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Biocompatibility and degradation mechanisms of predegraded and non-predegraded poly(lactide) implants: an animal study

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In all patients treated with as-polymerized poly(L-lactide) (PLLA), a swelling at the site of implantation was observed after three years of implantation. These swellings seem to be related with degrading PLLA and the formation of particles of high crystallinity. To avoid these complications, poly(96%L-, 4%D-lactide) (PLA96) was developed that possesses lower crystallinity that probably results in a faster and more complete degradation. To study the cause of the swelling of PLLA implants and to study the degradation of PLA96 long-term implantation studies are required. Considering the very slow degradation rate of as-polymerized PLLA, *in vitro* predegradation was performed at elevated temperatures (90 °C) to simulate long-term physiological degradation. In this study a comparison was made between the histopathological reaction to non-degraded and predegraded PLLA, PLA96 and polyethylene (PE) discs implanted subcutaneously in rats. Animals were sacrificed after a postoperative period varying from 4 to 52 weeks. Chemical, light- and electron microscopical analysis and semi-quantitative measurements were performed. Based on the chemical analysis, the degradation rate of PLA96 was higher compared with PLLA. The histological reaction to non-degraded PLLA and PLA96 discs was very mild. The histological reaction to the predegraded implants was qualitatively similar to the reaction to the non-degraded implants, however, quantitatively an increase was noted. A number of predegraded PLLA and PLA96 discs showed an increase of volume with implantation time caused by the formation of fields of polymer debris accompanied by a granulomatous inflammatory reaction. The debris zone was found to consist of both polylactide polymer fragments and small remnants of degenerated cells. From our results it can be concluded that, when compared to PLLA, the degradation of PLA96 is enhanced. Subcutaneously implanted predegraded PLLA and PLA96 discs can induce a swelling similar to that observed with PLLA implants in patients. So, *in vitro* predegradation followed by *in vivo* implantation might be used as a model to predict late complications during clinical use.

1. Introduction

Biodegradable polyesters such as polyglycolide or polylactide have been studied extensively in the past decades and are widely used in many surgical fields in the form of rods, screws and bone plates [1–4]. Both polymers are often considered to be fully biocompatible in their various applications [5–7]. However, polyglycolide pins used in orthopaedic surgery are known to cause foreign body reactions and in some cases osteolysis during degradation [8, 9]. In our department as-polymerized PLLA bone plates, used in a patient study for the fixation of zygomatic fractures, induced persistent swellings at the site of implantation from three years onwards [10–12].

The cause of this swelling is not completely understood, but a possible explanation might be a massive

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granulomatous reaction induced by small particles resulting after degradation and disintegration. In a study with *in vitro* predegraded poly(lactide) particles, a positive correlation between the mean particle size and the intensity of the cellular and tissue reaction was noted [13, 14]. However, a clear relation between predegraded poly(lactide) particles and a clinically detectable foreign body reaction could not be found.

Another explanation could be the fact that in a cross-section of an after 3.5 years explanted swelling in a patient, up to 65% of the surface area consisted of PLLA remnants [11]. This would suggest that the PLLA implant itself, through an increase in volume during degradation, could induce a swelling. To study this possibility, implants with dimensions comparable with the zygomatic bone plates should be used that have to be implanted for about three years. To avoid these long *in vivo* implantation periods, *in vitro* predegradation at elevated temperatures can be used to provide poly(lactide) implants that are comparable to physiologically degraded material [13, 14].

Similar to the study in which *in vitro* predegraded particles were used, polymers that are developed as an alternative material for PLLA, such as a copolymer of 96% L-lactide and 4% D-lactide can be tested on swelling behaviour during degradation [15].

The aim of the present study was to describe quantitatively and qualitatively the biocompatibilty and the late degradation of predegraded and non-degraded poly(100% L-lactide) (PLLA) and poly(96% L-, 4% D-lactide) (PLA96) implants, as controls polyethylene (PE) implants were used.

