JOURNAL OF MATERIALS SCIENCE: MATERIALS IN MEDICINE 4 (1993) 58–65

Evaluation of hydroxylapatite/poly(L-lactide) composites: physico-chemical properties

C. C. P. M. VERHEYEN *‡, C. P. A. T. KLEIN *§, J. M. A. DE BLIECK-HOGERVORST §, J. G. C. WOLKE *, C. A. VAN BLITTERSWIJN *¶, K. DE GROOT *

* *Biomaterials Research Group, Department of Biomaterials, School of Medicine, University of Leiden, The Netherlands*

‡ *Biomaterials Research Group, Division of Orthopaedic Surgery, School of Medicine, Leiden, The Netherlands*

§ *ACTA, Oral Implantology, Free University, Amsterdam, The Netherlands*

¶ *Biomaterials Research Group, Laboratory for Otobiology and Biocompatibility, School of Medicine, Leiden, The Netherlands*

The aim of this *in vitro* study was to examine the physico-chemical behaviour of hydroxylapatite/poly(L-lactide) (HA/PLLA) composites in solution tests. The polymer PLLA, the composites 30 wt% HA/PLLA (C30) and 50 wt% HA/PLLA (C50) and a one-side HA-coated PLLA (HAcP) were evaluated. Rectangular specimens were incubated in various acellular aqueous buffer solutions [citrate, Gomori's and phosphate-buffered saline (PBS)] up to 24 weeks. Data for cumulative release of calcium, phosphate and L-lactate release in solutions containing C30 or C50 showed linear patterns. Release data for solutions containing HAcP combined with scanning micrographs, X-ray microanalysis and X-ray diffraction patterns of the specimens in time showed that the plasma-sprayed HA coating on PLLA dissolves significantly, progressively in the first weeks and almost completely within the tested period of 24 weeks *in vitro*. A precipitate of scaly crystallites (calcium phosphates) was observed at the HA coating–PBS interface. After 24 weeks incubation all materials were still above their initial weight, indicating that swelling still exceeded dissolution. Application of C30, C50 and HAcP as implant materials seems interesting where initial stabilization through bone bonding is needed or where the linear release of constituents is a requirement. HAcP has the advantage that the HA coating acts as a hydrolysis barrier and consequently delays the degradation of PLLA *in vitro*.

1. Introduction

The calcium phosphate hydroxylapatite [$Ca_{10}(PO_4)_6(OH)_2$] has been widely investigated and is of fundamental importance in several areas, including biomaterials. Hydroxylapatite (HA) is the main inorganic (mineral) phase in bone, it participates in the calcification and resorption process *in vivo*. Natural HA crystals are relatively small, growing only up to 10–100 nm. Synthetic HA has well-defined reproducible properties [1] and is chemically and crystallographically closely related to the natural mineral component of bone, although the size and orientation of its crystals might differ. This material provides a bony contact and even bonds with host bone [2]. HA is used mainly as a bone filler [2, 3] or as a coating [4] in reconstructive surgery.

Poly(α-hydroxy acids) are one of the main polymer groups used in biomaterials research. Poly(L-lactide) (PLLA), one of its major representatives, is biodegradable, essentially non-toxic, elicits only a mild inflammatory response and the lactic acid yielded after hydrolysis is a normal intermediate of carbohydrate metabolism without accumulation in vital organs [5].

The combination of a bioactive ceramic (HA) and a bioresorbable polymer (PLLA) is expected to result in a promising composite because of its bone-bonding potentials and ability to resorb. The polymeric part is metabolized and excreted, and the ceramic constituents are assimilated in the body. This composite has possible prospects for application as implant material in restricted-load areas.

In order to examine physico-chemical properties such as solubility, surface reactions, swelling behaviour and degradation rate, *in vitro* studies are essential and are the subject of this paper.

2. Materials

2.1. Composite preparation

HA powder (< 125 μm), supplied by Merck (Darmstadt, FRG), was granulated, sintered, crushed, milled and sieved to < 45 μm particle size. PURAC biochem b.v. (Gorinchem, The Netherlands) prepared the

0957–4530

HA/PLLA composites by mixing HA and L(−)-dilactide before polymerization. The monomer [L(−)-dilactide] to initiator (stannous octoate) ratio, M/I, was set at 600 for all materials.

Three different materials were prepared: PLLA with 0, 30 and 50 wt % HA. All materials were delivered as cylinders. Out of these cylinders rectangular specimens (15 mm × 10 mm × 3 mm) were machined. Another material was prepared by plasma-spraying one side of the unfilled PLLA specimen with an HA coating of 50 μm with particle size < 125 μm. Therefore, in total four materials were prepared for testing (Table I).

2.2. Buffer solutions

Three different buffer solutions were used (Table II), the pH being set at 7.2: Sφrensen's citrate II [6], 0.1 M citric acid buffer; Gomori's Tris–maleate [6], 0.2 M tris(hydroxymethyl)-aminomethane and 0.2 M maleate; and calcium-free phosphate-buffered saline (PBS) of molarity equal to the classical Dulbecco's PBS [7].

3. Methods

All samples were sterilized in ethylene oxide (2 h at 52 °C, degassed for 72 h). This was done in order to mimic as closely as possible the procedures in future applications. To determine the molecular weight of the materials by viscosimetry, the samples were dissolved in chloroform and filtered to remove HA. Because only materials with same M/I ratios (600) were used, the initial molecular weights were different (Table I), an effect caused by mixing HA into PLLA before polymerization. The as-machined (on a lathe) surface was not altered and the average roughness of the samples (Tallysurf 4 and Surtronic 10) was measured. The average roughnesses (μm) of the PLLA, C30 and C50 specimens were similar, whereas HA-coated PLLA showed higher values. In the evaluation of results this difference has not been taken into account, because we wanted to study and compare the effect of applying an HA-coating on a polymer. The materials used in this experiment with corresponding molecular weights and surface roughnesses are listed in Table I.

