New composites of hydroxyapatite and bioresorbable macromolecular material

P. CERRAI, G. D. GUERRA, M. TRICOLI
CSMM-CNR, via Diotisalvi 2, I-56126 Pisa, Italy

A. KRAJEWSKI, S. GUICCIARDI, A. RAVAGLIOLI
IRTEC-CNR, via Granarolo 64, I-48018 Faenza, Italy

S. MALTINTI
Dipartimento di Ingegneria Chimica, via Diotisalvi 2, I-56126 Pisa, Italy

G. MASETTI
IB-CNR, via S. Lorenzo 26, I-56127 Pisa and Dipartimento di Fisica, Università di Pisa, Piazza Torricelli 2, I-56126 Pisa, Italy

Composite materials were prepared by mixing in different proportions of hydroxyapatite (HA) and poly(ε-caprolactone-oxyethylene-ε-caprolactone) block copolymer (PCL-POE-PCL) to produce a new resorbable material for biomedical applications. This material has proved to be very interesting for production of periodontal membranes. Mechanical properties are linearly proportional to the amount of HA introduced. Fourier transform infrared (FTIR) investigations have pointed out that HA is able to influence some close ε-caprolactone molecules to start its homopolymerization giving PCL with an end chain ionic bonding. HA grains are therefore surrounded by a film of PCL which grants close connection of HA grains within copolymeric matrix. This interface bond with PCL is, however, an interesting occurrence for preparations of HA/PCL composites.
© 1999 Kluwer Academic Publishers

1. Introduction

Composites and mixtures of hydroxyapatite (HA) and bioresorbable homo- and co-polymers were proposed for use in bone repair [1, 2], because they include bioactive osteogenesis. The production of a very biocompatible composite material, constituted with a biointegrable polymeric matrix and a ceramic, was considered to be useful for applications in which an initial shape is easily obtained through a molding-die. This technology should allow reproduction of a part of bone with standard devices at low temperatures, cheaply and with no host's need of close tolerances, because the final remodeling is left to the tissue itself which guests the implantation. Bone reconstruction should follow resorption of the polymer and should nucleate all around each grain of ceramic, in particular if the latter is bioactive.

The poly(ε-caprolactone-oxyethylene-ε-caprolactone) block copolymer (PCL-POE-PCL), which is obtained by a non-catalyzed synthesis in bulk [3, 4], between ε-caprolactone and poly(ethylene glycol) (PEG), was utilized for this work as a resorbable polymer, being a material both bioresorbable and bioactive [5]. When PEG is added to ε-caprolactone, a polymerization by transesterification reaction is promoted by the hydrogen of the alcoholic group on the ester lactonic group.

To have a better compatibility between the inorganic phase and the organic one in the obtaining of the composites, we tried to synthesize PCL-POE-PCL in the presence of bioactive ceramic powders of hydroxyapatite (HA), the theoretical chemical formula of which is $Ca_5(PO_4)_3OH$. Being some performances of HA dependent on heating treatment, it was used as prepared or after thermal annealing at the temperatures of 700, 900 and 1100 °C [6, 7].

2. Materials and methods

The materials were prepared by reacting ε-caprolactone monomer with preformed poly(ethylene glycol) (PEG) (20 000 Da), always in a 9 : 1 weight ratio, in the presence of HA granular ceramic powders, differently annealed as above described or not annealed. The organic substance, obtained by non-catalyzed synthesis in bulk, is the poly(ε-caprolactone-oxyethylene-ε-caprolactone) triblock copolymer (PCL-POE-PCL). Its chemical synthesis was carried out according to the general scheme shown in Fig. 1. The copolymer formation occurs through a ring-opening mechanism where the active hydrogen atoms of the preformed PEG determine a selective acyl-oxygen cleavage of the lactone ring [3]. After the initial formation of an intermediate bis-ε-hydroxy-diester, a step-by-step addition of monomeric lactone units occurs with formation of two external polyester blocks, the length of which depends on the amount of lactone monomer ($m_1 + m_2$ moles) used in the feed. The synthesis is carried out in bulk, with no
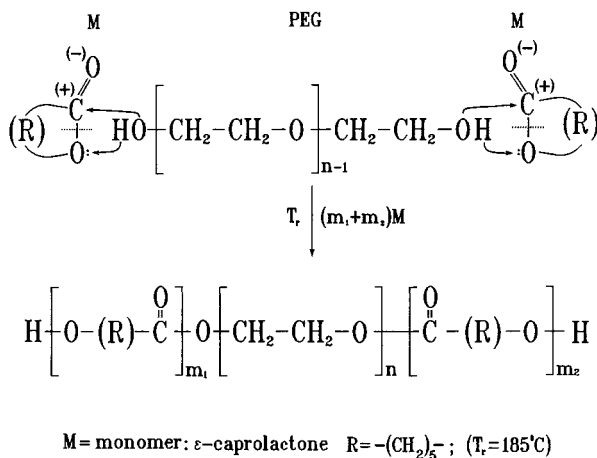
M = monomer: ε-caprolactone R = -(CH₂)₅- ; (Tᵣ=185°C)

