Research; development

Polyactive® as a bone-filler in a beagle dog model

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G. J. Meijer¹, A. van Dooren²,
M. L. Gaillard², R. Dalmeijer²,
C. de Putter¹, R. Koole¹,
C. A. van Blitterswijk^{2,3}

¹Department of Oral-Maxillofacial Surgery, Prosthodontics and Special Dental Care, Faculty of Medicine, University of Utrecht;
²Biomaterials Research Group, Faculty of Medicine, University of Leiden; ³Technical University Twente, The Netherlands

Abstract. Calcification is a crucial step in the bone-bonding mechanism of PEO/PBT hydrogel copolymers (Polyactive®), a new generation of bone-fillers. A beagle dog study was conducted to determine whether the preoperative presence of a calcium phosphate layer (precalcification) on a PEO/PBT 80/20 copolymer would further increase the bone-bonding rate. Standard bone cavities were filled with either precalcified or nonprecalcified porous cylindric PEO/PBT 80/20 implants, or hydroxyapatite granules held together with PEO/PBT 70/30, or were left unfilled. A significantly higher percentage of mineralized component was present in the cavities filled with the precalcified PEO/PBT 80/20 copolymer than in the control defects. As a result of swelling by fluid-uptake, the press-fit inserted copolymer implants showed a significant reduction in pore size, thus preventing optimal bone ingrowth. Both precalcification of the copolymer and underfilling of the defect, to create space for the copolymer to increase in diameter, stimulate postoperative calcification and bone ingrowth in PEO/PBT 80/20 copolymers.

Key words: osteoconductive material; hydrogel copolymers; bone ingrowth.

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The application of osteoconductive materials, which function as a scaffold and thus guide new bone formation, is an alternative approach to autogenic and allogenic bone grafts for restoration of bone defects. Research in this field has been prompted by the shortcomings of such autografts and allografts^{9,13,16,20}. Bioactive materials, such as tricalcium phosphate, hydroxyapatite, bioglass, glass ceramics, coral, and other materials, allow osteoconduction and have been used experimentally and clinically for oral and maxillofacial implantology. They have been applied either independently or in combination with autogenous bone and sometimes with freeze-dried bone^{4,19}. Generally, the relative brittleness, in the case of glasses and ceramics, associated with a low resistance to fatigue failure, and the risk of migration from the defect when applied as particles, constitute significant disadvantages for the application of these bioactive materials⁷.

In contrast to the brittle ceramics and glasses, flexible elastomeric poly(ethylene oxide) (PEO)/poly(butylene terephthalate) (PBT) segmented copolymers with a low modulus of elasticity, also referred to as Polyactive®^{2,3}, were recently introduced. Several studies have demonstrated the bone-bonding capacity of these materials and stressed its relation to PEO content and surface calcification^{26,27,36}. Bone was frequently in close contact with the surface of the implants, without an intervening fibrous tissue layer, and transmission electron microscopic evaluation of the bone/biomaterial interface demonstrated the presence of an electron-dense layer^{3,36}, which is considered to indicate bone-bonding^{5,12,17,34}. Further implantation studies showed postoperative deposition of carbonate-containing apatite crystals³¹, on top of and underneath the copolymer surface, which were in continuity with the apatite crystals in bone. This observation is also considered to

indicate bone-bonding, according to the accepted definition³⁷. The carbonate-containing apatite crystals in the PEO/PBT copolymers are very similar to the apatite layer that is formed on bioactive ceramics^{8,21,23–25} and, thus, provide a favourable surface for bone apposition by osteoblasts¹⁴.

Although PEO/PBT copolymers already show quite satisfactory bone-bonding properties^{29,30,36}, an effort was made to stimulate even further the bone-bonding behaviour of these copolymers^{10,11}. In the case of bioactive ceramics, several studies have demonstrated that release of calcium and phosphorus ions plays a crucial role in the formation of the apatite layer and in bone-bonding^{1,18,21}. PEO/PBT copolymers initially lack the presence of calcium and phosphorus ions and depend on postoperative calcification of the implant surface before bone-bonding can take place. In view of the calcification mechanism of bioceramics, it was ex-

pected that the preoperative presence of calcium and phosphorus ions in PEO/PBT copolymers might stimulate the postoperative calcification process, leading to more extensive bone-bonding. Recently, a method has been described in which a thin layer of calcium phosphate crystals, composed of a combination of hydroxyapatite (HA) and β -tricalcium phosphate (β -TCP), was formed on the entire surface of porous PEO/PBT copolymer implants¹⁰. This was also shown to be advantageous for bone-bonding¹¹.

