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Abundant postoperative calcification of an elastomer: matrix calcium phosphate–polymer composite for bone reconstruction

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In this experiment the behaviour of an 80/20 PEO/PBT copolymer in bone defects was assessed. Porous cylinders were press-fit inserted into the diaphyseal femur of goats and evaluated by light and electron microscopy and X-ray microanalysis. The most important finding in this study was that almost complete calcification throughout the implant was displayed after 26 weeks. The 80/20 PEO/PBT copolymer did not contain calcium and phosphorus prior to implantation, however, it apparently has the ability to take up considerable amounts of calcium and phosphate postoperatively, resulting in a calcium phosphate–polymer composite. As a consequence of the high calcification rate of 80/20 cylinders, bone-bonding (a continuum between calcification of the material surface and bone) was encountered as early as 3 weeks after implantation. Union of the 5 mm defect was observed at 6 weeks and ingrowth was frequently centripetal. After 26 weeks bone tissue occupied most of the pore area and was often seen in continuity with the calcified polymer. We conclude that an elastomeric matrix that is capable of abundant postoperative calcification behaves favourably with respect to the repair of bone defects. Porous 80/20 PEO/PBT copolymer is, therefore, a promising alternative for bone-replacement applications.

1. Introduction

An array of biological and synthetic materials is available for the reconstruction of osseous defects. Current research on the application of synthetics has largely focused on substrates with bone-bonding properties [1]. Such properties have mainly been attributed to calcium phosphate ceramics, glasses and glass-ceramics [2–4]. These materials form a strong interfacial bond with bone tissue through the generation of a carbonate–apatite surface layer [4–6].

In addition to the aforementioned, generally acknowledged bone-bonding biomaterials, a polyethylene oxide (PEO)/polybutylene terephthalate (PBT) segmented copolymer (Polyactive^{R}) has recently been introduced [7–10]. Postoperatively, calcium and phosphate ions precipitate within the materials surface and a continuum with the opposing bone tissue is established [7–10]. The degradation characteristics of PEO/PBT copolymers are advantageous over non-resorbable materials, where a second surgical intervention is frequently necessary in order to remove the implant [8, 9]. By variation of the contribution in weight of the two individual segments, a range of PEO/PBT proportions with different mechanical and biological characteristics can be synthesized [8–11]. Previous implantation studies in bone, with a range of PEO/PBT proportions (from 70 to 30% of the PEO component), demonstrated a direct relation between PEO content, water uptake, calcification, bone ingrowth rate and the occurrence of bone-bonding [8–11].

This preliminary study assessed the behaviour of porous implants of a recently synthesized 80/20 PEO/PBT proportion in bone defects. Special emphasis was placed on calcification rate, bone-bonding and bone ingrowth. *In vitro* studies have indicated a water uptake for the 80/20 ratio of more than 70% and a calcification percentage, in weight, of more than 20 [9]. Since water uptake and calcification are considered to be important determinants for bone-bonding, an 80/20 proportion is expected to be more bioactive in bone than the PEO/PBT copolymers evaluated to date [9].

2. Materials and methods

2.1. Implants

An 80/20 PEO (molecular weight (MW) = 1000)/PBT proportion was investigated. Porous cylinders (d = 5 mm, h = 7 mm) with a pore size of 300 ± 150 μm and an interpore connection of 150 ± 50 μm were sintered from granular starting material. The morphology of the pore structure was studied on random cross-sections using scanning electron microscopy (SEM, Phillips S525 Fig. 1). All implants were checked macroscopically and gamma-irradiated (2.5 MRad), prior to implantation.

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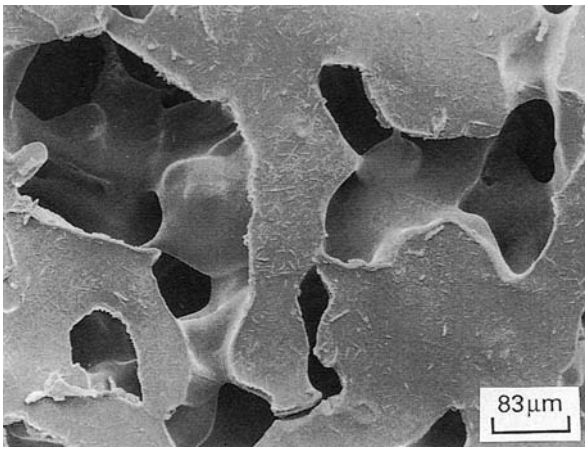

Figure 1 SEM showing an 80/20 implant in cross-section.

