Functionalized copolymers and their composites with polylactide and hydroxyapatite

S. JIN, K. E. GONSALVES*
Department of Chemistry & Polymer Program at the Institute of Materials Science, University of Connecticut, Storrs, CT 06269, USA

Synthetic copolymers poly(ε-caprolactone-*co*-vinylphosphonic acid) (P(MDOVPA) and poly(ε-caprolactone-*co*-dimethylvinylphosphoester) (P(MDOVPE)) were used to prepare composites with polylactide (PLac) and hydroxyapatite (HAp). The P(MDOVPA) is used as filler in PLac films, as it has pendant functional groups $P(O)(OH)_2$, providing nucleation sites for the deposition of HAp in simulated body fluid. HAp growth on P(MDOVPA) powder and PLac-P(MDOVPA) film was observed by Fourier transform infrared spectroscopy (FTIR), energy dispersive X-ray analysis (EDX) and X-ray diffraction (XRD). P(MDOVPE) and PLac blend are miscible, and the incorporation of hydrophilic P(MDOVPA) into PLac increased the hydrophilicity of the blend. Synthetic HAp was used to make multilayered, alternating organic–inorganic composites with porous PLac-P(MDOVPE) blends.
© 1999 Kluwer Academic Publishers

1. Introduction

The biomimetic growth of hydroxyapatite (HAp) on the surface of various organic and inorganic substrates has been a topic for both basic research and medical applications. It promotes a better understanding of the biomineralization process in the body and development of new materials for prosthetic applications in bone and teeth. Bone-like apatite can be coated on various substrates including ceramics, metals and organic polymers in simulated body fluid (SBF) solution using a plate of CaO, SiO_2-based glass as a source of nucleating agent of apatite on the surfaces of substrates [1]. Ionic groups on the polymer surface are essential for crystallization. Mucalo *et al.* used cationic or anionic ion-exchange resins presaturated with either Ca^{2+} or $HPO_4{}^{2-}$ as a means of stimulating calcium phosphate formation on the surface of resin spheres in 1.5 × SBF solution [2].

Phosphorus-containing polymers are biocompatible and they can provide nucleation sites for the deposition of inorganic minerals such as HAp. Phosphoprotein containing $-PO_3{}^{2-}$ side chain groups in collagen compartments can localize a microenviroment containing free mineral ions and serve to nucleate the mineral phase heterogeneously [3]. Phosphorylated Porapak-N, divinylbenzene copolymer and polystyrene polymers were used to induce the nucleation and growth of HAp [4]. Polymers containing phosphonate groups pendant to the polymer backbone chain can also adsorb onto HAp surfaces [5]. Calcium phosphate was observed on phosphorylated cotton fibers when immersed in 1.5 SBF solution [6]. Phosphoric acid PO_4H_2 functional group on the surface of self-assembled monolayers induced apatite formation, and the growth rate of PO_4H_2 is greater than that of other functional groups such as COOH, $CONH_2$, OH and NH_2 [7].

Polymer–inorganic hybrid materials have both the properties of polymers and inorganics. Although polymers are easy to process into a variety of end-use sizes and shapes, their moduli and strength are much lower than those of bone and dentine. Ceramics are hard, stiff and brittle materials. Ceramic materials, such as hydroxyapatite, the mineral phase of bone, are being studied because of their stability in a number of orthopaedic and dental applications as replacements for bone and dentine. Composites made up of two or more phases yield a material with properties that are between those of each phase. Ceramic and polymer composites have ductility and toughness approaching that of the polymer component and stiffness and strength approaching that of the ceramic [8]. In this study, we have designed copolymers containing the biodegradable segment poly(ε-caprolactone) and pendant functional groups $P(O)(OH)_2$ and $P(O)(OCH_3)_2$. The copolymers with $P(O)(OH)_2$ pendant functional groups provided nucleation sites for biomineralization in SBF solution. By mimicking the structure of nacre [9], multi-layered synthetic biocompatible polymer-HAp composites were also prepared.

