Cells and Materials

Volume 5 | Number 1 Article 5

1995

Post-Operative Carbonate-Apatite Formation in PEO/PBT Copolymers (Polyactive®)

A. M. Radder
University of Leiden, The Netherlands

J. E. Davies
University of Toronto

R. N. S. Sodhi
University of Toronto

S. A. T. van der Meer
University of Leiden, The Netherlands

J. G. C. Wolke
University of Leiden, The Netherlands

See next page for additional authors

Follow this and additional works at: https://digitalcommons.usu.edu/cellsandmaterials

 Part of the Biomedical Engineering and Bioengineering Commons

Recommended Citation

Radder, A. M.; Davies, J. E.; Sodhi, R. N. S.; van der Meer, S. A. T.; Wolke, J. G. C.; and van Blitterswijk, C. A. (1995) "Post-Operative Carbonate-Apatite Formation in PEO/PBT Copolymers (Polyactive®)," *Cells and Materials*: Vol. 5 : No. 1 , Article 5.
Available at: https://digitalcommons.usu.edu/cellsandmaterials/vol5/iss1/5

This Article is brought to you for free and open access by the Western Dairy Center at DigitalCommons@USU. It has been accepted for inclusion in Cells and Materials by an authorized administrator of DigitalCommons@USU. For more information, please contact digitalcommons@usu.edu.

UtahStateUniversity
MERRILL-CAZIER LIBRARY

Post-Operative Carbonate-Apatite Formation in PEO/PBT Copolymers (Polyactive®)

Authors

A. M. Radder, J. E. Davies, R. N. S. Sodhi, S. A. T. van der Meer, J. G. C. Wolke, and C. A. van Blitterswijk

This article is available in Cells and Materials: https://digitalcommons.usu.edu/cellsandmaterials/vol5/iss1/5

Cells and Materials, Vol. 5, No. 1, 1995 (Pages 55-62) 1051-6794/95$5.00+.25
Scanning Microscopy International, Chicago (AMF O'Hare), IL 60666 USA

POST-OPERATIVE CARBONATE-APATITE FORMATION IN PEO/PBT COPOLYMERS (POLYACTIVE®)

A.M. Radder*, J.E. Davies[1], R.N.S. Sodhi[1], S.A.T. van der Meer, J.G.C. Wolke and C.A. van Blitterswijk

Biomaterials Research Group, University of Leiden, Prof. Bronkhorstlaan 10, 3723 MB Bilthoven, The Netherlands
[1]Centre for Biomaterials, University of Toronto, Canada

(Received for publication June 7, 1994 and in revised form April 29, 1995)

Abstract

Previous studies have stressed the importance of calcification of the polyethylene oxide (PEO) / polybutylene terephthalate (PBT) copolymer surface by establishing a direct relation with the occurrence of bone-bonding. Here, we characterized the morphology as well as the composition of this post-operative reaction product in PEO/PBT copolymers. X-ray photoelectron spectroscopical results demonstrated the ability of PEO/PBT copolymers to rapidly adsorb calcium ions from fluids. After subcutaneous implantation in rats, it was shown that polymer calcification comprised plate-shaped crystals, whereas an electron-dense layer was frequently encountered at the interface. Cells with a characteristic morphology were directly apposed to abundantly calcified surfaces, indicating the biocompatible nature of the material. Subcutaneous calcification was composed of a carbonate-apatite structure with a crystallinity comparable to bone mineral as demonstrated by Fourier transformed infrared spectroscopy and X-ray diffraction.

It was concluded that the post-operative reaction product in PEO/PBT copolymers is, in morphology and composition, highly comparable to the carbonate-containing apatitic surface layer generated on acknowledged bioactive substrates and similar to the inorganic phase of bone apatite. This may, partially, explain the bone-bonding behaviour of PEO/PBT copolymers.

Key Words: Carbonate-apatite, calcification, bone-bonding.

*Address for correspondence:
A.M. Radder
Biomaterials Research Group
University of Leiden
Prof. Bronkhorstlaan 10, Bldg. 57,
3723 MB Bilthoven
The Netherlands

Telephone number: 31-(30)-288776
FAX number: 31-(30)-288430

Introduction

Bone-bonding is a feature attributed to an array of so-called bioactive materials. Although there is a considerable lack of information on the interfacial reactions preceding the establishment of bone-bonding, it seems generally acknowledged that the generation of a reactive calcium phosphate surface layer is essential. The formation of such an apatitic layer can be accomplished according to different mechanisms. For bioactive glasses and glass-ceramics, it has been demonstrated that mainly the diffusion or leaching of silicon and calcium ions from the bulk of these materials is involved in the formation of an apatitic surface layer (Hench, 1992; Kokubo, 1992). Recently, it was suggested that an hydrated silica layer is generated initially, providing nucleation sites for calcium and phosphate ions (Kokubo, 1992). This finding has directed investigations toward CaP-free silica gels and other hydrogels as bone-bonding substrates (Li *et al.*, 1992; Li and de Groot, 1993). A second mechanism, reported extensively for calcium phosphate ceramics, depends on dissolution and re-precipitation of calcium and phosphate ions and includes processes such as surface degradation and epitaxy (LeGeros *et al.*, 1991; Ducheyne *et al.*, 1992). Experiments designed to characterize the surface layers generated according to the mechanisms described above, reported a similar carbonate-containing apatite composition (LeGeros *et al.*, 1991; Kokubo, 1992). A third mechanism for the formation of a reactive surface layer is proposed for a specific subpopulation of polyethylene oxide (PEO) / polybutylene terephthalate (PBT) copolymers (van Blitterswijk *et al.*, 1992, 1993). This material, which does not contain calcium and phosphate ions initially, demonstrated the ability to absorb these ions from the surrounding fluids (Radder *et al.*, 1993; van Blitterswijk *et al.*, 1992). In this way, a calcified surface is produced which interacts favorably in a bony defect through the establishment of a continuity with the opposing bone (Bakker *et al.*, 1990; van Blitterswijk *et al.*, 1992, 1993). In contrast to the reactive layer of glasses and calcium phosphates, the detailed nature of

the calcified surface layer of PEO/PBT copolymers was not determined.

