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The polymer Polyactive™ as a bone-filling substance: an experimental study in rabbits

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The biocompatible, osteoconductive and resorbable polymer Polyactive™ (PA) was investigated for its performance as a bone-graft substitute. The model consisted of a 4 mm borehole, 1.5 cm distal of the major trochanter in both femurs of a rabbit, of which one was filled with a cylinder of porous PA. The other was left untreated. PA70/30 and PA60/40 were investigated, both before and after being incubated with allogenic bone marrow. Analyses were performed after 4, 8, 26 and 52 weeks and comprised dual energy X-ray absorptiometry (DXA) and image analysis of histological sections. DXA revealed an increased bone mineral density in the filled defects compared to the controls, both at the defect and immediately proximal and distal of the defect. Histology showed that gap-bridging had occurred within 8 weeks, with 80%–90% of the pores of PA70/30 and PA60/40 occupied by new bone, and an intimate bone–PA contact. PA70/30 seemed to be more suitable compared to PA60/40, in that the highest amount of bone was formed within the shortest period of time. Incubation of PA with allogenic bone marrow resulted in inflammatory reactions at the sites of implantation, which delayed bone growth, but did not prevent it. It was concluded that PA70/30 and PA60/40 are suitable bone-graft substitutes.

1. Introduction

Spontaneous repair of cortical bone defects, like screw holes, is limited in humans [1]. Therefore, these defects present a considerable risk of refracture with an incidence between 1% and 8% in forearm, tibia, femur or spine [2–5].

In this study a 4 mm defect in the femur of a rabbit, comparable to a screw hole, was used to compare spontaneous, new bone formation with bone formation after implantation of the biocompatible and osteoconductive polymer Polyactive™. A previous, pilot study revealed that the process of spontaneous repair of such a bore hole in the femur of a rabbit was not able to gap the defect within 8 weeks (unpublished work). Therefore, this model appears to be suitable for this study.

Polyactive is a segmented copolymer of poly(polyethylene oxide terephthalate) and poly(butylene terephthalate) (PEO/PBT), which was initially used to design a synthetic tympanic membrane. The material has been proven to be biocompatible and osteoconductive, and appeared to be a bone-bonding material [6–10]. In addition PA is easy to handle and biodegradable [10, 11]. Varying the ratio PEO over PBT results in materials with different properties. A relatively high concentration of PEO proved to be essential for the bone-bonding properties of the material. The bone-bonding mechanism of the material appeared to be related to the capacity of the material to absorb selectively calcium and phosphate [9, 12, 13].

The kinetics of bone growth inside the pores of the cylinders, both at the level of the cortex and in the medullary cavity, were investigated. PA60/40 was tested because of its slightly better mechanical properties than PA70/30. Furthermore, it was tested whether the bone growth in and near the Polyactive implant could be enhanced by incorporating osteoinductive factor(s) present in trabecular bone and bone marrow in the cylinders, as is seen when a polymer is implanted subcutaneously [14–16]. Because the osteoconductivity of Polyactive is caused by the ability of the material to absorb selectively calcium and phosphate [9, 12, 13], and incubation of Polyactive with bone marrow probably results in "pre-calcification" of the material, implantation of incubated Polyactive should also lead to enhanced bone formation.

2. Materials and methods

Polyactive:porous PA cylinders of composition (PEO/PBT) 70/30 and 60/40 used in this study were manufactured and supplied by HC Implants bv, Leiden, The Netherlands. The pores were interconnected. The average pore diameter was 300 $\pm$ 150 μm; the

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diameter of the interpore connections was 100 ± 50 μm. These diameters were shown to be optimal for growth of bone [12, 17, 18]. The cylinders were 4 mm diameter and 5 mm long. Half the number of cylinders were incubated with allogenic bone marrow: bone marrow was harvested from the femur of one New Zealand White rabbit, not belonging to the animals in this study, and diluted with Dulbecco's modified Eagle medium (DMEM culture medium) (1:1). PA cylinders were incubated for 24 h at 37 °C, then briefly washed in phosphate-buffered saline and freeze dried in order to kill allogenic cells and to shrink the implants to their original dimensions. All PA cylinders were sterilized by gamma irradiation (25 kGy) at Gammaster, Ede, The Netherlands.

