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A COMPARATIVE STUDY OF THE INTERACTIONS OF TWO CALCIUM PHOSPHATES, PEO/PBT COPOLYMER (POLYACTIVE) AND A SILICONE RUBBER WITH BONE AND FIBROUS TISSUE

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Abstract

In this study, hydroxyapatite, tetracalcium phosphate, HPEO/PBT 55/45 copolymer, PEO/PBT 55/45 copolymer (Polyactive) and silicone rubber were implanted as dense blocks, subcutaneously and into the tibia of rats. Biocompatibility and degradation were investigated but most attention was directed to the bone/biomaterial interactions. None of the materials showed any significant adverse tissue reactions. With exception of the silicone rubber, all materials showed bone bonding phenomena based on both morphological and mechanical evaluations. (H)PEO/PBT 55/45 copolymer is the first polymer reported to be bonded by bone and thus widens the spectrum of bone bonding materials with a low modulus, degradable, elastomer in contrast to the high modulus glasses and ceramics that are available to date. The possible associated bone-bonding mechanism is briefly discussed.

Key Words: Bioactive, bone, bone-bonding, calcium phosphate ceramics, degradation, interface, polyactive, subcutis, calcification.

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Introduction

To date, two different approaches have been used to obtain so-called bone-bonding or bioactive biomaterials. In the first approach, a silicate matrix either in the form of a glass or a glass ceramic, with the addition of several ions, provided a bone-bonding substrate [21, 26]. In the second approach, calcium phosphate ceramics were used [20, 23]. Initially hydroxyapatite [20, 23] and tricalcium phosphate [7, 24, 25, 29] were predominantly investigated as bone bonding agents followed by several other calcium phosphate crystal structures [17, 24]. Even biphasic calcium phosphate ceramics are currently being investigated [15, 16]. Most authors consider the above materials to be the only alloplastic bone bonding biomaterials available with the possible exception of titanium, to which a certain bone-bonding activity has been ascribed, but only after long term implantation [18].

All bone bonding alloplastic biomaterials that are available belong to the category of materials with a high elastic modulus and brittle fracture behavior. Therefore, the spectrum of bone bonding biomaterials is quite confined with regard to the mechanical properties. The manufacture of composites made of a matrix with a low elastic modulus, to which a bone bonding agent has been added as a filler or coating, has proven to be feasible in broadening the spectrum [12, 36]. The mechanical spectrum of bone-bonding biomaterials would be widened even further by the availability of a bone-bonding elastomeric polymer which would not obtain its bone-bonding properties from a filler or coating.

Most polymers do not bond to bone. On the contrary, several authors consider the presence of a fibrous tissue zone at the bone-biomaterial interface as a characteristic property of polymers [22]. Even though some authors have reported the apparent absence of such a fibrous tissue zone at the polymer/bone interface [39], such observations were usually based on light microscopy and not electron microscopy, which should be considered a prerequisite for determining a so called "direct contact" at the interface.

Recent studies by Bakker *et al.* [1], however, demonstrated that a Poly(ethylene oxide-hydantoin) Poly(butylene terephthalate) segmented copolymer

(HPEO/PBT 55/45) was characterized by a "direct contact" at the bone/biomaterial interface, as assessed by transmission and scanning electron microscopy, after the implantation of porous films of this polymer near the bone of the middle ear bulla of the rat. Furthermore, transmission electron microscopy demonstrated that decalcified sections of the bone/biomaterial interface were characterized by an electron dense layer, similar to and showing continuity with the lamina limitans of bone [1]. Such a "bonding zone" has been described by several authors for the hydroxyapatite/bone interface [8, 14, 19, 21, 31]. In addition to these electron microscopical findings, the light microscopical data seemed suggestive for bonding osteogenesis to occur, as described by Osborn [30], in contrast to the distance osteogenesis expected for a polymer.

Since the findings on porous films of this HPEO/PBT 55/45 copolymer in the rat middle ear were suggestive of a bone-bonding capacity, we decided to investigate these properties further. This report presents the data of such a study in which we compared the interactions in hard and soft tissue of HPEO/PBT 55/45 copolymer, PEO/PBT 55/45 (a similar polymer without hydantoin), two calcium phosphates (hydroxyapatite and tetracalcium phosphate) and silicone rubber. The calcium phosphates were considered as bone bonding controls whereas the silicone rubber served as a control with satisfactory biocompatibility but lacking bone bonding capacity. All materials were implanted as relatively smooth blocks, subcutaneously and into the tibia of rats. Bone bonding, degradation rate and general biocompatibility were assessed.

Materials & Methods

Implant materials

In this study, five materials were used. First, two types of calcium phosphate ceramic i.e., hydroxyapatite and tetracalcium phosphate. Second, two different poly-(ethylene oxide)/poly(butylene terephthalate) segmented block copolymers (Polyactive™, HC Implants bv) with (HPEO/PBT) and without (PEO/PBT) hydantoin segment. The molecular weight (MW) of the PEO segment was 1000 Dalton (D) and the overall MW, as determined by gel permeation chromatography, was ~ 100 kD. Third, silicone rubber (Dow Corning MDX 4-4210 clean grade elastomer). All materials were implanted as dense, smooth, blocks (1.5 x 1.5 x 1.5 mm). The polymer had no detectable porosity and the and the microporosity of the ceramic was less than 3%.

Implantation site and procedure

The materials were implanted in the rat, subcutaneously and through the cortex of the tibia. For the subcutaneous implantation procedure, a skin incision was made and subsequently a pocket was created by blunt preparation. In the tibia, a hole was drilled which was slightly wider than the actual size of the implant resulting in an initial loose fit. One implant was placed into each subcutaneous pocket and tibia. In every experimental animal used in this study, two subcutaneous pockets were created and in addition each tibia received an implant. The implants were evaluated after 3, 6, and 26 weeks. At each interval, 6 specimens of every material were implanted in both sites, resulting in a total of 180 implants in 45 rats.

Evaluation techniques

The implant/tissue interactions were assessed by light microscopy, transmission electron microscopy (Philips 201 and 400), scanning electron microscopy (Cambridge Stereoscan 180 and Philips SEM 525), backscattered electron microscopy and X-ray microanalysis (Tracor Northern 2000 and Tracor Voyager). Bone-bonding force was estimated by pull out testing using a Hounsfield 25 KN mechanical testing machine.

Implant evaluation: For light microscopy (LM), the implants and surrounding tissue were fixed in 1.5 % glutaraldehyde in 0.14 M sodium cacodylate, (4°C, pH 7.4), dehydrated in a graded ethanol series and embedded in glycomethacrylate (GMA). Sections (10 μm) were made using a Reichert Ultracut 2000 microtome, and were stained with toluidine blue, sudan black (stains the copolymers) and alizarin red (stains for calcium).