Material and methods

Material

In the application of Polyactive as a bone-filler, three types of implants, with a diameter of 4 mm and a length of 10 mm, were evaluated and compared to a control defect.

The first bone-filler (type E) comprised porous Polyactive with an 80/20 PEO/PBT ratio and an average porosity of $50 \pm 5\%$ (H.C. Implants BV, Leiden, The Netherlands). The average pore size diameter was $300 \pm 150 \mu\text{m}$, while the average interpore diameter was $100 \pm 50 \mu\text{m}$, as measured using VIDAS morphometry equipment.

For the second bone-filler (type F), samples similar to type E were given an extra treatment, in which a layer of calcium phosphate was deposited on the surface of the porous implant. This method of precalcification, which has been described in detail by GAILLARD et al. (1993), consisted of two steps. Briefly, the Polyactive implant was first rinsed in a calcium chloride solution and dried. In the second step, the implant was placed in a sodium phosphate solution¹⁰.

The third bone-filler (type G) comprised CAMCERAM® (>95% dense) HA granules with a >99% HA crystal structure (CAM Implants BV, Leiden, The Netherlands). The diameter of the granules was 0.45–1.0 mm. The granules were held together by a thin layer (15 μm) of Polyactive PEO/PBT 70/30, which served as a coating only. Between the coated granules, spaces of $225 \pm 75 \mu\text{m}$ were still present.

All implants were briefly rinsed in distilled water, dried for 16 h at 37°C, and sterilized by gamma irradiation (25 kGy). The pore size distribution and the presence of calcium phosphate depositions was checked in a selection of implants using scanning electron microscopy and X-ray microanalysis on carbon-coated samples.

Implantation procedure

Twelve beagle dogs, eight male and four female, were used in this study. They were approximately 2 years old and had an initial weight range of 9–14 kg. The first and second mandibular premolars were extracted

bilaterally, and, after a 3-month healing period, a mucoperiosteal flap was elevated and the alveolar crest was exposed. With a low-speed drill, a series of burrs with increasing diameters, and continual internal physiologic saline irrigation, defects of 4-mm diameter and 10-mm depth were created in the bone. Subsequently, the implants were inserted press-fit.

Three different bone-fillers (E-F) and one control defect (H) were allocated schematically, with a multiple Latin squares design, to ensure a balance of animal number, location, and implant type.

The dogs were killed 9 months after implantation. The implants and the control defects, with surrounding bone, were fixed with a solution of 4% paraformaldehyde and 5% glutaraldehyde in 0.1 M cacodylate buffer (Karnovsky's fixative) for 1 week at 4°C.

Light microscopy (LM)

The samples were dehydrated through a graded series of ethanol under vacuum and routinely embedded in methyl methacrylate (MMA). Light microscopic sections were processed on a histologic diamond saw (Leiden Microtome Cutting System) and were stained with basic fuchsin/methylene blue (staining for calcified and soft tissue, respectively), in order to study tissue ingrowth into the implants.

Back-scattered electron (BSE) microscopy and XRMA

After histologic sectioning, the remaining MMA blocks, with embedded specimens in cross section, were polished with silicon carbide paper and coated with a thin layer of carbon (Edwards carbon coater). The specimens were evaluated in a Philips S 525 scanning electron microscope working in the back-scattered mode at 20 kV. Radiographic elemental analysis was performed with an attached radiographic microanalysis unit (XRMA, Tracor Northern).

Histomorphometric analysis

For quantification, sections through the centre area of each implant were evaluated. With LM, it was difficult to distinguish between fibrous tissue and the Polyactive material. Therefore, it was decided to perform the quantitative analysis on photographs made during the BSE microscopy procedure. A composition of photographs with a magnification of $\times 40$ was made to reconstruct each bone defect. A line was drawn from one bone edge to the other, and the upper half of the original defect (Fig. 1) was scanned with the VIDAS Image Analysis System (Kontron Elektronik Bild Analyse, Munich, Germany). The percentages of bone ingrowth, fibrous tissue, and implant material were measured.