In total, 288 specimens (72 of each material) were manufactured for testing and 96 test tubes were used (24 for each material). Three specimens of one of the four materials were placed in each test tube. The mean starting weight of a group of three specimens was 1.68 ± 0.12 g. To each tube 10 ml of one of the three buffer solutions was added. The tubes were immersed in a reciprocal water-bath shaker at 37 ± 1 °C.

Measurements were taken at 0, 1, 2 and 4 days and every week up to 24 weeks. After each of these sequential periods all solutions were completely replaced by 10 ml fresh buffer solution. Buffer solutions were completely replaced after every incubation period when measurements were taken, 27 times in 24 weeks. This was done in order to maintain a constant volume and to imitate, to some extent, the *in vivo* flow model of continuously refreshing extracellular fluids. At this point, 27 times during the experiment, the pH and the Ca, P and lactate concentrations were measured. The Ca concentration in the solution was determined by atomic absorption (Varian SpectrAA-300, 423 nm), the phosphate concentration colorimetrically with an ultraviolet spectrophotometer [8] (Vitatron DCP, 820 nm) and L-lactate concentration by an enzymatic method which employs oxidation of lactate to pyruvate [9], measured by a discrete clinical analyser (ACA IV Du Pont, 340 and 383 nm). All results were corrected for the mean starting weight of the three samples.

At ten time intervals, up to 24 weeks, one group of three specimens of each material was used for the determination of weight (after drying in filter paper to remove surface water) and molecular weight (M_v, viscosimetry) and then discarded.

Scanning electron microscopy (SEM, Philips 525M), X-ray microanalysis (XRM, Voyager, Noran Institutes) and X-ray diffraction (XRD, Philips PW 1050) were performed on specimens of each material in each period.

4. Results

4.1. pH changes

The pH stability of the buffer solutions in relation to the different materials is shown in Fig. 1a–c. The citrate buffer containing one-side HA-coated PLLA (HAcP) specimens showed an initial increase in pH during the first few days of incubation, decreasing slowly to a stable pH after 4 weeks (Fig. 1a). Gomori's buffer maintained a stable pH (6.97 ± 0.17) for all of the materials throughout the entire period of incubation (Fig. 1b). The PBS solution demonstrated a constant pH value for the two composites and a substantial decrease in pH for the solutions containing the PLLA and HAcP specimen (Fig. 1c).

TABLE I The four materials (resulting from an M/I ratio of 600) listed with molecular weights after gas sterilization (M_v), and average surface roughness (R_a) measured on Surtronic 10 and Talysurf 4

Material		M_v	R_a (μm)	
			Surtronic 10	Talysurf 4
Poly(L-lactide)	(PLLA)	250 000	1.4 ± 0.3	1.9 ± 0.8
30 wt % HA/70 wt % PLLA	(C30)	125 000	1.2 ± 0.1	1.4 ± 0.5
50 wt % HA/50 wt % PLLA	(C50)	75 000	1.4 ± 0.4	1.1 ± 0.3
HA-coated PLLA	(HAcP)	200 000	8.0 ± 1.1	8.8 ± 0.8

TABLE II Contents of certain ions in the buffer solutions (in mM)

Buffer	Na^+	Cl^-	K^+	HPO_4^{2-}	Ca^{2+}
Citrate	156.7				
Gomori's	51.0				
PBS	156.2	142.7	4.5	9.9	

