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Treatments to induce the nucleation and growth of apatite-like layers on polymeric surfaces and foams

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In this work, a bioactive glass is used as a percusor of calcium-phosphate (Ca-P) film deposition onto several polymer-based materials. Both bioinert (high molecular weight polyethylene, HMWPE), and biodegradable (corn starch-based blends, SEVA-C) polymers, unreinforced or reinforced with hydroxylapatite (HA), were coated by the very simple proposed route. Also polyurethane (PU) foams, with an open-cell structure, were mineralized by the proposed method. In fact, it was possible to induce the growth of the Ca-P films not only at the surface, but also in the bulk of the PU foam. These cellular materials are intended for cancellous bone replacement applications. The morphology of the formed films was strongly dependent on the used substrate, its polar character, and on the presence of HA in its composition, as observed by SEM. Nevertheless, a well defined needle like structure was observed in all samples at high magnifications. The Ca:P ratios of the films were between 1.5 and 1.7, i.e. in the range of tricalcium phosphate-hydroxylapatite. Raman spectroscopy and thin-film x-ray diffraction (XRD) evidenced the formation of mostly amorphous calcium-phosphate films. After scraping the coating from the polymer surface and heat-treating the resulting powder at 1000 °C for 1 h, HA and β-tricalcium phosphate (TCP) typical peaks were found on XRD patterns.

1. Introduction

Mineralized tissues such as bones, tooth and shells have attracted considerable interest as natural anisotropic composite structures with adequate mechanical properties [1]. The main characteristics of the route by which these hard tissues are formed is that the organic matrix is laid down first and the inorganic reinforcing phase grows within this organic matrix. Oyster shells, coral, ivory, pearls, sea urchin spines, cuttlefish bone, are just a few of the vast variety of biomineralized materials engineered by living creatures [2–4]. Many of these biological structural materials consist of inorganic minerals combined with organic polymers. The study of these structures has generated a growing awareness in materials science that the adaptation of biological processes may lead to significant advances in the controlled fabrication of superior smart-materials [3]. To date, neither the elegance of the biomineral assembly mechanisms, nor the intricate composite microarchitectures, have been duplicated by nonbiological methods. However, substantial progress has been made in the understanding of how biomineralization occurs [5].

Bone formation is a particularly complex process, on which hydroxylapatite precipitation seems to be associated, initially, with matrix vesicles and subsequently with collagen fibres [1, 3]. Very small crystals are formed, parallel to one another, under the influence of the collagen fibrils structure, as a result of the composition of the inorganic salts present in the body fluids. Bone is synthesized as a complex composite and the organization and interfacial chemistry of the components are optimized for functional use. These mineralized tissues are bioceramic–biopolymer composites made by cell-mediated processes. Their production involves an exquisite level of control of both the spatial regulation of the nucleation and growth of mineral and the development of special microarchitectures during the formation of these structures [5].

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In the last few years it has become well established that the essential requirement for an artificial material to exhibit a bone-bonding behaviour is the formation on its surface of a calcium phosphate similar to bone apatite [6, 7]. In fact, the presence of an apatite-like layer on the surface of an orthopaedic biomaterial is considered as a positive biological response from the host tissues. So, it is expected that any material coated with such a film may show a bioactive behaviour when implanted. A typical example for a material that can form this type of layer on its surface and bond to living tissues is Bioglass® [8, 9]. Several works [10–12] have shown that hydrated silica, and particularly silanol groups, play a key-role on this mechanism, it being possible to observe the same type of behaviour on other silica containing bioceramics, and silica gel itself [10–12].

Furthermore, there is a need for the development of a material with a rapid rate of interfacial bonding and the biomechanical properties equivalent to those of bone [8]. This fact reflects the increasing importance of developing methods to assure effective bone-to-implant fixation of bone replacement materials. It is known that clinical success requires the simultaneous achievement of a stable interface with connective tissue and a match of the mechanical behaviour of the implant with the tissue to be replaced. Metals and ceramics are too stiff when compared with cortical bone and induce stress-shielding. Polymers and especially polymer matrix composites, may be engineered to present mechanical properties matching those of bone. However, only PEO/PBT block co-polymers (Polyactive®) have been claimed so far to be bone-bonding polymers [13].

Recently, it has been shown that by using special processing routes, it was possible to produce starch-based polymers reinforced with hydroxylapatite with a mechanical performance matching that of bone [14–16]. These polymers also present a very interesting degradation behaviour [16, 17] that makes them good candidates for being used on temporary orthopaedic applications. Polyethylene-based composites can also exhibit bone-analogue properties [18, 19]. However, the very high amounts of HA required to induce a bone-bonding behaviour to these types of composite lead to the embrittlement of the material and limit its application in load-bearing devices. This weakness may be overcome by developing pre-implantation routes for producing Ca–P films on the materials surface aimed at generating an *in vivo* bone-bonding behaviour.

Several works [20–27] have been tried, with success, to learn from the natural processes and to develop synthetic *in vitro* methodologies, whereby a mineral phase is deposited in a particular polymer matrix or on the surface of a polymer substrate. In all the works, a very important problem is to generate locally, at a particular surface, chemical conditions for inducing the mineral phase precipitation. The coatings have been produced on a wide variety of materials ranging from metals [20, 23], several types of nonbiodegradable polymers [21, 23–25], bioinert ceramics [22, 23], to natural materials such as bamboo [26, 27]. Polyurethane materials, which are known to be calcified *in vivo* [28], are usually not applied in orthopaedics, although their open-cell foams may constitute ideal cancellous bone-replacement materials, if pre-mineralized before implantation. Biodegradable polymers have not been used as substrates for nucleating these biomimetic Ca–P films, owing to the fact that their degradation induces more complexity to the phenomena occurring at the polymer/solution interface.