For this animal experiment, approval was obtained from the local committee for animal experiments. Female New Zealand White rabbits (128) were used, each about 6 months old, weighing between 3.5 and 4.5 kg. The rabbits were randomized and, at the time of operation, anaesthetized using ketamine hydrochloride (100 mg kg^{-1}) and diazepam (8 mg kg^{-1}). Both femurs were exposed laterally. The periosteum was put aside and, approximately 1.5 cm below the major trochanter, a borehole of 4 mm diameter was made. In the right femur, a PA cylinder was implanted; the borehole in the left femur was left untreated. Four groups of 32 rabbits were followed for, respectively, 4, 8, 26 and 52 weeks. Of the 32 rabbits, eight received a cylinder of Polyactive 70/30 (PA70/30); eight a cylinder of PA70/30 impregnated with bone marrow (PA70/30BM); eight a cylinder of PA60/40 and eight a cylinder of PA60/40 which was incubated with bone marrow (PA60/40BM) (Table I). The animals were housed in groups at a farm in cages of 4 m × 4 m. The animals were killed using an overdose of thiopental. Both femurs were dissected, placed in sterile PBS and transported to the dual energy X-ray absorbtiometer (DXA) (DPX 560, Lunar). The exact location of the borehole was measured relative to the femoral head and trochanter major. DXA scans were made in the lateral – medial direction. The bone mineral density (BMD, g cm^{-2}) was assessed in three regions of interest (ROI). Three ROIs of 0.2 mm^2 adjacent to each other were placed proximal to the defect, over the defect and distal to the defect, respectively. One 0.06 mm^2 ROI was placed in the centre of the 0.2 mm^2 ROI that was placed over the defect. Data given are from the 0.06 mm^2 ROI in the centre of the defect and both the 0.2 mm^2 ROI's proximal and distal of the defect. Then segments of the femur containing the borehole were fixed in 4% phosphate-buffered formaldehyde. Part of these segments were dehydrated through a series of acetone solutions and embedded in methylmethacrylate. Sections of 10 μm were cut using an especially developed histological inner lock diamond saw microtome cutting system (Leiden, The Netherlands) and stained with methylene blue and basic fuchsine [19].

Other segments were decalcified with a 10% solution of ethylenediaminetetraacetic acid (EDTA), dehydrated and embedded in glycol methacrylate. Sections of 4 μm were stained with trichrome according to Gomori. Image analysis (Quantimet 570C, Leica Cambridge Ltd, Cambridge, UK) was used to determine the course of the area occupied by implant material, and the percentage tissue occupied by exudate cells/fibrous tissue, and bone. Also, the percentage of the outline of the PA implant in intimate contact with new bone was determined. This is a measure for bone-bonding capacity of the biomaterials. The total area of the pores represented about 40%–50% of the field measured, and was taken as 100%.

For statistical analysis, data were compared with one-way ANOVA and Student's *t*-test.

TABLE I Distribution of rabbits over 16 groups: four groups of 32 rabbits had four different forms of PA implanted and were followed for 4, 8, 26 and 52 weeks. The data indicate the number of rabbits included in the study; the number of rabbits that were originally operated on is given in parentheses

	4 wk	8 wk	26 wk	52 wk
PA70/30	8 (8)	8 (8)	7 (8)	7 (8)
PA70/30BM	3 (8)	7 (8)	7 (8)	6 (8)
PA60/40	8 (8)	7 (8)	7 (8)	6 (9)
PA60/40BM	6 (8)	8 (8)	6 (8)	6 (7)

3. Results

From the 128 rabbits that were operated, 21 died or had to be killed before the end of the experiment (Table I). Cause of death was either a pneumonia or an unknown cause not related to the implants. One rabbit had a fractured femur at the level of the bore hole and four rabbits had developed an osteomyelitis.