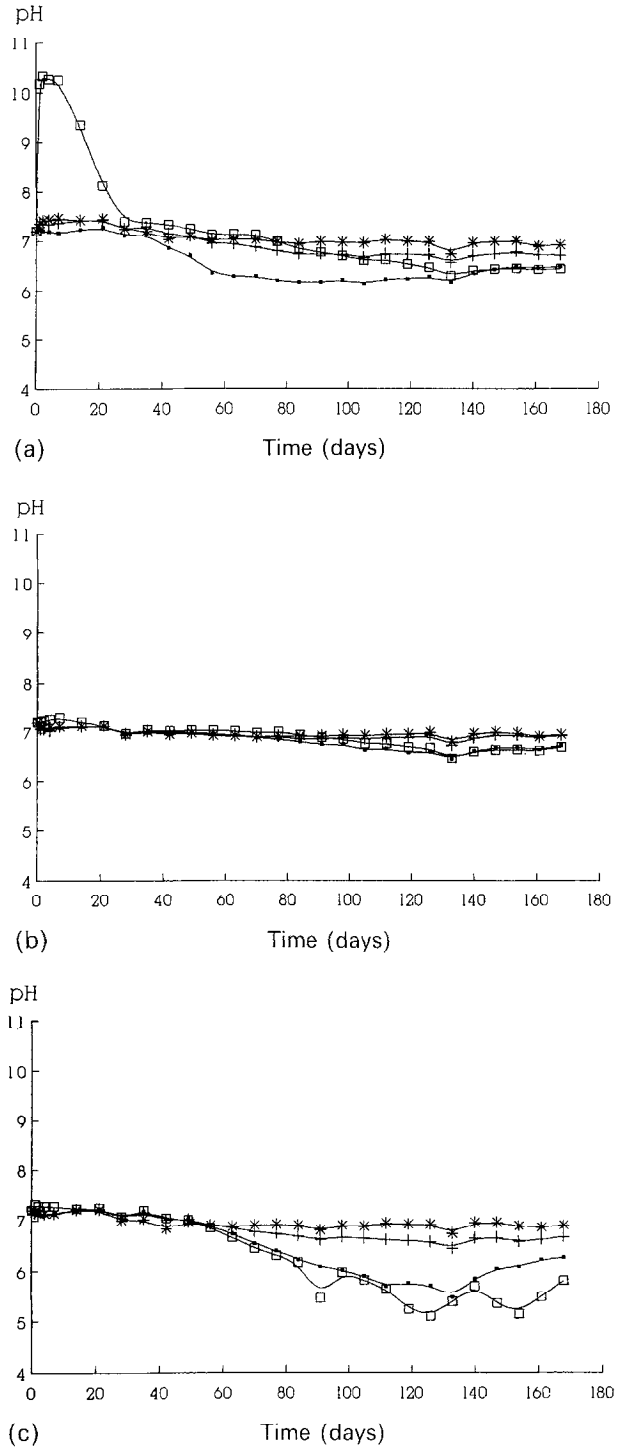


Figure 1 pH stability in time of three buffer solutions containing (■) PLLA, (+) C30, (*) C50 and (□) HAcP specimens: (a) citrate, (b) Gomori's and (c) PBS.

4.2. Calcium and phosphate release

The cumulative release of calcium ions into each of the three buffer solutions is shown in Fig. 2a and b. The patterns of cumulative release of calcium and phosphate ions (p.p.m.) proved to be similar for the same material incubated in citrate or Gomori's buffers, and therefore only the calcium release patterns are presented. They showed a linear increase for the two composites (C50 in Fig. 2a) and, after a peak release in the first week, no release after 7 weeks out of citrate and Gomori's buffers containing HAcP specimens (Fig. 2b).

Phosphate release of the four materials in the PBS solution, on the other hand, could not be measured sufficiently accurately because the released ions were only a fraction of the intrinsic content of phosphate ions in the PBS solution. Calcium release was still measured after 24 weeks incubation in PBS containing HAcP specimens rather than Gomori's and citrate buffers. There was obviously no release of calcium or phosphate ions detected in solutions containing PLLA specimens.

4.3. L-Lactate release

The cumulative release of L-lactate into the solutions gave the same patterns in all buffer solutions (Fig. 3a–c). This release was detectable for C50 almost from the beginning and for HAcP only after 7–10 weeks incubation. The figure also indicates that, after about 14 weeks, the curve for HAcP was almost parallel to that for PLLA.

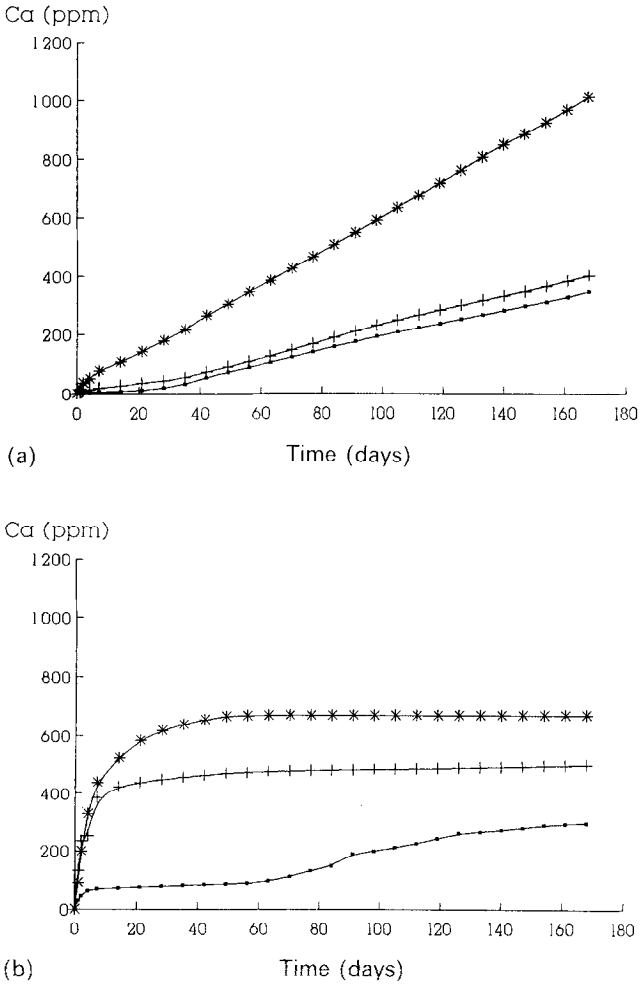
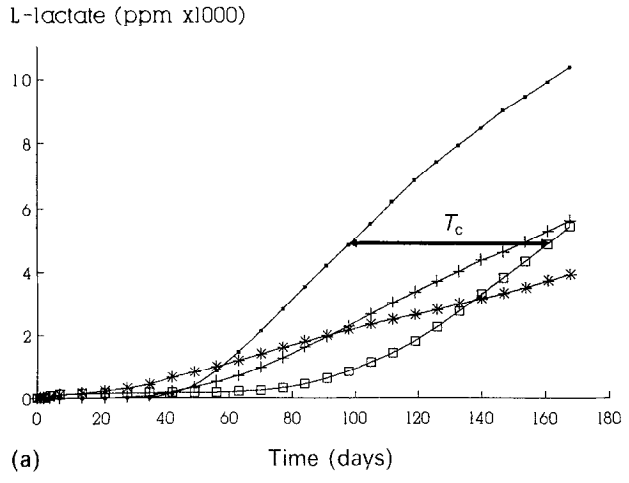
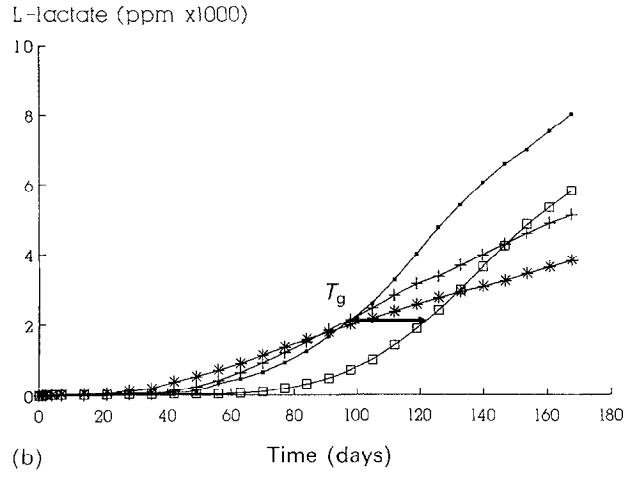


