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Biocompatibility of intraosseously implanted predegraded poly(lactide): an animal study

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During degradation of high molecular weight as-polymerized poly(L-lactide) (PLLA) late complications such as swelling of the subcutaneously implanted bone plates have been observed in patients. However, in the same patients the intraosseously implanted PLLA screws did not give rise to any complications. To investigate a possible difference in the degradation and histological response due to the site of implantation, subcutaneous and intraosseous implantation studies are necessary. In the present study rods of PLLA and a copolymer poly(96L/4D-lactide) (PLA96) and predegraded PLLA and PLA96 were implanted intraosseously in rabbit tibiae. *In vitro* predegradation at elevated temperatures was used to obtain an enhanced degradation rate to simulate long-term physiological degradation. Animals were sacrificed after a postoperative period varying from 2 to 26 weeks. The predegraded PLLA and PLA96 implants showed a high rate of fragmentation and internalization of particles by macrophages. In the medullary cavity large fields of macrophages and, with the predegraded PLA96 implants, haemorrhages and infiltrates were noted, but no osteolytic changes were observed in the cortical bone. It can be concluded that, based on the disintegration of the implants and the accompanying histological reaction, a reaction similar to that found with subcutaneous implants in former studies had occurred. This indicates that not only the site of implantation, subcutaneous or intraosseous, but a number of other factors will affect the degradation process and the corresponding histological response.

1. Introduction

In the last decade in orthopaedic and maxillofacial surgery there has been a wide and increasing range of applications of biodegradable biomaterials [1–3]. Although the use of both polyglycolide and polylactide has been reported to be very successful, a number of studies on intraosseously placed polyglycolide rods showed foreign body reactions, with discharging sinus formation and osteolytic foci [4–6]. No such complications have been reported so far using poly(L-lactide) (PLLA) intraosseously.

In our department as-polymerized PLLA has been successfully used for fracture fixation and orbital floor reconstructions [7–9]. Subcutaneously positioned PLLA bone plates used for fixation of unstable zygomatic fractures, however, caused clinically detectable swellings in all operated patients from 3 years onwards [10–12]. In the same patients the intraosseously positioned as-polymerized PLLA screws did not show any swelling or osteolytic changes. Moreover, as-polymerized PLLA orbital floor implants used in a goat study were covered with bone from 26 weeks and for up to 5 years of implantation did not show any swelling, osteolytic changes or foreign body reactions [9, 13]. Based on these findings, a difference in the long-term tissue reaction and implant degradation between subcutaneously implanted PLLA bone plates and intraosseously implanted PLLA screws seems possible.

To investigate the influence of the site of implantation on the long-term histological reaction and the degradation of PLLA, follow-up periods of at least 3 years will be necessary because of the slow degradation rate of PLLA and the late manifestation of the subcutaneous swelling in patients. *In vitro* predegradation at elevated temperatures, as described in previous studies, can be used to shorten the *in vivo* follow-up period [14–16].

To enable a good comparison with a previous subcutaneous implantation study, plugs made of non-degraded as well as predegraded PLLA and a copolymer poly(96L/4D-lactide) (PLA96) from the same batch were implanted in the tibiae of rabbits [16]. PLA96 was developed as a possible alternative

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material to PLLA; the copolymerization of L-lactide with 4% D-lactide will lead to a lower initial crystallinity and smaller and less perfect crystalline domains, which hopefully will result in an enhanced degradation rate and fewer clinical complications.

The aim of the present study was to describe quantitatively and qualitatively the degradation and biocompatibility of intraosseously positioned predegraded PLLA and PLA96 implants.

2. Materials and methods

Two types of high molecular weight as-polymerized polylactide were used for implantation: PLLA and a copolymer PLA96. Both polymers were as-polymerized in a block according to the method described by Leenslag and Pennings [17]. Polymerization of L-lactide and D-lactide was performed after purification of the monomer (L-lactide from CCA/Purac Biochem, The Netherlands) by recrystallization from toluene under N_2 atmosphere. Both PLLA and PLA96 were as-polymerized under vacuum at temperatures of 110 °C using 0.015 wt % stannous-2-ethyl-hexanoate as a catalyst. Molecular weights ($\overline{M}_w$ and $\overline{M}_n$) were determined by gel permeation chromatography (GPC) at 30 °C using THF relative to polystyrene standards. Thermal properties were evaluated by differential scanning calorimetry (DSC) on a Perkin Elmer DSC-7; 5–10 mg samples of PLLA and PLA96 were measured at a heating range of 10 °C min^{-1} to determine the heat of fusion (ΔH), the melting temperature (T_m) and the glass transition temperature (T_g). The initial molecular weight ($\overline{M}_W$)as determined by GPC of the undegraded PLLA and PLA96 disks was 880×10^3 and 1300×10^3, respectively. DSC analysis of the PLLA and PLA96 disks revealed a ΔH of 60 J g^{-1} and 26 J g^{-1}, a T_m of 187 °C and 152 °C, and a T_g of 60 °C and 56 °C, respectively.