This work reports a very simple, new, methodology, adapted from those proposed by Kokubo and coworkers [21–25], that uses a bioactive glass [29, 30] as a precursor of the nucleation and grow of Ca–P films on several material surfaces (compact polymers) and open-cells (foams). The basis for this procedure is consideration that polymer based materials may demand pre-implantation coating to stabilize the implant *in-vivo*. Bioinert (HMWPE), and biodegradable starch-based (SEVA-C) polymers were coated by the proposed route. Foamed polymers (PU) with an open-cell structure were also mineralized with the aim of being used as cancellous bone-replacement materials.

2. Materials and methods

The studied materials were high molecular weight polyethylene (HMWPE, Hostalen GM9255F, Hoescht, Germany), a biodegradable starch/ethylene–vinyl alcohol blend (SEVA-C, Mater-Bi 1128RR, Novamont, Italy) and polyurethane foams (PU,1: 1.25 polyol/isocyanate, produced in our Laboratories). Both HMWPE and SEVA-C and these materials reinforced with hydroxylapatite (HA. Plasma Biotal, UK), in amounts up to 50% by weight, were used as substrates for nucleating the apatite films. The HA was sintered at 1200 °C for 12 h in air. After sintering and crushing in a ball mill, the diameter of the HA particles was in the 3–9 μm (average of 6.5 μm) range. More information on these novel starch-based polymeric blends, and on their potential use as biomaterials, may be found in previous publications [14–17].

HMWPE, SEVA-C and their composites were injection moulded as rectangular section bars, from which 13 mm × 10 mm × 4 mm samples were obtained. These samples were obtained on an injection machine Klockner-Ferromatik Desma FM20. The samples were then carefully polished with a 4 μm diamond paste. PU foams were produced by reaction injection moulding (RIM). Samples 20 mm × 10 mm × 10 mm, in size were cut from the produced blocks. The composition (wt%) of the bioactive glass used to induce the formation of the apatite layers is 30.0 SiO_2–52.75 $3CaO{\cdot}P_2O_5$–17.25 MgO. The glass was produced from p.a. reagent grades in a platinum crucible according to routes published before [29, 30]. Previous work [29,30] has also shown that these glasses exhibit, *in vitro*, a bioactive behaviour being capable of nucleating Ca–P layers on its surfaces. The glass particles used to nucleate the Ca–P had a size between 30 and 50 μm (laser granulometry).

The samples were sterilized with ethylene oxide (EtO) before coating, in order to reproduce the

characteristics of the surface to be implanted. All subsequent handling was carried out in a laminar flow cabinet. The basis for this procedure was the consideration that the coating should act as a pre-implantation treatment, being nucleated as it would be *in vivo*. Samples from each material were etched with a 1 M hydrochloric acid (HCl) solution, for 15 min, according to a procedure proposed by Tanahashi *et al.* [24]. Samples were then rolled on a bed of wet bioglass particles, and immediately immersed, for 7 days at 37 °C and a pH = 7.4, in an acellular simulated body fluid (SBF) solution (composition similar to the human blood plasma) prepared according to the method described by Kokubo and co-workers [21–25]. After this nucleation stage, samples were moved to a 1.5 × SBF solution and remained there for various periods of time (up to 30 days). This solution was renewed every 2 days. Finally, they were cleaned in distilled water and dried in controlled environmental conditions (23 °C, 55% RH).

The coated surfaces were analysed by scanning electron microscopy and energy dispersive spectroscopy (SEM/EDS), thin-film X-ray diffraction (XRD, 1° incidence angle) and Raman spectroscopy. SEM/EDS was used to characterize the morphology of the produced films and to compute the Ca/P ratios. Raman was used to assess the presence of the typical PO_4^{3-} stretching bands and to evaluate the degree of crystallinity of the coatings. XRD was helpful for identifying the crystalline phases present, and to characterize the crystalline/amorphous nature of the produced films. XRD spectra were both acquired for the as-deposited films (thin-film) and for coating powder scraped from the polymer surface heat treated at 1000 °C for 1 h. In some of the XRD analyses, five consecutive scans were carried out for $2\theta = 30–35°$ in order to unambiguously identify the strongest apatite peaks.

3. Results and discussion

The formation of a continuous and adherent Ca–P layer on the surface of all the studied materials was observed by SEM/EDS. Figs 1 and 2 show the typical films formed respectively on HMWPE and SEVA-C substrates. In both cases, these films look extremely compact when observed at low magnifications, but evidenced a finer structure at higher magnifications, where needle-like crystals are agglomerated to produce a so-called *cauliflower* morphology. As discussed below, the observed morphologies are clearly different for HMWPE-based and SEVA-C-based substrates. To isolate any effect of surface roughness, claimed by some authors [21] to be related to a mechanical interlocking of the deposit to the substrate that would, in turn, be partially responsible for film formation and adhesion, all materials in this study were carefully polished down to 4 μm with diamond paste. Differences exhibited by the two types of materials would then depend mainly on the nature of the substrate, hence, on its surface chemistry.