2.2. Experimental design

The implants were unitranscortically inserted in the lateral cortex of the right diaphyseal femur of ten mature Dutch goats (the surgical procedure and animal specifications are described elsewhere [10]). The cylinders were press-fit implanted into the 5 mm defects by gentle tapping. At each survival time of 3, 6, 9, 12 and 26 weeks, two animals were sacrificed and the femora retrieved.

2.3. Microscopy

The retrieved implants were fixed at 4 °C in Karnovsky's fixative (5% paraformaldehyde, 4.5% glutaraldehyde, pH = 7.4) and embedded in methyl methacrylate (MMA) for light microscopy (LM). Undecalcified sections were prepared with a modified innerlock diamond saw, through the central area and along the longitudinal axis of the implants, and were subsequently stained with methylene blue and basic fuchsin. The remaining MMA-blocks were polished with diamond paste, carbon coated and evaluated using backscatter electron microscopy (BSE, Phillips S525) and X-ray microanalysis (XRMA, Tracor Northern).

The bone dynamics in the pore structure were investigated by fluorochrome labelling. Per goat, one dose each of tetracyclin (20 mg/kg i.v.) and calcein (10 mg/kg i.v.), was administered during the postoperative course, at different intervals.

3. Results

3.1. Animals and surgery

All goats recovered well from the surgical intervention and a rapid return to a physiological gait pattern was seen. Manipulation of the implants during surgery and insertion of the cylinders into the created defects was straightforward.

3.2. Morphology

3 weeks: The inflammatory reaction to the insertion of the implants was mild. The pores were filled with a combination of fibroblasts, fat cells and exudate. A high degree of contact between implant and the cortex was seen, although bone ingrowth into the pores was fairly limited and was restricted only to the periphery of the cylinders (Fig. 2a). Occasionally, confined focal areas, often composed of clusters of individual spots, were observed within the implant surface (Fig. 2b). These spots reflected in BSE and consisted of calcium and phosphorus, as indicated by XRMA, and were therefore identified as calcification. Such calcification spots within the implant surface were sometimes in contact with bone tissue (Fig. 2b). At those locations, a continuous calcium and phosphorus signal through the interface between these two compartments, was detected.

6 weeks: The intramedullar part of the implants was surrounded by adipose tissue and a layer of fibroblasts. The cortical reaction at 6 weeks was more extensive. Bone tissue occupied large parts of the peripheral pores and ingrowth extended into the central part of the cylinders, that is, union of the 5 mm defect was accomplished within 6 weeks (Fig. 3a, b). The direction of bone formation in the pores was frequently centripetally oriented, as observed morphologically with LM and confirmed with fluorochrome labelling (Fig. 3c, d). A higher degree of calcification within the surface of the material and focal contact between these calcification spots and bone tissue was encountered, in comparison to the 3-week survival time. The occurrence of calcification and bone formation at the material surface seemed closely associated, especially in the bulk part of the implants.

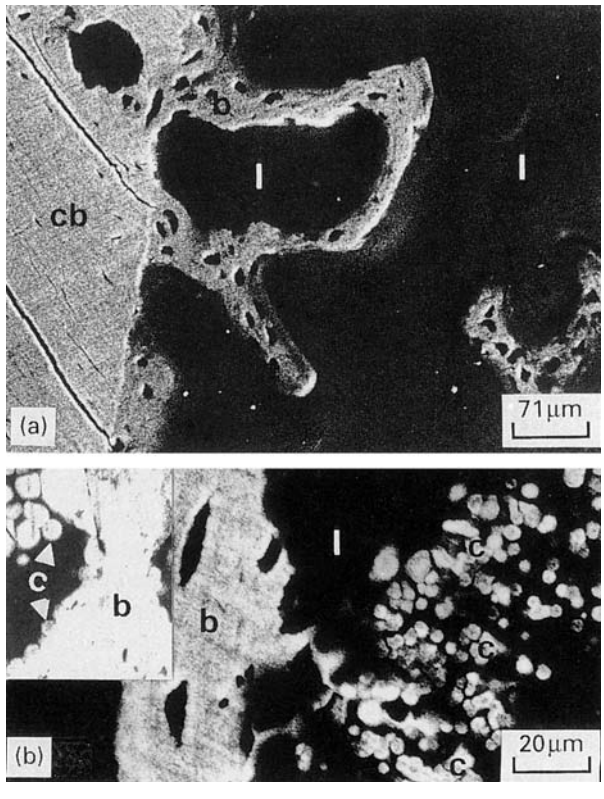

Figure 2 (a) BSE micrograph of bone (b) ingrowth at 3 weeks I = implant, cb = cortex. (b) Higher magnification of a similar area to that shown in (a). Calcification spots (c) are visible within the material (I) surface. Inset: contact between calcification and bone.