From the as-polymerized blocks, 50 cylindrical rods of each polymer were machined with a diameter of 2 mm and a height of 4 mm (mean weight for the PLA96 cylinders was 15.8 mg, and for the PLLA cylinders 15.5 mg). All rods were sterilized using regular steam sterilization. After sterilization the physical properties were measured. For *in vitro* predegradation, 25 PLLA and 25 PLA96 rods were immersed in a tube with phosphate buffer (pH 7.4) which was subsequently put in a temperature controlled basin containing 20 l of phosphate buffer at pH 7.4 and which was set at 90 °C. Based on the findings of previous studies, the PLLA and PLA96 rods were predegraded for 336 h ($PLLA_{336}$) and 168 h ($PLA96_{168}$), respectively, to obtain comparably degraded material. Five rods of each predegraded group were dried to a constant weight, after which mass loss was determined. After weighing, these rods were used for DSC and GPC measurements.

2.1. Animal study

Ten mature male rabbits were used in this study. All operations were performed under aseptic conditions. The rabbits were anaesthetized with Nembutal 0.5 ml kg^{-1} intravenously. The rear limbs were shaved and prepared using iodine. A vertical skin incision was made and the tibia accessed via blunt dissection. The periosteum was then incised and reflected. In both the left and right tibia, four equidistant holes with a diameter of 2 mm were drilled under constant saline cooling. In each tibia two non-degraded cylindrical rods (one $PLLA_0$ and one $PLA96_0$ rod) were inserted into the drilled holes with forceps. In the remaining two holes, predegraded $PLLA_{336}$ and $PLA96_{168}$ rods were carefully injected. The connective tissue and skin were closed in two layers with 3-0 Maxon. After 2, 4, 8, 12 and 24 weeks two animals were killed using Euthezate. The tibiae and the overlaying soft tissue were generously excised and fixed in 4% formaldehyde/1% glutaraldehyde. The inguinal and the iliaca medialis lymph nodes were also generously excised. Post-mortem radiographs of the tibiae were taken.

After fixation for at least 1 week at 4 °C, the tibiae were decalcified with 25% formic acid/10% trisodium citrate at room temperature for 4 weeks. Decalcification was checked using X-ray photographs. The decalcified tibiae were cut in a longitudinal plane and dehydrated in a graded ethanol series. The specimens were embedded in glycolmethacrylate (GMA) that was polymerized for 24 h at −20 °C, and 2 µm sections were cut (Jung microtome 1140/autocut) and stained with toluidine blue. The entire specimen was cut: sections were made every 0.2 mm.

2.2. Quantification of the predegraded material

Light microscopical sections of each tibia ($n = 4$) were used to determine the total polymer area fraction of PLLA and PLA96 implants per tibia and the mean cortical bone area fraction per frame area with a Quantimed 520 image analysis system (Cambridge Instruments, Cambridge, UK). All sections were analysed at a magnification of ten times. To determine the statistical significance of a decrease in the cortical bone area fraction, the regression with the implantation time was calculated. For the polymer area fraction a two-tailed student t test was used.

3. Results

The influence of both the sterilization procedure and the predegradation on the physical properties of the polymers are given in Table I. The sterilization procedure substantially altered the physical properties: the molecular weight showed a sharp decrease, with an increase in the polydispersity. The ΔH of the sterilized $PLLA_0$ and $PLA96_0$ implants increased from 60 J g^{-1} to 85 J g^{-1} and from 26 J g^{-1} to 46 J g^{-1}, respectively. The molecular weight of $PLLA_{336}$ and the $PLA96_{168}$ showed a further decrease, with a polydispersity of 1.85 and 1.4, respectively. The ΔH of the predegraded materials was 101 J g^{-1} and 79 J g^{-1} for the $PLLA_{336}$ and the $PLA96_{168}$, respectively. The T_m was 179 °C for the $PLLA_{336}$ and 145 °C for the $PLA96_{168}$.