Figure 1 Chemical process to obtain PCL-POE-PCL triblock copolymers.

addition of catalysts and by thermally activating the hydrogen atoms of PEG at a suitable reaction temperature T_r, the value of which is 185 °C for PCL-POE-PCL copolymers.

To have a better compatibility between the inorganic phase and the organic one of the composite, PCL-POE-PCL was synthesized in the intimate presence of the ceramic powders. HA was introduced as such or annealed in a laboratory kiln respectively at 700, 900 and 1100 °C. The shape of the grains of the powders was almost spherical. Increasing amounts of ceramic powders, namely 15%, 30% and 50% of the organic phase, were used. The Pyrex phials containing the reaction mixture, sealed under vacuum, were held in an oven at 185 °C for several days.

The composites so obtained were morphologically characterized by scanning electron microscopy (SEM) with a Jeol T-300 instrument.

A structural analysis of the composites was carried out by Raman and Fourier transform infrared (FTIR) spectroscopies. An accurate investigation was done by FTIR on differently prepared and specifically treated samples, with a Perkin-Elmer model 1600 apparatus. The samples were prepared either by casting on KBr discs or by sintering in KBr pellets, depending on the solubility of the materials. FTIR spectroscopy was carried out on the polymer alone, on HA alone (700 °C) and on the composite. To investigate a possible reaction between HA and PCL-POE-PCL a series of collateral tests was set-up. A first series of tests was the collection of spectra on the residue and soluble fraction obtained after an extraction with CHCl₃ from the composite. Details on the specific procedures will be reported in Section 3.

Raman spectra from solids were recorded at room temperature by using the 488 nm emission from an Ar⁺ laser (Innova 90 from Coherent) as the excitation source. The light scattered at right-angle with respect to the laser beam axis was analyzed by means of a Jobin Yvon HG-2S double monochromator equipped with a cooled photomultiplier (RCA mod. 31034A) and photon-counting electronics.

Physicomechanical tests were carried out, by an Instron model 6025, to emphasize some possible performances interesting to biomedical applications.

3. Results
3.1. Mechanical tests

As concerns the measurement of mechanical properties of the composites, Fig. 2 shows the compression strength resistance, Fig. 3 the flexural strength and Figs 4 and 5 the Young's elastic modulus of the composites containing ceramic powders of HA. All of these exhibit a significant increase of the property value with the amount of the ceramic present in the composite. This increase is linearly proportional to the HA concentration, in the interval of additions considered for all tested properties. However, it appears always independent of the annealing temperature of HA only for temperatures higher than 700 °C. Best-fitting plots of both graphics concerning compression versus concentration of added HA (Fig. 2) supply an extrapolated value (13.5 MPa) of hypothetical ultimate compression resistance of the adopted PCL-POE-PCL copolymer. The experimental point of the latter is absent because its rheological performances under compression do not allow to define a value of collapse load, due to a very large field of plastic deformations. The extrapolated value could be attributed to the starting point of molecular breakdown with formation of the first infrachain gaps.

As regards flexural strength, the samples filled with HA show a different behavior depending on the temperature of firing of the added powders. Actually, the samples containing powders either non-fired or fired only at 700 °C (both indicated by open square symbols) do not show any predictable trend; on the contrary, the samples containing powders thermally treated above 700 °C (closed square symbols) give rise always to a linear trend (Fig. 3).

Fig. 4 shows the linear increasing trend of the Young's modulus of the samples as a function of their content of HA ceramic powders, while Fig. 5 shows the position of the experimental Young's modulus points in the range predicted by the composite theory of Hashin and Shtrikman (H & S) where the upper and lower curves are reported [8].
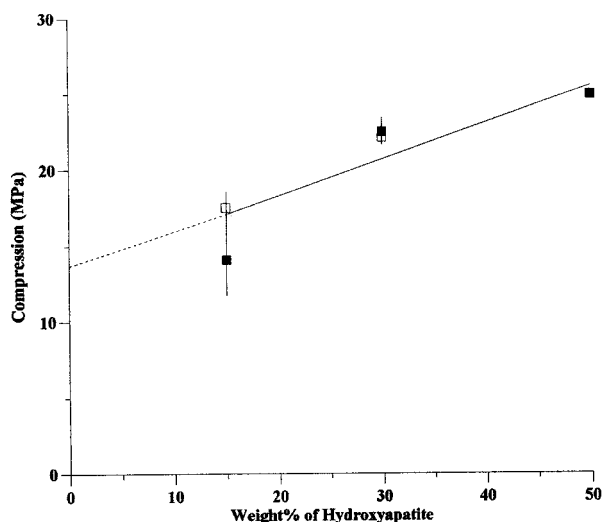