Figure 2 Cumulative release of calcium ions in time from (a) C50 and (b) HAcP specimens immersed in (*) citrate, (+) Gomori's and (■) PBS buffer solutions.





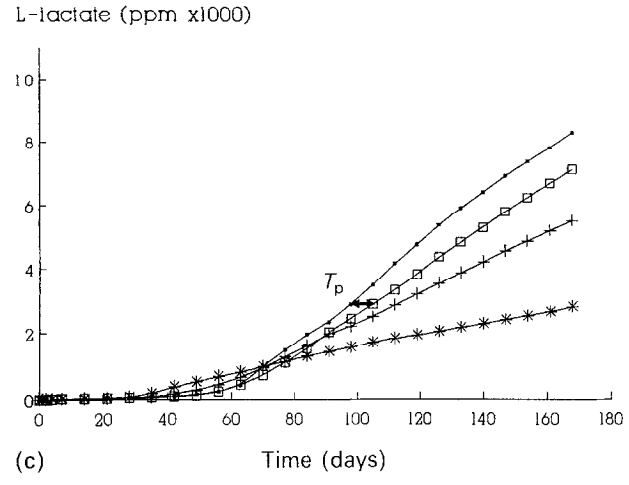


Figure 3 Cumulative release of L-lactate in time from (■) PLLA, (+) C30, (*) C50 and (□) HAcP specimens immersed in (a) citrate, (b) Gomori's and (c) PBS buffer solutions. *T*, Indication for dissolution rate of the HA coating.

4.4. Percentage of initial weight

After 24 weeks immersion in the buffer solutions, all specimens, after drying in tissue paper, had a higher weight than at the start of the experiment. There seemed to be no significant difference between buffers. The swelling of PLLA, C30 and C50 was maximum after about 9 weeks (2–3%) after which a steady decline in weight occurred (Fig. 4; C50 is exemplary for PLLA and C30). HAcP specimens behaved differently, but after 24 weeks of incubation swelling still increased.

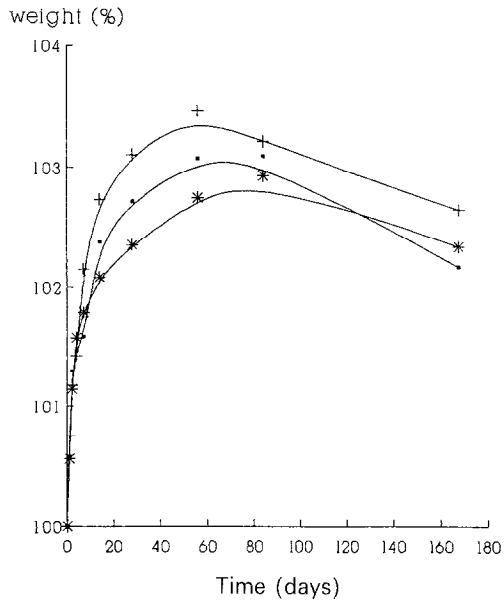


Figure 4 Percentage of initial weight, after drying in tissue paper, of C50 samples during 24 weeks of incubation in (*) citrate, (+) Gomori's and (■) PBS buffers.

4.5. Molecular weight

A fixed M/I ratio resulted in a difference in the initial molecular weights of the materials. The decrease in M_v in all of the buffer solutions in a 24 week period showed identical patterns, so Fig. 5 is exemplary for all of the buffer solutions. A higher initial M_v resulted in a sharper decline of M_v in the first 2–3 weeks. After 24 weeks immersion there was no significant difference in M_v between the four materials (10 000–25 000).

4.6. Scanning micrography and X-ray microanalysis

Compared with the original HA coating (Fig. 6a), after only 1 week incubation scanning micrographs revealed a significant dissolution of the plasma-sprayed HA coating on PLLA in all buffer solutions