Specimens destined for traditional scanning electron microscopy (SEM) were fixed and dehydrated as described above and were then critical point dried and sputter coated with gold.

Implants for transmission electron microscopical (TEM) examination were post- fixed with 1% osmium tetroxide for 30 minutes at room temperature after the initial glutaraldehyde fixation and were then dehydrated and embedded in Epon. Ultrathin sections were cut using a LKB ultramicrotome.

Samples for X-ray microanalysis were sputter coated with carbon in case scanning and/or back scattered electron microscopical analysis were required at a later stage. In the latter case, both Epon blocks and polished GMA blocks, already used for sectioning, were analyzed, as were light microscopical sections.

In studying samples in a bony implantation bed, most specimens were decalcified prior to embedding in 10% ethylenediaminetetraacetic acid, EDTA (pH 7.4) in a Biorad laboratory microwave oven. However, in order to study the non-decalcified interface, several specimens did not receive this decalcification procedure.

During the pull out study, the samples were continuously humidified in order to prevent shrinkage of the PEO/PBT hydrogels. Omission of this procedure (due to an initial underestimation of the swelling capacity of the polymer) at the 3 week interval caused mechanical failure of the bone/ PEO/PBT copolymer interface due to the shrinkage forces. A routine pull-out rate of 1 mm/min was used. The tip of the samples was mechanically fixed in a specifically designed clamp.

Results

Since biomaterial tissue interactions are largely affected by the surface texture of an implant, the surface of the five implant types was assessed by SEM prior to implantation. Essentially two different surface textures could be distinguished. The (H)PEO/PBT copolymers and silicone rubber implants revealed a smooth surface with very few irregularities at the ultrastructural level. In contrast, both calcium phosphates were characterized by a much rougher surface which, although apparently smooth at lower magnification, was composed of scaly structures caused by the milling procedure necessary to shape the implants (Figs. 1a and b).

Subcutaneous implantation

General tissue reactions: The general tissue reactions, as observed with light microscopy, near the different implant types were rather similar. At the three week interval, the implants were surrounded by a loosely organized fibrous tissue intermingled with inflammatory cells, predominantly macrophages. With the increase of time, the amount of inflammatory cells decreased and the tissue became more organized. In contrast to the surrounding tissue, which was still relatively loose, a thin zone was observed in the vicinity of the implant surface, in which collagen fibers were organized parallel to the biomaterial/tissue interface and fibroblasts assumed a similar orientation. Transmission and scanning electron microscopy confirmed these findings (Fig. 2) and showed no deviating morphology of the cells.

Interface reactions: All materials were characterized by confined areas with macrophages and multinucleated cells at their surface, alternated by areas without such a cell layer between the collagen network and the biomaterial. Although the thin intervening cell layer was generally visible by light microscopy, it was more clearly detected by transmission electron microscopy.

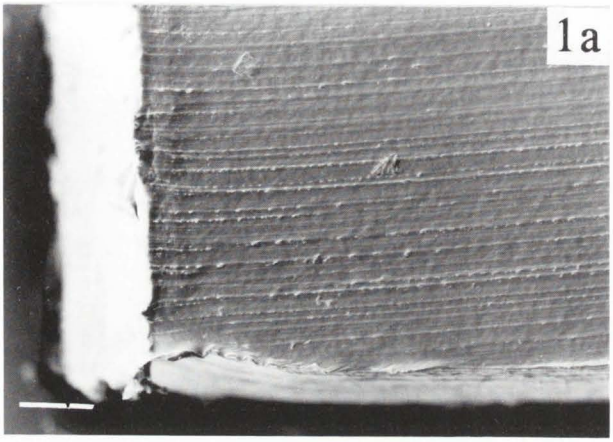
The cells at the silicone rubber interface showed the least phagocytic activity, that is, no implant derived material was found in the cytoplasm of these phagocytes by transmission electron microscopy. The situation was slightly different for both calcium phosphate ceramics (Fig. 3a). Here, albeit not very prominent, phagocytosis of electron dense material was seen. X-ray microanalysis showed peaks of both calcium and phosphorus in these inclusions demonstrating the implant-origin. The situation at the HPEO/PBT and PEO/PBT copolymer/fibrous tissue interface deviated from the previous findings in that phagocytosis, especially after longer implantation intervals, was a prominent finding. Quite different from the other three materials, both copolymers were characterized by a strong phagocytosis with foam-like cells, apparently filled with phagocytosed implant material, well visible with light microscopy. Transmission electron microscopy also showed many phagocytes loaded with polymer fragments (Fig. 3b). These fragments varied considerably in size and, on several occasions, the orientation and shape of the fragments was such that the scene was suggestive for fragmentation that continued intracellularly. The large quantity of intracellular fragments, although causing a change in cell shape, did not cause any morphological changes pointing to a toxic reaction. The ultrastructural morphology of the cell organelles such as nucleus, mitochondria and rough endoplasmic reticulum remained normal.

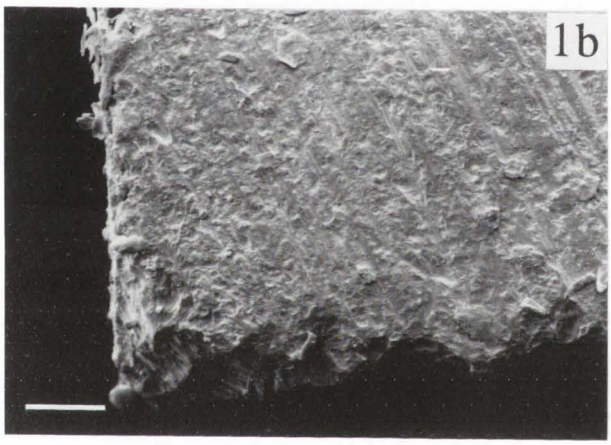
The difference in surface reactions of the five materials was indicative of a variation in degradation rate. The absence of phagocytosed material near the silicon interface already pointed to non-detectable degradation. This was confirmed by analysis of the surface by means of scanning (Fig. 4a) and transmission electron microscopy. None of these techniques revealed any noteworthy changes of the silicone rubber surface. The degradation phenomena at the implant surface was much more prominent for both copolymers. The outer zone of these polymers revealed fragmentation which became more prominent with the increase of time. Although these phenomena could be most clearly observed by scanning electron microscopy (Fig. 4b), they could also be observed by transmission electron microscopy since cell cytoplasm protruded into the interfragment spaces. In the case of the calcium phosphate ceramics, some changes could be observed, although large areas maintained their original ultrastructure. Scanning electron microscopy revealed that parts of both ceramics were covered with a granular material while in other areas the initially scaly structure had disappeared, revealing the outline of the particles of the starting powder (Fig. 5a). Transmission electron microscopy did not reveal any major changes of the hydroxyapatite surface but crevice formation at the tetracalcium phosphate surface seemed to indicate a higher degradation rate (Fig. 5b).