Figure 2 Ultimate compression resistance as a function of the HA content (□ = HA not annealed or annealed up to 700 °C; ■ = HA annealed over 700 °C).
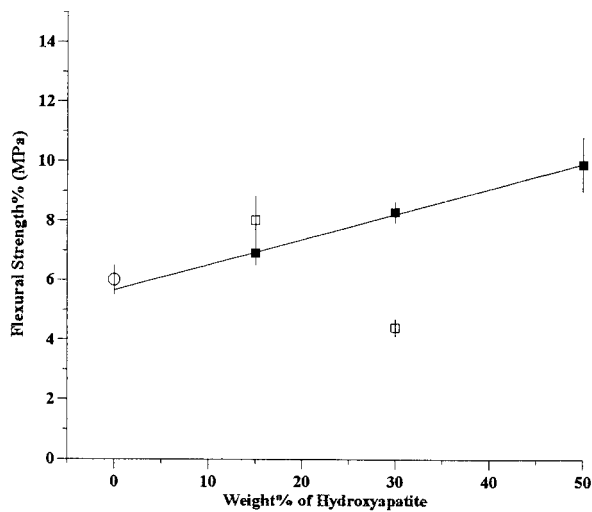
Figure 3 Flexural strength as a function of the HA content ($\circ$ = only copolymer; $\square$ = HA not annealed or annealed up to 700 °C; $\blacksquare$ = HA annealed over 700 °C).
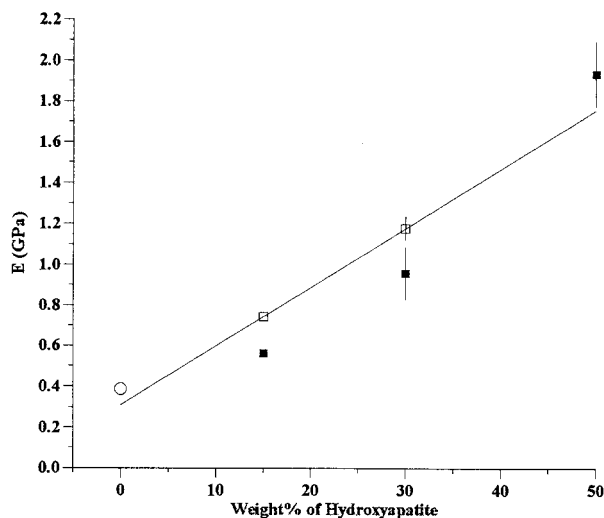

Figure 4 Young's modulus as a function of the HA content ($\circ$ = only copolymer; $\square$ = HA not annealed or annealed up to 700 °C; $\blacksquare$ = HA annealed over 700 °C).

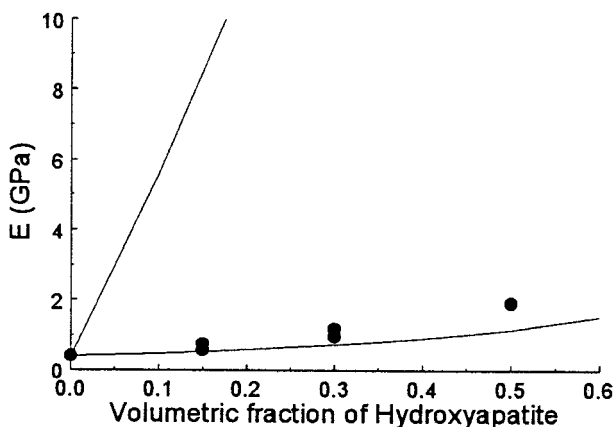

Figure 5 Position of the experimental Young's modulus points of the samples with HA inside the field of composite existence with the considered copolymers.