Since it has been stressed that the exact composition of the reactive surface layer is of importance with respect to the occurrence of bone-bonding (LeGeros *et al.*, 1991; Kokubo, 1992), this study aims to characterize the calcification in PEO/PBT copolymers. To meet this objective, we first carried out an *in vitro* experiment, employing a 55/45 PEO/PBT ratio, to assess the ability of this material to adsorb calcium ions from fluids. Second, an 80/20 PEO/PBT ratio was selected for the purpose of this study, because previous *in vitro* experiments pointed to a high calcification rate for this material (van Blitterswijk *et al.*, 1992). Porous 80/20 cylinders were subcutaneously inserted in the back of rats and the post-operative precipitation within the surface was morphologically, as well as compositionally, analyzed.

Materials and Methods

Materials

PEO/PBT block copolymers (Polyactive®, HC Implants b.v., Leiden, The Netherlands) comprise a soft segment, PEO, and a hard segment, PBT. The molecular weight of the soft (PEO) component was 1000 D. In this study, two different PEO/PBT ratio were used: 55/45 and 80/20.

In vitro evaluation

Granular 55/45 starting material was pressed into dense discs, with a thickness of 0.8 mm. These were evaluated by X-ray photoelectron spectroscopy (XPS) prior to and after rinsing in running tap water for 30 minutes. The analyses were performed by incident- and angle-resolved measurements, using a Leybold Max 200 XPS system, employing unmonochromatised Mg Kα X-ray radiation as an excitation source.

In vivo evaluation

Porous 80/20 cylinders (d = 5 mm, h = 2-3 mm) were sintered from granular starting material. They had a pore size of 300 $\pm$ 150 μm and an inter-pore connection of 150 $\pm$ 50 μm. All cylinders were checked macroscopically for irregularities and sterilized using gamma-irradiation (2.5 MRad). Sterilized implants were subcutaneously inserted in the back of male Wistar rats (weighing 200 grams) and left *in situ* for 6 weeks.

Morphology of the calcification Samples for microscopical analysis were fixed in 1.5% glutaraldehyde in 0.14 M sodium-cacodylate buffer (pH = 7.4). Light microscopic (LM) specimens were dehydrated in a graded series of ethanol and embedded in glycolmethacrylate. Sections, 2-3 μm in thickness, were cut on a Reichert Jung 2050 microtome and routinely stained with toluidine blue and alizarin red or a combination of these staining methods. The remaining blocks were polished with sandpaper and diamond paste, carbon coated, and studied using backscatter electron (BSE) mode in a Philips S525 scanning electron microscope (SEM). Samples for transmission electron microscopy (TEM) were, after fixation, postfixed in an aqueous solution of 1% OsO_4 and subsequently embedded in Epon. Ultrathin sections were processed on an LKB ultramicrotome and examined either unstained or contrasted with lead citrate and uranyl acetate in a Philips EM 400 transmission electron microscope.

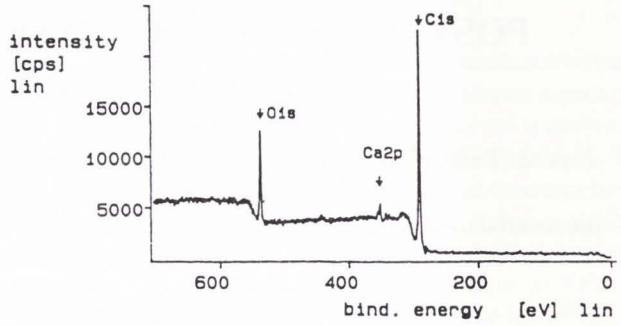


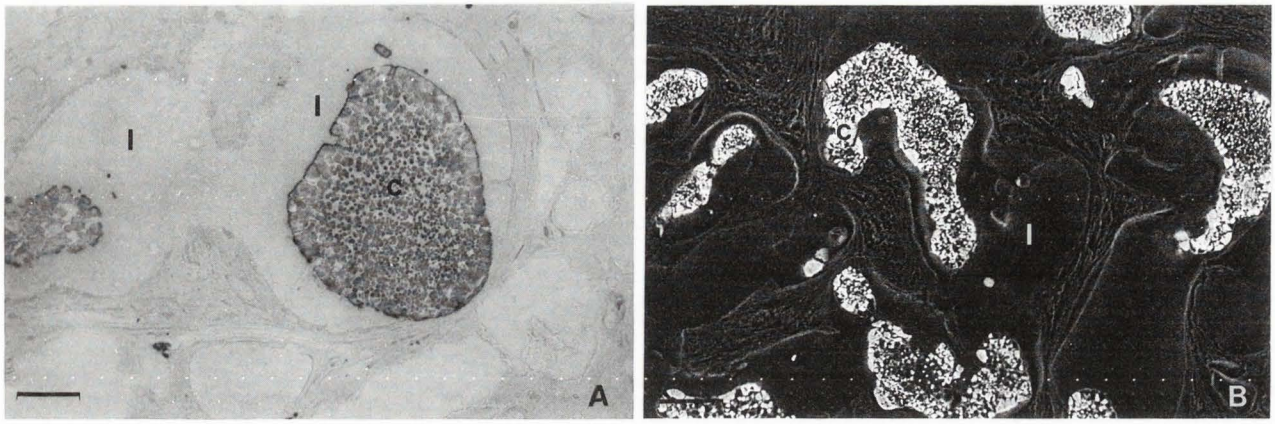
Figure 1. X-ray photoelectron spectrum of a 55/45 disc, incubated in tap water, shows the absorption of calcium ions.