TABLE I Chemical characterization of the sterilized and predegraded polymers

Type	Mass loss	$\overline{M}_w$	$\overline{M}_n$	$\overline{M}_w/\overline{M}_n$	T_m (°C)	T_g (°C)	ΔH (J g^{-1})
$PLLA_0$	—	280000	58400	4.8	190	57	85
$PLA96_0$	--	85000	25500	3.3	160	47	46
$PLLA_{336}$	48.3%	2000	1100	1.85	179	45	101
PLA_{168}	47%	300	200	1.4	145	41	79

TABLE II Changes in the surface areas of the cortical bone and predegraded implants with implantation time.

Weeks of implantation	Surface area cortical bone (mm^2/frame area)	Total surface area $PLA96_{168}$ implants (mm^2)	Total surface area $PLLA_{336}$ implants (mm^2)
2	6.01 ± 0.86	41.3 ± 10.6	20.3 ± 1.9
4	5.89 ± 0.13	38.9 ± 13	13.4 ± 0.4
8	5.50 ± 0.1	37.7 ± 9.8	14.3 ± 2.1
12	6.09 ± 0.88	34.4 ± 11.2	12.8 ± 3.6
24	6.14 ± 0.55	35.2 ± 5.2	8.88 ± 0.5

Table II shows that the mean total surface area of the measured cortical bone per frame area did not show any significant decrease with implantation time. The mean surface area of the predegraded PLA96 implants was significantly higher ($P < 0.01$) compared with predegraded PLLA implants.

All animals recovered uneventfully from the operation, and the rear limbs seemed to be totally functional. In no animals were clinically detectable inflammatory reactions seen. On radiographical examination at 2 weeks the drilled holes were clearly visible. From 12 weeks on, the holes with the predegraded material became less visible, and were no longer detectable 24 weeks postoperatively. The radiographs of these tibiae suggested a complete healing of the cortex (Fig. 1). Around and on top of each of the undegraded rods a radio-opacity was seen with PLLA as well as the copolymer PLA96 from 8 weeks on. The holes remained visible up to 24 weeks.

Microscopically, at 2 weeks the $PLLA_0$ and $PLA96_0$ implants were encapsulated by a thin and immature fibrous tissue capsule with a monocyte layer directly adjacent to the implants. The drilled holes in the cortex were fully covered by fibrous tissue. With longer implantation periods a mature fibrous capsule of three cell layers, orientated parallel to the surface, was observed. In some parts reactive immature bone was seen, starting at the endosteal cortex and growing to the apical end of the cylinder. Some $PLA96_0$ rods showed cracks and ingrowth of cells. Over the total implantation period the surface area of the implants did not decrease significantly ($P < 0.05$).

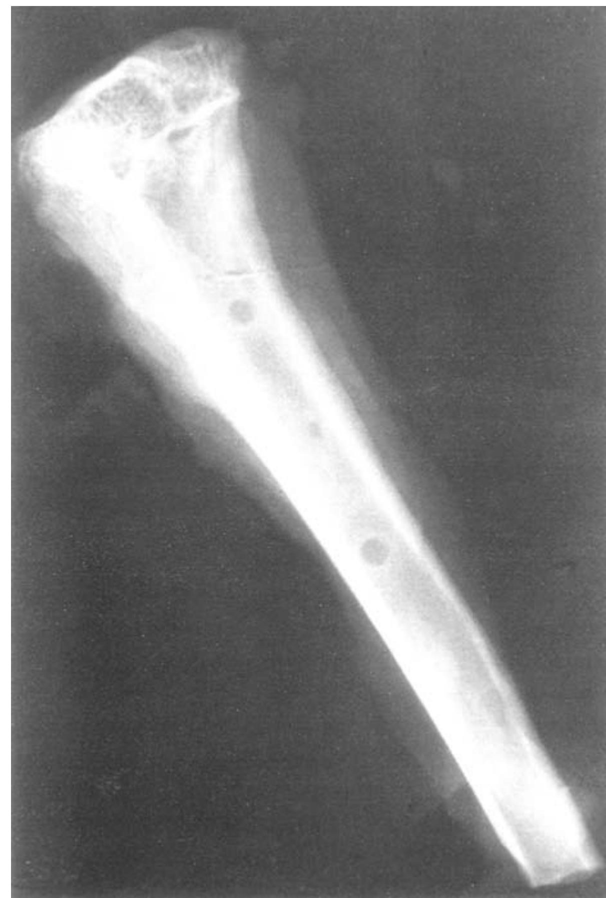

Figure 1 Radiograph of a tibia after 24 weeks of implantation.