2. Materials and methods

2.1. Implants

Poly(100% L-lactide) (PLLA) and poly(96% L-, 4% D-lactide) (PLA96) were as-polymerized according to the method described by Leenslag *et al.* [15]. Polymerization of L-lactide and D-lactide was performed after purification of the monomer (CCA/Purac Biochem, the Netherlands) by recrystallization from toluene under N_2 atmosphere. Both PLLA and PLA96 were polymerized under vacuum at 110 °C with 0.0015 wt % stannous-2-ethyl-hexanoate as a catalyst. Molecular weights (weight average molecular weight $\bar{M}_w$, and number average molecular weight $\bar{M}_n$) were determined by gel permeation chromatography (GPC) at 30 °C using in THF relative to polystyrene standards. Thermal properties were evaluated by differential scanning calorimetry (DSC) on a Perkin Elmer DSC-7. Five to 10 mg samples of PLLA and PLA96 were measured at a heating range of $10\,^{\circ}C\,min^{-1}$ to determine the heat of fusion, the melting temperature and the glass transition temperature.

PLLA discs (mean weight 193 ± 1.6 mg, $n = 116$), PLA96 discs (mean weight 197 ± 2.1 mg, $n = 76$) with a diameter of 10 mm and a thickness of 2 mm were prepared from blocks of PLLA and PLA96. As a non-degradable control material, ultra high molecular weight polyethylene (PE, Goodfellow, UK) discs were used (mean weight 147 ± 2.5 mg, $n = 76$). All discs were sterilized by regular hospital steam-sterilization. 80 PLLA discs, 40 PLA96 and 40 PE discs were predegraded, the remaining 36 discs of each polymer (PLLA, PLA96 and PE) were implanted as non-degraded discs. For predegradation, the discs were separately immersed in phosphate buffer of pH 7.4 in a glass tube with a permeable cap. Each disc was enveloped by stainless steel mesh (interstices 40 μm × 40 μm) to facilitate the removal of the degraded discs from the glass tube. Subsequently, the glass tubes with the discs were placed in a temperature controlled basin containing 20 litres of phosphate buffer of pH 7.4 which was set at 90 °C. The 40 PLA96 discs were predegraded for 168 h ($PLA96_{168}$), the first group of 40 PLLA discs were also predegraded for 168 h ($PLLA_{168}$), and second group of 40 PLLA discs were predegraded for 336 h ($PLLA_{336}$). The predegradation periods were based on results of previous studies [14]. Four discs of each predegraded group were dried to constant weight, and mass loss was determined with a Mettler PJ360. After weighing, these discs were used for DSC and GPC measurements to determine the material alterations during predegradation. The various implants used in this study were designated as follows:

PLLA	non-degraded poly-L-lactide
PLA96	non-degraded poly-DL-lactide (96% L, 4% D)
PE	non-degraded poly-ethylene
$PLLA_{168}$	predegraded poly-L-lactide (168 h at 90 °C)
$PLA96_{168}$	predegraded poly-DL-lactide (168 h at 90 °C)
PE_{168}	predegraded poly-ethylene (168 h at 90 °C)
$PLLA_{336}$	predegraded poly-L-lactide (336 h at 90 °C)

2.2. Animal study

The implantation procedure was performed according to good laboratory practice (GLP) regulations. A total of 42 male Wistar Albino rats, weighing approximately 300 g, were operated on. The rats were anaesthetized with a nitrous oxide-oxygen-fluothane mixture. The dorsal hair was clipped and the skin was prepped with iodine. Subsequently six incisions were made on the back of the rat and in bluntly created subcutaneous pockets one sample of each implant as mentioned above was inserted. The implant position was rotated for each group of implants, with the exception of $PLLA_{336}$ of which six samples were implanted into an animal. The predegraded discs were inserted with a specially designed instrument to prevent damage of the feeble discs during insertion in the pocket. After implantation the wounds were closed with Dexon® sutures.