2. Experimental procedure

2.1. Materials and characterization

Functionalized copolymers poly(ε-caprolactone-*co*-vinylphosphonic acid) (P(MDOVPA), 32 mol% of MDO), poly(ε-caprolactone-*co*-dimethylvinylphosphonate) (P(MDOVPE), 60 mol% of MDO, M_W/M_n is 100 000/27 000) were synthesized in our laboratory [10].

*Author to whom correspondence should be addressed.

Poly(L-lactide) (PLAc) and hydroxyapatite (HAp) were also synthesized in our laboratory [11]. PLAc has an M_W of 58 000 and M_n of 47 000, respectively. Other chemicals were obtained from Aldrich. The pH of the solution was measured by a Fisher pH meter (model 107). Contact angle was measured under water by a NRL C.A. goniometer (model 100-00 115). X-ray diffraction (XRD) was recorded by using CuK_α radiation (20 kV, 15 mA) with a Ni filter. Energy dispersive X-ray analysis (EDX) was performed on an Amray 1000 A SEM, equipped with Kevex quantum EDX system with an ultralight window. Fourier transform infrared (FTIR) spectra were obtained on a Nicolet 60SX FTIR spectrometer. Differential scanning calorimetry (DSC) of the polymers were performed using a TA Instrument, DSC 2929, in N_2 at a heating rate of 20 °C min^{-1}.

2.2. Preparation of PLac-P(MDOVPA) film

PLac 0.2 g was dissolved in 10 ml of CH_2Cl_2. Then 0.2 g of ground P(MDOVPA) powder was added into the PLac solution. The mixture was then cast into a film on a glass plate. After drying, the film was peeled off from the glass and then hot pressed at 80 °C and a pressure of 2 metric tons. P(MDOVPA) was used as filler in the PLac film.

2.3. Biomineralization of P(MDOVPA) in 1.5 SBF solution

Copolymer P(MDOVPA) 0.3 g was presaturated in a 1 mol l^{-1} $Ca(NO_3)_2$ solution. It was stirred in a 1 mol l^{-1} aqueous solution of $Ca(NO_3)_2$ for 24 h. The polymer swelled in the solution and formed a hydrogel. The hydrogel was filtered and washed with water, and it was used without drying (SBF0). SBF0 was then put in a plastic flask with 20 ml of 1.5 SBF (simulated body fluid) solution for crystallization, setting in a water bath with a stable temperature of 37.5 °C. SBF solution (1.5 ×) was prepared by adding 15 ml each of 2.74 mol l^{-1} NaCl, 0.06 mol l^{-1} KCl, 0.05 mol l^{-1} $CaCl_2$, 0.03 mol l^{-1} $MgCl_2$, 0.0895 mol l^{-1} $NaHCO_3$, 0.02 mol l^{-1} K_2HPO_4 and 0.01 mol l^{-1} Na_2SO_4 to a 200 ml volumetric flask along with 25 ml each of 0.4 mol l^{-1} TRIS ($(CH_2OH)_3CNH_2$) and 0.36 mol l^{-1} HCl with the remainder of the volume being made up with distilled water. The pH of 1.5 SBF solution was 7.20. The solution was refreshed each day. A small amount of polymer was taken out every 2 days. The crystallization was stopped after 6 days. Each sample taken out from the flask every two days was labeled as SBF1, SBF2 and SBF3. All these samples were dried at 50 °C overnight for characterization.

2.4. Biomineralization of PLac-P(MDOVPA) film

The film was presaturated in a 1 mol l^{-1} $Ca(NO_3)_2$ solution for 24 h. The filler P(MDOVPA) swelled in the solution and formed a hydrogel. The presaturated film (film0) was then removed from the solution and washed with distilled water. For biomineralization, film0 was placed in a plastic flask with 20 ml of 1.5 SBF, setting in a water bath with a stable temperature of 37.5 °C. The 1.5 SBF solution was refreshed every day. The biomineralization time was 2, 4 and 8 days, respectively. The film after biomineralization (film1, film2 and film3 for 2, 4 and 8 days biomineralization, respectively) was taken out from the solution and rinsed with distilled water and then dried.