After 2 weeks the injected predegraded $PLA96_{168}$ implants still had a cylindrical form but severe fragmentation was seen. Implanted material was observed in the medullary cavity and underneath the periosteum. In the medullary cavity some fields of foamy macrophages were seen, but also areas of haemorrhage and cellular infiltrate (Fig. 2). Active bone formation and remodelling was found in close contact with the implants (Fig. 3). The $PLLA_{336}$ implants showed a much higher rate of fragmentation and internalization by macrophages compared with the $PLA96_{168}$ implants. In the medullary cavity large fields of macrophages were present without clear signs of fibrous encapsulation. Around the $PLLA_{336}$ implants no necrotic zones or haemorrhages were observed. In the subperiosteal region, fields of densely packed macrophages interlaced with newly formed capillaries were seen.

Figure 2 Predegraded $PLA96_{168}$ after 4 weeks of implantation. Low power view (original magnification × 2.5) of a drilled hole in the cortex with PLA96 particles (★) and areas of haemorrhage (arrrows).

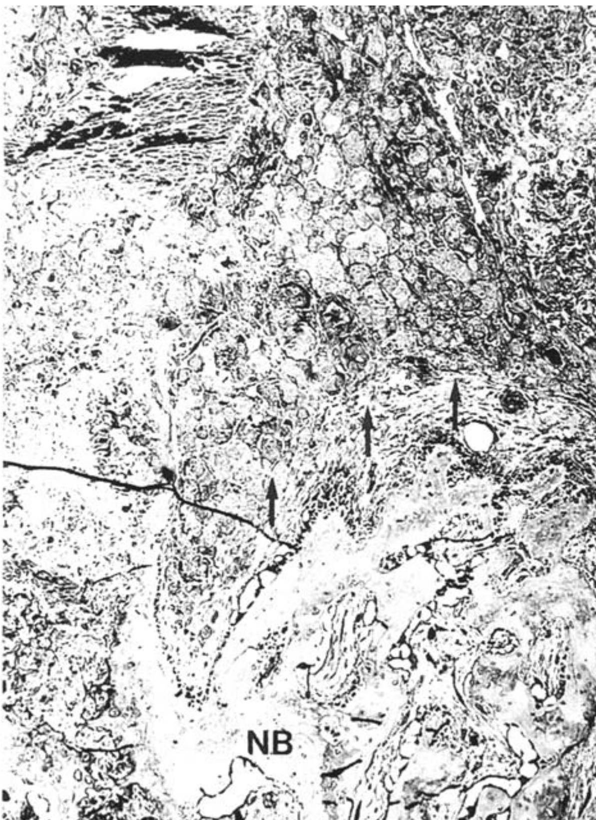

Figure 3 Low power micrograph (original magnification × 2.5) of predegraded $PLLA_{336}$ after a postoperative period of 4 weeks. Active new bone formation (NB) and remodelling was found in close contact with fields of foamy macrophages (arrows).