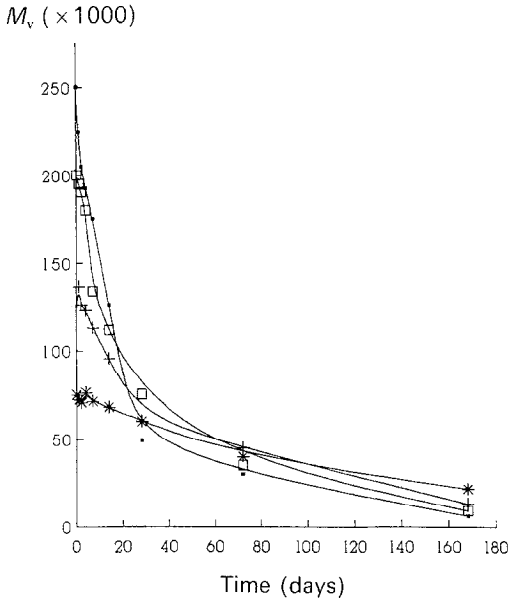
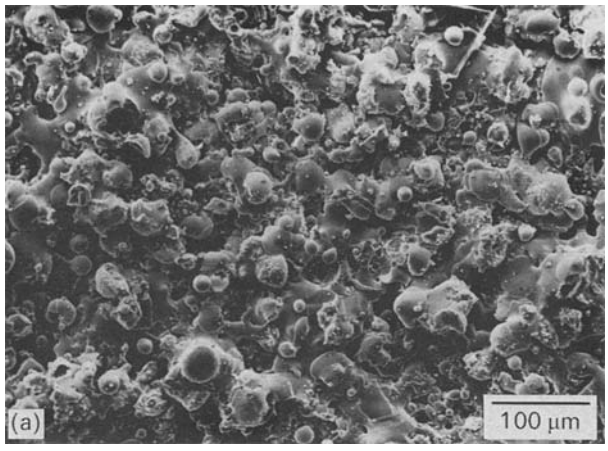
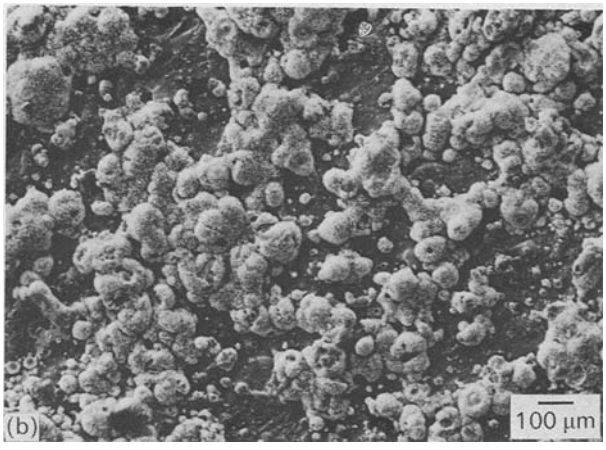
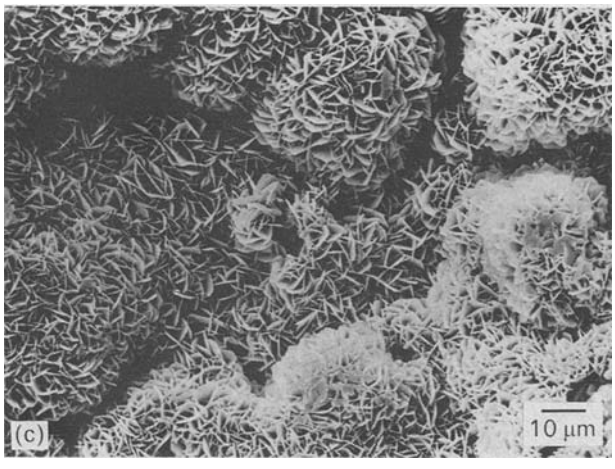


Figure 5 Molecular weight (M_v) distribution in time of (■) PLLA, (+) C30, (*) C50 and (□) HAcP specimens incubated in PBS.







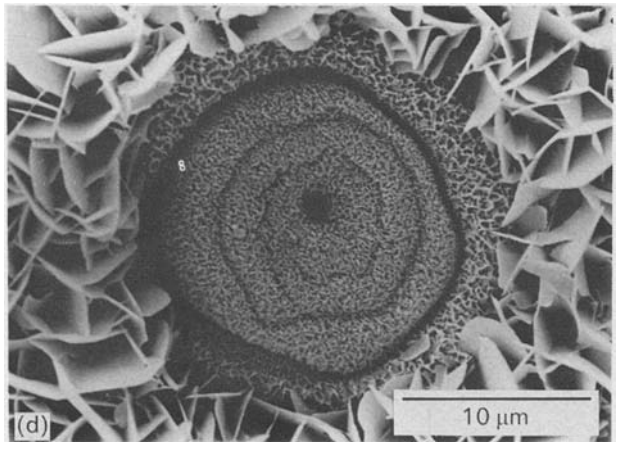
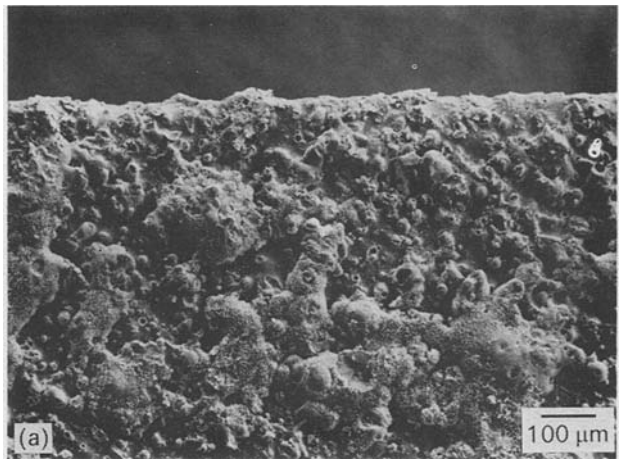
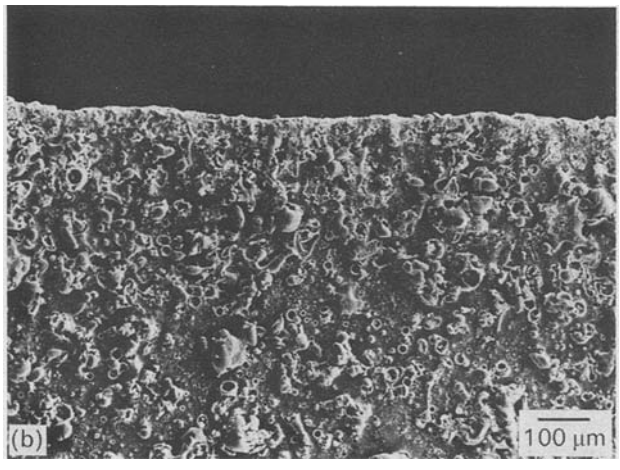


Figure 6 Scanning micrographs of the plasma-sprayed surface of (a) HA-coated PLLA and (b) after 4 days incubation in PBS buffer. (c) Detail of the thin layer formed by calcium phosphate crystallites shown in (c). (d) A detailed view of the area with the rosaceous pattern indicated in (b).

