 d. This was probably due to leaching out of calcium from the composite surface in the initial period of immersion and reprecipitation after 12 d. This seems to agree with previous results¹⁶. Sodium detected on the surface at 1072.5 eV most probably indicates adsorption of sodium sulphate¹⁸ from the solution, as no sodium was detected on the control surface during the quantitative elemental analysis.

O 1s, C 1s, P 2p, Ca 2p³ and Si 2p peaks were detected at 531.6, 285.0, 133.2, 347.6 and 101.4 eV respectively, for the S1 control. The peak positions did not change with immersion time. However, the Ca/P ratio decreased after 2 d immersion and increased after 6 and 12 d immersion. An additional peak of Na 1s was also detected on the specimen surface after 6 and 12 d immersion. This indicates that along with reprecipitation of calcium phosphate, sodium sulphate was adsorbed on the surface. As sodium was not detected in the control, the adsorbed sodium sulphate on the surface originated from the plasma solution only. An Si 2p peak, found at 101.8 eV on the composite surface, probably indicates the presence of wollastonite¹⁹.

The O 1s peak of the Si composite was deconvoluted using gaussian curve-fitting, as shown in Figure 6. It may be seen from this figure that oxygen was formed in two chemical states on the surface. The main peak of O 1s was found at 531.0 eV, with a small peak at 533.1 eV on the control. The O 1s peak at 531.0 eV corresponded to the oxide constituent of the HA composite whereas the peak at 533.1 eV corresponded to bound water²⁰. More interestingly, the main O 1s peak at 531.6 ± 2 eV was found on the immersed samples. The peak height at 531.6 ± 2 eV was found to diminish whereas that at 533.2 ± 0.1 eV was found to increase with immersion time. This augments the fact that oxygen in the form of oxide became reduced

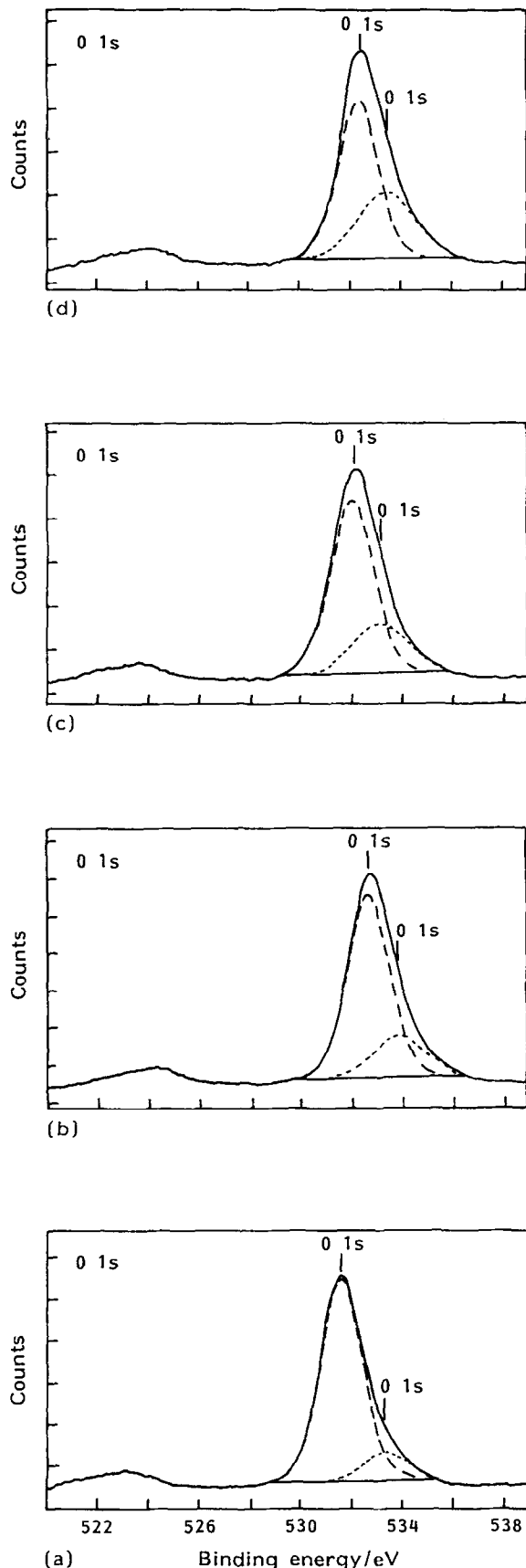


Figure 6 X-ray photoelectron spectra of S1 sample: **a**, before immersion and after immersion of **b**, 2 d, **c**, 6 d and **d** 12 d. The O 1s peak of the oxide form decreased (dashed line) whereas bound water increased with immersion time (dotted line).

whereas the bound water content on the surface increased with respect to immersion time.