3.2. Spectroscopic tests

The investigations carried out by FTIR, Raman and SEM techniques have shown interesting results. The FTIR spectrum carried out on composite (Fig. 6) was compared with those of HA alone and of PCL-POE-PCL copolymer alone. The spectrum exhibits the typical bands of both phosphate and PCL-POE-PCL. The triplet at 1088, 1048 and 962 cm^{-1} of HA lies in a spectral region which is already full of PCL-POE-PCL bands. This superposition of bands (between 1100 and 962 cm^{-1}) makes this spectral region less significant for such a comparison; however, the aspect of this spectral region of the composite is more similar to that of HA alone. Common peaks of adsorption between composite and HA alone are the sharp peak at 3572 cm^{-1} on the shoulder of the –OH band at 3436–3434 cm^{-1} and the triplet at 632, 604–602, 572–566 cm^{-1}. The more significant common peaks of adsorption between composite and copolymer alone are at 2946–2944 cm^{-1} (–CH– band), and at 1726–1724 cm^{-1} (typical ester band); all peaks between 1500 and 1100 cm^{-1} are copolymer peaks.

To investigate a possible interaction between HA and polymeric organic material, the material was extracted with CHCl$_3$ and centrifugated twice; the soluble fraction, cast on KBr discs, gives a spectrum (Fig. 7) showing only the typical PCL-POE-PCL bands. The spectrum of the solid residue showed instead all the HA bands (very strong) together with some weak absorption peaks attributable to poly(ε-caprolactone) homopolymer (PCL), in particular the methylene bands at 2936 and 2860 cm^{-1}, as well as the typical aliphatic polyester band at 1736 cm^{-1}. This result indicates that some homopolymeric PCL chains, somehow bonded to the HA structure, are formed during the copolymerization reaction. Since ε-caprolactone does not homopolymerize at 185 °C in the absence of suitable catalyst, its homopolymerization reaction is attributed to OH^{-} ions present in the HA structure which initiate the polymerization process with the formation of –COO^{-} ended PCL chains, ionically linked to Ca^{2+} ions of HA. To check this hypothesis, the residue was treated twice with 0.2 N HCl solution, sufficiently diluted to avoid an acid hydrolysis of the polyester. HCl acts as a neutralizer of the groups –COO^{-} end groups which bond the organic part to HA; therefore, free polymer molecules are obtained which are soluble with CHCl$_3$. The solid was dried under vacuum and at last extracted with CHCl$_3$; the soluble fraction, cast on KBr disc after double centrifugation, gives the spectrum of Fig. 8, identical to that of a preformed PCL sample (MW = 20 000 Da); the spectrum of the insoluble fraction is that of an inorganic phosphate.

The same procedure was carried out by mixing HA and PEG and trying to react them up to 185 °C. In this case the simple extraction with CHCl$_3$ is able to separate completely (as confirmed by FTIR) the HA alone in the residue and the PEG alone in the solution, as expected. PEG can in fact put at disposal only ether (–O–) bridges and two end alcoholic (–OH) groups. The only kind of interaction possible is the formation of weak dipolar hydrogen bonds. These combined results confirm the hypothesis of a bond formation between PCL molecules formed and HA.
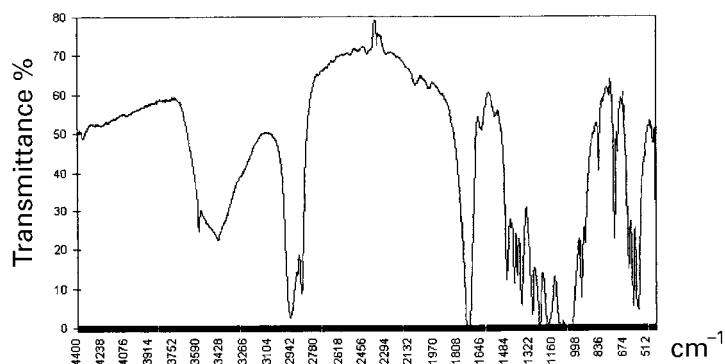
Figure 6 FTIR spectrum of typical composite HA/PCL-POE-PCL.

To confirm that HA is able to initiate the homopolymerization of ε-caprolactone, giving polymer chains bonded to the inorganic HA grains, the monomer and HA powders were heated *in vacuo* at 185 °C for some days, in the absence of PEG. A solid mass was obtained which gave FTIR spectra showing both HA and PCL bands. The extract with CHCl₃, cast on KBr, supplied a weak spectrum practically identical to that of poly(ε-caprolactone); in particular, no phosphate peaks were found. This fact would indicate that only a very little fraction of all formed PCL is not linked to HA; its formation is probably due to some chain transfer reactions during the overall polymerization, since ε-caprolactone does not polymerize when heated alone. However, the amount of the PCL unlinked to HA is negligible.