2.5. Preparation of PLac-P(MDOVPE) blend

A total of 0.5 g of a mixture of the two polymers PLac and P(MDOVPE) (25, 50 and 75 wt% of PLac, respectively) was dissolved in 10 ml of CH_2Cl_2. The 0.5% solution of the polymer blend mixture was cast into a film on a glass plate. Glass transition temperatures (T_g) of the blend (blend1, blend2 and blend3 for 25, 50 and 75 wt% PLac, respectively) were measured by DSC, and the hydrophilicity was assessed by contact angle measurement.

Porous PLac-P(MDOVPE) film was also prepared by adding 0.5 g of NaCl powder (particle size < 150 μm) into 10 ml 5% CH_2Cl_2 solution of P(MDOVPE) and PLac. The mixture was cast into a film and then NaCl was leached out from the film by placing into distilled water for 48 h, leaving pores in the film [12].

2.6. Preparation of multilayered blend-HAp composite

HAp powder 0.5 g was dispersed in 10 ml of distilled water and sonicated for 4 h. Then the HAp dispersion was sprayed onto the porous blend film. Due to the hydrophilic nature of the polymer blend surface, the water dispersion covered the entire surface of the film. After drying, the layered blend-HAP was peeled off from the glass plate and then cut into even pieces. These pieces were then piled up with alternating layers of blend and HAp, respectively. These piled pieces were then placed between two layers of Teflon and hot pressed at 80 °C and a pressure of 2 metric tons.

3. Results and discussion

3.1. Biomineralization of P(MDOVPA) in 1.5 SBF solution

The structure of copolymer P(MDOVPA) is shown in Fig. 1. It has a biodegradable poly(ε-caprolactone) segment as well as pendant phosphonic acid groups from a vinyl monomer, vinylphosphonic acid. P(MDOVPA) has characteristics of a hydrogel with high swelling ratio in water. Presaturation of

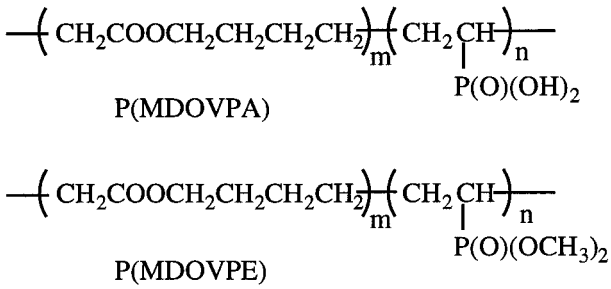
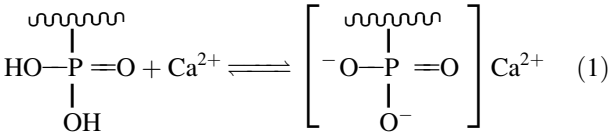


Figure 1 Chemical structure of functionalized copolymers P(MDOVPA) and P(MDOVPE).

P(MDOVPA)-PLac in $Ca(NO_3)_2$ solution included the ionization of protons in phosphonic acid groups and the formation of a polymer-Ca^{2+} compound, as shown in the equilibrium 1



(1)

The formation of $-PO_3^{2-}Ca^{2+}$ was observed by both FTIR and EDX. Fig. 2(a) is the FTIR spectrum of the copolymer P(MDOVPA) including ester group ($1734\,cm^{-1}$) and pendant $-P(O)(OH)_2$ groups (3400–$2400\,cm^{-1}$). After presaturation, the phosphonic acid still exists, but part of the acidic group was exchanged by Ca^{2+}. This was confirmed by the appearance of a new peak at $901\,cm^{-1}$ in the FTIR spectrum of the presaturated polymer (Fig. 2b), which corresponds to the P–O stretching of a $—PO_3^{2-}Ca^{2+}$ salt [11, 13]. The absorption of $P—O^-$ at $901\,cm^{-1}$ is also present in HAp [10]. Furthermore, EDX analysis showed a significant amount of Ca in SBF0 (Table I), confirming the presence of Ca in SBF0.