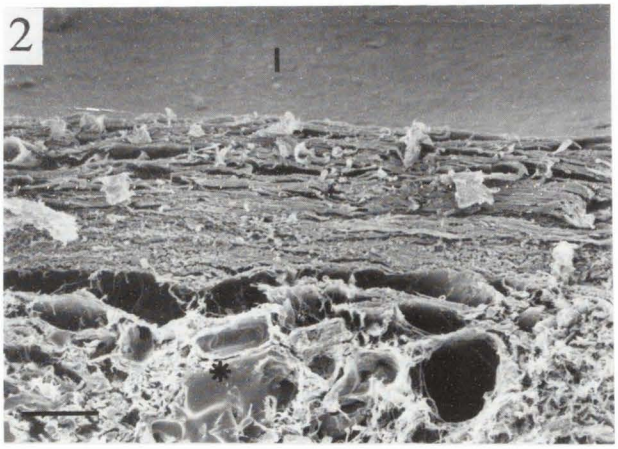
Calcification: The use of toluidine blue frequently revealed positive staining within the HPEO/PBT and PEO/PBT copolymers. With silicone rubber, such staining was never seen. The use of alizarin red on the copolymers demonstrated that these areas contained calcium (Fig. 6a). Analysis of similar light microscopical sections by back-scattered electron microscopy showed a white area (Fig. 6b) which gave distinct calcium and phosphorus peaks as indicated by X-ray microanalysis using single spot measurements and line scans (Figs. 6c, d). This was observed at each of the implantation intervals, predominantly in the form of calcified zones parallel to the surface of the implant and mostly at several micrometers away from the interface with the surrounding tissue. Towards the interior of the blocks, relatively dense calcified zones were observed which developed into calcified spots, suggesting that calcification started by spot-formation.

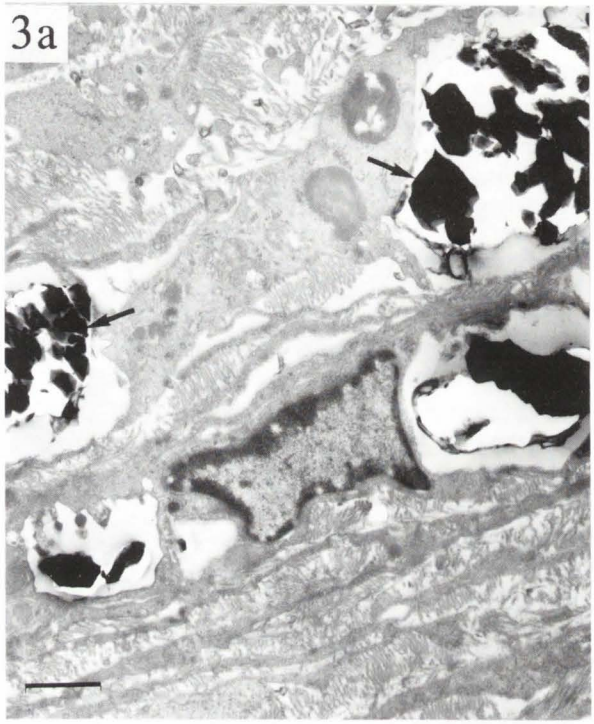
Implantation into the tibia

General tissue reactions: In the course of time, the reactions of bone to the five implant materials showed some general phenomena. Using light microscopy, new bone deposition was observed at the edge of the created defect and after only three post-operative weeks, bone was present near the surface of the implants. In









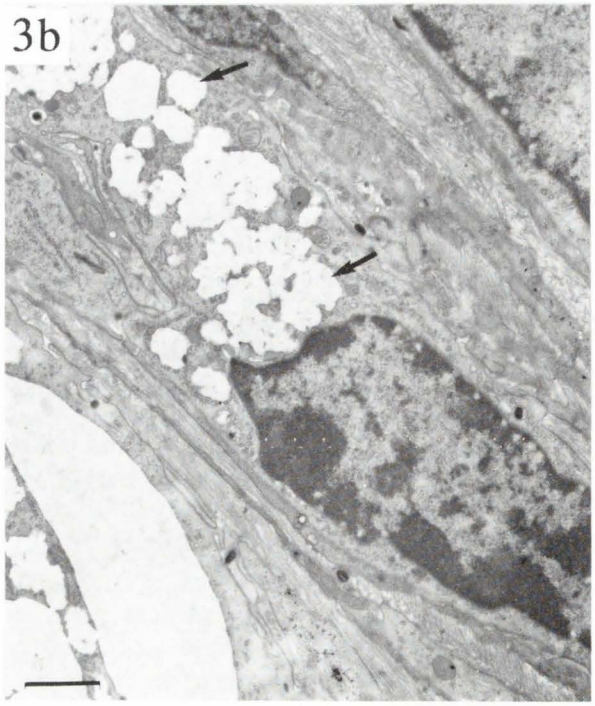
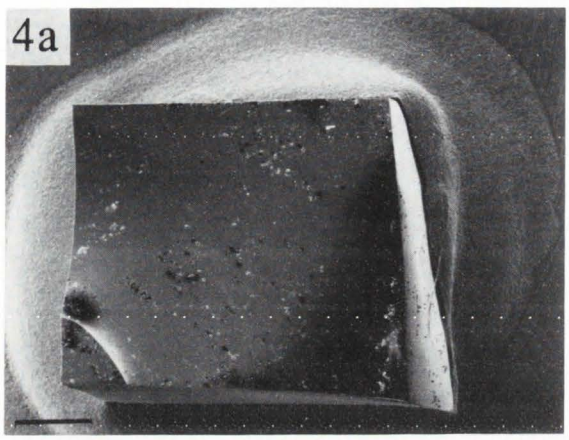
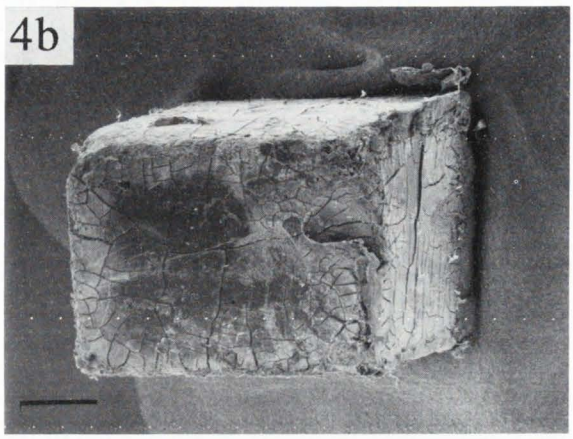