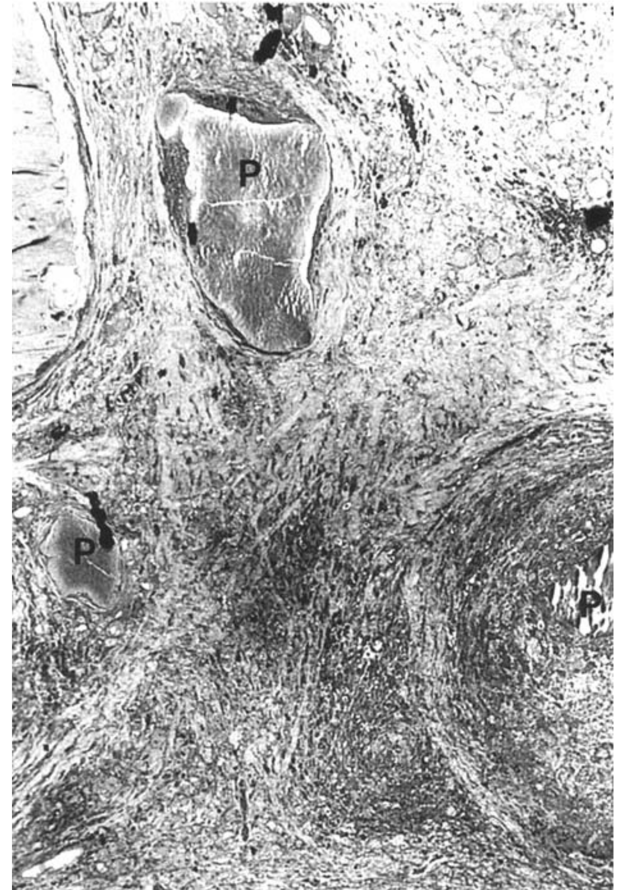

Figure 4 $PLA96_{168}$ particles (P) encapsulated by a cellular infiltrate and a fibrous capsule after 12 weeks of implantation. Original magnification × 4.

At 12 weeks there was a continuous fragmentation of the $PLA96_{168}$ into fragments that could be internalized; the number of foamy macrophages therefore seemed to have increased. There were still large areas of particles and necrotic cells that were encapsulated by fibrous tissue (Fig. 4). Some of the drilled holes were filled with newly formed bone (Fig. 5). The $PLLA_{336}$ implants were encapsulated in the subperiosteal region and large fields of foamy macrophages were seen in the vicinity of active new bone formation (Fig. 6). In the intramedullary cavity, circular areas of macrophages were seen without a clear fibrous demarcation. At 24 weeks of implantation both the $PLA96_{168}$ and the $PLLA_{336}$ implants had totally fragmented and large fields with densely packed macrophages were noted (Fig. 7). The $PLA96_{168}$ implants no longer showed large fields of necrosis and haemorrhages. Reactive non-woven bone was found both on the endosteal and the periosteal side, mostly in close contact with large fields of macrophages. In none of the animals extensive osteoclastic activity was noted, although active bone remodelling was observed (Fig. 8). $PLLA_{336}$ or $PLA96_{168}$ particles were not observed in the inguinal or the iliaca medialis lymph nodes in any of the animals, irrespective of the implantation time.

4. Discussion

The histological reaction to the $PLLA_0$ and $PLA96_0$ implants was comparable to the findings in the

Figure 5 Some of the holes implanted with predegraded material were filled with newly formed reactive bone (arrows) at 12 weeks. Original magnification ×6.

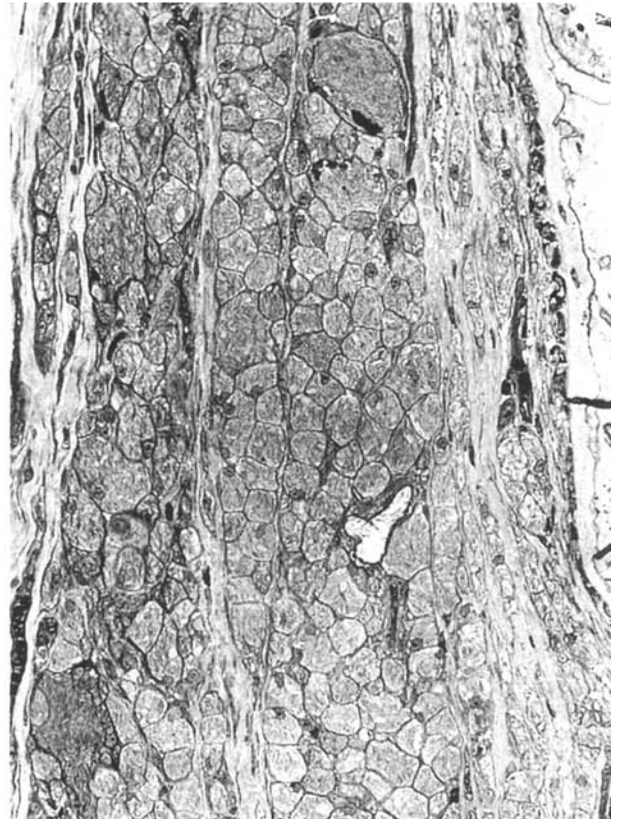

Figure 7 At 24 weeks $PLA96_{168}$ implants no longer showed large fields of necrosis or haemorrhages, and resembled the $PLLA_{336}$ implants with large fields of foamy macrophages (arrows). Original magnification ×16.