The $P—O^{-1}$ absorption at $901\,cm^{-1}$ was also observed in all the biomineralization products. The relative intensity in this absorption of SBF3 was larger than that of SBF0, indicating the increased amount of $P—O^-$ content in SBF3. This absorption in SBF3 was caused partly by the calcium salt formation, as SBF0 did. But the absorption from apatite was also possible. The presence of Ca in the biomineralization samples was also examined by EDX. All the biomineralization samples showed the presence of Ca. EDX was generated from different areas of the samples. Analysis of the three areas per sample indicated the sample's inhomogeneousness (Table I). The hydrophilic phosphoric acid groups were present not only on the surface of the polymer but also inside the hydrogel which was filled with SBF solution. Because the deposition of apatite was induced by the phosphonic acid group [7], thus biomineralization occurred in different areas of the copolymer. The initial step of biomineralization of apatite involved the ion-ion interaction of phosphonic acid anion with Ca^{2+}, and then the formation of calcium phosphate (apatite) by the ion–ion interaction of Ca^{2+} and HPO_4^{2-}. The heterogeneous growth of apatite was probably caused by the local high concentration of Ca^{2+}, as found in some other systems [4, 7].

TABLE I Ca/P of SBF0 and SBF3 in three different areas

Sample	SBF0			SBF5		
	Area 1	Area 2	Area 3	Area 1	Area 2	Area 3
Ca/P	0.3	0.3	0.8	0.4	0.4	0.9

FTIR spectra of the biomineralization products (Fig. 2) also showed the degradation of the polymer P(MDOVPA) when in 1.5 SBF solution. P(MDOVPA) showed an ester peak at $1734\,cm^{-1}$. After presaturation in $1\,mol\,l^{-1}$ of $Ca(NO_3)_2$ for 24 h, the polymer (SBF0) showed a degraded product with an absorption at $1645\,cm^{-1}$, which corresponds to the acidic carbonyl stretching [10]. From SBF0 to SBF3, the relative intensity of the acidic carbonyl stretching increased, indicating the increasing amount of degradation product.

Fig. 3 is the XRD of the copolymer P(MDOVPA), SBF0 and the biomineralization product SBF3. The copolymer P(MDOVPA) and SBF0 are totally amorphous and showed a broad peak at $2\theta = 20°$ (Fig. 3a and b, respectively). SBF3 showed a small sharper peak at $2\theta = 31.5°$, indicating the formation of apatite. Thus calcium is not only from the formation of a calcium salt but also from the deposition of apatite [11, 15].

3.2. Biomineralization of PLac-P(MDOVPA) film

As shown in Fig. 4, P(MDOVPA) powder was used as filler in the PLac film. P(MDOVPA) has pendant hydrophilic functional groups $P(O)(OH)_2$ in the SBF solution. The ionic functional groups act as the nucleation sites for the formation of apatite, similar to the biomineralization of the pure P(MDOVPA) powder

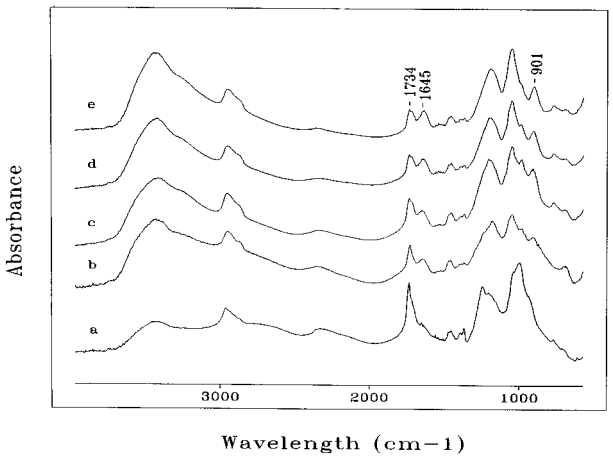


Figure 2 FTIR spectra of copolymer P(MDOVPA) and its biomineralization products: (a) P(MDOVPA), (b) SBF0, (c) SBF1, (d) SBF2, (e) SBF3.