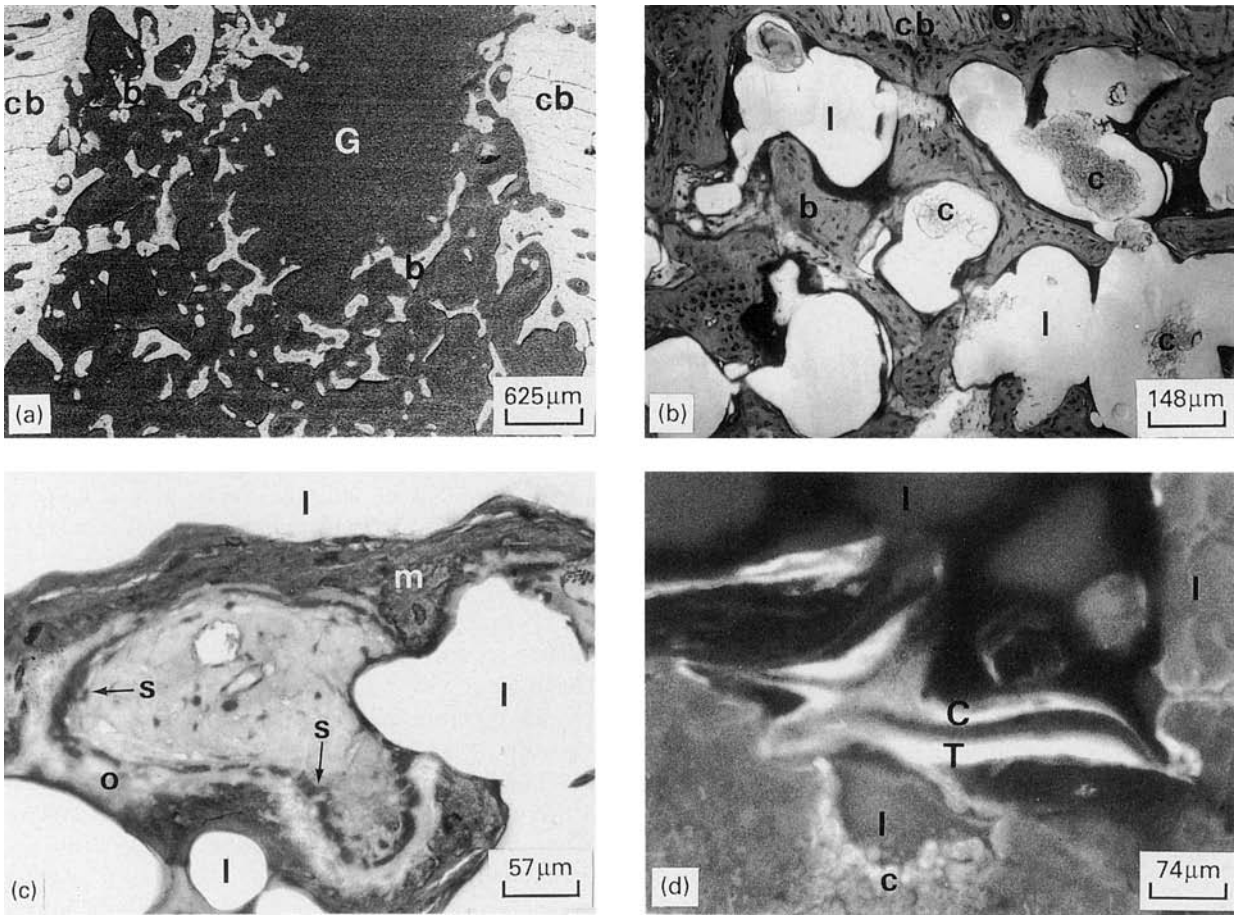

Figure 3 (a) Low power BSE micrograph showing the extent of bone ingrowth (b) from the cortical sides (cb) at 6 weeks. A gap (G) indicates a partial loss of the implant during the processing procedure. (b) LM-overview of the peripheral part of the implant (I) in Fig. 3a. Bone (b) occupies most of the pores. Note the dense calcification (c) areas within the material surface. cb = cortex. (c) Higher magnification of a centrally located pore. Mineralized tissue (m) is lining the implant (I) surface, while towards the centre of the pore osteoid tissue (o) and a seam (s) of osteoblasts are visible. (d) Fluorochrome labelling shows tetracyclin (T, 4 weeks) near the implant (I) surface and calcein (C, 5 weeks) towards the centre of the pore, c = calcification of the implant.

9 weeks: The 9-week results corroborated the 6-week observations, although the bulk part of the implants showed a more excessive ingrowth pattern. The first signs of material degradation, restricted to the part of the implants that penetrated the marrow cavity, were observed. Peripheral fragmentation, in combination with surface erosion, was evident. Loose fragments were encountered in fibrous tissue and surrounded by macrophages.

12 weeks: A substantially higher degree of bone tissue was observed in the pores, compared to 9 weeks postoperatively (Fig. 4a, b), and the material showed extensive calcification. Bulk particles were densely calcified at their periphery, while calcification in the centre was more granular (Fig. 4b, e). Bone-bonding, a continuous