Composition of the calcification BSE-specimens were additionally analyzed by X-ray microanalysis (XRMA, Tracor Northern). Some subcutaneous samples were collected in distilled water, immediately incinerated at 600°C; the remaining ashes were ground for study by Fourier transformed infrared spectroscopy (FTIR) and X-ray diffraction (XRD). Analyzed samples (FTIR and XRD) were then subjected to heat-treatment at 900°C and 1200°C (4 hours each) respectively to obtain information on crystal structure. For both XRD and FTIR, rat femoral bone was used as a control and consequently, put through the same regime as described for the implants.

Results

In vitro experiment

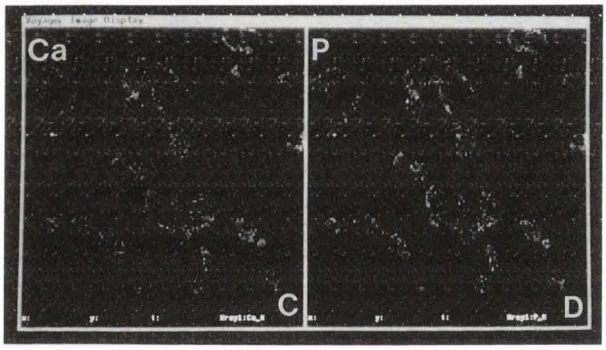
XPS showed the presence of calcium in the spectrum of 55/45 samples that were exposed to tap water (Fig. 1). Such a signal was, in contrast, not detected in control "as received" discs. Furthermore, angle-resolved measurements at 45°C suggested that calcium was actually incorporated in the material rather than a precipitate on the surface.



In vivo experiment

Morphology The toluidine blue stained histological sections revealed distinct peripheral areas within the surface of the 80/20 implants. These areas occurred occasionally as dense zones, but frequently as confined focal spots, which were composed both of dense structures as well as concentrations of individual granules. They stained positively with alizarin red (Fig. 2A) and appeared white in BSE images (Fig. 2B). Elemental analysis by XRMA confirmed the presence of calcium (Fig. 2C) and also demonstrated the presence of phosphorus (Fig. 2D). TEM observations demonstrated a plate-shaped electron-dense crystal composition of the calcified material (Fig. 2E). These crystals varied in length from 25-60 nm and in thickness from 2-3 nm. In unstained and contrasted sections, an electron-dense layer, occasionally a multilayered structure, was frequently observed at the soft tissue interface (Fig. 2E).

Composition XRD-spectra of both the calcified implant material and the control bone samples showed an apatite structure (Fig. 3A). Peak sharpening and peak separation were more evident in implant specimens than in bone mineral. These results were confirmed in FTIR-analysis which revealed the major PO_4^{3-}-absorption bands in the 565-605 and 1030-1040 cm^{-1} regions (Fig. 4A). In addition, we observed v_3 (1400-1500 cm^{-1}) and v_2 (870-880 cm^{-1}) CO_3^{2-}-absorption bands, indicative of a carbonate-containing apatite structure, both for the material calcification and the control bone (Fig. 4A). Furthermore, the spectra were in the v_2 as well as in the v_3 area composed of two characteristic bands. When control and implant samples were subsequently subjected to heat-treatment at 900°C, these carbonate bands were absent in the FTIR-spectra (Figs. 3B and 4B). In contrast, however, tricalcium phosphate (TCP)-bands appeared in all the evaluated specimens. The intensity of these bands varied considerably, although they were in general more prominent in control rat bone spectra (Fig. 4B).



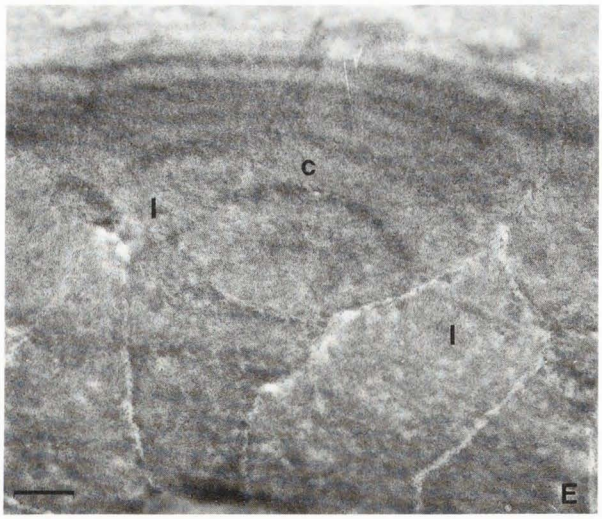
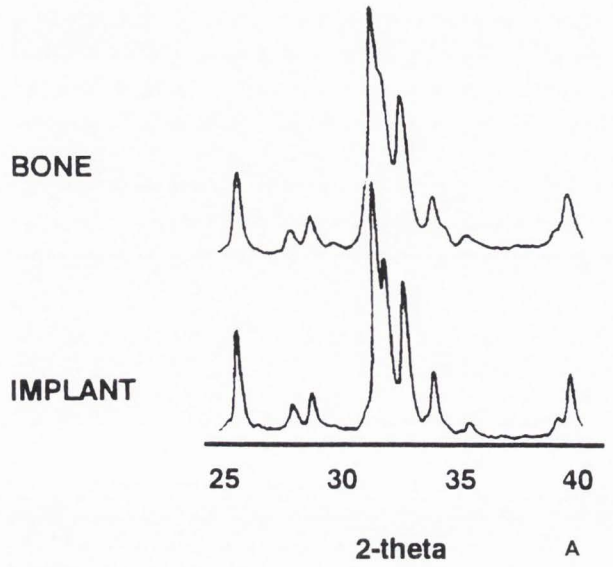