At each of the intervals, 2, 4, 8, 12, 26 and 52 weeks postoperatively, animals were sacrificed. The dorsal hair was clipped and the implants were generously excised and subsequently fixed in 4% commercial formaldehyde/1% glutar-aldehyde (4CF1GA) solution for at least one week. Lymph nodes draining the implantation sites (Lnn inguinalis, Lnn brachiallis,

and Lnn axillaris) were also excised. The size of the implant plus capsule was measured with vernier callipers in three perpendicular directions.

2.3. Histology

After fixation for at least one week in 4CF1GA, tissue samples were excised and dehydrated in graded series of ethanol. Tissues were embedded in 2-hydroxyethylmethacrylate (GMA) using infiltration and embedding solutions (Technovit 7100, Heraeus Kulzer GmbH, Wehrheim, Germany). Tissue sections of 1–2 μm were prepared and stained with toluidine blue (0.1%). Localization and spread of polylactide implants and/or degradation products were determined light microscopically using polarized light induced by crossed Nicol prisms, resulting in birefringent polymer material. For transmission electron microscopy (TEM) postfixation with OsO_4 was done, and sections were prepared routinely for EM evaluation.

Tissue reactions to the implants were scored semiquantitatively according to the following criteria: + mild reaction, + + moderate reaction, and + + + marked reaction.

Capsule	+	Presence of predominantly macrophages directly against implant plus several fibroblast connective tissue layers.
	+ +	Capsule consisting of three layers, macrophages and fibroblasts next to the implant, several layers of fibroblasts, and a third layer of macrophages.
	+ + +	Presence of extensive third layer of macrophages.
Phagocytosis	+	A few macrophages containing polymer.
	+ +	A few areas of macrophages containing polymer.
	+ + +	Many and large conglomerates of macrophages containing polymer.
Implant-degradation	+	A few pieces of polymer detectable outside implant.
	+ +	Several pieces of polymer outside implant.
	+ + +	Major part of implant degraded, presence of debris zone.
Cellular invasion in implant	+	A few cells within implant.
	+ +	Several groups of cells in implant or deep infiltration of cells.
	+ + +	Many groups of cells in implant or deep and broad tissue cord formation.

3. Results

After *in vitro* predegradation for 168 h the mass loss of the $PLLA_{168}$ was about half compared with the $PLA96_{168}$. After 336 h the mass loss of the $PLLA_{336}$ was similar to the $PLA96_{168}$ (Table I). The molecular weight showed a decrease for all three materials, although the values for the PLLA remained slightly higher. Opposite to the molecular weight, the heat of fusion showed an increase to respectively 78.8 $J g^{-1}$, 92.4 $J g^{-1}$ and 101 $J g^{-1}$ for the $PLA96_{168}$, the $PLLA_{168}$ and the $PLLA_{336}$. The melting temperature of the PLA96 and the PLLA samples decreased with degradation time and is also presented in Table I.

At autopsy, macroscopically, no significant changes in volume of the non-degraded implants could be observed during follow-up (Table II). Incidentally, at several explanation times in each group of implants for one implant an increase in volume was measured. The $PLLA_{168}$ implants did not reveal any changes in volume with implantation time. From eight weeks onwards, some $PLLA_{336}$ implants showed a clinically detectable increase in volume. At each follow-up interval some discs had clearly swollen, although some discs still had a normal appearance. The $PLA96_{168}$ discs showed at 52 weeks after implantation a significant increase in volume compared with $PLA96_{168}$ discs at week two ($p < 0.001$, Student t-test) and with PLA96 discs at week 52 ($p < 0.01$, Student t-test).