The insoluble fraction was examined in the same way as that obtained from the HA/PCL-POE-PCL composite: treating with diluted HCl, drying *in vacuo* and double extraction with CHCl₃. The extract, cast on KBr, gave a spectrum practically identical to that in Fig. 8, which is a typical PCL spectrum.

Samples of composites constituted with copolymer and HA annealed at different temperatures were investigated by Raman scattering, as a support of that obtained by FTIR. To have suitable references, spectra were also recorded from PCL-POE-PCL copolymer and from HA annealed at different temperatures. All spectra, with the only exception of those from fired hydroxyapatite, showed an intense fluorescence background which could be reduced to an acceptable level after irradiation at 448 nm for 2 h before measurements. The relative HA content in the composites was estimated by the intensity ratio of the signal at $963 \, cm^{-1}$, due to a stretching mode of the phosphate group in HA, to the bands at either 1110 or $1726 \, cm^{-1}$ which are assigned to vibration involving the ether and ester groups in the copolymer, respectively. In Fig. 9 the Raman spectrum of the composite synthesized from HA annealed at 1100 °C is reported. As it is shown in the figure, though the original fluorescence of this specimen is not completely quenched, the Raman bands of interest are prominent and sharp enough to allow a reliable valuation of their relative intensities.

SEM microscopy on fracture surface of samples containing hydroxyapatite is substantially represented by the micrographs of Figs 10–12. Such series of micrographs show that the intimate dispersion of HA crystalline grains inside the organic matrix diminishes with the increase of the temperature of HA thermal treatment. It shows also that HA crystallite size increases with firing temperature, as expected. HA shows very small crystallites in the powders not annealed or annealed at only 700 °C, whereas crystallites with a size up to 0.5 μm are present in the powders annealed at 1100 °C. According to previous papers [6, 7], the logarithm of crystallite volume is proportional to the fourth power of the absolute temperature (T^4).

On the other hand, SEM micrographs obtained on different samples of composites HA/copolymer also display a formation of clusters. Only single HA crystal-
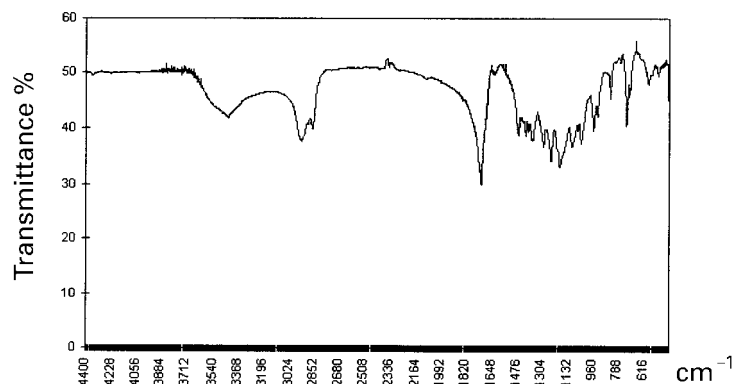

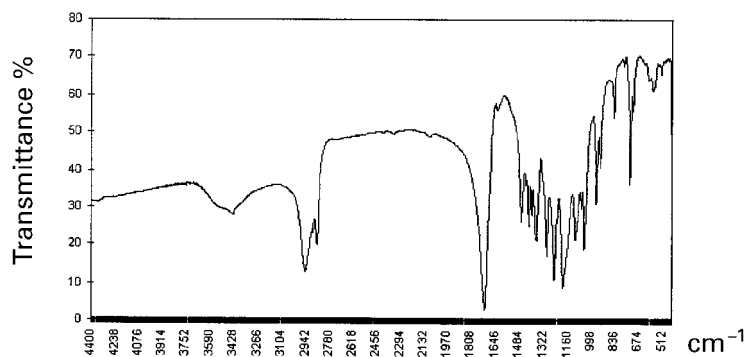*Figure 7* FTIR spectrum of the composite fraction soluble in CHCl₃.

286

Figure 8 FTIR spectrum referred to the composite fraction insoluble in CHCl$_3$, after HCl acidification and CHCl$_3$ extraction: the spectrum concerns the fraction soluble in CHCl$_3$.

lites appear to be dispersed in the copolymeric material when the composite is obtained with HA not annealed or annealed at 700 °C (Fig. 10); possible clusters do not exceed a size of 3–4 μm. In the composites obtained from HA annealed at 900 or at 1100 °C there is an additional clear presence of clusters of HA grains, the dimension of which increases with the temperature of thermal treatment of HA. These clusters assume an average size of 9–12 μm with HA treated at 900 °C (Fig. 11) which increases up to 15–20 μm with HA treated at 1100 °C (Fig. 12). The number of these clusters also increases with concentration.