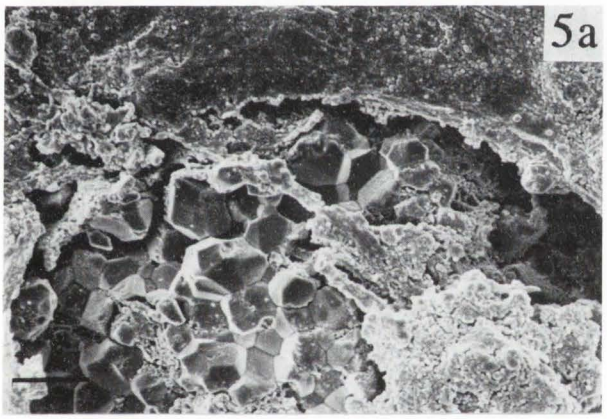
Figure 1. **a)** Scanning electron micrograph of the surface of a 55/45 PEO/PBT copolymer prior to implantation. **b)** The surface of hydroxyapatite before implantation as seen by scanning electron microscopy. Bars = 0.1 mm.

Figure 2. Scanning electron micrograph of HPEO/PBT copolymer 26 weeks after subcutaneous implantation. Note the fibrous tissue reactions near the implant/tissue interface. * = implant. Bar = 60 μm.

Figure 3. Transmission electron micrographs showing: **a)** phagocytosed electron dense material (arrows) within the cytoplasm of a phagocyte near a hydroxyapatite implant; and **b)** cytoplasm of a phagocyte filled with PEO/ PBT fragments (arrows). Bars = 1.3 μm (in a) and 1.6 μm (in b).







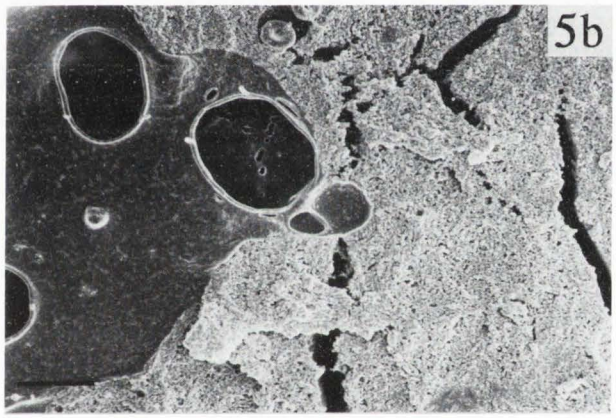


Figure 4. Scanning electron micrographs of: **a)** the surface of Silastic 26 weeks after subcutaneous implantation, no significant changes can be observed; and **b)** the surface of PEO/PBT copolymer 26 weeks after subcutaneous implantation; note the prominent crack formation. Bars = 0.5 mm.

Figure 5. Scanning electron micrographs of the two types of calcium phosphate implants. **a)** Hydroxyapatite implant showing the outline of the particles of the starting powder. Bar = 5.9 μm. **b)** Tetracalcium phosphate showing distinct crack formation at its surface. Bar = 10.5 mm.

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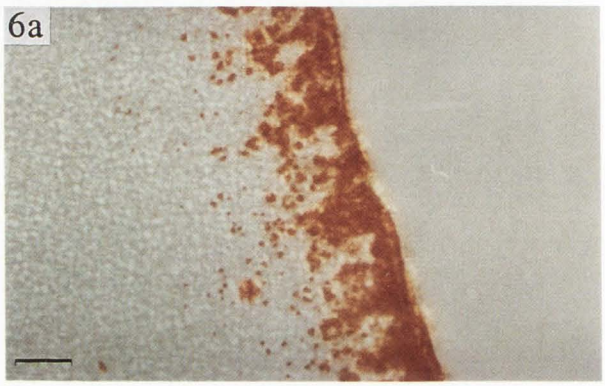
the medullary cavity, seams of new bone ran along the interface alternated by areas of fibrous tissue or sometimes marrow. The amount of bone near the implants increased with time and at the 26 week interval, large parts of the implants were covered by bone (Figs. 7, 8).

Interface reactions: Although the general bone reactions versus the various implant materials seemed to be rather similar, the reactions at the interface with bone showed several characteristic differences. In case of the silicone rubber, higher light microscopical magnifications frequently demonstrated an intervening fibrous tissue zone with some inflammatory cells in areas where, at lower magnifications, bone seemed to be directly deposited onto the silicone rubber surface. This finding was confirmed by transmission electron microscopy which clearly demonstrated a fibrous zone which varied in thickness from several cells separated by collagen fibers to only collagen fibers. Areas where bone seemed to be in intimate contact with the implant surface were confined and only seldom encountered. The situation at the interface of the two calcium phosphates was quite different. In spite of the fact that areas with an interposed fibrous tissue layer were also seen, an intimate contact between bone and the ceramic was often seen when using transmission electron microscopy. The extent of such zones increased with implantation time. In most cases, the decalcified bone/ceramic interface was characterized by an electron dense layer that could either be a monolayered or multilayered structure (Figs. 9a, b). A continuity of this electron dense zone and the lamina limitans of bone was sometimes observed. No noteworthy differences between the bone/biomaterial interactions of hydroxyapatite and tetracalcium phosphate were seen other than that the interface with bone of the latter was more prominently covered with a bilayered electron dense layer.