CONCLUSIONS

From the point of view of biomedical applications, glass-reinforced HA composites exhibited bioactive behaviour in a simulated physiological solution. The apatite layer formed on the surface most probably contained sodium sulphate which came from the physiological solution. Bound water on the surface increased with respect to immersion time.

ACKNOWLEDGEMENTS

The authors would like to thank Dr Carlos Sá for his technical assistance and helpful discussions about the XPS results.

REFERENCES

- 1 Santos JD, Knowles JC, Hastings GW, Bonfield W. Sintered hydroxyapatite compositions and method for the preparation thereof. *British Patent 9213774.4*, 29 June 1993.
- 2 Santos JD, Reis RL, Knowles JC, Hastings GW, Monteiro FJ. Microstructural characterization of glass-reinforced hydroxyapatite composites. *Biomaterials* 1994; **15**: 5–10.
- 3 Santos JD, Knowles JC, Monteiro FJ, Hastings GW. Development of a glass reinforced hydroxyapatite with enhanced mechanical properties: physical properties and *in vitro* studies. In: Yamamuro T, Kokubo T, Nakamura T, eds. *Bioceramics 5*. Kyoto, Japan: Kokunshi Kankokai, 1992: 35–41.
- 4 Kitsugi T, Yamamuro T, Kokubo T, Ono M. Mechanical properties of sintered hydroxyapatite for prosthetic applications. *J Mater Sci* 1981; **16**: 809–812.
- 5 Best S, Bonfield W, Doyle C. A study into the preparation of dense hydroxyapatite ceramics using powders of different morphologies. In: Oonishi H, Aoki H, Sawai K, eds. *Bioceramics 1*. Kyoto, Japan: Ishiyaku EuroAmerica Inc., 1989: 68–73.
- 6 Aoki H. In: Oonishi H, Aoki H, Sawai K, eds. *Science and Medical Applications of Hydroxyapatite*. JAAS, 1981.
- 7 Burnie J, Gilchrist T. Controlled release glass (CRG)—a new biomaterial. In: Vincenzini P, ed. *Ceramics in Surgery*. Amsterdam: Elsevier, 1983: 169–176.
- 8 Kitsugi T, Yamamuro T, Kokubo T, Ono M. Bonding behavior between two bioactive ceramics *in vivo*. *J Biomed Mater Res* 1987; **21**: 1109–1123.
- 9 Ono K, Yamamuro T, Nakamura T, Kokubo T. Mechanical properties of bone after implantation of apatite-wollastonite containing glass ceramic-fibrin mixture. *J Biomed Mater Res* 1990; **24**: 47–63.
- 10 Ono K, Yamamuro T, Nakamura T, Kokubo T. Surface-active biomaterials. *Science* 1984; **226**: 630–636.
- 11 Hyakuna K, Yamamuro T, Kotoura Y, Kokubo T, Kushitani H, Ono M. Surface reactions of calcium phosphate ceramics to various solutions. *J Biomed Mater Res* 1990; **24**: 471–488.
- 12 Kokubo T, Kushitani H, Kitsugi T, Yamamuro T. Solutions able to reproduce *in vivo* surface-structure changes in bioactive glass-ceramics. *J Biomed Mater Res* 1990; **24**: 721–734.

- 13 Kokubo T, Kushitani H, Kitsugi T, Ohtsuki C, Yamamuro T. Effects of ions dissolved from bioactive glass-ceramic on surface apatite formation. *J Mater Sci Mater Med* 1993; **4**: 1–4.
- 14 Santos JD, Knowles JC, Reis RL, Monteiro FJ, Hastings GW. Liquid phase sintering of hydroxyapatite by phosphate and silicate glass additions. Structure and properties of the composites. *J Mater Sci* (in press).
- 15 Kodri M, Miki K, Waite D, Okabe T. *In vitro* stability of biphasic calcium phosphate ceramics. *Biomaterials* 1993; **14**: 299–304.
- 16 Bruijn J. Calcium phosphate biomaterials: bone bonding and biodegradation properties. PhD thesis. University of Leiden: 1993.
- 17 Landis WJ, Martin JR. X-ray photoelectron-spectroscopy applied to gold-decorated mineral standards of biological interest. *J Vac Sci Technol* 1984; **A2**: 1108–1111.
- 18 Turner NH, Murday JS, Ramaker DE. Quantitative determination of surface composition of sulphur bearing anion mixtures by Auger-electron spectroscopy. *Anal Chem* 1980; **52**: 84–92.
- 19 Wagner CD, Hillery HF, Jansen WT, Passoja DE, Taylor JA. Auger and photoelectron line energy relationship in aluminium–oxygen and silicon–oxygen compounds. *J Vac Sci Technol* 1982; **21**: 933–944.
- 20 Wagner CD, Zatko DA, Raymond RH. Use of the oxygen KLL Auger lines in identification of surface chemical states of electron spectroscopy for chemical analysis. *Anal Chem* 1980; **52**: 1445–1451.