3.1. DXA

Both experimental and control femurs were subjected to DXA analysis. With the ROI over the defect, after 4 weeks implantation the BMD was 0.073 g cm^{-2} (18.2%; $p < 0.02$) higher when PA70/30 was implanted as compared to the contralateral control site (Table II). For the other implants, in general the experimental sites at 4 weeks implantation time contained 10%–20% more mineral than the control site, but the differences were not significant. After longer implantation times, differences between experimental and control sites had not changed: the experimental site contained 10%–25% more mineral than the control site. Occasionally the differences were significant (Table II). The BMD was also assessed proximal and distal to the defect. Proximal to the defect, the BMD at the experimental site was 10%–20% higher than at the control site after 4 and 8 weeks. After 26 weeks implantation, the experimental site contained 5%–10% more mineral than the control site and after 52 weeks the differences were eliminated. The same was seen just distal to the defect.

The BMD at sites where PA pre-incubated with bone marrow was implanted showed larger variability than when PA without bone marrow was implanted. DXA analyses did not reveal differences between PA70/30 and PA60/40, whether or not these materials were pre-incubated with bone marrow.

TABLE II DXA analysis: relative differences between experimental side and control side assessed in an ROI of 0.06 mm^2 over the defect, an ROI, of 0.2 mm^2 proximal to the defect and an ROI of 0.2 mm^2 distal to the defect. Data from experimental and control sides were compared using the Student's *t*-test for paired data: * = $p < 0.05$; ** $p < 0.02$

	4 wk			8 wk			26 wk			52 wk		
	(%)	*n*	*p*	(%)	*n*	*p*	(%)	*n*	*p*	(%)	*n*	*p*
0.06 defect												
PA70/30	18.2	7	**	11.6	5	ns	15.2	7	ns	14.3	6	ns
PA70/30BM	9	5	ns	21.8	5	*	26.3	7	**	0.8	5	ns
PA60/40	15	7	ns	− 8.6	5	ns	19.6	8	**	2.3	5	ns
PA60/40BM	20.1	4	ns	− 1.2	3	ns	14.8	6	ns	19.8	6	ns
0.2 proximal												
PA70/30	12.4	7	*	18.5	5	*	9.3	7	*	8.6	6	*
PA70/30BM	22.0	5	*	4.0	5	ns	16.5	7	**	− 2.2	5	ns
PA60/40	17.0	7	ns	6.2	5	ns	11.6	8	ns	− 5.8	5	ns
PA60/40BM	17.2	5	ns	14.9	3	ns	14.5	6	ns	5.6	6	ns
0.2 distal												
PA70/30	24.1	7	**	22.4	5	ns	8.1	7	*	− 2.8	6	ns
PA70/30BM	19.1	5	ns	10.5	5	ns	7.5	7	ns	1.3	5	ns
PA60/40	14	7	*	14	5	ns	8.2	8	*	2.5	5	ns
PA60/40BM	19.4	5	**	19.3	3	ns	5.4	6	ns	18.5	6	**

3.2. Histology

3.2.1. Cortex of the femur

At 4 weeks after implantation of the PA70/30 and the PA60/40 cylinders, the pores of the plugs within the cortex of the femur were mainly filled with bone and cell-dense fibrous tissue. Bone growth was from the edges of the cylinder, where the PA was in contact with the cortical bone, towards the centre. Polyactive served as scaffold for deposition of bone (Fig. 1a). Assessment of the percentage of pore surface occupied by bone revealed that after 4 weeks, PA70/30 contained 53% bone. This was more than for PA60/40 cylinders (40%; not significant) and for PA70/30BM or PA 60/40BM cylinders (27% and 8%, respectively; significant) (Fig. 2). After 8 weeks, 70%–95% of the total surface area of the pores of all types of PA cylinders were filled with newly synthesized calcified bone (Figs 1b, 2). Again, pores of PA70/30 or PA60/40 plugs contained significantly more bone (95% and 91% respectively) than pores of PA70/30BM or PA60/40BM cylinders, respectively (82% and 66%). After 26 weeks, the pores in PA70/30, PA70/30BM and PA60/40 were filled to the same extent, approximately 87%, with new bone, while 69% of the pores of PA60/40BM was occupied with bone at that time. After 52 weeks, in all types of PA, over 83% of the total surface area of the pores was filled with new bone (Fig. 2). The remaining part of the pores was filled with fibrous connective tissue and bone marrow.