An accurate morphological investigation on the quality of the interfaces between ceramic grains and copolymeric matrix has indicated that each grain of HA, even inside the clusters, is closely surrounded by the matrix.

4. Discussion

All the composites coming from different mixtures of ceramic HA and PCL-POE-PCL copolymer fall inside the area of predicted range of composites [8], mechanically tested. It is possible to say that such composites correspond to physically integral materials (e.g. without pores, microcracks and any other physical discrepancy, and with ceramic filling material well bounded to polymeric matrix, etc.) in which the ceramic particles

Figure 9 Raman spectrum in the 50–1850 cm^{-1} shift range of the composite between PCL-POE-PCL copolymer (66% by weight) and HA annealed at 1100 °C. Excitation wavelength: 488 nm; power at the samples: 55 mW; spectral slit width: 3 cm.

should be intimately bound to the surrounding matrix. The bond might be physical and even chemical. Literature reports [9] that the shape and dimensions of the particles present inside a matrix in a composite play a role in determining the position in the field between the upper and lower limits of the H & S theory. The experimental points are close to the lower boundary curve (Fig. 5) and this is correct because the dispersed phase is constituted of roundish fine particles or clusters (Figs 11 and 12). The first part of this lower H & S curve can be reasonably approximated by a linear relationship and the experimental points close to this part of the curve follow in their turn a linear trend (Fig. 5).

In Raman spectra of both HA and composites several multiplets are observed whose resolution increases as long as the HA annealing temperature increases. This fact suggests that high annealing temperatures favor the regular organization of HA in a crystalline lattice which is not destroyed during the reaction with the organic phase. Raman intensity measurements on composites obtained from HA, unannealed and annealed at 700, 900 and 1100 °C, indicate that the HA content in the sample increases with respect to the copolymer when the annealing temperature increases. Finally, comparison with the spectra of the single components reveals that the Raman spectrum of the composites is a simple sum of contributions by HA and copolymer. Indeed, no experimental evidence for chemical linkages between HA and PCL-POE-PCL copolymer or PCL homopolymer in the composite was found in the data coming from this spectroscopic technique. This result very likely is due to the fluorescence contribution as well as to the low concentration of such sites with respect to the composite bulk. However, the deductions coming from mechanical tests, seem to indicate an enhancement of the compatibility between two phases with increasing HA annealing temperature.

The experimental results coming from FTIR analysis and by the procedure needed for the separation of the composite components, show that some homopolymeric PCL chains, bound to the HA structure, are formed together the PCL-POE-PCL copolymer. That such PCL chains are linked to HA through ionic bonds between –COO$^-$ end groups in the polymer and Ca^{2+} ions in the ceramic particles is proved by the need of acidification to separate the two phases. The formation of PCL chains ionically bonded to the ceramic structure at the surface of the grains, which are likely responsible for the observed
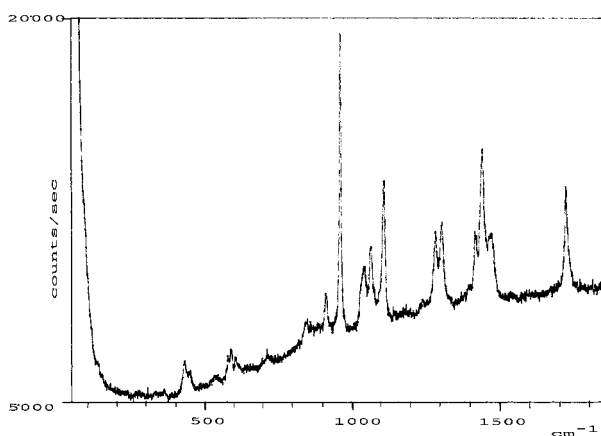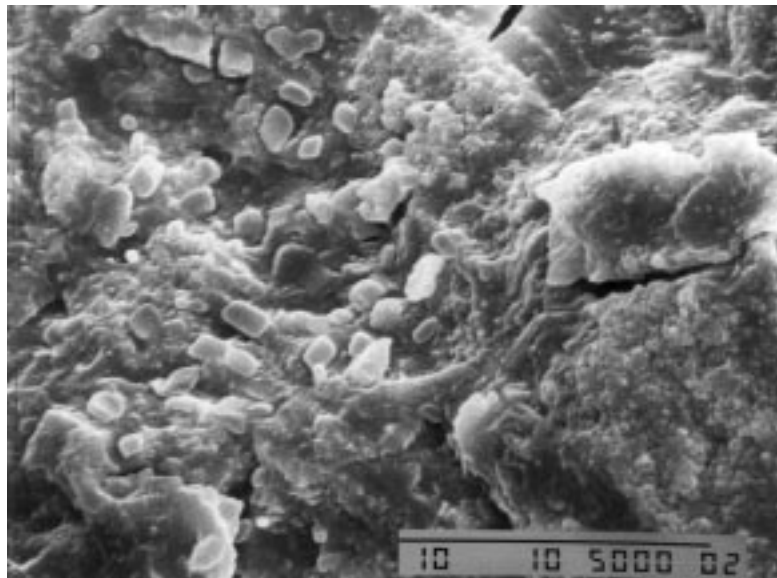
287