(Fig. 7a–c). In PBS (Fig. 7a) the remains of the coating were covered with a scaly thin cover (up to 10–15 μm). These small crystallites could already be seen after 1 day incubation and covered almost the entire coating after 4 days (Fig. 6b). These platelets measured up to 15 μm in diameter and had a thickness of about 0.5 μm (Fig. 6c). Scattered rosaceous patterns could be observed with very small crystallites regularly arranged (Fig. 6d). After 1 month almost no crystallites were detected. XRM revealed that this precipitation consisted of calcium phosphates. In Gomori's buffer only a few crystallites were observed





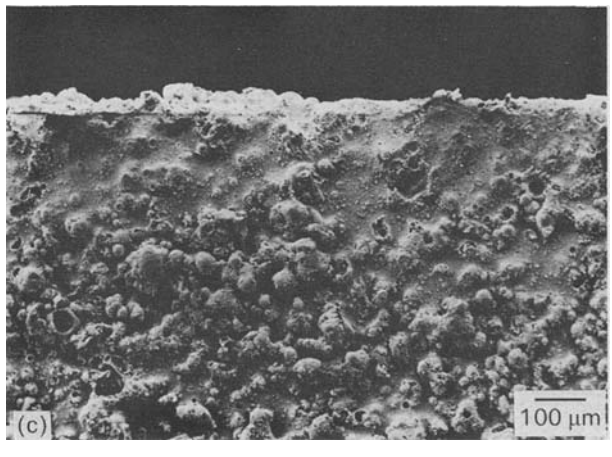


Figure 7 Scanning micrographs of HAcP specimen after 1 week of incubation in (a) PBS, (b) Gomori's and (c) citrate buffer. Note the partially covered HA coating with precipitate (CaP) when incubated in PBS (a) and the sandy aspect of the specimen incubated in citrate (c).

and in citrate buffer none. In citrate buffer a striking aspect was the sandy precipitate partly covering the remains of the coating (Fig. 7c), possibly due to precipitation of calcium citrate complexes. HAcP was the only material that showed this surface reaction.

4.7. X-ray diffraction

XRD patterns were measured for several specimens over time. Comparing the XRD patterns of the HA coating on metal (Fig. 8a, HAc T_0) with HA-coated PLLA at the beginning of the experiment (HAcP T_0) shows the PLLA peaks in the diffraction pattern. After 1 week incubation in PBS the HAcP samples already showed a predominant loss of the characteristic HA pattern, which was progressive after 6 months (Fig. 8b). Even the major HA peak at $2\theta = 31.74°$ (composed of two coincident reflections, viz. 2 1 1 and 1 2 1) could not be detected after 1 week incubation in PBS.

5. Discussion

Citrate ions are believed to play a major part in bone solubilization and remodelling [10]. Citrate forms a number of complexes with calcium ions in solution and enhances the dissolution of HA [11]. By exceeding the buffering capacity of citrate, these complexes have an alkaline effect on the pH in this buffer solution with HA-coated PLLA (Fig. 1a). After about 7 weeks incubation, when no detectable release of calcium or phosphate ions from the HA coating was measured, the pH was stable throughout the remaining time of incubation. The moderate drop in pH after 8 weeks in buffers containing PLLA specimens, especially in PBS, is possibly caused by the hydrolysis of PLLA where H^+-ions come into the solution, exceeding the buffering capacity of PBS (Fig. 1c). Gomori's buffer, contrary to citrate buffer and PBS, is a very strong, pH-stable buffer solution, as the pH pattern for all of the materials indicates (Fig. 1b).

The detectable initial release of calcium ions in PBS containing HAcP specimens in the first weeks was lower than in the other two buffer solutions. The dissolution patterns were similar. Unlike the other buffers, PBS is supersaturated with phosphate ions and this affects strongly the surface reactions at the coating–fluid interface. Phosphate ions which first absorb on to the surface of the coating form, in combination with calcium from the HA coating, a calcium phosphate layer of small crystallites (as shown by XRM). This accounts for the moderate concentration of calcium in the PBS solution (Fig. 2b).

There are interesting parallels between the surface reactions on this HA coating and those on bioactive glass-ceramics, both in supersaturated solutions. In the case of glass-ceramics the formation of a thin calcium phosphate layer by successive deposition of chemical reaction products at the interface with the fluid is essential for the bonding ability with living bone [12]. Because this layer has similar compositional and structural characteristics to the apatite of natural bone [13], it forms a tight bond with it. Therefore, the formation of this "apatite surface" *in vitro* has been said to be indicative of the bone-bonding ability [14].