Between 5% and 90% of the PA surface was in contact with bone, depending on the kind of PA and the implantation period (Fig. 3). In general, a higher percentage of the surface of PA implants that had not been pre-incubated with bone marrow, was in intimate contact with bone, as compared with implants that were pre-incubated. PA70/30 showed the highest area of intimate contact with bone after 8 weeks (92.5%). At longer implantation times the area of intimate contact decreased to $< 80\%$ (Fig. 3).

PA cylinders that were pre-incubated with allogenic bone marrow, evoked an inflammatory reaction characterized by infiltrations of polymorphonuclear and mononuclear cells (data not shown). There were large fluctuations between different animals in the extent of inflammation.

At all implantation times, the bone content of the pores of the PA that had been pre-incubated with bone marrow, was lower compared to the bone content of the non-treated PA cylinders. However, after 1 year, differences in bone content between implants that had been pre-incubated and those that had not been pre-incubated were no longer significant. Repair of the defect at the control site showed large variations. After 4 or 8 weeks, defects were still visible and only limited filling of the defect with new bone had occurred. After 52 weeks, control defects in general were almost fully repaired (Fig. 1c).

3.2.2. Medullary cavity

The implants extended into the medullary canal. The PA implants were predominantly filled with bone marrow and stroma. At 4 weeks after the implantation, 17% of the pores of PA70/30 were filled with newly synthesized bone (Fig. 1d, Table III). In the other types of PA implants the amount of bone was 10% or less after that implantation period. Of the pores of PA 70/30, 6% were filled with bone after 8 weeks, and $< 1\%$ after 26 and 52 weeks. In all other PA implants, the amount of bone decreased with time, to zero after 26 weeks (Table II).

4. Discussion

In this study, the biocompatible and bone-bonding polymer Polyactive™ was tested for its performance as a bone-graft substitute in a rabbit model. The results after 4, 8, 26 and 52 weeks were analysed by DXA and image analysis of histological sections. The