A semiquantitative score of the histologic evaluation with implantation time is presented in Table III. The histologic reaction towards the non-degraded implants was characterized by a fibrous tissue capsule with an intermediate layer of predominantly macrophages between capsule and implant surface. With longer implantation periods the fibrous capsule increased somewhat in thickness and had a more mature appearance with long slender fibrocytes. For the PLLA and especially the PLA96, the formation of small cracks and fissures was associated with infiltration of fibroblasts and macrophages (Fig. 1). Occasionally, at 26 and 52 weeks with the PLA96 implants, under polarized light a layer of foamy macrophages with internalized birefringent polymer fragments was

TABLE I Molecular weight and thermal properties

	Degradation (h)	Mass loss (%)	$\bar{M}_w$	$\bar{M}_n$	$\bar{M}_w/\bar{M}_n$ (Jg^{-1})	ΔH (°C)	T_m (°C)	T_g (°C)
PLLA	0	–	88×10^5	35×10^5	2.5	60	187	59
PLA96	0	–	13×10^5	62×10^5	2.1	26	152	55
$PLLA_{168}$	168	22.5	4000	2800	1.6	92.4	183	55
$PLA96_{168}$	168	46.5	1400	800	1.6	78.8	140.5	42
$PLLA_{336}$	336	50.2	1800	900	2	101	177	47

TABLE II Volume of PLA polymer implants at several time points after implantation

Time (weeks)	N PLLA non-degraded	N PLA96 non-degraded	N PE nondegraded
2	6 262 ± 62 (0)[a]	6 258 ± 20 (0)	6 259 ± 14 (0)
4	6 330 ± 145 (1)	6 316 ± 89 (1)	6 260 ± 34 (0)
8	6 286 ± 39 (0)	6 291 ± 19 (0)	6 270 ± 28 (0)
12	6 339 ± 50 (1)	6 311 ± 56 (1)	6 326 ± 77 (1)
26	6 290 ± 84 (1)	6 356 ± 64 (1)	6 266 ± 47 (0)
52	5 245 ± 81 (0)	5 277 ± 80 (0)	6 262 ± 50 (0)
Time (weeks)	N $PLLA_{168}$ pre-degraded	N $PLA96_{168}$ pre-degraded	N PE_{168} pre-degraded
2	6 249 ± 25 (0)	6 240 ± 16 (0)	6 272 ± 47 (0)
4	6 318 ± 163 (1)	6 251 ± 57 (0)	6 267 ± 27 (0)
8	6 269 ± 22 (0)	6 251 ± 45 (0)	6 293 ± 39 (0)
12	6 256 ± 6 (0)	5 286 ± 75 0)	6 282 ± 35 (0)
26	6 261 ± 33 (0)	6 319 ± 97 (1)	6 283 ± 87 (1)
52	6 287 ± 63 (0)	5 565 ± 115 (5)[b]	5 270 ± 36 (0)

[a] Mean volume in mm^3 ± standard deviation, within parentheses number of animals with volume of implant $\geq 400\ mm^3$
[b] Statistically significant (Student's t-test), $p < 0.001$ compared to $PLA96_{168}$ week 2, and $p < 0.01$ compared to PLA96 week 52

TABLE III Semiquantitative evaluation[a] of local reactions to PLA polymers after subcutaneous implantation in rats

Implant	Time (weeks)	N	Capsule	Phagocytosis	Degradation	Cell in growth
PLLA	2 + 4	12	+ / + +	—/ +	—	+ / + +
	8	6	+	—/ +	—	+
	12	6	+	—	—	+
	26	6	+	—/ +	—	+
	52	5	+	—	—/ +	+ / + +
PLA96	2 + 4	11	+	—	—	—
	8	6	+ / + +	—/ +	—/ +	+
	12	6	+ / + +	—/ +	+	+ / + +
	26	6	+ / + +	—/ +	+ / + +	+ +
	52	5	+ / + +	+	+ / + +	+ + / + + +
PE	2 + 4	12	+ / + +	—	—	—
	8	5	+	—/ +	—	—
	12	4	+	—	—	—
	26	5	+	—	—	—
	52	4	+	—	—	—
PLLA 168 h	2 + 4	12	+ +	+ +	+	+ / + +
	8	6	+ +	+ +	+	+ / + +
	12	6	+ +	+ +	+ / + +	+ +
	26	6	+ +	+ +	+ / + +	+ +
	52	5	+ +	+ +	+ / + +	+ + / + + +
PLA96 168 h	2 + 4	12	+ +	+ + / + + +	+ +	+ / + +
	8	5	+ + / + + +	+ +	+ +	+ / + +
	12	5	+ +	+ +	+ / + +	+ / + +
	26	5	+ + +	+ + +	+ + +	+ +
	52	5	+ + / + + +	+ + +	+ + +	+ / + +
PE 168 h	2 + 4	11	+	—	—	—
	8	5	+	—	—	—
	12	4	+	—	—	—
	26	5	+	—	—	—
	52	4	+	—	—	—
PLLA 336 h	2 + 4	12	+ + / + + +	+ + +	+ +	+ +
	8	6	+ +	+ + / + + +	+ + / + + +	+ + / + + +
	12	5	+ + / + + +	+ + / + + +	+ + / + + +	+ / + +
	26	4	+ + +	+ + +	+ +	+ +
	52	—	nd	nd	nd	nd