Considering calcium release patterns in solutions containing HAcP specimens (Fig. 2b), the release of calcium and phosphate ions in citrate buffer solutions was higher because calcium citrate complexes probably prevented precipitation. It seems only that HA coating dissolved slower in the first days of incubation in PBS. Because calcium phosphates precipitations on the coated surface had already occurred in the first days of incubation (Fig. 6b–d), this calcium was not measured by atomic absorption. It is also imaginable that calcium release of the covered coating is blocked. After about 8 weeks the pH dropped substantially in the PBS solution containing HAcP specimens (Fig. 1c). It is possible that hydrolysis of the polymer releases H^+-ions, causing a decrease in the pH which increases the solubility of calcium phosphates, therefore calcium release out of the covered coating increases again giving rise to this biphasic release pattern of calcium in PBS (Fig. 2b). The solubility of the HA coating was not reflected in the calcium release patterns because dissolved HA coating reprecipitated as calcium phosphate or calcium citrate complexes. At the end of the 24 week incubation period the levels of cumulative calcium release were not equal for all of the buffer solutions. An explanation could be that some of the precipitated calcium is "soaked" into the

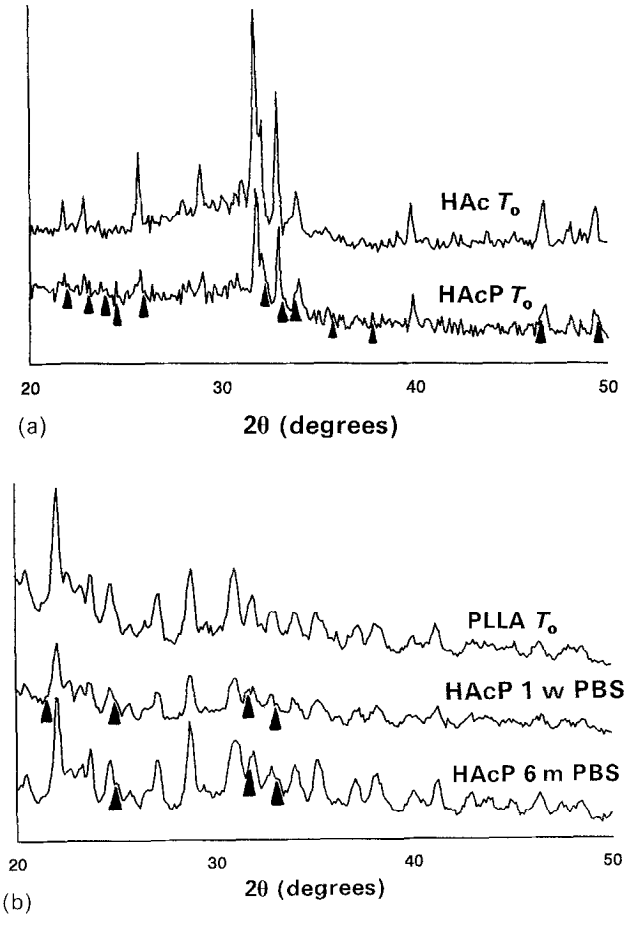


Figure 8 XRD patterns of HA plasma-sprayed on a titanium (HAc T_0) and on a PLLA (HAcP T_0) surface (a) at the beginning of the experiment. (b) The patterns of PLLA at T_0 (PLLA), and HA-coated PLLA after 1 week and 6 months incubation in PBS.

polymer, as indicated by scanning micrographs revealing precipitations in the gaps developing when degradation proceeds and becomes physically apparent. It is also possible that precipitations attach to the wall of the test tube or sedimentate, and therefore its calcium is not measured by atomic absorption.

On scanning micrographs it appeared that the dissolution of the HA coating on PLLA is rather abrupt in nature. The patterns shown in Fig. 7a–c indicate a dissolution of the plasma-sprayed coating by fragments of considerable size. No gradual decrease in the coating thickness was observed. A possible explanation is that the release of the crystalline beads by dissolution of the surrounding amorphous substances of the coating results, in those spots, in the disappearance of the HA coating on the polymer as seen in the scanning micrographs (Fig. 7a–c).

A detectable release of L-lactate on all materials started only after 4–7 weeks. In the first weeks water uptake and consequently swelling took place, then hydrolysis became apparent. In the second half of the incubation period the release of L-lactate in solutions containing PLLA was parallel with the release in solutions containing HAcP specimens. The explanation for this shifted pattern is probably that the physical presence of the coating prevents partially the initial L-lactate release. This release exhibits the same pattern as PLLA at the moment that the coating is largely dissolved (Fig. 2b). An indication for the dissolution rate of the HA coating is reflected by the length of the time interval T (Fig. 3a–c) between the PLLA and HAcP curves showing L-lactate release: slowest in citrate buffer and fastest in PBS. The pattern of L-lactate release from the composite materials was similar in all three buffer solutions; a higher release for C30, which contains more L-lactate than C50.

After 24 weeks incubation all materials were still above their initial weight, which is in contrast with some studies [15] and in partial agreement with others [16, 17]. In this study only surface water was removed to mimic the *in vivo* situation in terms of swelling of the materials. This may account for differences from other studies in which the samples were first dried by heat treatment. On the other hand, it is also hazardous to compare studies that differ in specimen size, preparation and initial molecular weight. After 7 weeks swelling was at its maximum except for HAcP, in which the increase in weight was less because the coating prevents partial swelling on its side of the polymer. Apparently, after about 9 weeks water uptake was maximal in PLLA, C30 and C50 specimens, after which dissolution started (Fig. 4). It seems that dissolution of the hydrolysis products was possible only at molecular weights lower than about 50 000. The decrease in molecular weight reflected the common pattern of steeper decreases when initial molecular weights were higher, known from other studies [18], and indicates that hydrolysis of the chains takes place from the very beginning (Fig. 5).

In summary, hydrolysis became apparent after about 9 weeks incubation for PLLA and HAcP specimens. Hydrolysis caused a drop in pH in PBS because the H^+-concentration exceeded the buffering capacity (Fig. 1c). This increased solubility of calcium phosphates resulted in the biphasic pattern of calcium release in PBS (Fig. 2b). At the same point hydrolysis equalled or even exceeded swelling (Fig. 4) and L-lactate release started (Fig. 3c). XRD patterns support the progressive dissolution pattern of the HA coating (Fig. 8b).

It is remarkable that plasma-spraying only one side of the polymer with HA already serves as a hydrolysis barrier, causing considerable differences in the solubility and degradation of PLLA.