Light microscopy at lower magnifications suggested that large areas of the copolymers were covered with bone and that the extent of these areas increased with time. Evaluation of these areas at higher magnifications, however, showed that part of these zones still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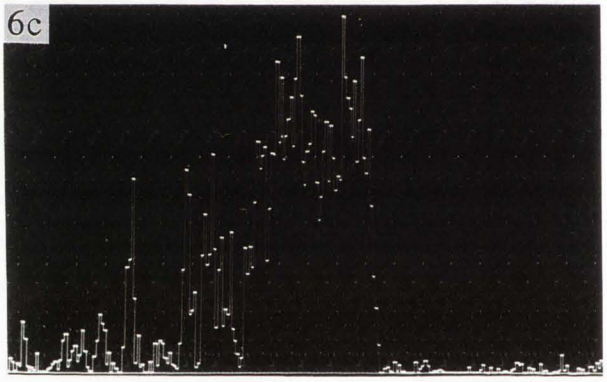
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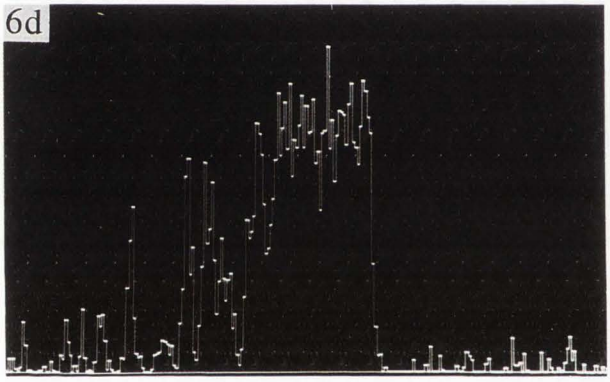
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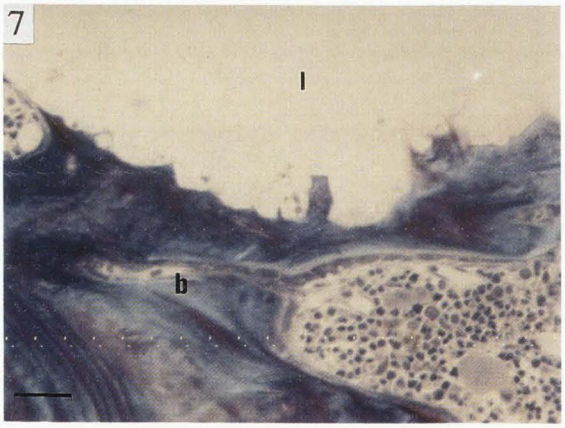
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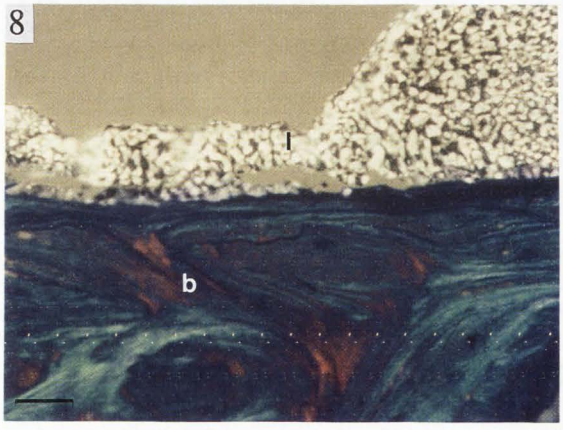
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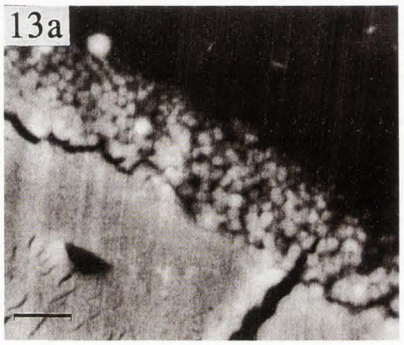
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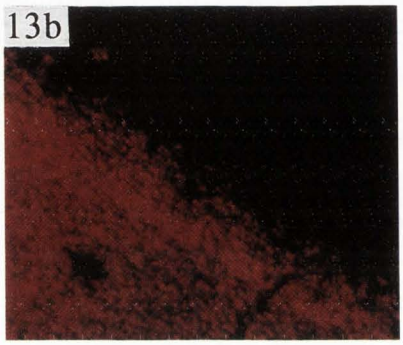
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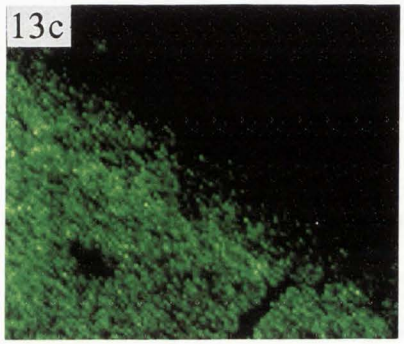
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Figure 6 (facing page top). **a)** Light micrograph revealing the positive alizarin red staining for calcium within 55/45 PEO/PBT copolymer. Bar = 25 μm. **b)** Back-scattered electron micrograph of a light microscopical section of HPEO/PBT copolymer at its tissue interface. Note the white deposits. Bar = 5.9 μm. **c)** Line scan of the area shown in Fig. 6b revealing the presence of calcium. **d)** Similar linescan as in Fig. 6c revealing the presence of phosphorus.

Figure 7 (facing page middle left). Light micrograph of the decalcified bone/tetracalcium phosphate interface (toluidin blue staining). Note the irregular implant surface. Bar = 70 μm. I = implant; b = bone.

Figure 8 (facing page middle right). The decalcified PEO/PBT-bone interface as seen by light microscopy. An intimate contact between bone (b) and the copolymer (I) is observed (toluidin blue stainging and polarized light). Bar = 70 μm.

Figure 9 (at right). Transmission electron micrographs of the decalcified calcium phosphate/bone interface. I = implant; b = bone. **a)** The hydroxyapatite/bone interface 3 weeks after implantation. An electron dense layer can be seen (arrows). **b)** A multilayered electron dense structure at the tetracalcium phosphate/bone interface 3 weeks after implantation. Bars = 0.6 μm.

Figure 13 (facing page bottom). Back-scattered electron micrograph (**a**) and X-ray maps, indicating the presence of calcium (**b**) and phosphorous (**c**), of a 55/45 PEO/PBT copolymer after 26 weeks. Bar = 7.7 μm.