The non-polar hydrophobic structure of HMWPE tends to create an epitaxial non-matching with

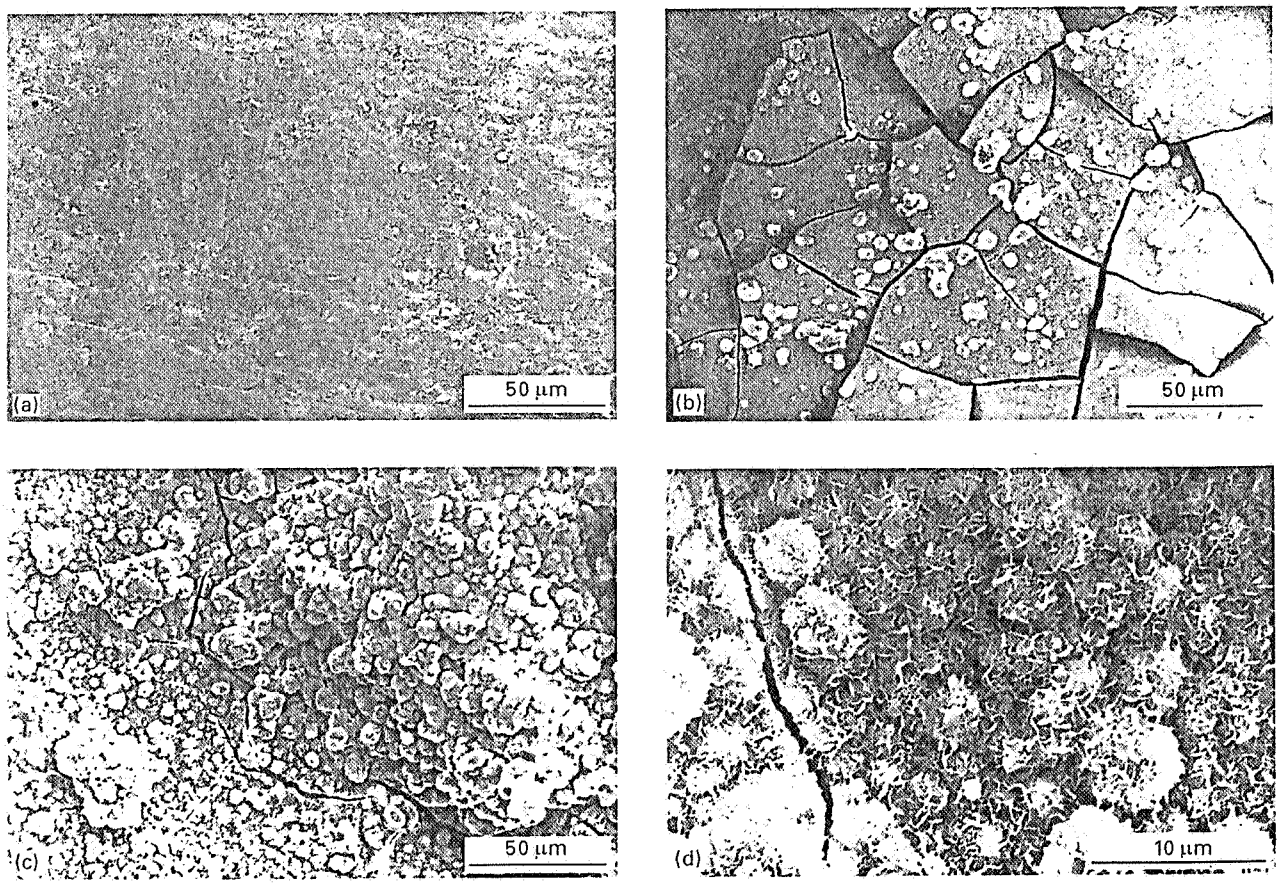

Figure 1 (a) HMWPE matrix, uncoated (as processed), and Ca–P coatings obtained on (b) HMWPE, (c) HMWPE + 30% HA composite, (d) magnification showing a detail of the structure presented in (c).