calcium and phosphorus signal through the interface, as shown by XRMA, was markedly increased (Fig. 4c, d). At this survival time, degradation of the material had progressed. Larger fragments had detached from the material surface and were embedded in fibrous tissue. Macrophages in the fibrous capsule were often foamy in appearance, indicative of a high phagocytotic activity, while foreign body giant cells were not observed at locations of degradation.

26 weeks: Within the intracortical space, the implants displayed almost complete and dense calcification of their surface (Fig. 5). The pores were largely occupied by bone tissue, while the non-reflecting areas in BSE correspond mainly to vascularization (Fig. 5b). At confined locations, the material had condensed, most probably due to the press-fit implantation and water-uptake, in such a way that an open pore structure was no longer discernable. Consequently, bone was not present in these areas. A remarkable finding was that bone ingrowth was largely restricted to the natural femoral contours and seldom extended into the marrow cavity. A continuum at the interface between bone and calcified Polyactive^{R}, bone-bonding, was often revealed.

4. Discussion

This study assessed the ability of 80/20 PEO/PBT copolymer to repair bone defects. In our opinion, the most remarkable finding was the dense calcification of the implants after 26 weeks. It was shown that a synthetic substrate that contained no calcium or phosphorus prior to implantation, is able to take up substantial amounts of calcium phosphate postopera-