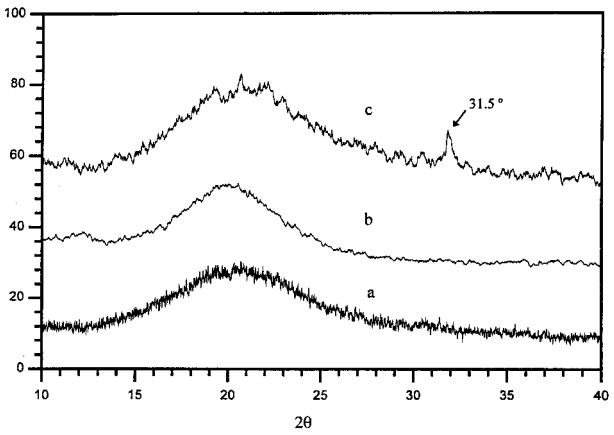


Figure 3 XRD pattern of copolymer P(MDOVPA) and its biomineralization products: (a)P(MDOVPA), (b) SBF0, and (c) SBF3.

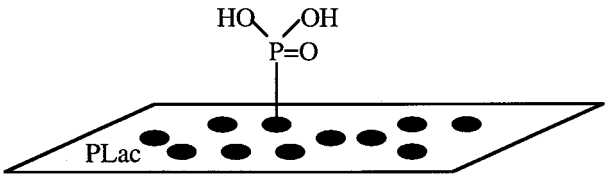


Figure 4 P(MDOVPA) serves as filler in PLac film. The filler has $P(O)(OH)_2$ functional groups acting as nucleation sites.

in SBF solution as discussed above. The formation of apatite on the P(MDOVPA)-PLac film was observed by XRD (Fig. 5). XRD of the film before biomineralization only showed a broad peak at 20° (Fig. 5a, indicating the amorphous properties of the film. After biomineralization for 2 and 4 days, a small peak showed at $2\theta = 31.5°$ (Fig. 5b and c, respectively). After 8 days, this peak increased (Fig. 5d), showing the increasing amount of apatite formed.

3.3. Multi-layered PLac-P(MDOVPE) blend-HAp composites

Blends of PLac and P(MDOVPE) polymers were made by a solution technique. The latter has been used to incorporate water soluble polymers, such as polyethylene oxide into the surface of the polymeric biomaterials, such as poly(methylmethacrylate) [15]. The polymer blend formed a transparent film while spin coating. If the two polymers are compatible, a single glass transition temperature should be observed, intermediate between the T_gs of the two separate polymers at a value determined by the weight fraction of the two polymers and approximated by the Fox equation [16]. The DSC thermograms of all the blends showed a broadened T_g while the T_m of PLac still remained (Fig. 6). P(MDOVPE) is totally amorphous with a low and broad T_g (due to its high molecular weight distribution) of $-18\,°C$. PLac is a highly crystalline material with a higher T_g of 67 °C and a T_m of 178 °C. After blending, a

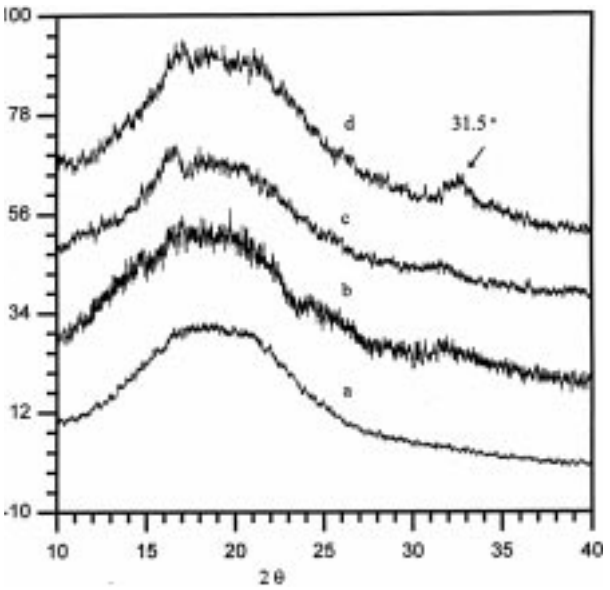


Figure 5 XRD pattern of PLac-P(MDOVPA) film and its biomineralization products: (a) PLac-P(MDOVPA) film, (b) film1, (c) film2 and (d) film3.