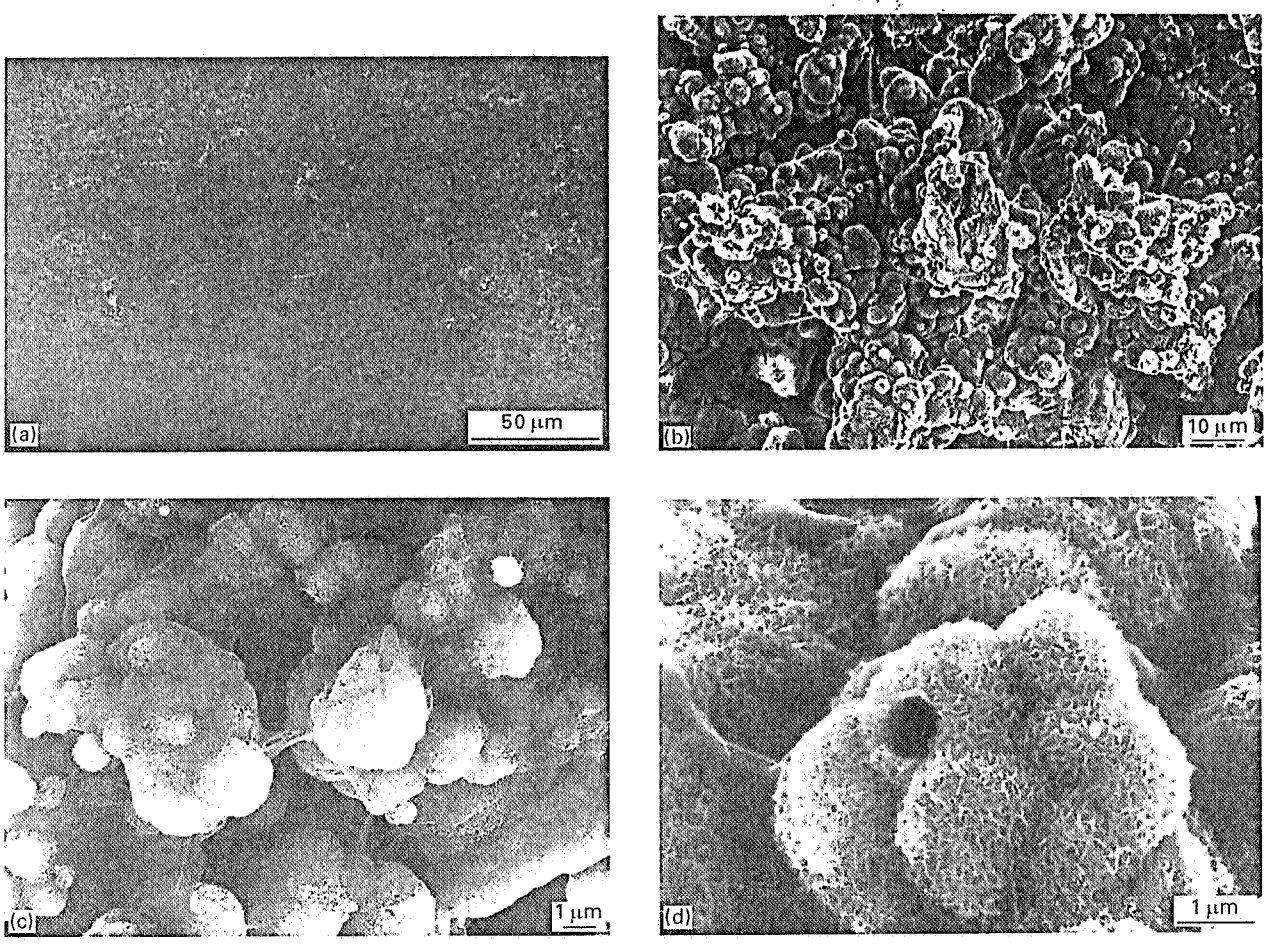

Figure 2 (a) SEVA-C matrix, uncoated (as-processed), and Ca–P coatings obtained on (b) SEVA-C, (c) a detail of the structure of the coating, (d) needle-like appearance of the Ca-P films.

hydrated apatite leading to the formation of a layer that tends to break into several very compact agglomerates of Ca–P globules (Fig. 1b). The fracture of these films is the result of the stress-field imposed by the adjustment of the Ca–P agglomerates network, grown from different nuclei, upon the interaction of the respective fronts. The film formed on polymer filled with HA (Fig. 1c) presents a finer microstructure, with smaller Ca-P globules, presumably because of the presence of surface HA particles that act as additional nuclei and anchoring points for the film. At higher magnifications (Fig. 1d) the needle-like structure may be clearly observed.

It should also be noted that in non-reinforced samples of both HMWPE and SEVA-C without the 7-day incubation treatment no film could be observed for HA amounts lower than 40 wt%, which confirms the reported [21–24] influence of the incubation stage. For lower HA amounts the reinforcing particles may act as additional nuclei, as described, but they cannot nucleate the coating by themselves.

The coatings formed on SEVA-C substrate, a polar and quite hydrophilic substrate, exhibit a very distinct morphology (Fig. 2b). In this case the coatings do not fracture and they seem to be much more adherent to the substrate. In this case the presence of OH groups on the substrate seems to facilitate the connection with the apatite layer. Similar results were obtained by other authors [21] studying polyvinyl alcohol (PVA) polymers. The water-uptake capability of PVA generated a higher adhesive strength of the Ca–P when compared with other polymeric substrates. In fact, it seems that a fairly strong bond could be formed between the polar groups of the polymer and calcium ions of the apatite layer. It has been shown by Li *et al.* [10,11] and Cho *et al.* [12] that heterogeneous nucleation of apatite can be induced from metastable solutions, including physiological solutions on those specific superficial sites, where there are OH-containing groups. These observations may explain the better results obtained for SEVA-C based materials. Furthermore, the Ca-P needles are much smaller (Fig. 2c and d) reinforcing the idea that the substrate chemistry has a deep effect over the film morphology, which is again *cauliflower*-like.

Fig. 3 presents an example of film thickness evolution withtime for a HMWPE substrate. The relationships between immersion time, solution temperature and concentration, coating thickness, film adhesion, and chemistry of the substrate is being investigated in more detail and will be published in future works. The film's growth rates obtained with the proposed method are, at 37 °C with the solution being renewed each 2 days, around 0.3 $\mu m\, d^{-1}$ for HMWPE and 1.3 $\mu m\, d^{-1}$ for SEVA-C. Fig. 3c shows an aspect of a film layer that was peeled off from the substrate with an adhesive tape. It may be seen that the lower part of the film (that we are looking at) was adherent to the