Statistics

Differences in bone-filler type (E-H) performance were determined by multiple regression analysis in incomplete block design with missing data. Bone-filler effects were corrected for interdog variations.

Results

Initial healing was generally uneventful, although it was observed that 1 week after implantation, one implant of type E and one of type F had perforated the mucosa, probably due to insufficient primary wound closure. As a result, these two implants were lost and could not be evaluated. In contrast to the three bone-filler types, it was difficult to section precisely through the middle of the sham preparations, since the original outline of these cavities was difficult to define. For this reason, one sham preparation was also excluded from the experiment.

Microscopy

Bone-fillers: preoperative results

Fig. 2 shows the three implant materials in cross section preoperatively. Scanning electron microscopic images demonstrated that the PEO/PBT 80/20 implants had a relatively smooth surface and an equal distribution of pores in the implant (Fig. 2A).

Scanning electron microscopic evaluation of precalcified PEO/PBT 80/20 copolymer implants demonstrated the presence of 5–10- μm -thick areas on the implant surface (Fig. 2B and C). Radiographic microanalysis demonstrated the presence of calcium and phosphorus in these surface areas. The pore distribution in these precalcified implants was comparable to the nonprecalcified implants.

Evaluation of the third bone-filler type with scanning electron microscopy showed the HA granules to be covered by a thin layer of PEO/PBT Polyactive (Fig. 2D). The spaces between the granules were only partially filled by the Polyactive material, thus leaving sufficient pore size (diameter: $225 \pm 75 \mu\text{m}$).

Bone-fillers: postoperative results (9 months) PEO/PBT 80/20 implants

Both LM (Fig. 3A) and back-scattered scanning electron (BSE) microscopy (Fig. 4A) showed relatively large globular spots in the implants, with a diameter ranging from 5 to 40 μm . Radiographic

Fig. 1. Schematic illustration of bone-filler type G. Upper half (5 mm) of bone defect was scanned by Vidas Image Analysis System.

microanalysis showed the presence of calcium and phosphorus in these calcification spots, which were usually more densely packed near the surface than in

the centre of the copolymer granules. Calcification was present only from the periphery of the porous cylinder to approximately 1 mm from the original outline, and was not usually present in the centre of the implants.

A reduction in pore size was observed as compared to the original implants before implantation. This phenomenon was most prominent in the central part of the implants. The pores in these calcification-free central zones were very small ($\leq 50 \mu\text{m}$) and were often filled with soft tissue, which was partially composed of phagocytotic cells with a foamy appearance. Larger pores were observed in the peripheral part of the implants. Calcification of the implant and ingrowth of new bone were restricted to the periphery of the implant. Bone was usually in contact with the calcified copolymer surface, without an intervening fibrous tissue layer. Radiographic microanalysis line scans in these areas demonstrated a continuity in

Ca and P signals across the interface. Some calcified copolymer granules were covered with a thin layer of new bone, while others were completely incorporated in bone tissue.

Degradation was observed with light microscopic techniques. The original contour of the implants was hardly affected by the degradation after 9 months of implantation. However, copolymer fragments of various sizes were observed in the soft tissue around the implants. Adjacent to these fragments, phagocytotic cells with a foamy appearance, suggestive of vacuoles filled with phagocytosed polymer particles, were observed.

Precalcified PEO/PBT 80/20 Implants

The postoperative calcification in precalcified PEO/PBT copolymers was composed of a layer of fine granular calcium phosphate depositions, as is shown with LM (Fig. 3B) and BSE (Fig. 4B). The calcification was restricted to the

Fig. 2. Scanning electron micrographs: a) PEO/PBT 80/20 copolymer, field width: 1.49 mm; b) precalcified PEO/PBT 80/20 copolymer (note calcification areas), field width: 1.49 mm; c) calcification spot in precalcified PEO/PBT 80/20 copolymer, field width: 90 μm ; d) HA granules held together with PEO/PBT 70/30 copolymer, field width: 1.49 mm.