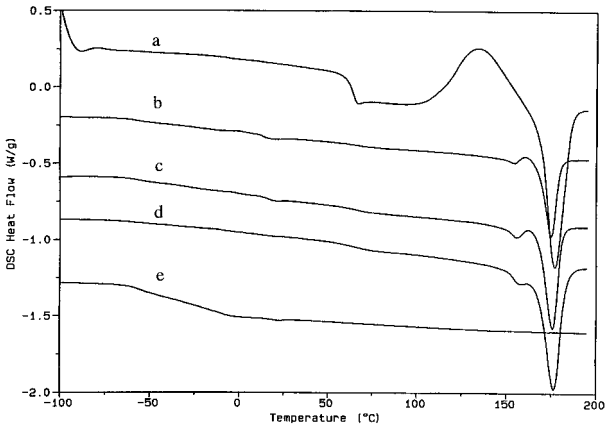


Figure 6 DSC thermograms of PLac-P(MDOVPE) blends (a) PLac, (b) blend1, (c) blend2, (d) blend3, and (e) P(MDOVPE).

broadened T_g occurred in the region of 0 °C, in between the T_g of P(MDOVPE) and PLac. The single T_g indicates the phase-mixed or interpenetrating P(MDOVPE) and PLac chains [17]. The blending of the two polymers also changed the physical properties of the polymers. The copolymer P(MDOVPE) is sticky at room temperature due to its low T_g, but after blending, the film can be taken off the substrate. Furthermore, blending of the biodegradable PLac and potentially biocompatible P(MDOVPE) would maintain the biodegradability and biocompatibility of the materials.

Measurement of contact angle on the blend surface gave an indication of the relative hydrophilicity of these surfaces before and after blending. Decreasing contact angles indicate increasing hydrophilicity, and a better attachment of the HAp water dispersion on the surface of the blends than on PLac, as depicted in Fig. 7. Fig. 8 shows that the contact angle decreases with the increasing amount of P(MDOVPE). PLac is hydrophobic, but P(MDOVPE) has a hydrophilic pendant functional group $P(O)(OCH_3)_2$ which imparts hydrophilicity to the blends.

The porous structure of the blend film was observed by SEM, (Fig. 9a). Pore size is below 150 μm, as determined by the particle size of NaCl. Porous polymeric materials have been utilized for various applications [18–20]. Macroporous matrices are structurally similar to cancellous bone in their porosity [21]. Pores allow sufficient space to promote cell growth and transport of nutrients for maintenance of growth in matrix environment [12]. The multilayered alternating structure of blend-HAp was observed by SEM (Fig. 9b). HAp layer is the white powder layer between two layers of films. It has a depth of about 30 μm. The film layer is approximately 5 μm thick. The thickness of the polymer layer can be controlled by the concentration of the polymer solution while casting film and the hot press pressure, and the load of HAp on the polymer film can also be adjusted by varying the HAp concentration in the water dispersion.

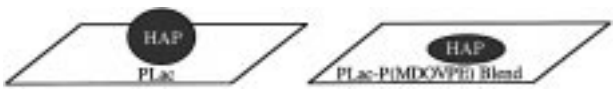


Figure 7 HAp water dispersion has a better adhesion with the blend film than with the PLac film.

