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Quantitative histological analysis of bony ingrowth within the biomaterial Polyactive™ implanted in different bone locations: an experimental study in rabbits

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The quantity of bone formed in cylinders of a newly developed erodible copolymer, Polyactive™ (PA60/40) was examined. PA60/40 was implanted in three different bone locations in the rabbit: in the cortex, in bone marrow and in trabecular subchondral bone. Bony ingrowth was assessed after 4, 8, 26 and 52 w after the operation and investigated by histology and image analysis. The ingrowth of bone was observed in PA60/40 placed in the cortex from 4 w onwards. After 8 w, more than 90% of the pores of the biomaterial were filled with dense bone. In bone marrow, initially some bone formation was seen. After 26 w, all newly formed bone was resorbed. Subchondral bone formation was less than in the cortex of the femur, but somewhat comparable to the amount of bone found in healthy trabecular bone. Bone formation appeared not to be affected by the degradation of the biomaterial. It was concluded that Polyactive™ is a suitable bone graft substitute. Bone formation within PA60/40 is site-dependent and this follows Wolff's law. © *1998 Chapman & Hall*

1. Introduction

Autogenous bone grafts are widely used in orthopaedic practice. These grafts are needed to fill bone defects, to induce an arthrodesis or to heal a pseudarthrosis. The quantity of these grafts, however, is limited. Allogenous bone grafts are often used to overcome this problem. Disadvantages of these allografts are the risk of transmitting infections [1, 2]. Allografts are more prone to infection, fracture and non-union several years after implantation [3, 4]. Furthermore, the costs of sampling, work up and storage are high.

In order to overcome these limitations, research has been directed towards artificial materials that may be useful as bone substitutes. Such a material should be mechanically stable, bio-erodible, biocompatible and, most of all, osteoconductive or osteo-inductive [3, 5–8].

A number of ceramic materials are suitable; however, they are difficult to manipulate and, in general, resorption is low [8–10]. Recently, a new material was discovered that proved to be biocompatible, bio-erodible and osteo-conductive [11–14]. This material, trademarked Polyactive™ (PA60/40), is a polyether-polyester segmented block copolymer, composed of a soft segment, polyethylene oxide (PEO), and a hard segment polybutylene terephthalate (PBT).

Based on the same compounds, a whole range of polymers with different degradation characteristics can be obtained by varying the PEO/PBT ratio.

The PEO component stands for the bone-bonding properties of the material, provided it is used in relatively high amounts: 60% PEO or higher. With increasing amounts of PEO, the degradation is faster [11, 14, 15]. Polyactive™ was originally used as a synthetic tympanic membrane. PA60/40 functions as a hydrogel and selectively absorbs calcium from the vicinity [14]. PA60/40 has a spongy appearance and it is easy to manipulate [13, 14, 16]. In other studies, PA60/40 proved to be biocompatible and osteoconductive [11–13, 16].

In this study Polyactive™ 60/40 was used to fill the defects in trabecular bone and the cortex of rabbit femora. The amount of bone growth in the material at the two different locations as well as in the bone marrow was measured semi-quantitatively.

2. Materials and methods

Polyactive™ 60/40 (PA60/40) cylinders were manufactured and supplied by HC Implants bv., Leiden, The Netherlands. The average pore diameter was $300 \pm 150\,\mu m$; the diameter of the interconnecting pores was $100 \pm 50\,\mu m$. These diameters have been

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shown to be optimal for the ingrowth of bone [14, 17, 18]. The cylinders were 4 mm diameter and had a length of 5 mm. All PA60/40 cylinders were sterilized by gamma irradiation (25 kGy) at Gammamaster, Ede, The Netherlands.

These experiments were approved by the local committee for animal experiments. Female New Zealand White Rabbits (32), about 6 mon old, were used, weighing between 3.5 and 4.5 kg. The rabbits were anaesthetized using ketamine hydrochloride ($100\,mg\,kg^{-1}$) and diazepam ($8\,mg\,kg^{-1}$). The rabbits were in a supine position.

2.1. Trabecular bone

The left knee was opened through a medial parapatellar incision and the patella was dislocated laterally. A borehole of 4 mm diameter was made in the facies patellaris of the femur. A PA60/40 cylinder was placed in the borehole. In the right knee, a sham operation was performed without drilling the borehole.

2.2. Cortex

The right femur was exposed laterally by pushing off the musculature ventrally. The periosteum was moved aside and approximately 1.5 cm below the major trochanter, a borehole of 4 mm diameter was made. The PA60/40 cylinder was placed here. Again, the contralateral site was used as a control; a borehole was made without placing a PA60/40 cylinder.