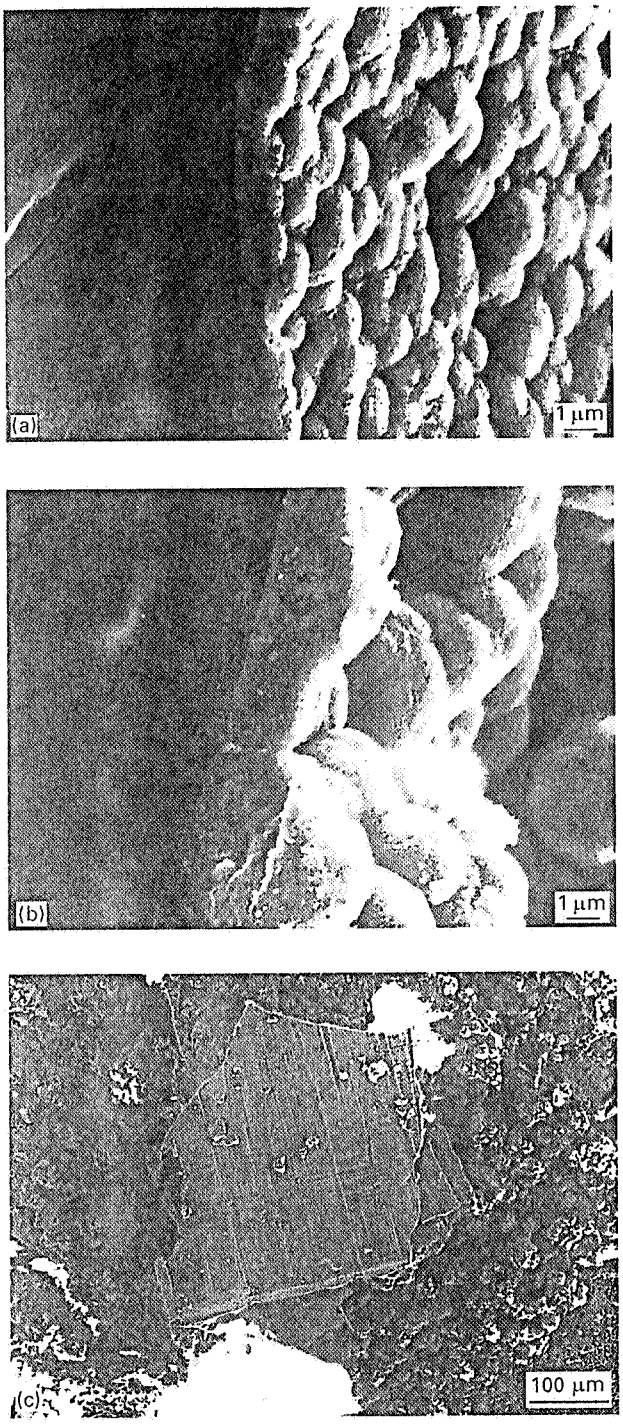

Figure 3 An example of the coating's thickness evolution in HMWPE substrates (a) after 7 days immersion in 1.5X SBF, (b) after 14 days immersion in 1.5X SBF. (c) The topography of a fragment of film peeled off from the surface evincing the good adhesion to substrate and replicating the substrate surface scratches.

substrate. Fig. 3c shows an aspect of a film layer that was peeled off from the substrate with an adhesive tape. It may be seen that the lower part of the film (that we are looking at) was adherent to the substrate. It may be seen that the film wetted the substrate in such a way that scratches were replicated.

Preliminary data, originating from mechanical tests, also indicate that the coatings obtained on SEVA-C substrates exhibit a higher adhesive strength, which is in accordance with the morphological observations, and with data published by Kokubo's group for other hydrophilic polymers [21]. Furthermore, the HCl treatment proved to have a more pronounced effect over polar structures (such as that of SEVA-C) that can be hydrolysed. The film morphology is not significantly changed but its adhesion to the substrate is increased. This result is in accordance with those obtained by Tanahashi [24], who found an increase in adhesive strength for adequate treatments with HCl in polar polymers and detected no consequences of the treatment for non-polar polymers such as polyethylene. The etching effect is described as an oxidation of C containing superficial groups to carbonyls (as concluded from XPS analysis), providing more favourable sites for silicate ions to bond to the substrate and, therefore, to stimulate formation of Ca-P nuclei. The effect of polar groups, including hydroxyl and carbonyl, created by glow-discharge treatments, was also considered as being determinant to the adhesive strength of biomimetic coatings produced on several polymeric substrates [25]. Other works [20] have shown the importance of surface functionalization, for instance hydroxylation, on the *in vitro* induction of biomineralization on several substrates.

In this work we have also tried, with success, to mineralize open-cell PU foams. Fig. 4 presents the typical aspect of a PU foam before (Fig. 4a) and after (Fig. 4b–d) the pre-implantation treatment. It may be observed that it was possible not only to nucleate and grow a Ca–P film at the surface of the PU foam, but also to induce its growth into the bulk of the foam (see Fig. 4b). As expected, the Ca–P concentration is higher at the surface (lower region of Fig. 4b) but the internal cells are also coated with a Ca–P film (Fig. 4c). In the bulk of the foam (Fig. 4d) there are some cell walls that after 14 days immersion are not fully covered with a Ca–P layer. Again, the polar structure of PU seems to increase the adhesion of the film to the polymer cell walls. However, in this case, this assumption corresponds only to a speculation from SEM observations, as there are, as yet, no data available for characterizing the attained adhesion. This biomimetic mineralization of open-cell foams, which to our knowledge has not been reported before, may be very useful in the development of materials for cancellous bone replacement. New starch-based foams will also be proposed for the development of biodegradable replacement materials for repairing bone defects.