Figure 2. Morphology of the calcification (c) in porous 80/20 implants (I). (**A**) Light microscopy, staining alizarin red, bar = 45 μm. (**B**) Appearance in BSE, indicating a high density of hard material, bar = 125 μm. (**C**) and (**D**) Eemental maps for calcium and phosphorus corresponding to the BSE micrograph in B. (**E**) An electron-dense (multi) layer is observed at the soft tissue interface. In the area of calcification, plate-shaped crystals were easily observed at higher magnification (not shown). Unstained section, bar = 714 nm.



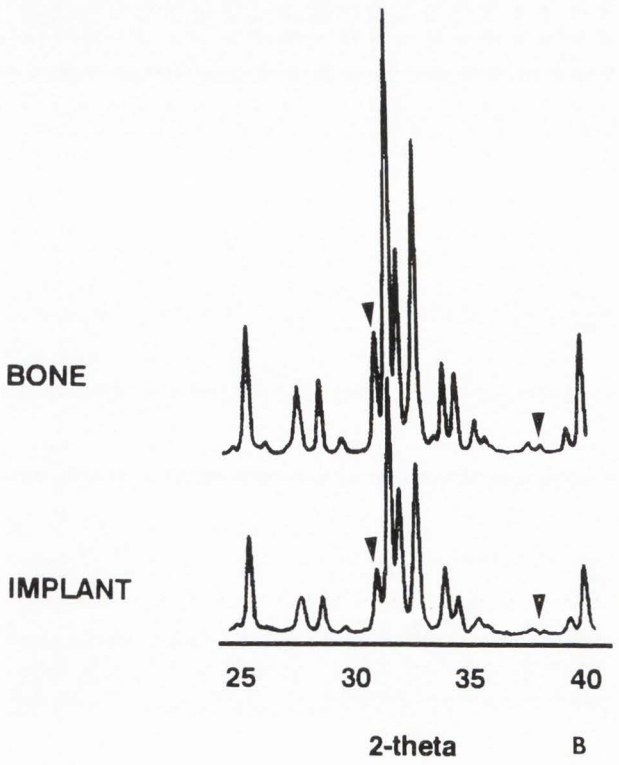


Figure 3. (**A**) XRD-spectra of calcified 80/20 and of rat control bone after incineration at 600°C, showing an apatite structure. The peak separation and peak broadening is more clear in the upper spectrum. (**B**) After heat treatment at 900°C, a TCP-phase (arrows) is observed in both spectra. This phase was calculated at 20% for bone spectra and at 10% for the implant calcification.