2.3. Marrow

The cylinders were placed in the defect of the femur onto the contralateral cortex. Thus information could be obtained both from the growth of bone within the PA60/40 in the marrow and in the cortex.

Four groups of eight rabbits were sacrificed after respectively 4, 8, 26 and 52 wk. The animals were killed using an intravenous overdose of thiopental. Both knees and femurs were dissected and fixed in 4% phosphate-buffered formaldehyde. The specimens were dehydrated through a series of acetone/alcohol solutions and embedded in methylmethacrylate. Sections of 10 μm were cut using an innerlock diamond saw and stained with methylene blue and basic fuchsine [19].

To determine the percentage of area occupied by implant material, exudate cells, fibrous tissue and bone, we used an image analyser (Quantimet 570 C, Leica Cambridge Ltd, Cambridge, UK). For statistical analysis, data were compared using the one-way ANOVA test.

3. Results

From the 32 rabbits that were operated on, three died or had to be killed before the end of the experiment. Causes of death were a meningitis in one case and fractures at the level of the borehole in two cases.

3.1. Trabecular bone

Already 4 w after implantation, some bone formation was seen in the PA60/40 cylinders placed in the trochlea (Table I). Here, 14.3% of the total pore area was filled with bone. Besides bone formation, a fibrous cell-dense tissue and bone marrow were seen. At the periphery, trabecular bone and normal bone marrow made contact with the PA cylinder and from there on bone growth proceeded. Some bone formation also took place in the centre of the cylinder. After 8 weeks, 12.5% of the pores were filled with bone (Table I, Figs 1 and 2). After 26 w, significantly more bone was seen; both at the periphery and in the centre of the cylinder (28.2%, $p < 0.039$). The amount of fibrous tissue had diminished. At 52 weeks the extent of bone formation had diminished to 14.5%.

3.2. Cortex

In the cortical level of the femur, the pores of the PA60/40 were mainly filled with a cell-dense fibrous tissue at 4 wk. Here, also, bone formation started from the margins of the cylinder and progressed inwards. At 4 wk, significantly less bone was detected in the centre of the PA60/40 cylinder compared to 8, 26 and 52 wk (Table I, Fig. 1, $p < 0.001$). After 8 wk, 90.7% of the pores of the PA60/40 cylinder was filled with newly synthesized calcified bone (Fig. 3). After 26 and 52 wk, respectively, 87.6% and 83.1% of the PA60/40 pores were filled with bone (Table I). The remaining part of the pores was filled with fibrous connective tissue and bone marrow.

The control site in the cortex showed marginal bone formation. After 4 or 8 wk, the defects were still visible and only a relatively thin neocortex was present at the defect site (Fig. 4). After 26 and 52 wk, in most rabbits the amount of bone matched the control defects.

TABLE I Percentage of bone in pores of Polyactive™ in the cortex, the trabecular bone and the marrow at different implantation times

Implantation time (wk)	Percentage of pores filled with bone							
	Cortex	S.D.[a]	S[b]	Trabecular bone	S.D.	S[b]	Marrow	S[b]
4	39.5%	31.5	*	14.3%	11.4	–	10.4%	*
8	90.7%	14.0	–	12.5%	6.5	–	2.6%	–
26	87.6%	20.6	–	28.2%	4.3	‡	0.2%	–
52	83.1%	16.7	–	14.5%	6.2	–	0.0%	*

[a]S.D. = standard deviation.
[b]S = significance: *$p < 0.001$; ‡$p < 0.039$.

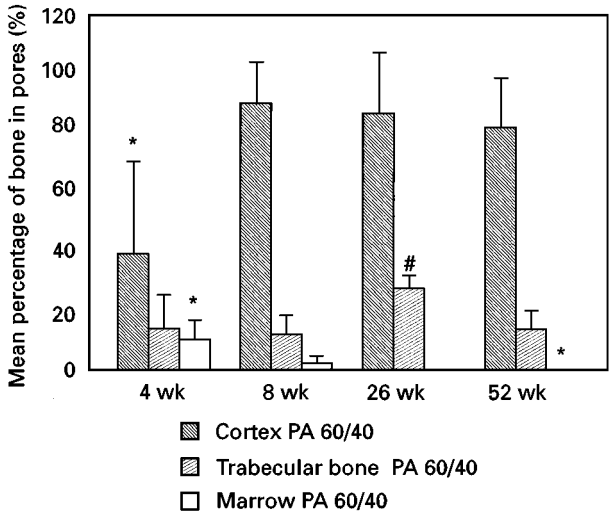

Figure 1 Graph showing the mean pecentages of the pores in Polyactive 60/40 filled with bone at different implantation times.* $p < 0.001$, = $p < 0.039$.