In the films nucleated on HMWPE and SEVA-C, silicon and magnesium were residual or absent, as testified by the EDS spectra (Fig. 5a), indicating that they were dissolved or released to the solution after creating a silica gel [20–27] that was responsible for nucleating the films. These elements, especially magnesium, could instead, be incorporated in the apatite structure and replace calcium. On the PU foams (Fig. 5b), some sodium and chlorine, from the SBF solution, were detected. Magnesium was also detected, but silicon was again almost fully dissolved, confirming the mechanism based on the hydration, formation of silanol groups and consequent nucleation of the Ca–P particles. The mechanism for the formation of these films is, according to Kokubo and co-workers

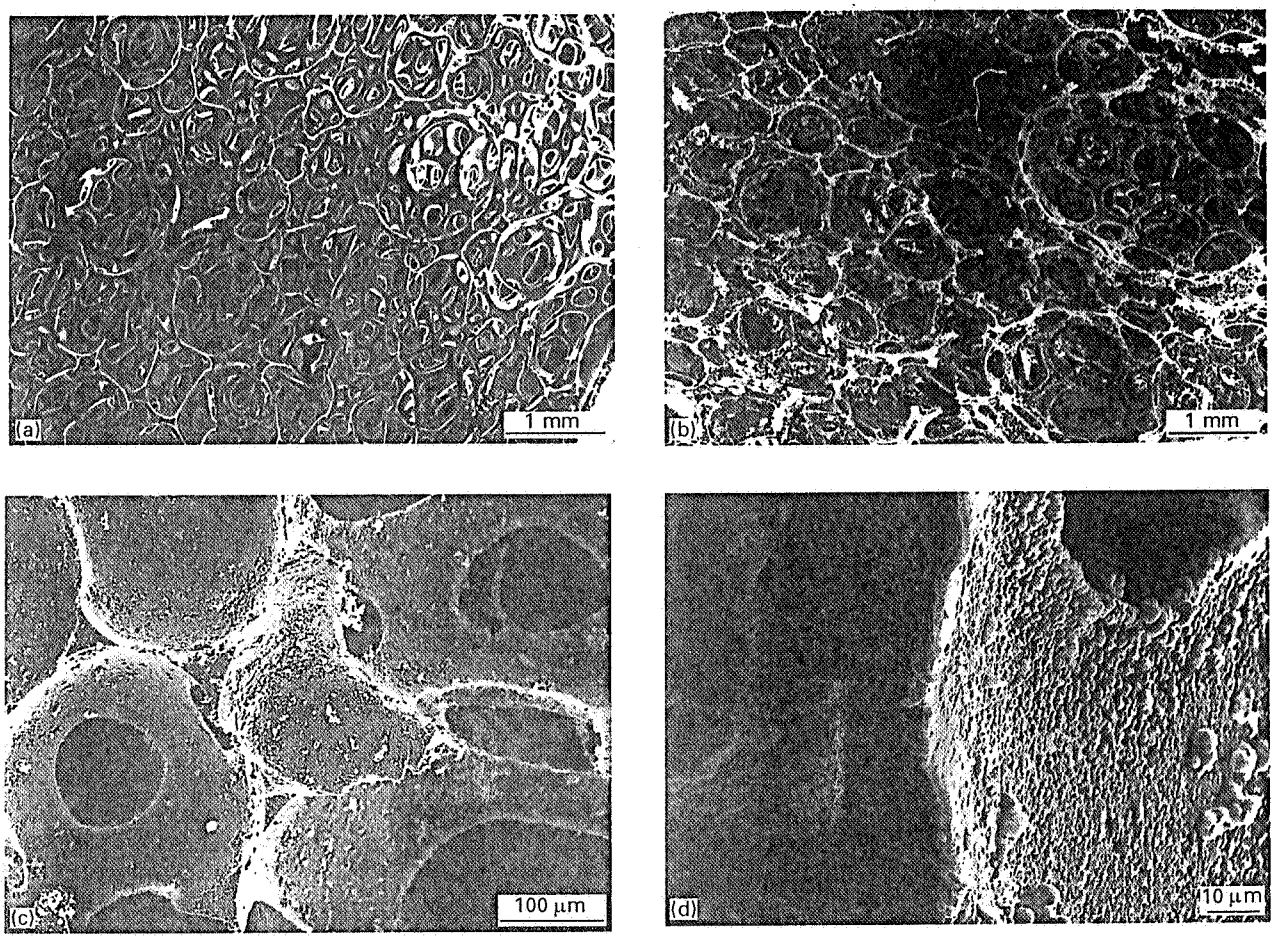

Figure 4 PU foams that were calcified using the method proposed in this work. (a) Reference as-produced (untreated) PU foam, (b) calcified PU foam (after 14 days in 1.5XSBF) surface (lower region) and bulk (upper region), (c) detail showing the formation of a Ca–P film on the walls of the PU cells, (d) higher magnification evincing the globular structure of the Ca–P particles nucleated on the PU cell walls.