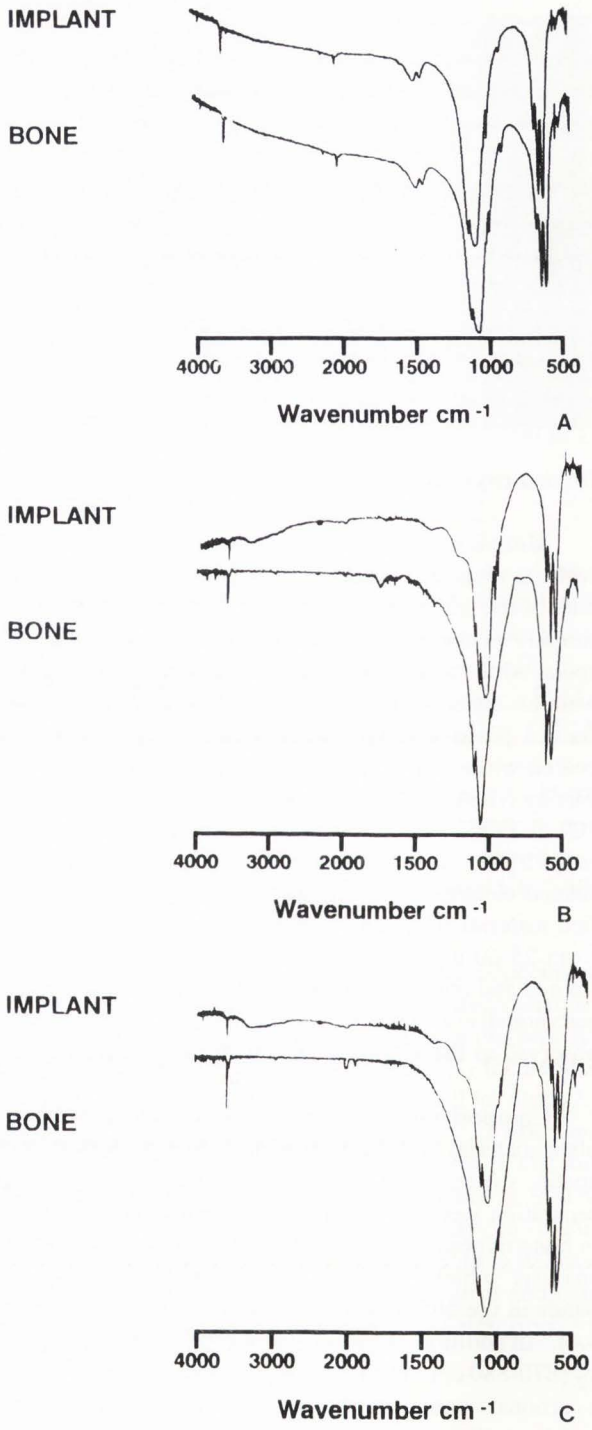
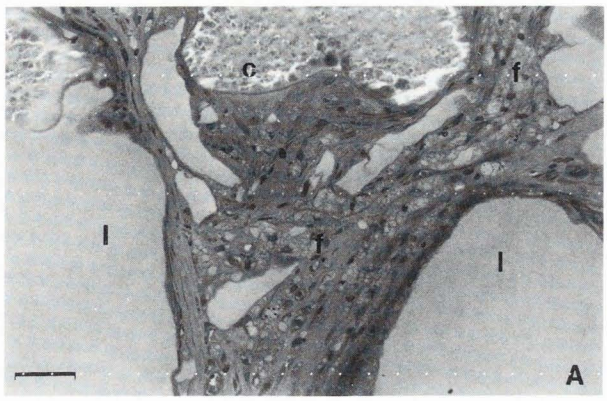
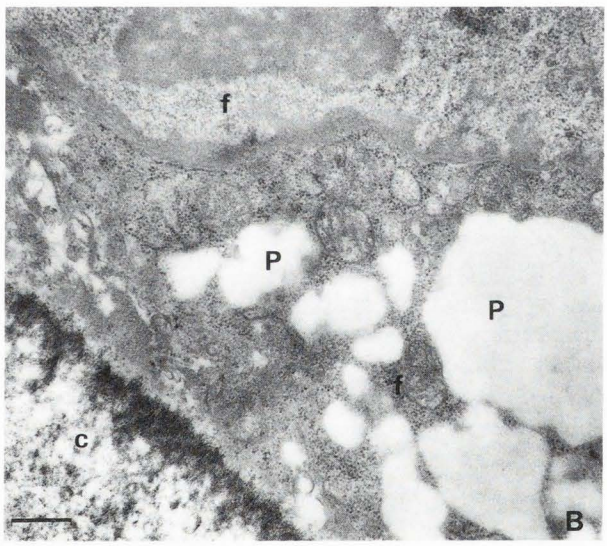


Figure 4. (**A**) FTIR demonstrates a carbonated apatite structure in bone and 80/20 incinerated specimens. Both the v_2 and v_3 CO_3^{2-} absorption bands are shown with a dual character. (**B**) Spectra of the same samples after heat treatment at 900°C. TCP-absorption bands are visible, whereas the CO_3^{2-}-bands have disappeared. (**C**) Heat treatment at 1200°C confirmed the TCP presence. In addition, a small peak is observed at 3540 cm^{-1}, indicative of a fluor-phase.





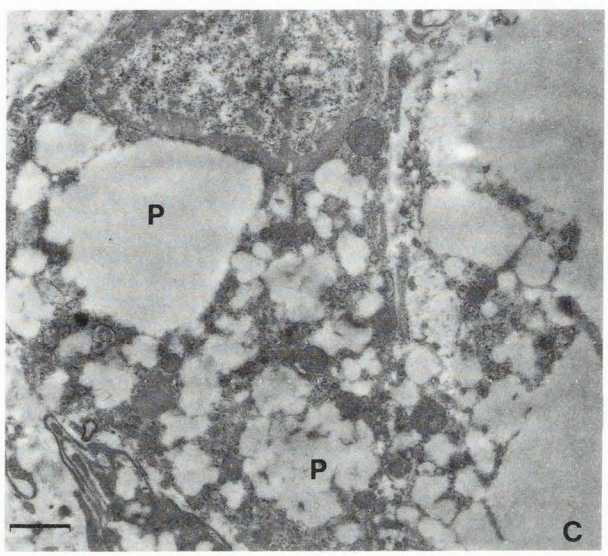


Figure 5 (at right). (**A**) This light micrograph shows the soft tissue reaction to abundant calcification of 80/20 implants (I). Both fibroblastic and cells with a "foamy" appearance (f) are lining the calcified (c) and non-calcified surface. Staining toluidine blue, bar = 45 μm. (**B**) Transmission electron micrograph of an area opposing the calcified surface. "Foamy" cells (f) have phagocytozed large amounts of polymer particles (P), whereas they are in direct contact with the calcified surface (c). Note that polymer particles do not contain electron-dense crystals. Bar = 450 nm. (**C**) Transmission electron micrograph of an area distant from the 80/20 surface, mainly composed of material fragments located at intracellular locations (P). The amount of phagocytozed particles is frequently extensive, but the morphology of the cells is satisfactory. Bar = 1.3 μm.