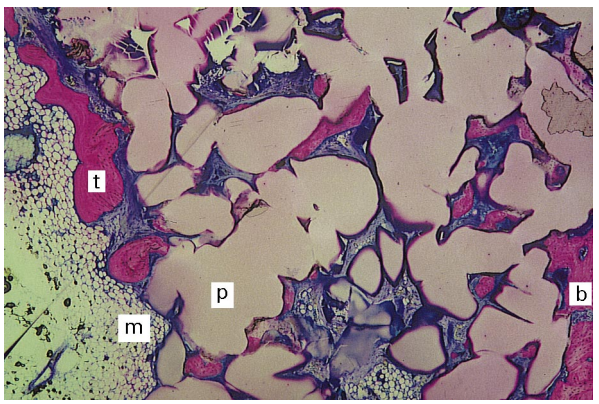

Figure 2 Light micrograph of a section from a sample 8 wk after implantation of a PA60/40 cylinder in the trabecular bone. p, Polyactive 60/40; b, newly synthesized bone; m, marrow; t, trabecular bone (10 μm; methylene blue and basic fuchsine; × 25).

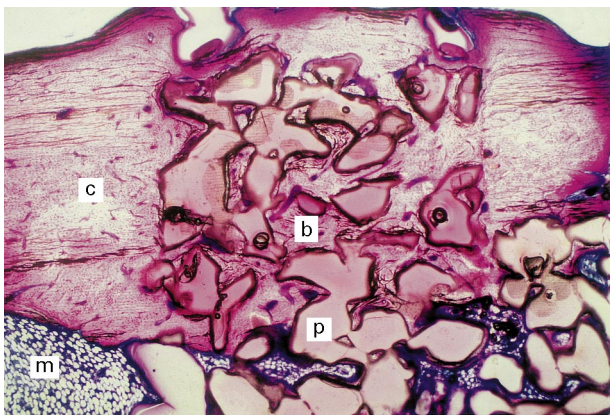

Figure 3 Light micrograph of a section from a sample 8 wk after implantation of a PA60/40 cylinder in the cortex. Note the thickened neocortex at the site of the cylinder. p, Polyactive 60/40; b, newly synthesized bone; c, cortex; m, marrow cavity (10 μm; methylene blue and basic fuchsine; × 25).

3.3. Marrow

In the marrow, the PA60/40 cylinders were predominantly filled with bone marrow cells and some connective tissue. At 4 wk, 10.4% of the pores was filled with bone. At later stages, this percentage decreased significantly to 2.6% (Fig. 5) and below 1% after, respectively, 8 and 26 wk ($p < 0.001$).

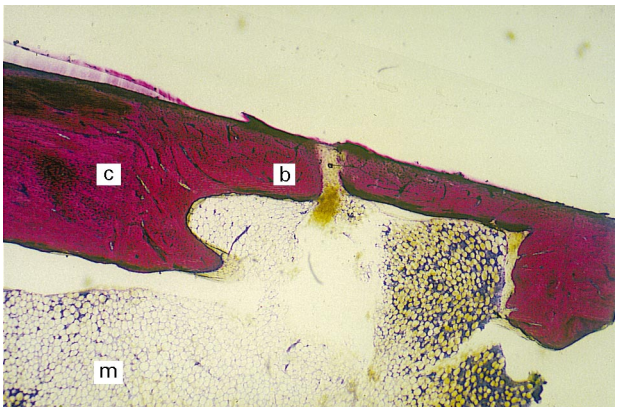

Figure 4 Light micrograph of a section from a sample 8 wk after making the control defect. Note the relatively thin neocortex. b, newly synthesized bone; c, cortex; m, marrow cavity (10 μm; methylene blue and basic fuchsine; × 25).