Comparing these results with *in vivo* experiments puts them in another perspective. Implantation studies in goats using subcutaneous specimens of the same dimensions as in this study and plugs placed in the femoral cortex did not show any substantial degradation of the HA coating on PLLA after 3 months [19]. This outcome is not in accordance with this *in vitro* study. Although this study does present indications about the physico-chemical properties of the tested materials, it should be realized that this kind of *in vitro* testing is one of accelerating type and results cannot be simply translated into predictions of *in vivo* behaviour.

The rapid dissolution of a substantial part of the HA coating on the PLLA in all buffer solutions tested *in vitro* may indicate that this polymer is a less suitable substrate for the application of an HA coating when the aim is a long-lasting coating–substrate bond. However, when the assumption that the objective of applying a coating is to ensure initial stabilization at the substrate–bone interface through bone bonding is valid, this model of an HA-coated polymer could be of interest.

6. Conclusions

In conclusion, HA/PLLA composites with 30 or 50 wt % HA (equal to, respectively, 15 and 25 vol %) showed a linear release of calcium and phosphate ions and L-lactate when incubated into different buffers (citrate, Gomori's or Ca-free PBS). Considering the results of the release of calcium and phosphate ions and the evaluation of scanning micrographs and XRD patterns, it can be concluded that the HA coating dissolves substantially within the tested incubation period of 24 weeks.

Scaly crystallite particles (calcium phosphates) were observed to cover the HA coating, especially in the first weeks of incubation, when soaked in (Ca-free) PBS. These crystallites appeared to be similar to those found at glass-ceramic–fluid interfaces, referred to as apatite surfaces.

This study supports the ideas that the *in vitro* solubility of calcium phosphates is as dependent on the surrounding medium as on the materials themselves [2, 20], and that generally *in vitro* studies have problematic predictive value for extrapolation to *in vivo* behaviour. A point of interest for the future is the possible employment of the composites as drug carriers because of the linear releases of the tested constituents.

Acknowledgements

The authors thank I. Kangasniemi for his valuable advice in the preparation of the manuscript and PURAC biochem b.v. for preparation of the materials and analysis of the molecular weights.

The research was founded by a grant-in-aid from the Dutch Ministry of Economics Affairs (STIPT) and the companies PURAC biochem b.v. and HC-Implants.

References

1. M. JARCHO, C. H. BOLEN, M. B. THOMAS, J. BOBICK, J. F. KAY and R. H. DOREMUS, *J. Mater. Sci.* **11** (1976) 2027.
2. *Idem, Clin. Orthop.* **157** (1981) 259.
3. P. K. BAJPAI, in "Biomaterials in reconstructive surgery", edited by L. R. Rubin (Mosby, St Louis, Missouri, 1983) p. 312.
4. R. G. T. GEESINK, K. DE GROOT and C. P. A. T. KLEIN, *J. Bone Joint Surg.* **B70** (1988) 17.
5. R. K. KULKARNI, K. C. PANI, C. NEUMAN and F. LEONARD, *Arch. Surg.* **93** (1982) 839.
6. H. A. SOBER (editor), "Handbook of biochemistry, selected data for molecular biology", 2nd Edn (CRC Press, Boca Raton, Florida, 1970) p. J-234.
7. T. C. DULBECCO and M. VOGT, *J. Exp. Med.* **99** (1954) 167.
8. P. S. CHEN, T. J. TORIBARA and H. WARNER, *J. Biol. Buccale* **12** (1953) 59.
9. E. P. MARBACH and M. H. WEIL, *Clin. Chem.* **13** (1967) 314.
10. W. F. NEUMAN and M. W. NEUMAN, in "The chemical dynamics of bone mineral" (University of Chicago Press, Chicago, 1958).
11. C. Y. C. PAK and E. C. DILLER, *Calcif. Tissue Res.* **4** (1969) 69.
12. T. NAKAMURA, T. YAMAMURO and S. HIGASHI, *J. Biomed. Mater. Res.* **19** (1985) 685.
13. A. ENGSTROM, in "The biochemistry and physiology of bone", edited by G. Bourne (Academic Press, New York, 1972) p. 237.
14. T. KOKUBO, T. HAYASHI, S. SAKKA, T. KITSUGI, T. YAMAMURO, M. TAKAGI and T. SHIBUYA, in "High tech ceramics", edited by P. Vicenzini (Elsevier Science, Amsterdam, 1987) p. 175.
15. K. JAMSHIDI, S.-H. HYON, T. NAKAMURA, Y. IKADA, Y. SHIMIZU and T. TERAMATSU, in "Biological performance of biomaterials", edited by P. Christel, A. Meunier and A. J. C. Lee (Elsevier Science, Amsterdam, 1986) p. 227.
16. A. S. CHAWLA and T. M. S. CHANG, *Biomater. Med. Dev., Artif. Organs* **13** (1985–86) 153.
17. R. R. M. BOS, F. R. ROZEMA, G. BOERING, A. J. NIJENHUIS, A. J. PENNINGS, A. B. VERWEY, P. NIEUWENHUIS and H. W. B. JANSEN, *Biomaterials* **12** (1991) 32.
18. J. W. LEENSLAG, A. J. PENNINGS, R. R. M. BOS, F. R. ROZEMA and G. BOERING, *ibid.* **8** (1987) 311.
19. C. C. P. M. VERHEYEN, J. R. DE WIJIN, C. A. VAN BLITTERSWIJN and K. DE GROOT, *Br. J. Surg.* **79** (1992) S148.
20. C. P. A. T. KLEIN, J. M. A. DE BLIECK-HOGERVORST, J. G. C. WOLKE and K. DE GROOT, *Biomaterials* **11** (1990) 509.

Received 12 August
and accepted 20 December 1991