Figure 10 SEM micrograph of a section of a copolymer sample with HA annealed at 700 °C (bar = 10 μm).

enhanced compatibility between HA and this specific PCL-POE-PCL copolymer matrix, have also been confirmed by the homopolymerization of ε-caprolactone in the presence of HA alone. The modification of HA structure by the substitution of some hydroxyl ions with poly(ε-caprolactone)carboxylate ions in the HA ionic lattice is still under investigation, as well as the influence of the HA annealing temperature on the homopolymerization reaction.

Morphological results coming from SEM investigations on all these samples seem to indicate a high physicochemical compatibility between the inorganic (HA) and organic (copolymeric matrix) phases with increasing firing temperature of the HA powders utilized. The number of clusters formed inside the matrix body increases with concentration, but not their average size which instead slowly decreases. That agrees well with the displacement of the points in Fig. 5. The cluster size increases with temperature of thermal treatment of HA and this is still not well explained. On the other hand, the

formation of the observed wider clusters agrees with the progressive deviation from the lower boundary curve of Fig. 5 with concentration of HA. A possible answer is an interference between molecules of ε-caprolactone and molecules of HA. Differently from utilized PEG (MW = 20 000), which is a wax-like hard solid (m.p. $\approx$ 65–70 °C), ε-caprolactone is liquid at room temperature and wets ceramic HA well. Therefore, it is highly likely to think that ε-caprolactone is the first compound which contacts HA and both have the possibility to promote reciprocal interaction which at last brings to a chemical end chain ionic bond of locally produced PCL with specific Ca^{2+} sites present on the surface of the HA grains. This PCL layer, which surrounds the HA grains and interposes between them and the PCL-POE-PCL matrix, is an extremely positive occurrence since it chemically bonds HA surface of the grains and its chains are perfectly miscible with the surrounding copolymer, so granting a strong overall connection between grains and PCL-POE-PCL matrix.
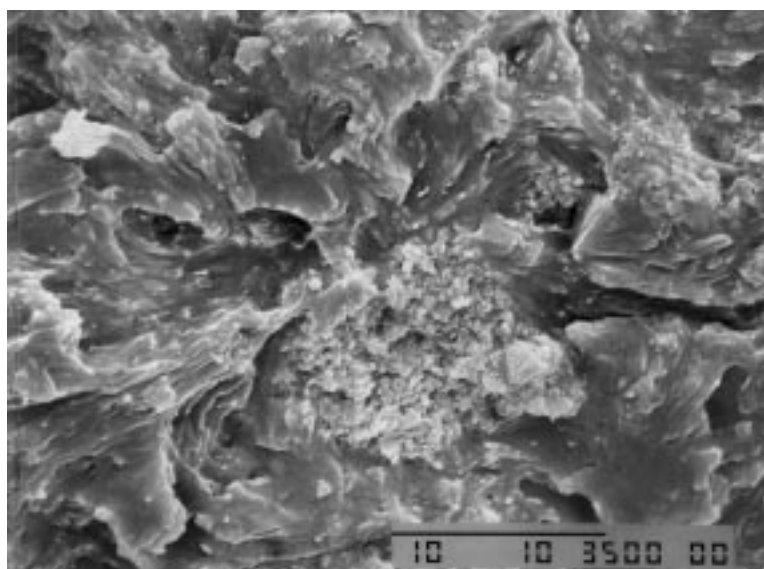