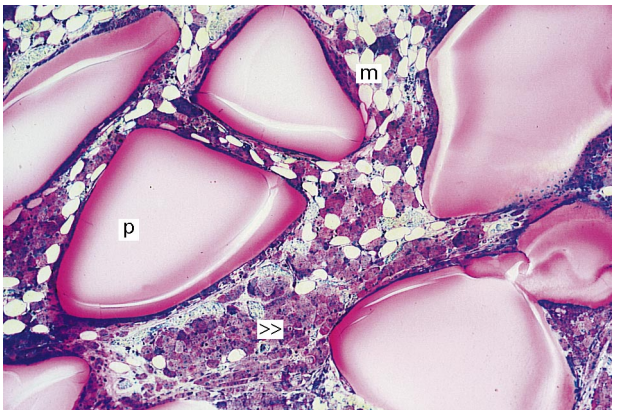

Figure 5 Light micrograph of a section from a sample 4 wk after implantation of a PA60/40 cylinder in the marrow. b, newly synthesized bone; m, marrow cavity; p, Polyactive 60/40; ≫, macrophages filled with Polyactive 60/40 (10 μm; methylene blue and basic fuchsine; × 100).

4. Discussion

Polyactive™ 60/40 was tested as a bone graft substitute in a rabbit model. Image analysis of histological sections was performed on samples yielded at 4, 8, 26 and 52 wk after the implantation of PA60/40. The results suggest that bone formation in the presence of PA60/40 followed Wolff's law of bone architecture: the structure in bone is the result of a dynamic regulatory process, controlled by mechanical loads. Bone formation occurs depending on the direction and the amount of stress induced in the bone [20–24]. Osteocytes, located within the bone, sense mechanical signals and these cells mediate osteoclasts and osteoblasts in their vicinity to adapt bone mass [25].

At the cortex, within 8 wk, >90% of the pores of PA60/40 were filled with bone. When PA60/40 was implanted in the cortex of the femur or within the bone marrow, the data were, respectively, 12.5% and 2.6%. After initial calcification and slight bone formation in the marrow, new bone formation stopped,

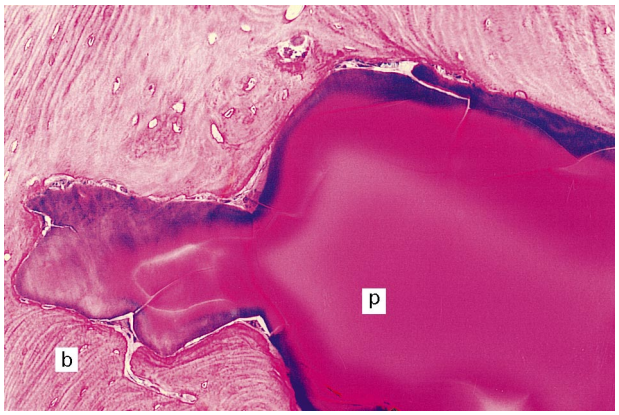

Figure 6 Light micrograph of a section from a sample 8 wk after implantation of a PA60/40 cylinder in the cortex. Note the close contact between the bond and PA60/40. p, Polyactive 60/40; b, newly synthesized bone (10 μm; methylene blue and basic fuchsine; × 200).

while already synthesized bone was resorbed and replaced by normal bone marrow.

Bone formation not only occurred from the margins of the cylinder towards the centre, but new bone formation was also found in the centre of the cylinder. This was found both in the cortex and in the trabecular bone. These results are indicative for the osteoconductive properties of PA60/40 [11, 14, 15, 26]. The amount of new bone formed in PA60/40 is somewhat comparable to hydroxylapatite (HA) or even better [3, 5, 7]. An important difference is the absence of new bone formation in the centre of HA, where bone formation occurs over a maximal distance of 2 mm. Like in porous HA, other bone graft substitutes, such as demineralized bone matrix or coral, only show a peripheral creeping substitution, suggesting osteoconduction [27, 28].

Bone formation in PA60/40 was in direct contact with the biomaterial (Fig. 6) as in contrast to apatite- and wollastonite-containing glass-ceramic. In the latter, the radiolucent line, as seen on X-ray examination, only tended to decrease with time [29].

HA provides, in some respects, for immediate mechanical stability, whereas PA60/40 is expected to give the necessary mechanical stability within 8 wk. Of course, the actual and relative size of the defect are important, and mechanical studies are needed to establish these. In comparison with HA, which is brittle and breaks easily, PA60/40 is very easy to manipulate in the operating room. Biomechanical studies are needed to compare the mechanical properties of these biomaterials and other ceramics after being incorporated by bone.

5. Conclusion

It can be stated that Polyactive™ is a suitable substitute for allografts. It is bio-erodible, fully biocompatible and, moreover, bioactive, stimulating new bone formation without the need of additives.

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