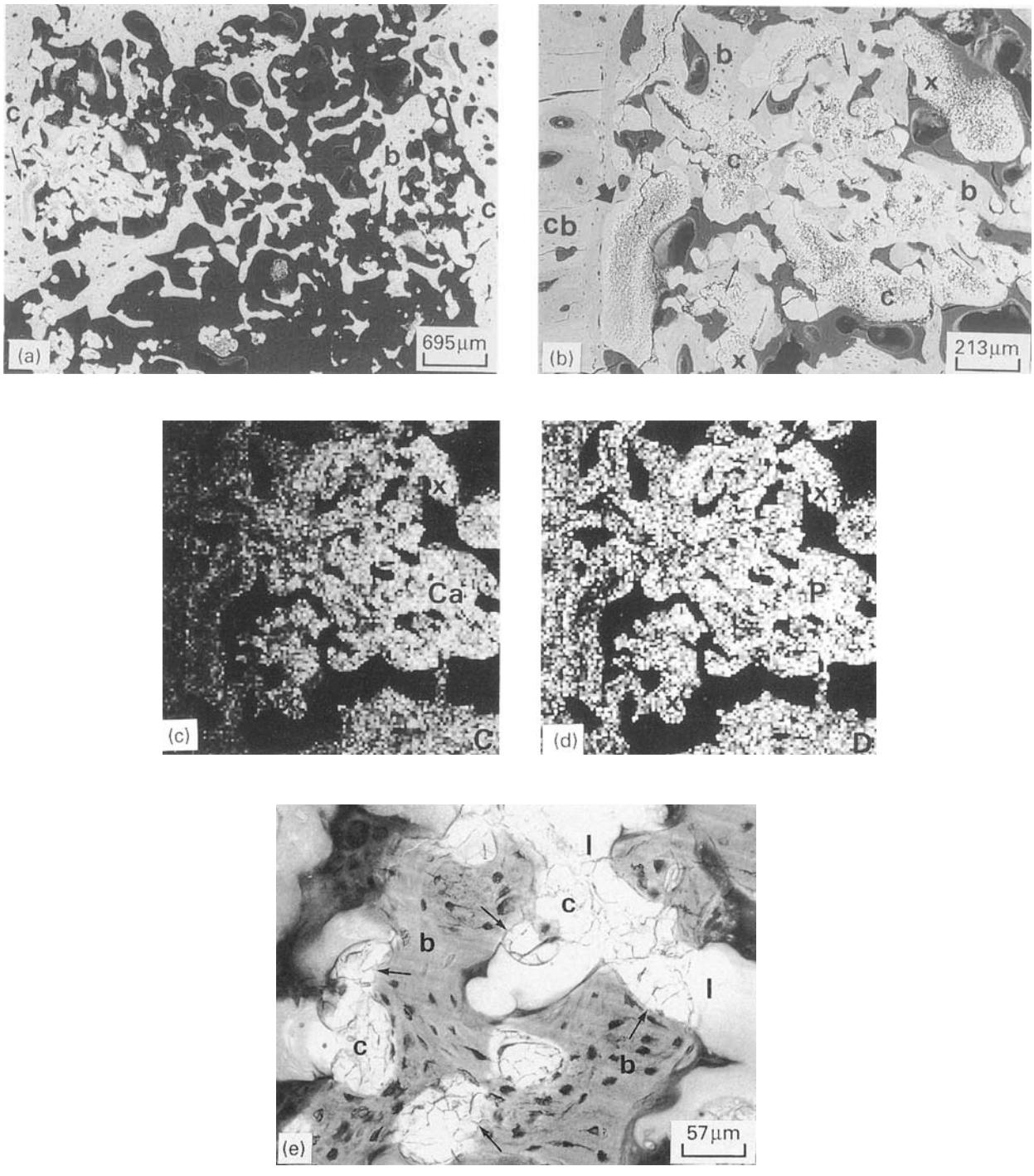

Figure 4 (a) Overview, at 12 weeks, in BSE of the 5-mm defect showing extensive bone (b) ingrowth. c = cortex. (b) Detail from Fig. 4a (large arrow in (b) corresponds with the arrow in (a)). Bone (b) has infiltrated from the cortex (cb). Note the extensive calcification (c) of the implant and the high contact (small arrows) rate between bone and calcified material. (c) X-ray mapping for calcium of the area shown in Fig. 4b (× correspond in (b), (c) and (d)). X-ray mapping for phosphorus The X-ray mapping in (c) and (d) confirmed the morphological continuum in calcium and phosphorus signal at the bone/calcified implant interface. (e) LM-morphology of the bone (b)/implant (I) interface. Arrows point to continuity at the interface with calcified material (c).

tively, resulting in a calcium phosphate–polymer composite. This preliminary study demonstrated a favourable effect on the repair of bone defects and will therefore form the basis of the design of future experiments which will aim to determine the exact implications of this phenomenon for bone reconstruction.

Although bone ingrowth was limited to a few peripheral pores at 3 weeks, union of the defect within the porous 80/20 matrix was observed 6 weeks postoperatively. Comparison of the results presented here with the available data in the literature is complicated, because of the considerable variation in implant geometry and location, defect size and animal model (especially critical, because of varying degree of bony repair along the phylogenetic scale) [12]. We can state, however, that in the same animal model, the rate of bone ingrowth in 80/20 implants was higher than previously reported for stiffer PEO/PBT proportions, although this was not a quantitative observation [11]. A drawback of this preliminary study was that sham defects were not incorporated. However, com-