Fig. 3. Light micrographs: a) PEO/PBT 80/20 copolymer, field width: 2.25 mm; b) precalcified PEO/PBT 80/20 copolymer. Field width: 2.25 mm; c) HA granules held together with PEO/PBT 70/30 copolymer, field width: 2.25 mm; d) sham preparation, field width: 2.25 mm. Arrow-head shows border between old lamellar (above) and new (below) bone. b: bone; c: calcification; h: HA granules; i: implant.

outermost 150 μm of the polymer matrix. In contrast to the periphery, the centre of the bulk of the implants was not calcified.

Bone ingrowth was observed in pores in both the periphery and centre of the implant, and new bone had usually bridged the 4-mm defect. Although not as prominent as with the nonprecalfied Polyactive, a reduction in pore size was observed, especially in the central part of the porous implant (diameter pore size $\leq 100 \mu\text{m}$). Intimate contact was seen between bone and the calcified implant surface, without an intervening fibrous tissue layer. The continuity in calcium and phosphorus signal across the bone/Polyactive interface, which was observed with radiographic microanalysis, indicated bone-bonding in these regions. Some pores were completely filled with bone tissue, except for a few gaps that contained foam-like phagocytotic cells. The total amount of postoperative calcification, bone ingrowth in the pores of the implant, and bone contact with the

implant surface was much higher in precalcified than nonprecalfied PEO/PBT 80/20 implants. As in the nonprecalfied implants, degradation was observed by light microscopic techniques. Copolymer fragments of various sizes, surrounded with a layer of foam-like phagocytotic cells, were present in the soft tissue around the implants.

HA granules with Polyactive PEO/PBT 70/30 coating

At the periphery of the defect, the HA granules (Figs. 3c and 4c) were often surrounded by, and in close contact with, bone. Radiographic microanalysis line scans in these areas demonstrated a continuous Ca and P signal across the interface, which confirmed bone-bonding. At the centre of the defect, most of the HA granules were enveloped by fibrous tissue, although bridging of the defect with bone was also observed. Remnants of the Polyactive coating were rarely seen; however, phagocytotic cells, which had a foamy appearance,

probably due to phagocytosed polymer fragments, were still present.

Sham preparation (no bone-filler)

In general, bridging of the defect with bone was seen (Figs. 3d and 4d). At the top of the defect, the cortical bone was restored, although, in one sample, the defect was completely filled with bone fragments. These were probably produced during the drilling procedure and prevented the cortical bone from bridging the defect. Although the contour of the mandibular bone was restored, the new cortical bone showed a less dense structure than the old lamellar bone.

Histomorphometric analysis

To interpret the measurements of bone and fibrous tissue ingrowth, the mean percentages of mineralized component present in the bone cavities, i.e., bone, calcified Polyactive, and HA, were defined.

Fig. 4. Back-scattered electron micrographs: a) PEO/PBT 80/20 copolymer, field width: 0.90 mm; b) precalcified PEO/PBT 80/20 copolymer, field width: 0.90 mm; c) HA granules held together with PEO/PBT 70/30 copolymer, field width: 1.43 mm; d) sham preparation, field width: 1.43 mm. Arrowhead shows border between old lamellar (above) and new (below) bone. b: bone; c: calcification; h: HA granules; i: implant.

Fig. 5 shows the results for the precalcified and nonprecalfied Polyactive bone-fillers. In the case of nonprecalfi-

fied Polyactive bone-fillers, 13.5% bone and 9.9% calcified Polyactive were present.

Precalcification of the copolymer material raised the bone ingrowth to 24.4% and the presence of postoperatively calcified Polyactive to 45.4%. In these cavities, a total of 69.8% mineralized component was present.

In the HA granules held together by Polyactive 70/30, a bone ingrowth of 29% and the presence of 34.1% HA material were observed (Fig. 6).

In the sham preparation, 54.9% of the original bone cavity was refilled with bone (Fig. 6).

Statistics

Bone-filler effects, corrected for interdog variations, were significant ($F_{3,7}$ value: 11.87, $P=0.004$).