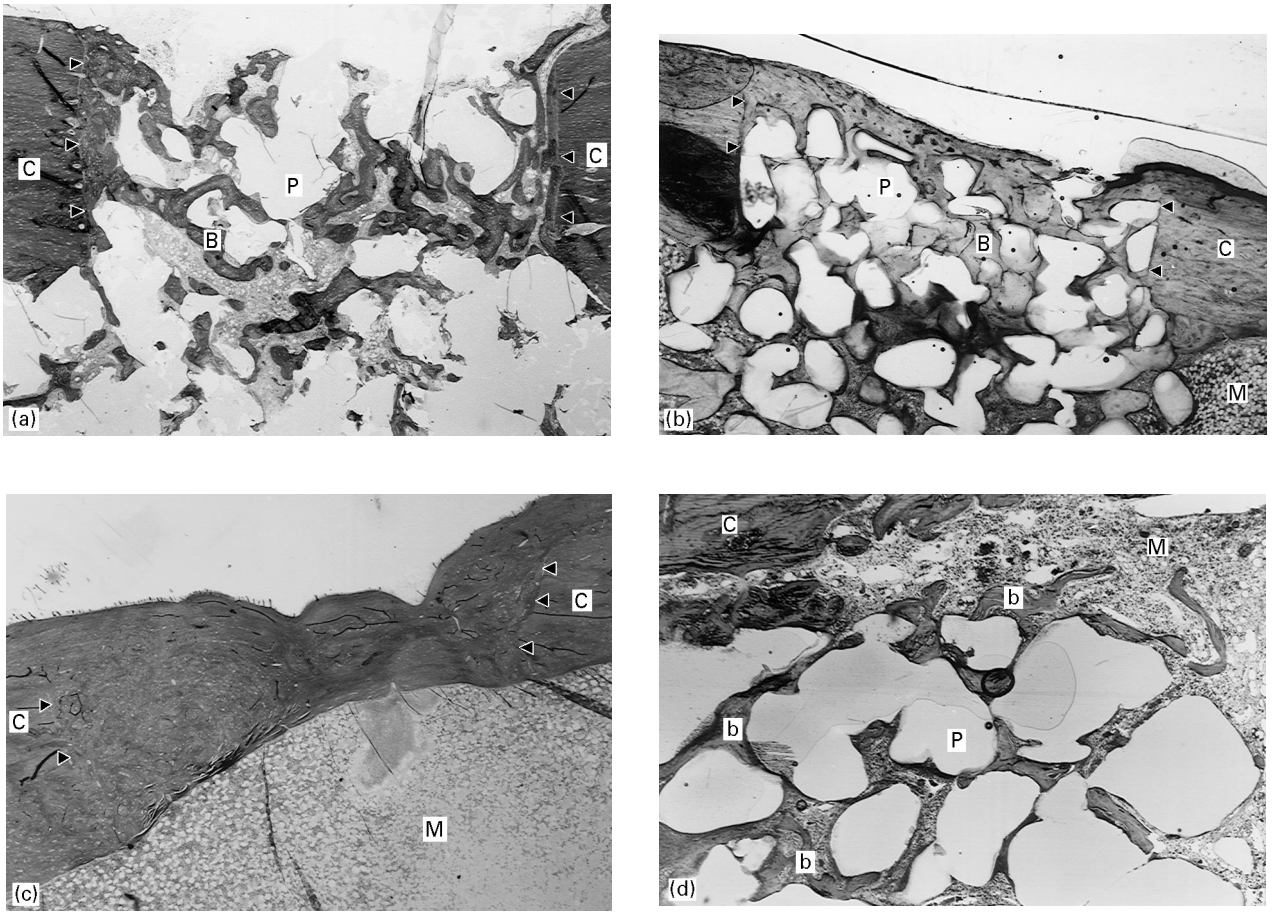

Figure 1 Histological sections of bone defects filled with Polyactive and after spontaneous "repair". Undecalcified sections (10 μm) were prepared using a modified innerlock diamond saw. These sections were stained with methylene blue and basic fuchsine. Decalcified sections were stained with trichrome according to Gomori. (a) A decalcified section showing a defect filled with PA70/30, 4 weeks after implantation. P, Polyactive; B, newly synthesized bone; C, cortex; (△) the margins of the borehole. (b) An undecalcified section showing a defect filled with PA70/30 8 weeks after implantation, P, Polyactive; B, newly synthesized bone; C, cortex; M, marrow cavity; (△) the margins of the borehole. (c) An undecalcified section showing a control defect 52 weeks after transplantation. C, cortex; M, marrow cavity; (△) the margins of the borehole. (d) A decalcified section showing bone present in the pores of PA70/30 in the medullary cavity after 4 weeks implantation. P, Polyactive, b, newly synthesized bone; C, cortex; M, marrow cavity.

results obtained after analysis of the DXA data were indicative of enhanced bone formation in the cortical region at the site where the bone defect was filled with PA, when compared to the non-implant control site. We were not able to measure the BMD at time zero, due to technical problems.

Owing to the limited number of samples, not always statistically significant differences could be obtained. As time progressed, decreasing differences between experimental and control sites were observed, due to spontaneous repair of the control bone defects. The increases in BMD proximal and distal to the filled defect are noteworthy. This may be caused by the defect closure with PA, which seems favourable compared to no treatment. Closure of the defect with PA resulted in a reaction of periosteum and endosteum resulting in a thickening of the cortex proximal and distal to the defect. It seems unlikely that these differences in BMD between control and experimental site were caused by differences in loading between both hind-legs, i.e. more atrophic (osteoporotic) control site. However, the data from this study are not conclusive for this issue.

The histological data showed that bone formation in the cortex of the femur proceeded from the periphery of the PA cylinders towards the centre. This is indicative for bioactivity of PA. Within 8 weeks, the defect was bridged by new calcified bone which filled the pores of the PA almost completely. At this time, an intimate contact was seen between bone and PA at the light microscope level, which is indicative of the bone-bonding properties of these materials [6, 7, 12, 16, 20].

The decrease in bone–PA contact with time is probably caused by the process of degradation of PA and by the normal process of bone turnover.