This observation was semi-quantitatively supported in XRD, which indicated TCP-traces ranging from 20% for bone to 10% for calcified PEO/PBT samples (Fig. 3B). Further heat treatment more clearly demonstrated the TCP-phase and thus underlined the 900°C findings (Fig. 4C). Both bone and implant spectra indicated a band at 3540 cm^{-1}, after heating to 1200°C, which appeared either as a shoulder or as a distinct peak and was not seen at lower temperatures (Fig. 4C). This seems suggestive of a fluor-phase (possibly fluorapatite), but this observation was not further confirmed.

Biocompatibility The partially calcified subcutaneous implants were histologically surrounded by fibrous tissue although, inflammatory cells, frequently with a "foamy" appearance, were also observed at the surface (Fig. 5A). When areas containing such "foamy" cells were viewed in TEM, varying amounts of 80/20 fragments, which also differed in size and shape, were found at intracellular locations (Figs. 5B and 5C). Phagocytozed particles were frequently invaded with electron-dense material, possibly of organic origin. In the same field of analysis, larger fragments were observed concurrently in extracellular spaces (Fig. 5C). Fibroblasts, as well as "foamy" cells, were directly apposed to the calcified outer surface of bulk 80/20 substrates (Fig. 5B). Although cells with apparent phagocytotic capacity were found close to the calcified bulk, intracellular fragments were not crystalline (Fig. 5B). The high number of phagocytozed polymer particles and the abundant presence of calcified material did not result in an adverse cellular reaction. Cells, in contact with the 80/20 implants, displayed intact mitochondria, well-developed Golgi apparatus and a heterogenic chromatin nuclear distribution (Figs. 5B and 5C).

Discussion

In a series of experiments, we have stressed the importance of the relationship between the calcification of the PEO/PBT copolymer surface and the occurrence of bone-bonding (van Blitterswijk *et al.*, 1992, 1993; Okumura *et al.*, 1992). In view of recent reports that have demonstrated the carbonate-apatite composition of the reactive layer formed on acknowledged bone-bonding substrates (LeGeros *et al.*, 1991; Hench, 1992; Kokubo, 1992), this study aimed to assess the morphology and composition of the post-operative reaction product in PEO/PBT copolymers. Copolymers rich in PEO, associated with rapid and abundant calcification, were the materials of choice to meet the objectives set (van Blitterswijk *et al.*, 1992).

It is generally believed that the generation of a reactive surface layer by bioactive materials is an essential determinant for bone-bonding to occur. XPS demonstrated that PEO/PBT copolymers have the ability to rapidly capture and incorporate calcium ions from fluids (Radder *et al.*, 1993). Previously, it had been postulated that the hydrogel properties of PEO/PBT copolymers facilitate this calcium uptake (van Blitterswijk *et al.*, 1992, 1993; Radder *et al.*, 1993). Thoma *et al.* (1987) showed that a molecular weight of 1000 D of the PEO segment, as used in this study, accomplishes an optimal absorption through a chelating effect. This is especially relevant since the surface of polyether-ester copolymers are, compared to the bulk, enriched in the hydrophilic PEO component (Burrell *et al.*, 1990). Absorbed calcium ions may subsequently provide nucleation sites for the growth of calcium phosphate crystals, resulting in the observed calcification. Although calcification behaviour is commonly reported for polymers at soft tissue sites (Thoma and Phillips, 1985) and is therefore independent of mineralization processes, a reactive surface layer seems a critical pre-requisite for bonding in a bony implantation bed (LeGeros *et al.*, 1991; Kokubo, 1992). Thus, while we cannot yet describe the exact mechanisms by which bonding of bone to PEO/PBT copolymers occurs, calcification of the polymer matrix is an essential preparatory step toward bone-bonding.

The calcification of 80/20 implants ultrastructurally comprised electron-dense plate-shaped crystals, which were in morphology and dimensions comparable to the crystals of bone mineral (Weiner and Traub, 1992) and the crystals constituting the surface layer of glasses, glass-ceramics and calcium phosphates (LeGeros *et al.*, 1991; Kokubo, 1992; Neo *et al.*, 1992). A remarkable finding here was the electron-dense structure(s) at the soft tissue interface, since the occurrence of such a layer at bony sites is often considered a definite proof of bone-bonding. This is in line with observations on hydroxyapatite coatings (de Bruijn *et al.*, 1994) and implicates that electron-dense layers are not entirely specific for a bony environment and necessitate careful examination when related to bone-bonding. Cells were found lining the abundantly calcified 80/20 substrates. Several of these cells contained polymer fragments within their cytoplasm which were frequently invaded by organic material suggestive of intracellular resorptive activity. Such cells had maintained their morphological characteristics, indicative of a satisfactory biocompatibility of these implants. Currently, we have no information on whether a high degradation rate influences calcification phenomena.