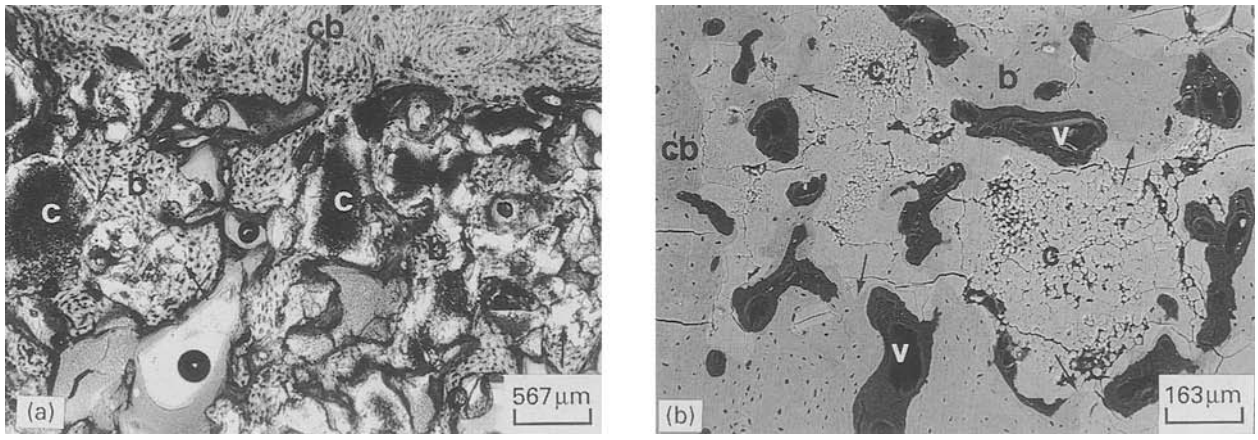

Figure 5 (a) Light micrograph showing the 26-week morphology. The continuity between calcified implant (c) and bone (b) is indicated by arrows. Cb = cortex. (b) BSE showing almost completely calcified (c) implant, with bone tissue (b) occupying the pore structure. Arrows mark the continuity at the interface between these two compartments. Note the high extent of vascularization (v) in the non-reflecting areas. Cb = cortical bone, 26 weeks.

parable experiments in rabbits showed faster healing in the presence of 70/30 and 60/40 PEO/PBT proportions than in unfilled defects [13]. A striking finding, with respect to the extent of bone ingrowth, was the maintenance of preoperative femoral contours, since bone proceeded only marginally into the marrow cavity. This might be attributed to a better stress transfer through PEO/PBT implants in bone tissue.

Upon insertion of 80/20 implants, several favourable conditions for bone ingrowth were met [14, 15]: (1) close contact between implant and bone [14, 15] as a consequence of water uptake; and (2) a minimal amount of relative movement between implant and bone [15], initially due to the swelling behaviour and possibly a better stress transfer through the implants. Despite these beneficial factors, enhancement of bone ingrowth can be achieved by presenting a more appropriate, postoperative, pore structure or combining Polyactive^{R} with osteoinductive substances. The latter can be accomplished in two ways. First, Polyactive^{R} can be mixed with autogenous marrow or demineralized bone matrix. Secondly, PEO/PBT copolymers can be loaded with soluble osteoinductive factors, in view of earlier reports that demonstrated an uptake of proteins from body fluids [13]. If properly calibrated, these factors may be released or diffuse from the implant and influence phenotypic expression and cell differentiation. This is still largely speculative, although the hypothesis is currently under investigation.

Calcification within the material surface appeared at 3 weeks and progressed to almost complete density. Bone-bonding, defined here as a continuity between bone tissue and calcification following the recently formulated definition [16], increased concurrently. This is in line with previous experiments that stressed the importance of material calcification for the occurrence of bone-bonding [7–10]. The calcification in PEO/PBT copolymers has been identified as carbonate–apatite, the mineral component of bone [17]. This finding is of interest in view of the fact that other synthetics bond to bone through the generation of a carbonate-apatite surface layer [4–6]. According to a recent theory on bone-bonding, the postoperative precipitation of such a layer is considered of more importance than the initial presence of calcium and phosphate [5,6,18]. We conclude that the cascade of events at the PEO/PBT copolymer interface complies with the acknowledged bone-bonding substrates and that abundant carbonate–apatite formation within an elastomeric matrix is favourable for bone-bonding.

The behaviour of a synthetic bone-bonding material, in bone defects, was evaluated. We feel that the excessive postoperative calcification of an elastomer, in analogy to the mineral–elastic organic matrix that composes bone, results in an optimal integration with and a relatively rapid healing of a defect. The 80/20 PEO/PBT copolymer is therefore a valuable alternative for use in bone-replacement surgery.

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