Bone was initially formed in the pores of the PA plugs at the level of both the cortex and the medullary cavity. After 8 weeks, however, the bone insite the medullary cavity had almost completely been resorbed, whereas the pores of the PA plugs in the defect at the level of the cortex were filled with increasing amounts of new bone. From this moment on bone formation apparently proceeded according to Wolff's law of bone architecture: It depended on the direction and the amount of stress induced in the bone [21–25]. From the data of this study, the most suitable material to use for treatment of bone defects appeared to be PA70/30 without the addition of allogenic bone marrow. The incubation of PA with allogenic bone/bone marrow, which induced bone formation when

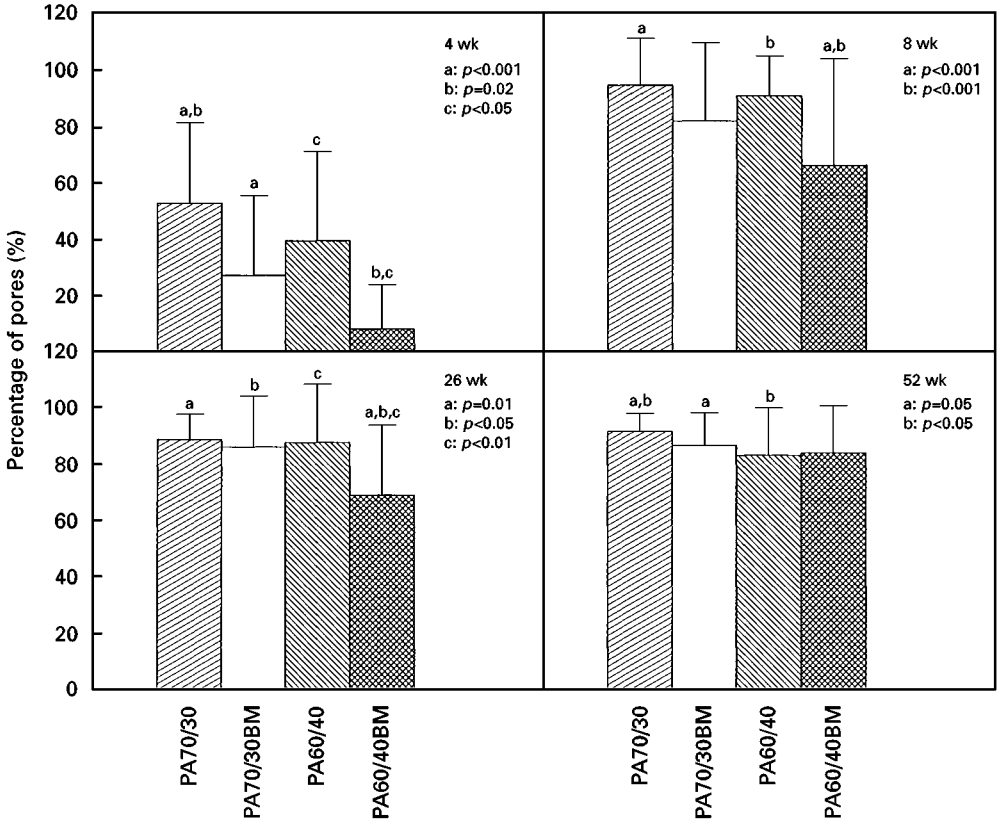

Figure 2 The percentage of pores of different Polyactives filled with bone within the cortical gap at different implantation times. Bars that share the same letter (within one quarter of the figure) differ significantly (Student's t-test $p < 0.05$).