In a preliminary study, the calcification of 70/30 PEO/PBT implants was evaluated at room temperature by FTIR. This required a subtraction procedure resulting in complex spectra, although both CO_3^{2-} and PO_4^{3-} absorption bands were undoubtedly present (Radder *et al.*, 1993). In view of this finding, it was decided to incinerate retrieved subcutaneous samples prior to compositional measurements. XRD-spectra of control bone and implant calcification showed an apatite structure, with a superior peak separation in the implant spectra. This might be due to a difference in crystallinity and crystal size or possibly to a variation in distribution of organic moieties in the inorganic phase. This last explanation is supported by the fact that the broadening of XRD reflections is only directly related to crystal sizes of perfect crystals (Rey *et al.*, 1991). Especially in the case of solid solutions, like carbonate-apatites, a heterogeneous distribution may create domains with different unit-cell dimensions with maintenance of crystal size (Rey *et al.*, 1991). Differences might alternatively simply be related to a higher TCP component in bone samples, as indicated after heat-treatment. The presence of a TCP and, the suggestion, of a fluor-phase was also encountered after heat-treatment of synthetic non-stoichiometric rod-shaped crystals (Li Yubao, perrsonal communication, 1993). FTIR identified carbonate in the apatite structure. The carbonate bands in both the ν_2 and ν_3 CO_3^{2-} domain show two components. In the determination of carbonate type, especially the ν_2, CO_3^{2-} domain is of interest because the presence of organic substances may interfere with ν_3 domain absorption bands (Rey *et al.*, 1989). The dual character of the ν_2 domain points to the substitution of CO_3^{2-}, both at monovalent OH^- (type A) and trivalent PO_4^{3-} (type B) sites, which was earlier shown in studies on bone mineral (Rey *et al.*, 1989). The CO_3^{2-} absorption bands vanished after heat-treatment due to decomposition, possibly related to the preferential association of CO_3^{2-} with lower crystal sizes (Yubao, 1993). Both the XRD and FTIR data demonstrated a composition of the

post-operative precipitation in 80/20 copolymer which is, as far as the inorganic phase is concerned, comparable to naturally formed bone apatites.

The results obtained in this study led us to conclude that the post-operative reaction product in PEO/PBT copolymers bears a morphological and compositional resemblance to the reactive surface layer formed on acknowledged bioactive substrates and is similar to the inorganic phase of bone mineral. Hence, a third mechanism, calcium and phosphate adsorption by an initially CaP-free (and silica free) material, can be held responsible for the generation of a carbonate-containing apatite layer. The carbonate-apatite layer generated by PEO/PBT copolymers may partially account for the bone-bonding behaviour and the similarities, to glassy and calcium phosphate substrates, suggest an analogy in the mechanisms preceding this phenomenon.

References

Bakker D, van Blitterswijk CA, Hesseling SC, Daems WTh, Grote JJ (1990) Tissue/biomaterial interface characteristics of four elastomers: a transmission electron microscopical study. J Biomed Mater Res **24**: 277-293.

Burrell MC, Bhatia QS, Chera JJ, Michael RS (1990) Surface studies of polyether-ester copolymers and blends. J Vac Sci Technol **A8**: 2300-2305.

de Bruijn JD, Bovell YP, Klein CAPT, de Groot K, van Blitterswijk CA (1994) Structural arrangement att he interface between plasma-sprayed calcium phosphates and bone tissue *in vivo*. Biomaterials **15**: 543-550.

Ducheyne P, Radin S, Ishikawa K (1992) The rate of calcium phosphate precipitation on metal and ceramics and the relationship to bioactivity. In: Bone-Bonding Biomaterials. Ducheyne P, Kokubo T, van Blitterswijk CA (eds.). Reed Healthcare Communications, Leiderdorp, Netherlands. pp. 213-218.

Hench LL (1992) Bioactive bone substitutes. In: Bone Grafts and Bone Graft Substitutes. Habal MB, Reddi AH (eds.). WB Saunders Company, Philadelphia, USA. pp. 263-275.

Kokubo T (1992) Bioactivity of glasses and glass-ceramics. In: Bone-Bonding Biomaterials. Ducheyne P, Kokubo T, van Blitterswijk CA (eds.). Reed Healthcare Communications, Leiderdorp. pp. 31-46.

LeGeros RZ, Orly I, Gregoire M, Daculsi G (1991) Substrate surface dissolution and interfacial biological mineralization. In: The Bone-Biomaterial Interface. Davies JE (ed.). University of Toronto Press, Toronto, Canada. pp. 76-88.

Li P, Ohtsuki C, Kokubo T, Nakanishi K, Soga N, Nakamura T, Yamamuro T (1992) Apatite formation induced by silica gel in a simulated body fluid. J Am Ceram Soc **75**: 2094-2097.

Li P, de Groot K (1993) Calcium formation within sol-gel prepared titania *in vitro* and *in vivo*. J Biomed Mater Res **27**: 1495-1500.

Neo M, Kotani S, Fujita Y, Nakamura T, Yamamuro T, Bando Y, Ohtsuki C, Kokubo T (1992) Difference in ceramic-bone interface between surface-active ceramics and resorbable ceramics: a study by scanning and transmission electron microscopy. J Biomed Mater Res **26**: 255-267.

Okumura M, van Blitterswijk CA, Koerten HK, Bakker D, Hesseling SC, de Groot K (1992) Bone formation process in porous PEO/PBT copolymer (Polyactive®): a histological study of ectopic bone formation induced by rat bone marrow cells. In: Bone-Bonding Biomaterials. Ducheyne P, Kokubo T, van Blitterswijk CA (eds.). Reed Healthcare Communications, Leiderdorp. pp. 189-200.

Radder AM, Davies JE, Leenders H, van der Meer SAT, van Blitterswijk CA (1993) Post-operative carbonate-apatite formation in a polymer matrix: characterization and relation to bone-bonding. In: Bioceramics 6, Proceedings 6th Int Symp on Ceramics in Medicine. Ducheyne P, Christiansen D (eds.). pp. 335-341.