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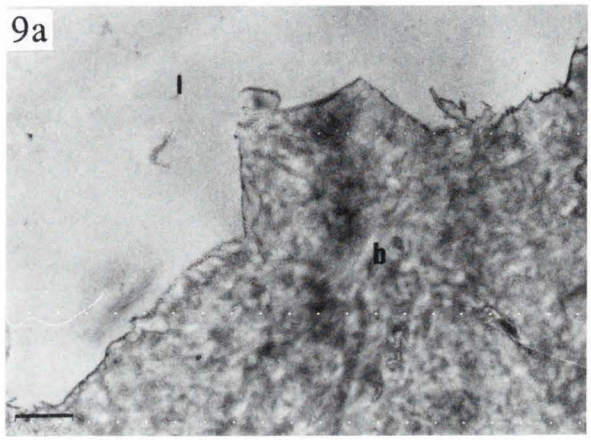
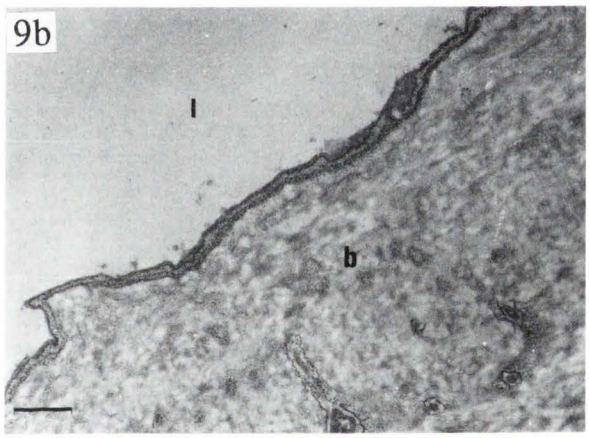
contained an interposed cellular zone. It should be emphasized however, that in contrast to the silicone rubber, such an intervening zone seemed to be absent quite frequently which was confirmed by transmission electron microscopy that revealed an intimate bone/ copolymer contact in many cases. Furthermore, the cellular zone at the bone/copolymer interface differed in morphology from that seen near silicone rubber. In the latter situation, the zone consisted of cells with typical fibroblast morphology, collagen fibers, and inflammatory cells. With the copolymers, however, the cell density was much higher, lacking the relatively large amount of collagen, and the morphology of the cells deviated from normal fibroblasts and/or phagocytes. The cell cytoplasm was much more electron dense than normally observed with normal fibroblasts or macrophages and the cells showed large quantities of rough endoplasmic reticulum (Fig. 10). The cells were not dissimilar in morphology from the osteoblasts seen at areas of bone deposition activity. With increasing implantation time, the amount of copolymer fragments in this cellular zone increased. In the absence of a cellular layer at the bone/ copolymer interface, the situation resembled that found for the calcium phosphate ceramics. Bone was in intimate contact with the copolymer surface and an electron dense layer was frequently seen similar to that at the calcium phosphate ceramic / bone interface (Figs. 11, 12, 13; note Fig. 13 is on color plate at page 16).





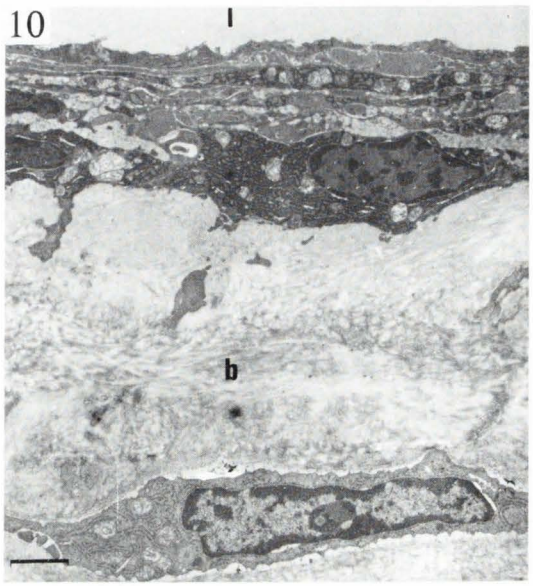
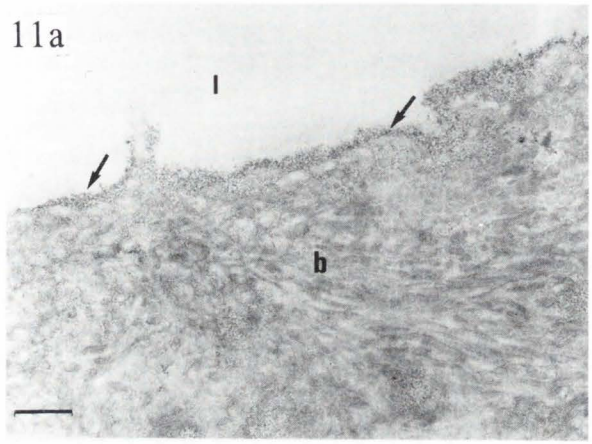
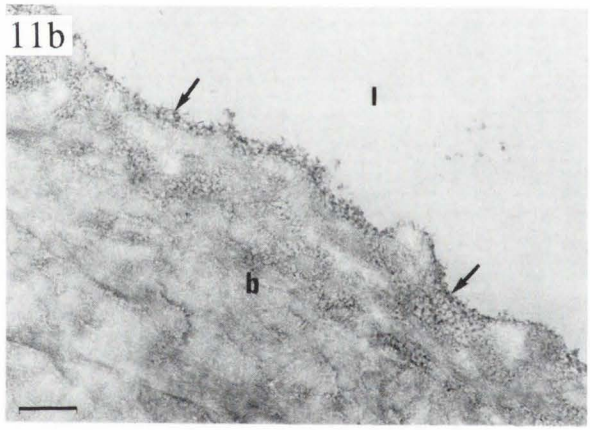
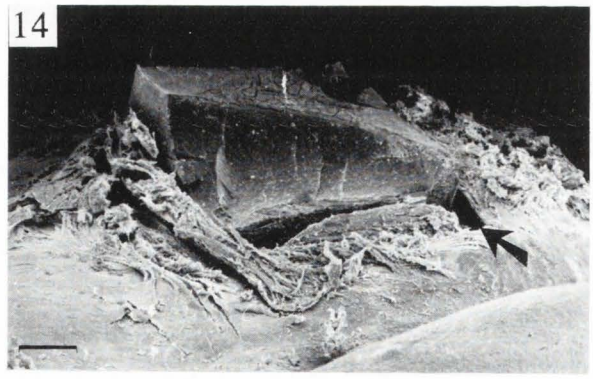


Figure 10. Transmission electron micrograph of the bone/PEO/PBT copolymer interface with an interposed cellular layer. Note the abundant rough endoplasmic reticulum. I = implant; b = bone. Bar = 1.4 μm.