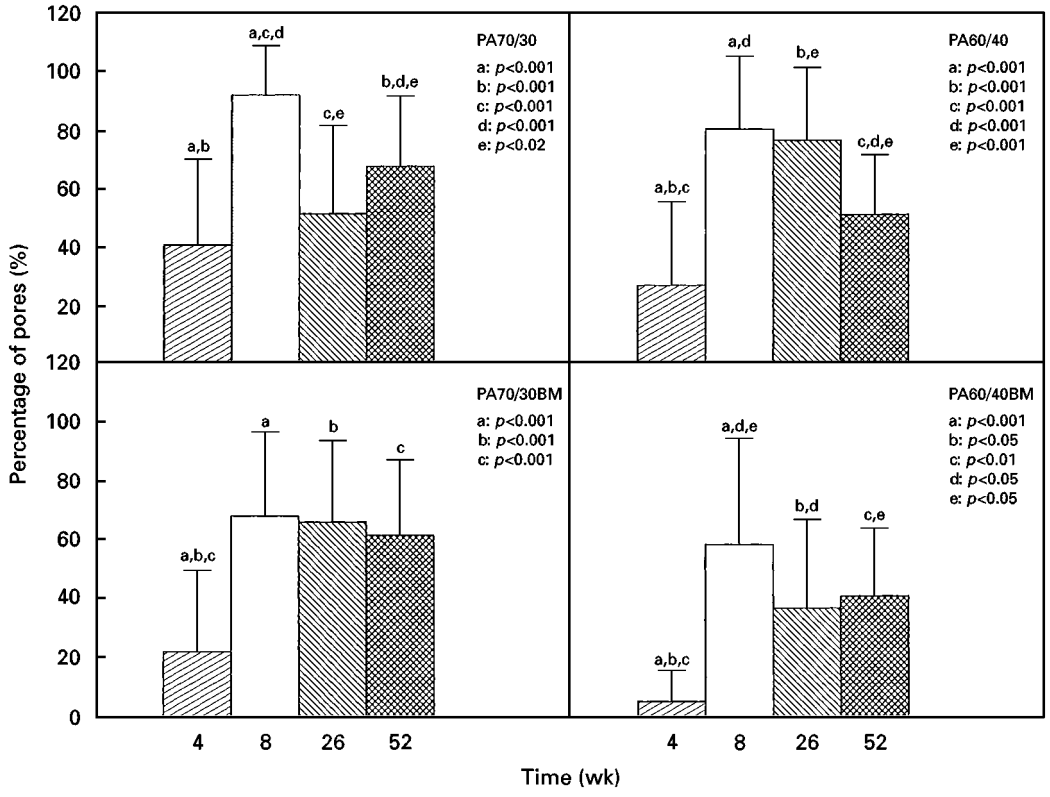

Figure 3 The percentage of Polyactive in direct contact with bone within the cortex of the femurs versus the graft material. Bars that share the same letter (within one quarter of the figure) differ significantly (Student's t-test, $p < 0.05$).

implanted subcutaneously in syngeneic Fisher rats [16], did not enhance bone formation when implanted in bone defects. In contrast to non-pretreated polyactive, an inflammatory reaction occurred. This inflammation, which had to be caused by the allogenic bone marrow, only delayed the formation of new bone tissue; bone formation was not prevented. So, bone formation guided by Polyactive was even present in an

TABLE III Percentage of bone in pores of Polyactive inside the medullary cavity at different implantation times

Implantation time (wk)	PA70/30		PA70/30B		PA60/40		PA60/40B	
	(%)	S.D.[a]	(%)	S.D.[a]	(%)	S.D.[a]	(%)	S.D.[a]
4	17.4	10.1	4.1	2.0	3.9	2.5	2.3	0.3
8	6.3	4.7	0.6	0.4	1.0	0.8	2.5	1.3
26	0.9	0.8	2.4	1.8	0.1	1.1	2.4	0.8
52	0.03	0.04	0.03	0.04	0	0	0.07	0.12

[a]S.D. = standard deviation.

inflamed area. Further studies are needed to establish the suitability of Polyactive implanted in inflamed environments.

Pretreatment of Polyactive with bone marrow was also suspected to result in some precalcification of the material, which was expected to be advantageous for the kinetics of new bone formation. Probably due to the inflammatory reaction, this was not the case. In a later study, it was established that precalcification of Polyactive by subsequent incubations with calcium and phosphate significantly enhanced the amount of new bone formed within the first 4 weeks after implantation [26].

A major advantage of PA over a number of ceramic materials is that the material is very easy to handle in the operating theatre and, in most instances, does not need additional fixation. Furthermore, polyactive does not produce wear particles and can be used in or near a joint surface. Ceramic materials, like hydroxyapatite or tricalcium phosphate, produce wear particles and when used in or near a joint surface additional care has to be taken to avoid wear of the material.

Polymers like polyactive do not provide immediate mechanical stability. However, it is assumed that mechanical stability is reached within 8 weeks, in case of PA70/30 or PA60/40. Further biomechanical studies are necessary to test the suitability of PA in different anatomical locations.

5. Conclusion

It can be stated that PA is a biocompatible, bone-bonding material, suitable to fill cortical bone defects in a stable environment, like screw holes, bone cysts or benign tumours. The behaviour of this material in a less stable environment is the subject of another study. Biomechanical studies will be needed to assess the strength of PA implants filled with bone.

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