Rey C, Collins B, Goehl T, Dickson IR, Glimcher MJ (1989) The carbonate environment in bone mineral: a resolution-enhanced fourier transform infrared spectroscopy study. Calcif Tissue Int **45**: 157-164.

Rey C, Renugopalakrishnan V, Shimizu M, Collins B, Glimcher MJ (1991) A resolution-enhanced Fourier transform infrared spectroscopic study of the environment of the CO_3^{2-} ion in the mineral phase of enamel during its formation and maturation. Calcif Tissue Int **49**: 259-268.

Thoma RJ, Phillips RE (1985) Calcification of poly-(ether)urethanes. In: Proceedings of the 13th Annual Meeting of the Society for Biomaterials (Minneapolis, MN). pp. 245 (abstract).

Thoma RJ, Hung TQ, Nyilas E, Haubold AO, Phillips RE (1987) Metal-Ion complexation in polymer urethanes. In: Advances in Biomedical Polymers. Plenum Press, New York, USA. pp. 131-137.

van Blitterswijk CA, Bakker D, Leenders H, van den Brink J, Hesseling SC, Bovell YP, Radder AM, Sakkers RJB, Gaillard ML, Heinze PH, Beumer GJ (1992) Interfacial reactions leading to bone-bonding with PEO/PBT copolymers (Polyactive®). In: Bone-Bonding Biomaterials. Ducheyne P, Kokubo T, van Blitterswijk CA (eds.). Reed Healthcare Communications, Leiderdorp. pp. 13-30.

van Blitterswijk CA, van den Brink J, Leenders H, Bakker D (1993) The effect of PEO ratio on degradation, calcification and bone-bonding of PEO/PBT copolymer (Polyactive®). Cells Mater **3**: 23-36.

Weiner S, Traub W (1992) Bone structure: from angstroms to microns. FASEB J **6**: 879-88.

Discussion with Reviewers

T. Kokubo: Both the silica and titania gel also form the carbonate-containing hydroxyapatite on them in the body environment, although they contain neither the calcium nor the phosphate ion, similar to the PEO/PBT copolymers. Do you think that the mechanism of apatite formation is the same for these three kinds of materials?
Authors: It is our opinion that these mechanisms may be similar. In the case of silica gels, a negatively charged OH-rich surface precedes apatite formation. These two factors are considered essential for the formation of carbonate-apatite. In the case of PEO/PBT copolymers, a negatively charged COOH-rich surface will be formed post-operatively. This suggests mechanistic similarities in apatite formation between these materials.

M. Neo: Have you found any collagen fibres on the surface of the materials in this *in vivo* study? If any, what kind of relationship between collagen fibres and the material surface was observed?
Authors: In this study, we have only occasionally found collagen at the interface with the material. From this, we could only conclude that the orientation was mainly parallel to the surface and interdigitation was not seen. We do not know if collagen is important with respect to the bioactivity of PEO/PBT copolymers for two reasons: first, this experiment was executed in subcutaneous conditions and second, collagen interdigitation seems according to recent theories only a secondary effect of bone-bonding; initially, an afibrillar layer of mineralized matrix is deposited on the implant and collagen fibres become anchored into this initial layer.

C.C. Rey: The proposed mechanism of formation of the apatitic layer at the surface of the polymer is indeed very remarkable as, in a first stage, calcium is taken up from the solution and penetrates the copolymer subsurface to continue what seems to be an ion reservoir. This first stage of the reaction is the reverse of what has been proposed by Hench (1992) and Kokubo (1992) for Ca-containing bioglasses where there is a release of calcium ions from the material which causes a local increase of the supersaturation in the solution at the material surface and the precipitation of the apatite. Do phosphate ions also penetrate the polymer as it is briefly mentioned in the introduction? Is the formation of the apatite always associated with a degradation of the polymer surface and is there any evidence of crystal formation (or possibly amorphous Ca-P phase) inside the polymer?
Authors: We have not obtained absorption data on phosphorus as we have them on calcium. It is certainly possible that phosphate penetrates the polymer in ionic form or conversely as a calcium phosphate complex. Clearly, phosphate is present in the calcified areas of the polymer. If a COOH-rich surface is indeed important for the generation of a calcified PEO/PBT implant as stated in the answer to Dr. Kokubo, degradation mechanisms may be helpful. Oxidation processes and hydrolysis, occurring post-operatively, result in such groups. It can therefore be expected that degradation is involved or associated with apatite formation, but we cannot be absolutely sure at the moment. All the crystal formation will take place within the polymer.

C.C. Rey: In their procedures for analyzing the apatitic layer, the authors ash the samples at different temperatures. Although this is a radical way to get rid of the organic matter, extensive alterations of the mineral phase are also produced. In particular, it has been shown by Young *et al.* (1981) that heating type B carbonate apatite above 400°C leads to a partial redistribution of carbonate ions in A sites. Although the interpretation of the authors concerning the carbonate location in both sites in the original crystal appears likely, it cannot be rigorously inferred from the data they present.
Authors: We are aware of a possible redistribution of carbonate ions due to heating. Our conclusions have therefore been limited to stating that we have a carbonate-apatite structure. For the determination of carbonate-apatite type (A or B), improved preparation methods are required. Currently, it is not known if the type of carbonate-apatite is influential in bone-bonding processes.

Additional Reference

Young RA, Bartlett ML, Spooner S, Mackie PE, Bonel G (1981) Reversible high temperature exchange of carbonate and hydroxyl ions in tooth enamel and synthetic hydroxyapatite. J Biol Phys **9**: 1-26.