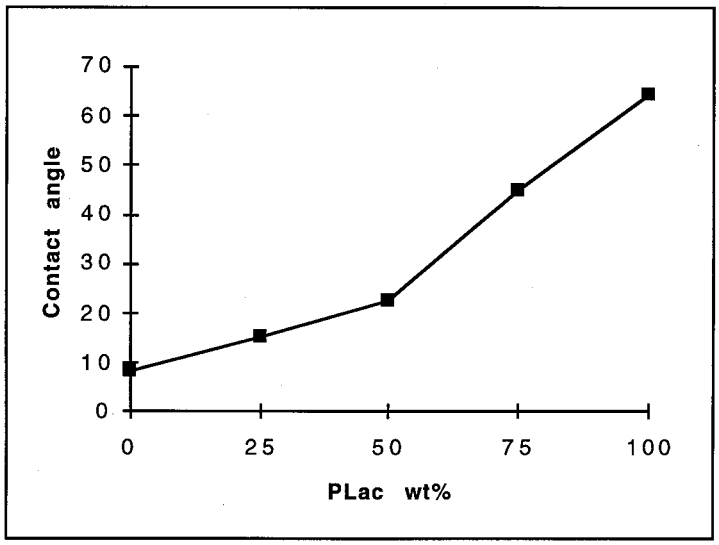


Figure 8 Contact angle of PLac-P(MDOVPE) blend versus PLac wt%.

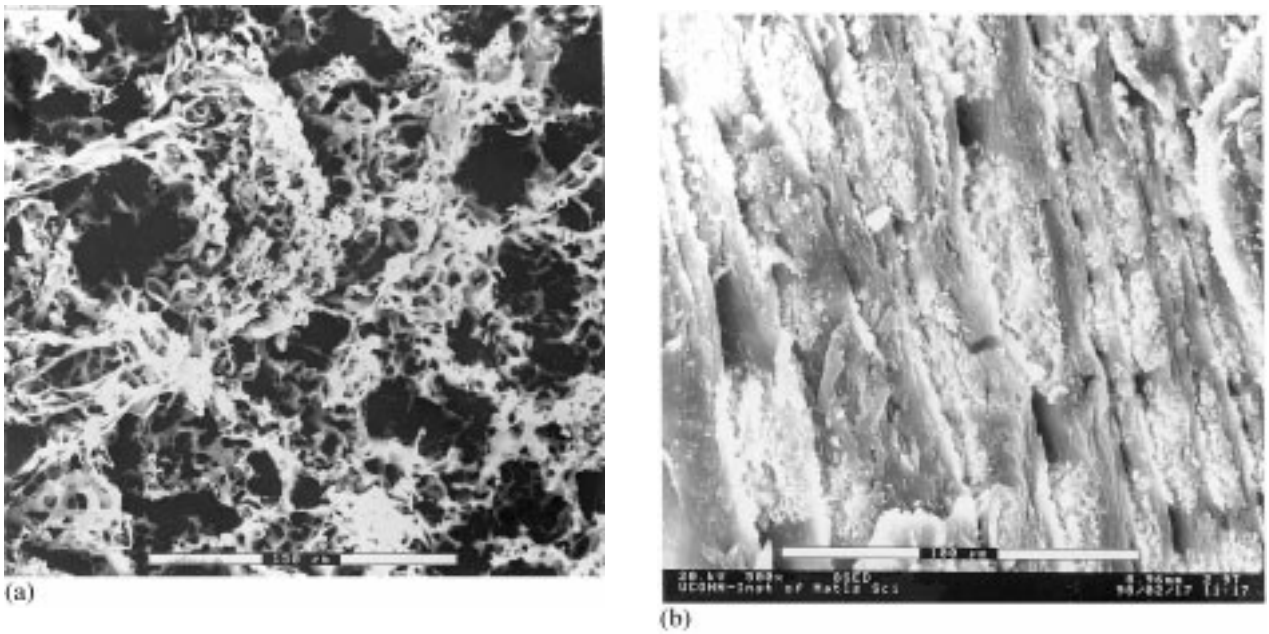


Figure 9 SEM of PLac-P(MDOVPE) blend-HAp composites: (a) porous structure of PLac-P(MDOVPE) film, and (b) multi-layered alternating structure of composite.