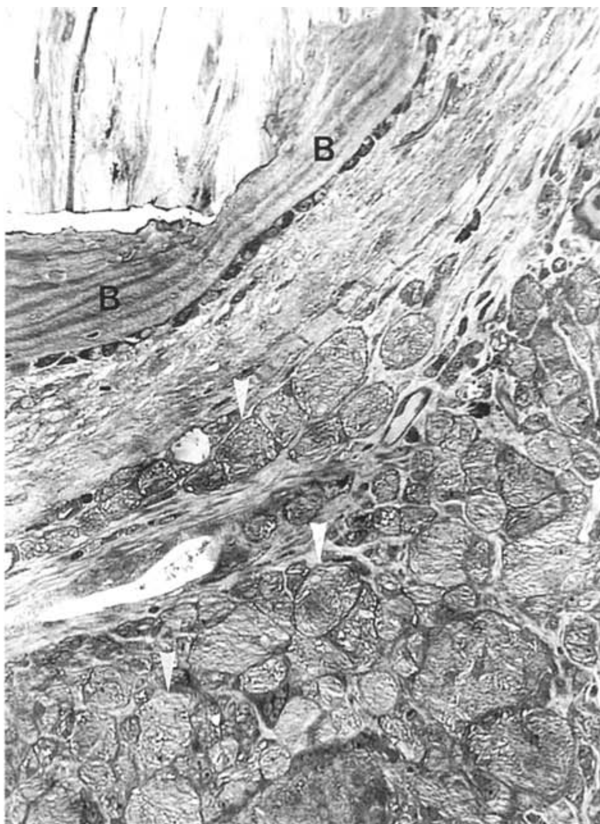

Figure 6 In the subperiosteal region large fields of foamy macrophages (arrows) were observed in the vicinity of active new bone formation (B). Original magnification ×16.

Figure 8 After a postoperative period of 24 weeks, the screw holes were filled with newly formed woven and lamellar bone. Original magnification ×4, with crossed Nicol prisms.

literature. PLLA hardly ever shows a direct contact between implant and bone; usually, a thin cellular or fibrous layer is present between the implant and the bone surface [18–20]. As-polymerized PLLA implanted in goats for orbital floor reconstructions also showed a cellular or fibrous intermediate layer between the implant and the bone surface [9, 13]. No elevated cellular or inflammatory reactions were observed during the follow-up period, which confirms the good osseous tissue biocompatibility of $PLLA_0$ and $PLA96_0$. After 4 weeks of implantation some cracks in both the $PLLA_0$ and $PLA96_0$ rods, with ingrowth of cells, was observed. PLLA and especially PLA96 are moisture-labile polymers, which implies that regular steam sterilization can have a substantial influence on the physical properties. The PLLA as well as the PLA96 rods showed a steep decrease in the molecular weight and an increase in the heat of fusion with sterilization. This clearly indicates degradation of the polymers, which could explain the early cracking.

The implantation of *in vitro* predegraded material was used to study the long-term degradation of PLLA and PLA96 implants and the histological reaction in an osseous environment. In the present study, we used predegraded $PLLA_{336}$ and $PLA96_{168}$ from the same batch as in a subcutaneous implantation study [16]. In both the present study and in the subcutaneous implantation study, the $PLA96_{168}$ implants showed an almost similar, although slightly retarded, fragmentation into small particles when compared with the $PLLA_{336}$ implants. The surface area of the $PLA96_{168}$ was significantly ($P < 0.01$) higher compared with $PLLA_{336}$. The reason for this difference is not quite clear; a possible explanation could be that the $PLA96_{168}$ particles become much more scattered due to haemorrhages and infiltrate during the first weeks of implantation. With longer implantation times, macrophages become the major cellular factor, but the initial scattering probably remained. In contrast with the previous subcutaneous implantation study with $PLA96_{168}$ disks, in this study the intramedullary implanted $PLA96_{168}$ plugs evoked a nonspecific inflammatory reaction [16]. The reason for this difference is not known, although contamination during implantation or predegradation may be a possible cause, and further research is needed. Based on the results of the present and previous studies, it can be expected that PLA96 implants will have qualitatively the same, and quantitatively perhaps an increased, histological reaction and degradation characteristics as PLLA implants.