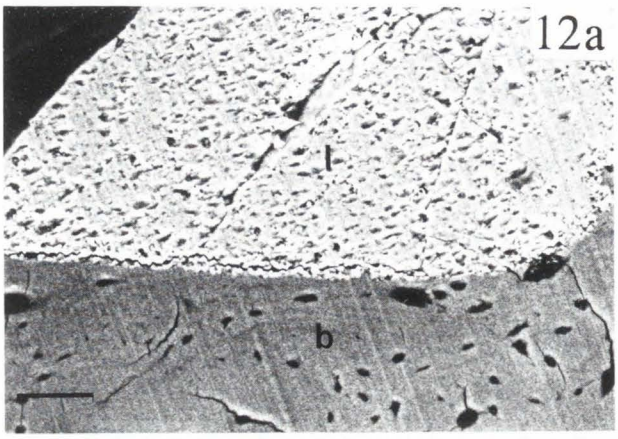






Pull out experiment: The pull out experiment could only be properly performed at the 6 week interval as the 3 week implants were not continuously humidified during the pull out tests. This resulted in shrinkage of the (H)PEO/PBT copolymers which ruptured the biomaterial/tissue interface at three of the four sides of contact with bone. Although minor pull out forces could still be assessed they were not considered to be representative of the actual situation. Based on previous experiments, the degradation of the (H)PEO/PBT copolymers was expected to be such that pull out studies were no longer feasible at the 6 month period. Extensive fragmentation of

Note: Figure 13 on color plate, page 16.



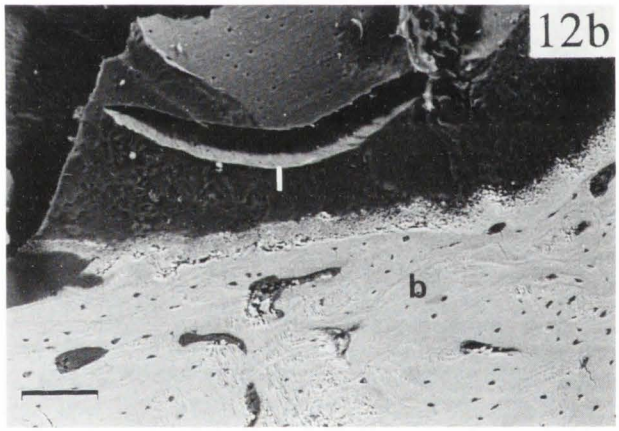


Figure 11 (top left). Transmission electron micrographs of the decalcified (H)PEO/PBT copolymer/bone interface. I = implant; b = bone. **a)** Electron dense layer (arrows) at the interface of HPEO/PBT copolymer with bone. Bar = 0.4 μm. **b)** A similar electron dense structure as shown in Fig. 11a but now for PEO/PBT copolymer. Bar = 0.2 μm.

Figure 12 (above). Back-scattered electron micrographs of the non-decalcified bone/biomaterial interface. I = implant; b = bone. **a)** The hydroxyapatite/bone interface. Bar = 47 μm. **b)** The PEO/PBT copolymer/bone interface. Bar = 91 μm.

Figure 14 (at left). Scanning electron micrograph revealing a dense 55/45 PEO/PBT implant in the tibia of the rat 6 weeks after implantation. Note the site of implant failure (arrows) caused by the pull-out experiment. Bar = 0.4 mm.

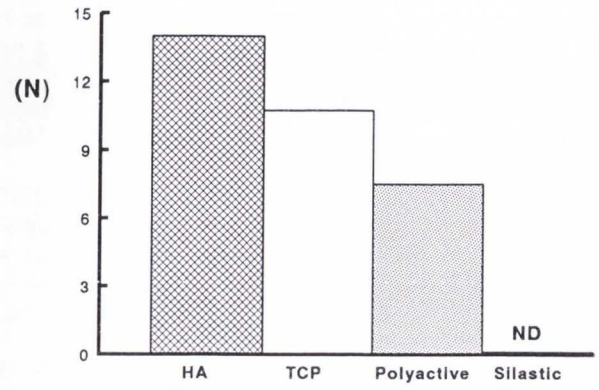


Figure 15. Pull-out values obtained for the different biomaterials at six weeks post-operation. ND = non-detectable.

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the polymers during handling for embedding confirmed this presumption. Furthermore, no new information was expected on the calcium phosphates at this interval.

The pull out experiment performed on the 6 week specimens is summarized in Figure 15 and essentially showed that no mechanically tight bonding was obtained with the silicone rubber while all other materials showed substantial bone-bonding. The pull-out forces, with exception of the silicon rubber, represent the values obtained at the point of implant failure i.e., the implant failed before it detached from its interface with bone (Fig. 14).

Discussion

All materials investigated in this study showed satisfactory biocompatibility as far as the inflammatory response was concerned. This observation was valid for both the implantation in the tibia and the subcutis. In the case of hydroxyapatite and silicone rubber [2], this finding was in accordance with several reports in the literature [9, 11, 16, 23, 25]. The amount of data on tetracalcium phosphate [10, 24] is more confined but the composition of the material and its similarity with other calcium phosphate ceramics seem to explain the favorable biocompatibility found in this study. In the case of HPEO/PBT 55/45 and PEO/PBT 55/45 copolymer, few references are available which allow comparison of the data derived from this study. However, Bakker *et al.* [1-4] performed several studies directed towards biocompatibility assessment of porous films of HPEO/PBT 55/45 and did not find any significant adverse effects and in general a similar behavior as seen with estane 5714 F1 polyetherurethane and Dow Corning silicone rubber (Silastic™). Comparable findings were shown by Beumer *et al.* [6] when investigating bilayers of PEO/PBT 55/45 copolymer as a substrate for cultured keratinocytes [6]. Also Wagener *et al.* [37] confirmed the biocompatibility findings by means of a tissue culture agar overlay test. Most of these reports concerned HPEO/PBT 55/45 copolymer and not PEO/PBT 55/45 copolymer. The study reported here suggests that omitting the hydantoin segment did not affect general biocompatibility in a noteworthy way.

Study of the phagocytosis of particles by cells near the implant surface combined with an analysis of the material surface structure predominantly by scanning and transmission electron microscopy, revealed different reactions. The silicone rubber showed virtually no visible degradation as indicated by the continuing smoothness of its surface and the absence of material fragments in surrounding phagocytes. These findings correspond with reports by several authors describing the absence of degradation with silicone rubber, however, some reports describing degradation have been published [27, 35]. The slight degradation of hydroxyapatite found in this study is in general accordance with findings in the literature [8, 11] although deviating results can be found. The apparently higher degradation rate of tetracalcium phosphate, as indicated by an increasing surface roughening, was noteworthy and may have been caused by impurities such as a high calcium oxide content. Both HPEO/PBT 55/45 and PEO/PBT 55/45 showed significant degradation at their surface during the evaluated interval. Crack formation occurred and fragments of material detached leading to an extensive phagocytosis of particles by phagocytes in the vicinity of the implant. This degradation did not result in a prominent inflammatory response and the intracellular morphology remained intact in spite of the sometimes abundant presence of phagocytosed polymer fragments. These findings were to be expected since earlier studies showed an intermediate degradation rate of HPEO/PBT copolymer as compared to other biomaterials [5].