Compared with the sham preparation, significantly ($P=0.04$) more mineralized component was present in the cavities filled with precalcified Polyactive and significantly less mineralized component was present in the nonprecalfied Polyactive-filled cavities. No differences were observed between the cavi-

Fig. 5. Percentage of tissue components in defects filled with precalcified and nonprecalfied PEO/PBT 80/20 copolymer.

Fig. 6. Percentage of tissue components in defects filled with HA granules held together with PEO/PBT 70/30 copolymer, as compared with defects, which were left unfilled.

ties filled with HA granules held together with PEO/PBT 70/30 Polyactive and the sham preparations ($P=0.19$).

Discussion

The relatively high bone ingrowth (54.9%) observed in the sham preparations is partially explained by the fact that a critical size defect was not used. The major goal in this study was to assess whether bone ingrowth was affected by precalcification and not what defect size could be bridged.

PEO/PBT copolymers have hydrogel properties, and tissue fluid uptake is influenced by the PEO/PBT ratio and PEO molecular weight. As a consequence, the hydrogel material swells 43% for the 80/20 PEO/PBT copolymer (molecular weight PEO: 1000). During tissue fluid sorption, calcium ions are bonded by the chelating effect of the soft PEO segment^{32,33,35}. This leads to calcium phosphate crystal formation within the PEO/PBT implant, the extent of which is considered an important parameter in relation to the bone-bonding capacities of PEO/PBT copolymers^{35,36}.

A positive effect of the swelling behaviour of the PEO/PBT copolymers is the formation of a tight seal in the bone cavity²⁹. However, press-fit insertion of the implant prevents the copolymer from increasing in diameter. As a consequence, implant swelling is bound to reduce the porosity of the implant. It is probable that this reduction in pore diameter was responsible for the unexpectedly low amount of bone ingrowth. In contrast to our findings, RÄDDE et al. demonstrated abundant bone ingrowth in both the periphery and centre of 80/20 PEO/PBT copolymer implants after 6-week implantation in the femora of goats³⁰. However, they used low-speed orthopaedic drilling equipment with externally cooled drills, a method considerably less precise than our implantation procedure, which used low-speed dental drilling equipment and internally cooled precision implantation drills with increasing diameters. Efforts to insert the implants optimally press-fit, as mentioned above, did evoke a negative effect, and recent experiments have indicated that bone defects should be underfilled when PEO/PBT copolymers are applied.

Precalcification increased the bone

ingrowth and calcification rate of the PEO/PBT 80/20 copolymer considerably. POLLOCK et al. have already demonstrated that the preoperative presence of calcium in polyurethanes acts as an initial nidus for calcification²⁸. A similar mechanism has also been described for the formation of the apatite layer on bioactive calcium phosphate ceramics by epitaxial crystal growth^{6,22}.

The third bone-filler in this study comprised HA granules held together with a PEO/PBT 70/30 copolymer layer. In general, HA granules are difficult to handle, and spillage of the granules beyond the implantation area gives rise to mucositis and pain¹⁵. For prevention of spillage, the HA granules were held together with a PEO/PBT 70/30 copolymer layer. Unfortunately, LM and BSE revealed that loose HA granules were present in the oral mucosa covering the bridged defects. The percentage of mineralized component (29% bone and 34.1% calcified Polyactive) was comparable to the sham preparation (54.9% bone). However, it should be stressed that reconstructing defects with brittle HA particles inhibits future dental implant placement.

In conclusion, the results of this study quantitatively showed that precalcification of the PEO/PBT copolymer induced more abundant postoperative implant calcification in the implant surface, which increased the total amount of bone in the pores. Care should be taken, however, not to insert the copolymer implants press-fit. Sufficient space should be present to allow the copolymer to swell. Underfilling the defect is essential and makes the surgical procedure easier. Studies on critical size defects are necessary to demonstrate the advantage of applying precalcified PEO/PBT copolymers as a bone reconstructive material in large bone defects.

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Address:

G. J. Meijer

Department of Oral-Maxillofacial Surgery
 Prosthodontics and Special Dental Care
 University of Utrecht
 Universiteitsweg 100
 3584 CG Utrecht
 The Netherlands