[a] See Materials and methods

observed in the periphery of the capsule. Indications for a beginning of further degradation/dissolution are present for PLA96 at 52 weeks after implantation (Table III, Fig. 2). For the reference material PE, at each interval investigated a mild histologic response was found consisting of a fibrous encapsulation with a few macrophages directly adjacent to the implants (Table III). No indications for a preference of the

implantation site with regard to the degree of capsule formation or infiltrate was noted.

For the predegraded $PLA96_{168}$ and the $PLLA_{336}$ a similar fibrous encapsulation was seen but the number of macrophages directly adjacent to the implants and in the periphery of the capsule was higher when compared with the non-degraded implants (Table III).

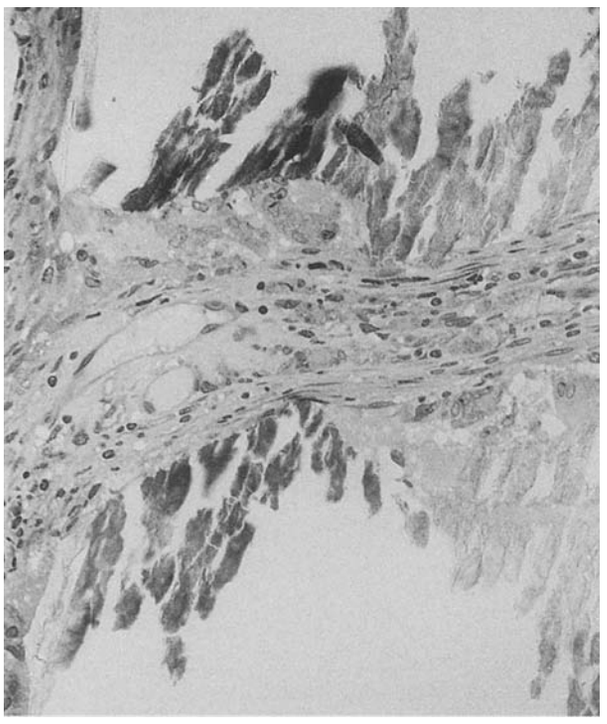

Figure 1 PLA96 implant 26 weeks after implantation with cellular invasion in fissure of implant by macrophages and connective tissue (including blood vessels). × 20.

$PLLA_{168}$ induced a mild histological reaction which was comparable with the non-degraded PLLA disks, degradation and particle formation were observed after 12 weeks onwards. Fig. 3 shows the mild reaction to $PLLA_{168}$ at 52 weeks after implantation. For the $PLLA_{168}$ and $PLLA_{336}$, fragmentation, particle formation and internalization by cells, as indicated by microscopy using polarized light, was already observed in the first weeks of implantation (Table III). Larger particles of degraded material were surrounded by macrophages, which sometimes seemed to form multinucleated cell syncytia (Fig. 4). As shown in Table III (score + + + for degradation) for the $PLLA_{336}$ from eight weeks on, and for the $PLA96_{168}$ at weeks 26 and 52, fields of polymer debris surrounding the fragmented implants were noted (Figs 5 and 6). Generally, there was a sharp boundary between the debris and the original implant. For the $PLA96_{168}$ at 52 weeks, and $PLLA_{336}$ from eight weeks and onwards indications were found for a more complete breakdown of the implant, as besides the debris zone also fragmentation of the implants in large pieces was noted (Fig. 7). The cellular influx around these implants and the number of cells with internalized polymer fragments had increased. Light microscopically, the debris material seemed to be acellular (Fig. 8), only incidentally cells were noted in such areas. Major tissue reactions outside the capsule were only noted with the predegraded ($PLLA_{168}$, $PLLA_{336}$, and $PLA96_{168}$) polymers with exception of PE_{168}. In the lymph nodes draining on the implantation sites (Lnn inguinalis, Lnn brachiallis, and Lnn axillaris) no polymer material was noted.