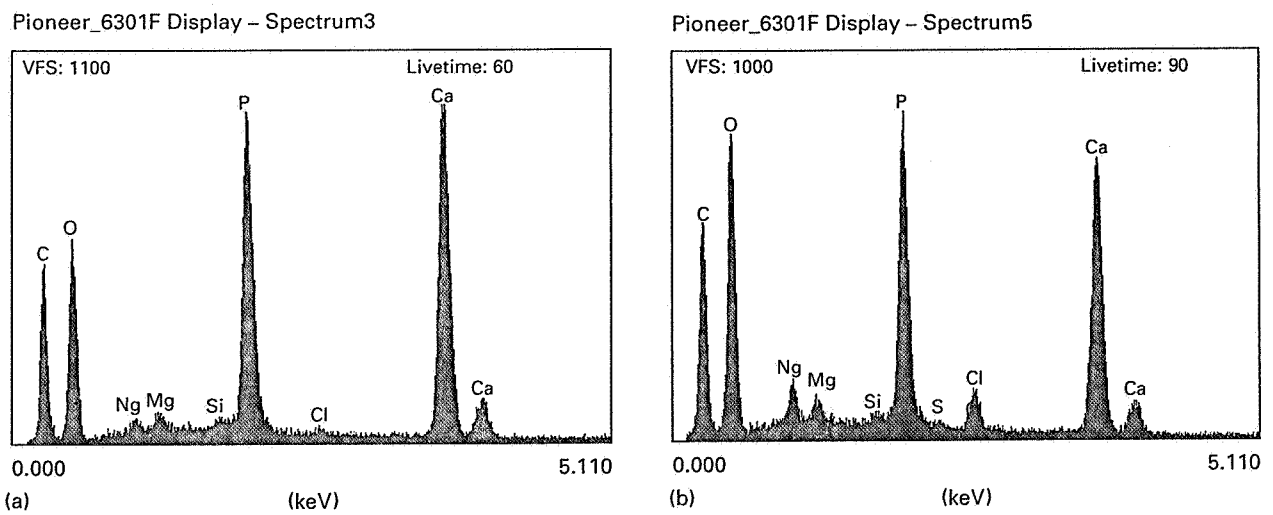

Figure 5 EDS spectra of (a) film deposited on HMWPE after 14 days immersion, (b) a cross section through the bulk of treated (30 days in 1.5XSBF) PU foam showing calcium and phosphorous and also some residual NaCl from the SBF. In both cases, only traces of silicon and magnesium from the glass precursor were found, indicating that the glass was dissolved or released and act as a Ca–P nucleator.

[21–25], as follows. Calcium ions are dissolved from the materials and increase the ionic activity product of the apatite in the surrounding fluid. Then the hydrated silica (silanol groups Si–O–OH) formed on their surfaces provides specific favourable sites for the apatite nucleation. As a result, a large number of apatite nuclei are rapidly formed on their surfaces, and subsequently they grow spontaneously by consuming the calcium and phosphate ions from the surrounding fluids, because the body fluid is already supersaturated with respect to the apatite. The local conditions during reactions, occurring at the polymer/glass particles/

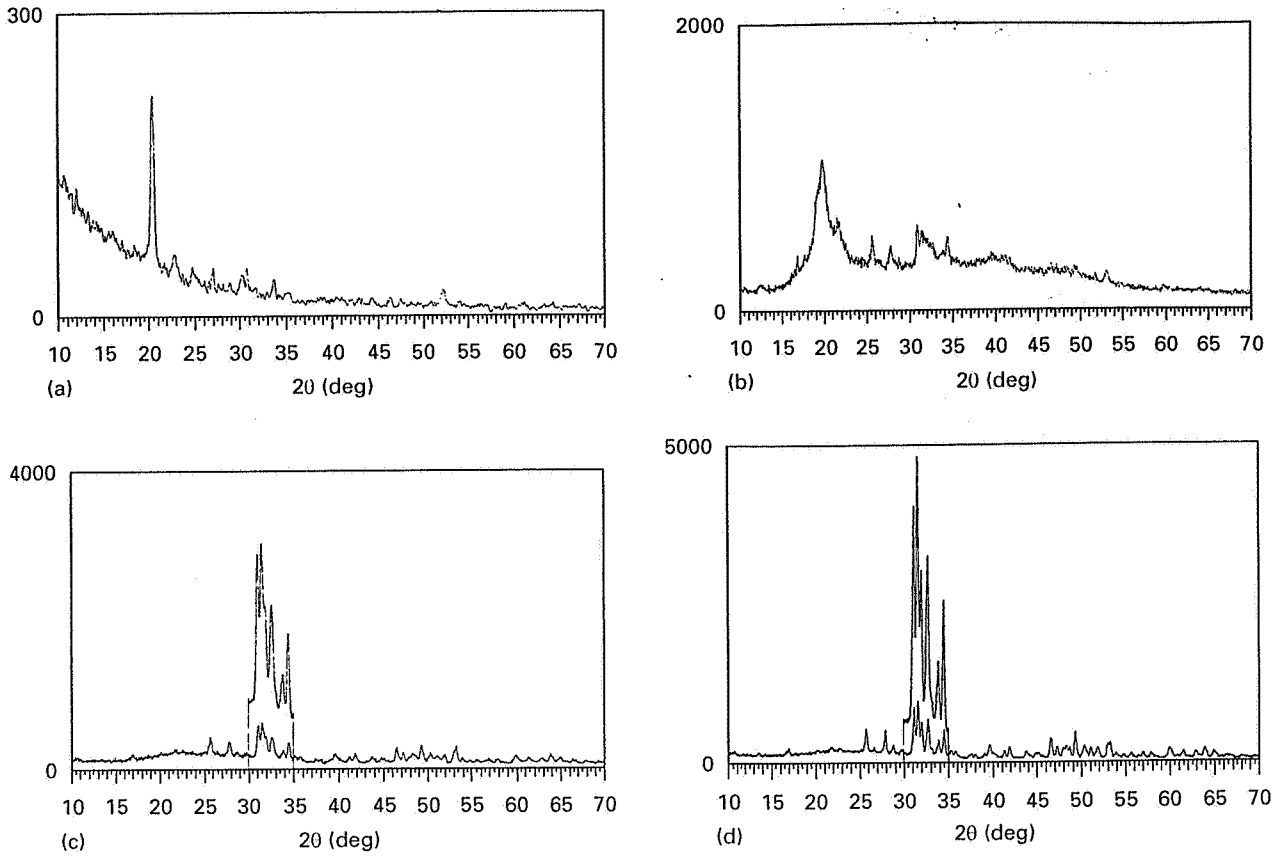

Figure 6 Thin-film XRD spectra of as-deposited films produced on (a) HMWPE, (b) SEVA-C. XRD spectra of powders scraped from (c) HMWPE and (d) SEVA-C surfaces. For all the materials an almost perfect match with the standard HA spectra (see. (e)) was found, as may be observed in (e) for a powder scraped from a substrate HMWPE + 50% HA.

solution interface, may be very distinct in the bulk of the foams, where very particular environmental conditions may turn out. Also the degradation of the starch-based polymers, that changes continuously not only the composition of the solution adjacent to the interface, but also the pH and the polymer surface itself, induces a greater degree of complexity to the reactions. The obtained Ca/P ratios were in the 1.5–1.7 range, i.e. between tricalcium phosphate (TCP) and HA for all materials. These values were determined using well-established sub-routines for EDS semi-quantitative analysis.