Figure 11 SEM micrograph of a section of a copolymer sample with HA annealed at 900 °C (bar = 10 μm).
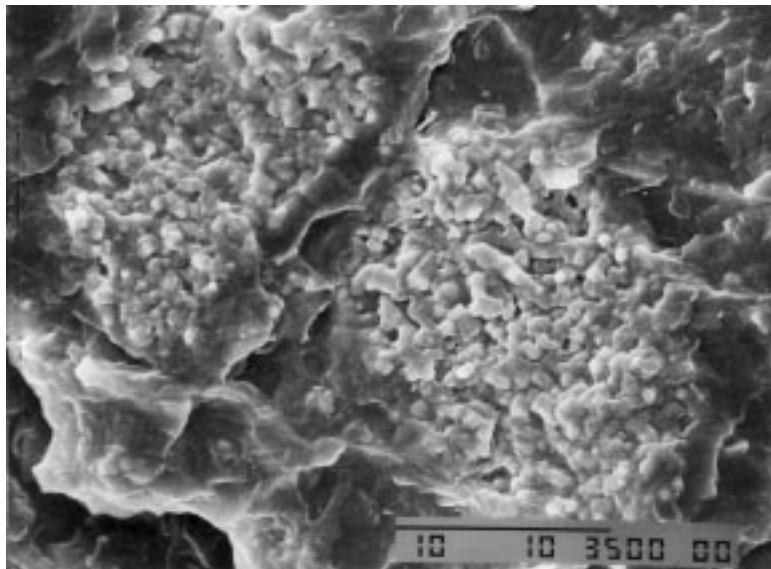
288

Figure 12 SEM micrograph of a section of a copolymer sample with HA annealed at 1100 °C (bar = 10 μm).

5. Conclusions

The composite PCL-POE-PCL copolymer containing ceramics show remarkable mechanical improvement, at least in the presence of HA calcined above 700 °C and appear suitable for different biomedical applications.

The observed mechanical improvements are explained on the basis of the formation of a close bonding between polymeric matrix and HA grains, not only of a physical nature, but also chemical. In detail, the interaction takes place with molecules of ε-caprolactone or PCL thanks to the presence of –OH groups at the surface of HA grains which act both as chain-forming promoters and as their traps in forming a bond. The HA agglomeration in clusters is probably due to a demixing between the PCL surrounding the HA grains with which it is linked and the PCL-POE-PCL that mainly forms in greater and greater amounts as the reaction proceeds at the temperature of synthesis. The reasons for the formation of these clusters are of different nature in respect to the ones purely physical (e.g. wettability).

The obtained product can be considered as a temporary substitute for bone parts to preserve the volume integrity, assuring a sufficient mechanical compression and is bioresorbable. The calcium phosphate grains, beyond their function of reservoir of chemical components, can be used by the organism as suitable nucleation sites for new-bone forming, just when the polymer is locally resorbed.

A possible use of these new composites is the production of biomembranes for guided bone regeneration surgery [10] which is now under study. It is to be underlined that the obtained composite is surely very cheap both for the low cost of the starting materials and for the ease in obtaining the copolymer.

References

1. C. C. P. M. VERHEYEN, J. R. DE WIJN, C. A. VAN BLITTERSWIJK, K. DE GROOT and P. M. ROZING, *J. Biomed. Mater. Res.* **27** (1993) 433.
2. J. E. DEVIN, M. A. ATTAWA and C. T. LAURENCIN, *J. Biomater. Sci. Polym. Edn* **7** (1996) 661.
3. P. CERRAI, G. D. GUERRA, L. LELLI, M. TRICOLI, R. SBARBATI DEL GUERRA, M. G. CASCONE and P. GIUSTI, *J. Mater. Sci. Mater. Med.* **5** (1994) 33.
4. R. SBARBATI DEL GUERRA, C. CRISTALLINI, N. RIZZI, R. BARSACCHI, G. D. GUERRA, M. TRICOLI and P. CERRAI, *ibid.* **5** (1994) 891.
5. R. SBARBATI DEL GUERRA, P. GAZZETTI, G. LAZZERINI, P. CERRAI, G. D. GUERRA, M. TRICOLI and C. CRISTALLINI, *ibid.* **6** (1995) 824.
6. A. KRAJEWSKI, A. RAVAGLIOLI, G. CELOTTI and A. PIANCASTELLI, *Cryst. Res. Technol.* **30** (1995) 843.
7. A. KRAJEWSKI, G. CELOTTI, A. RAVAGLIOLI and M. TORIYAMA, *ibid.* **31** (1996) 637.
8. Z. HASHIN, Proceedings of the Third International Symposium on Ceramic Microstructures: their Analysis, Significance and Production, edited by R. M. Fulrath and J. A. Pask (John Wiley & Sons Inc. Publishers, New York, USA, 1966).
9. G. ONDRACEK, *Mater. Chem. Phys.* **15** (1986) 281.
10. R. MARTINETTI, A. RAVAGLIOLI, A. KRAJEWSKI and C. MANGANO, *Biomateriali* **9** (1995) 43.