The interactions with bone of the five materials are most clearly described by the pull out study performed at six weeks postoperatively. Hydroxyapatite, tetracalcium phosphate, HPEO/PBT 55/45 and PEO/PBT 55/45 showed significant pull out forces while silicone rubber did not bond to bone. It should be emphasized that the four bone bonding materials did not detach at their interface with bone but fractured before detachment could occur. Unfortunately, it is not possible to compare the bone bonding capacity of the different materials based on the pull out values. First, because the materials themselves fractured and not the interface with bone and second because the different mechanical properties of the polymers as compared to the ceramics (low elastic modulus versus high elastic modulus) did not allow such a comparison. Interpretation of the pull out data is also complicated by the different degradation rates observed for the various materials, that may have affected the implant surface texture and thereby increased the mechanical attachment factor in this study.

Investigation of the bone/biomaterial interface did confirm the pull out data presented in this study. With exception of the silicone rubber, all materials showed an intimate contact with bone at their surface. Furthermore the morphology of the interface was very similar to that

described for hydroxyapatite [8, 23, 31]. Hydroxyapatite is characterized by an electron dense layer at its surface which shows continuity with the lamina limitans of bone [32] and is composed of an organic matrix incorporating calcium phosphate crystals [8, 28] . This layer was observed for both hydroxyapatite, tetracalcium phosphate, HPEO/PBT 55/45 and PEO/PBT 55/45 copolymer. It varied in thickness and, in the case of tetracalcium phosphate, a multilayered structure was mostly seen.

Although the bone bonding mechanism of calcium phosphates is still not clear, it seems to be related to the presence of calcium and phosphate in the ceramic which may lead to an epitaxy between biological and alloplastic calcium phosphate crystals [13, 34]. A similar phenomenon occurs with Bioglass and glass ceramics [21, 26, 29]. It is therefore interesting to note that, in spite of the initial absence of calcium and phosphorus in the HPEO/PBT and PEO/PBT copolymer, bone-bonding occurred. This is most probably related to the calcification within the polymer surface, which was seen after both subcutaneous implantation and implantation in bone. The impregnation of the polymer surface with calcium phosphate crystals, as described in this study, may partially explain the mechanical strength of the bond with bone since in this way a micromechanical interlocking between the polymer surface and crystals occurs. Calcification may have been induced by the PEO fragment. Polyethers with a molecular weight of 1000 Dalton have been shown to absorb calcium ions [37]. It is questionable, however, whether calcification of the polymer matrix is the only driving force in the bone bonding that was observed. Although many polymers calcify, few reports on bone bonding of such polymers have been published. Winter described ectopic bone formation in poly(hema) gels after subcutaneous implantation in pigs [38]. Poly(hema) is a hydrogel known to calcify and although this study has never been repeated it may be that a combination of swelling (due to water uptake) and calcification would create favorable conditions for bone/biomaterial interactions relating to bone-bonding since PEO/PBT 55/45 is also known as a hydrogel.

In summation, it can be concluded that both HPEO/PBT 55/45 and PEO/PBT 55/45 copolymer bond to bone. Since the hydantoin segment is not necessary for bone bonding and is reported to have teratogenic effects, it should be omitted in future studies. Although the morphology of the bone/biomaterial interface of both polymers was comparable to that of the calcium phosphates, the bonding mechanism must have been different due to the initial absence of calcium and phosphate in the copolymers prior to implantation. Both polymers showed an intermediate degradation rate which did not cause a prominent inflammatory response or other noteworthy adverse effects.

Our future studies will be dedicated to the role of PEO contents in the polymer both as far as weight fraction and molecular weight is concerned.

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Authors' late addition: Sautier *et al.* have also observed an electron dense layer between dextran beads and bone in culture which might be representative for bonding, although lack mechanical data (Biomaterials, 1992, **13**, 400-402; and Calcif. Tissue Int. 1992, **50**, 527-532).

Discussion with Reviewers

J.M. Sautier: The electron dense layer observed at the interface between bone and Ca-P ceramic have been postulated to be composed of remnant of an organic matrix incorporating apatite. Do you have some information concerning the composition of this organic matrix and its possible role in bone/bonding mechanisms?

Authors: In some studies that were performed by our group in the past, we demonstrated that the organic matrix was at least partially composed of glycosaminoglycans. This was assessed on both hydroxyapatite

ceramic and PEO/PBT copolymers. The exact role of this structure in bone-bonding is not as yet clear. However, as demonstrated in the accompanying paper in this issue, the morphology of the structure seems to be related to the amount of polymer calcification, suggesting that its morphology somehow reflects the extent and speed of bonding.

J.M. Sautier: In Figure 9b, you describe a multilayered structure. Did you observe this structure only at the tetra-calcium phosphate / bone interface; and how do you explain this particular organization? In addition, the electron dense layer observed at the copolymer/bone interface seems to be more granular than the one observed on the Ca-P ceramic. Could you please further elaborate on this point?
Authors: In this study, the multilayered structure was indeed mostly characteristic for the tetra-calcium phosphate. It should be emphasized, though, that such a multilayered structure has also been seen with other types of ceramics (like hydroxyapatites) and PEO/PBT copolymers in other studies. Due to the relative complexity of this structure, and the variation in morphology depending on the location, it is difficult to state whether one zone is more granular than the other or not. We currently feel that a more prominent electron dense layer is indicative of more intense interfacial interactions related to bonding (a wider exchange zone).

T. Kitsugi: It is necessary to provide detailed characterization of starting materials. How much is the roughness of the surface, and the mechanical strength of the copolymer? It is very difficult to define the irregularities of the surface from the morphological observation of SEM.
Authors: In this paper we did not specifically measure the roughness of the implant surface. Due to the difference in modulus of elasticity between the polymers and the ceramics, a thorough comparison of the push-out data was almost impossible to begin with. Therefore, we considered a superficial scanning electron microscopic analysis of the surface more than sufficient. The surface roughness of this class of polymers, as prepared by various processing techniques, is currently the subject of another specific study and the preliminary data suggest that the polymers are substantially smoother as compared to the ceramics that are usually used in these implantation studies.

T. Kitsugi: It is very difficult to define the crevice formation. There is a possibility that the crevice was made during the processing of samples. There is a possibility of artifact.
Authors: This might be true. But even if it is an artifact it is characteristic for tetra-calcium phosphate after long-term implantation.