In the previous study the subcutaneously implanted $PLLA_{336}$ and $PLA96_{168}$ disks were encapsulated by fibrous tissue and were relatively form-stabile, although fragmentation and phagocytic activity was observed with longer implantation periods [16]. The intraosseously implanted material, however, showed a much higher level of fragmentation, migration and cellular activity after only 2 weeks of implantation. The implants were clearly recognizable but no longer had their original shape; material had migrated out of the medullary cavity underneath the periosteum. The high fragmentation and migration level could be explained by the fact that there might have been a positive intramedullary pressure just after implantation, which caused movement out of the medullary cavity and perhaps extensive fragmentation of the already fragile implants. The fragmentation could be increased through pressure on the implants generated by the tibial muscles. In a number of studies an increase in the intramedullary pressure was measured after reaming and nailing in the tibia [21, 22]. The insertion of the pins used in this study may have a comparable effect, resulting in extrusion and fragmentation of the feeble predegraded implants.

In many studies in the orthopaedic literature the formation of large amounts of polymer or metal fragments is associated with osteolysis around the implant [23–25]. Goodman *et al.* [26] observed a more intense histological reaction, mainly involving macrophages, to polymethylmethacrylate (PMMA) particles when compared with bulk PMMA implants. PMMA particles phagocytozed by macrophages can cause a frustrated reaction because the digestion or resorption of these particles is impossible. These frustrated cells can release a number of enzymes and mediators such as interleukin-1, resulting in the activation of osteoclasts. Murray and Rushton [27] state that macrophages that have incorporated large quantities of particles stimulate bone resorption. They also state that this stimulation depends on the kind and the amount of internalized particles; 100 times more latex particles are needed to cause bone resorption when compared with zymosan particles. Maguire *et al.* [28] conclude that the size, surface area and rate of particle production are more important factors than physical composition, since both polyethylene and PMMA can induce bone lysis. In the present study no osteolysis or bone resorption was observed at any time with either the $PLLA_{336}$ implants or the $PLA96_{168}$ implants, although large fields of fully packed macrophages in close contact with bone were seen. It is therefore possible that other factors mentioned in the literature, such as micromotion or external stresses, may be important in inducing osteolytic changes.

In a previous study on a group of patients treated with bone plates and screws for the internal fixation of zygomatic fractures, no massive disintegration and internalization of intraosseously placed PLLA screws was seen [10, 12]. In patients reoperated on 3 and 5.8 years after implantation, the monocortical screws still had their original shape and were not interlaced with cells and collagen [10, 12]. In a study on goats, the PLLA orbital floor implants had not disintegrated into small particles and no cellular influx was observed, even after 5 years of implantation [13]. This may indicate that, unlike in the present study, in the zygomatic region and at the orbital floor region there is probably no substantial pressure during physiological degradation on the intraosseous screws to cause extensive disintegration followed by internalization by cells. In contrast, in the same patients the subcutaneously implanted bone plates did show a high degree of fragmentation and swelling during degradation, and the histological reaction was similar to that to the predegraded implants in the rabbit tibiae. In the

present study the total surface area of the implanted particles and cells was measured and after only 2 weeks a large increase in the area fraction was seen compared with the undegraded implants. The increased area fraction can be explained by a massive disintegration of the predegraded implants that will lead to extensive particle formation, resulting in an increase in the implant volume. A secondary factor will be an extensive reactive cellular influx, resulting in a further increase in the area fraction. In the intramedullary cavity, perhaps just like in soft tissue, this expansion is not limited by the high resistance of the environment. In a bony environment, however, expansion could be limited due to the higher resistance of cortical bone, which acts as an obstacle to extensive particle formation and the proliferating macrophage response. This could be the main reason for the absence of any fragmentation of the monocortical screws or increased cellular proliferation in the patients studied.

The present study used *in vitro* predegraded implants as a model to simulate long-term degradation in an osseous environment. A disadvantage of predegraded material is that the implants have lost their mechanical properties and cannot withstand external forces without deformation and fragmentation. In our model the combination of external forces in the intramedullary cavity and the low mechanical properties led to extensive degradation and it therefore does not seem to be comparable to the intraosseously implanted monocortical PLLA screws in our patient group. However, it can be concluded that if biodegradable polymers are implanted in areas with a low resistance which allow expansion during normal physiological degradation, it is possible that a similar reaction to that observed in this study will be seen. This indicates that, besides the physical composition of the implant, the surface structure, the amount of debris formation, micromotion and external stresses are all factors that will influence the degradation process and the severity of the tissue reaction, which may explain the observed differences in degradation and tissue response between the subcutaneous and the intraosseous implantation sites.

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