4. Conclusions

Copolymers P(MDOVPA) and P(MDOVPE) containing biodegradable poly(ε-caprolactone) segment and pendant functional groups $P(O)(OH)_2$ and $P(O)(OCH_3)_2$, respectively, were used to make composites with PLac and HAp. HAp can grow on the P(MDOVPA) powder and PLac-P(MDOVPA) film in SBF solution. Degradation occurred in the process of biomineralization. The blend of PLac and P(MDOVPE) has good hydrophilicity, and was used to make a multi-layered, alternating, porous structure of HAp-blend. These materials, combining the biodegradable organic polymer components and inorganic biomaterial, have potential application as implants.

References

1. P. LI, C. OHTSUKI, T. KOKUBO, K. NAKANISHI, N. SOGA, T. NAKAMURA and T. YAMAMURO, *J. Mater. Sci.: Mater. Med.* **4** (1993) 127.
2. M. R. MUCALO, M. TORIYAMA, Y. YOKOGAWA, T. SUZUKI, Y. KAWAMOTO, F. NAGATA and K. NISHIZAWA, *ibid.* **6** (1995), 409.
3. M. J. GLIMCHERE, in ''The chemistry and biology of mineralized connective tissues,'' edited by A. Veis (Elsevier, Amsterdam, 1981) p. 618.
4. E. DALLAS, J. KALLITSIS and P. G. KOUTSOUKOS, *Langmuir* **7** (1991) 1822.
5. H. R. RAWLS, T. BARTELS and J. ARENDS, *J. Colloid. Interface Sci.* **87** (1982) 339.
6. M. R. MUCALO, M. TORIYAMA, Y. YOKOGAWA, T. SUZUKI, Y. KAWAMOTO, F. NAGATA and K. NISHIZAWA, *J. Mater. Sci.: Mater. Med.* **6** (1995) 597.
7. M. TANAHASHI and T. MATSUDA, *J. Biomed. Mater. Res.* **34** (1997) 305.
8. F. H. SILVER, ''Biomaterials, medical devices and tissue engineering,'' (Chapman & Hall, London, 1994).
9. M. SARIKAY and I. A. AKSAY, in ''Structure cellular synthesis and assembly of biopolymers,'' edited by S. T. Case (Springer-Verlag, Berlin, 1992).
10. S. JIN and K. E. GONSALVES, *Macromolecules* **31** (1998) 1010.
11. S. ZHANG and K. E. GONSALVES, *J. Mater. Sci.: Mater. Med.* **8** (1997) 25.

12. C. T. LAURENCIN, S. F. AMIN, S. E. IBIM, D. A. WILLOUGHBY, M. ATTAWIA, H. R. ALLOCK and A. A. AMBROSIO, *J. Biomed. Mater. Res.* **30** (1996) 133.
13. D. LIN-VIEN, N. B. COLTHUP, W. G. FATELEY and J. G. GRASSELLI (eds), ''The handbook of infrared and Raman characteristic frequencies of organic molecules,'' (Academic Press, New York, 1991).
14. K. TSURU, C. OHTSUKI, A. OSAKA, T. IWAMOTO and J. D. MACKENZIE, *MRS Sym. Proc.* **435** (1996) 403.
15. N. P. DESAI and J. HUBBELL, *Biomaterials* **12** (1991) 144.
16. L. H. SPERLING, ''Introduction to physical polymer science,'' 2nd edn, (John Wiley & Sons, New York, 1992) p. 357.
17. N. P. DESAI and J. A. HUBBELL, *Macromolecules* **25** (1992) 226.
18. H. L. WALD, A. G. MIKOS, G. SARAKINOS, M. D. LYMAN, J. P. VACANTI and R. LANGER, *Biomaterials* **14** (1993) 270.
19. A. G. MIKOS, G. SARAKINOS, S. M. LEITE, J. P. VACANTI and R. LANGER, *ibid.* **14** (1993) 353.
20. A. G. MIKOS, A. J. THORSEN, L. A. CZERWONKA, Y. BAO, D. N. WINSLOW, J. P. VANCANTI and R. LANGER, *Polymer* **35** (1994) 1068.
21. J. E. DEVIN, M. A. ATTAWIA and C. T. LAURENCIA, *J. Biomater. Sci.: Polym. Ed.* **7** (1996) 661.

Received 10 March
and accepted 31 August 1998