The obtained XRD spectra suggest a poorly crystalline structure for the films (Fig. 6a, b). The polymer substrate standard peaks are dominant and the HA typical peaks are not well defined. In order to confirm if the deposits were disordered phosphates, they were scratched from the sample surfaces, and heat treated for 1 h at 1000 C. The XRD analysis of the calcined powders (Fig. 6c, d) evidenced, in both cases, the presence of the JCPDS 9-0432 characteristics peaks of HA and β–tricalcium phosphate, TCP (JCPDS 9-169). Fig. 6e presents the almost perfect match with the standard file for HA. However, some TCP peaks could also be observed. As it is well known, bone apatite also presents a high amorphous content. The films formed are consequently mimicking bone apatite structure. The presence of some TCP may be an advantage, as this ceramic is known to be more reactive than HA and may enhance the coating rate of bone-bonding.

Fig. 7 compares the Raman spectra of uncoated (Fig. 7a) and coated non-reinforced SEVA-C (Fig. 7b). The formation of an amorphous apatite layer on the polymer surface is clear. The typical PO_4^{3-} stretching bands may be observed in Fig. 7b. The much stronger signal, which emanated from the ceramic coatings, makes the polymer peaks almost disappear. It is also important to note that the Si–OH stretching vibration at 980 cm^{-1}, could not be observed, confirming the absence of silicon in the grown film. Raman spectroscopy (Fig. 8) also showed that the films present a spectrum (Fig. 8b) that closely matches that of pure standard HA, though (Fig. 8a) much more amorphous.

The use of these pre-implantation treatments on HA-reinforced starch-based composites, processed by shear-controlled orientation in injection moulding (Scorim) [15, 16] may allow for the development of

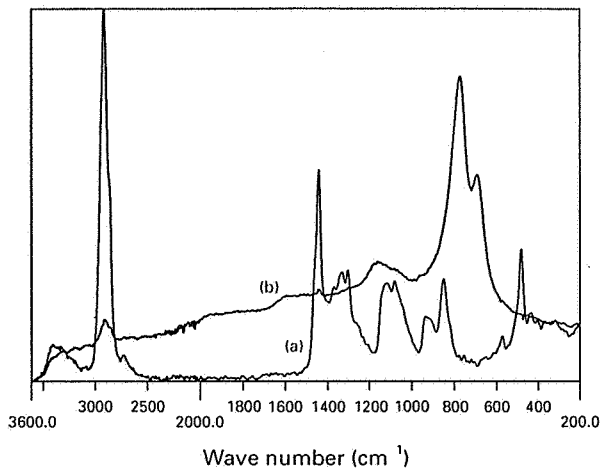

Figure 7 Raman spectra of (a) uncoated SEVA-C, (b) coated SEVA-C, presenting the typical PO_4^{3-} stretching bands.

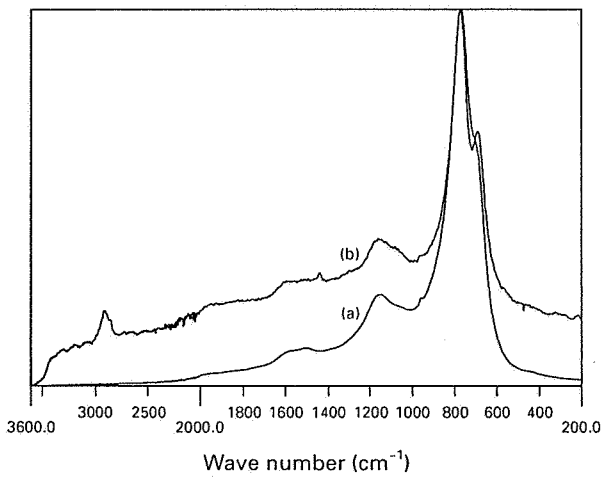

Figure 8 Raman spectra of (a) standard pure crystalline HA, (b) Ca–P film nucleated and grown on the SEVA-C substrate. The similarity of the spectra is clear, although the coating evinces a much more amorphous structure.

biodegradable bone-bonding polymer-based composites with a mechanical performance matching that of bone. In fact, it is possible to produce, by this route, highly bioactive materials with very distinct mechanical properties for applications as varied as load-bearing and soft-tissue replacement implants. Furthermore, it is possible to coat by the proposed routes, both complex geometries and porous or open-cell materials.

4. Conclusion

A new, more straightforward, methodology was developed to grow Ca–P layers on polymeric surfaces and foams. It was possible not only to coat bioinert materials, such as HMWPE, but also biodegradable starch-based blends and PU open-cell foams. The production of biomimetic films, prior to implantation, on biodegradable polymers, may allow for the development of bone-bonding, bioabsorbable implants and fixation devices. Furthermore, the elaboration of ways of pre-mineralizing open-cell foams may allow the introduction of novel bone-repair and bone-filler materials with tissue-tailored properties. The reported process may be very useful as a pre-implantation treatment of cortical and cancellous bone-replacement materials.

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