TEM analysis of $PLLA_{336}$ implants retrieved 12 weeks after implantation revealed that the cellular

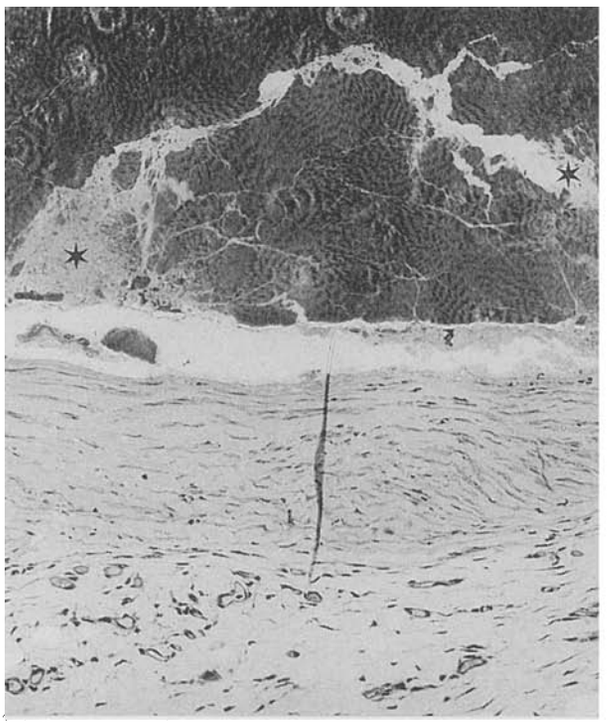

Figure 2 PLA96 implant 52 weeks after implantation, limited degradation and disintegration (asterisks) of implant, presence of connective tissue capsule. × 4.

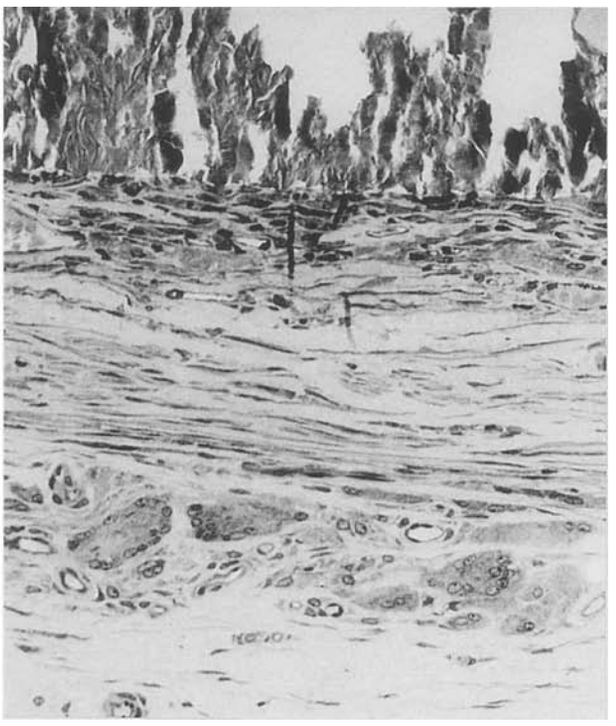

Figure 3 $PLLA_{168}$ implant 52 weeks after implantation with a mature connective tissue capsule and multinucleated giant cells. × 20.

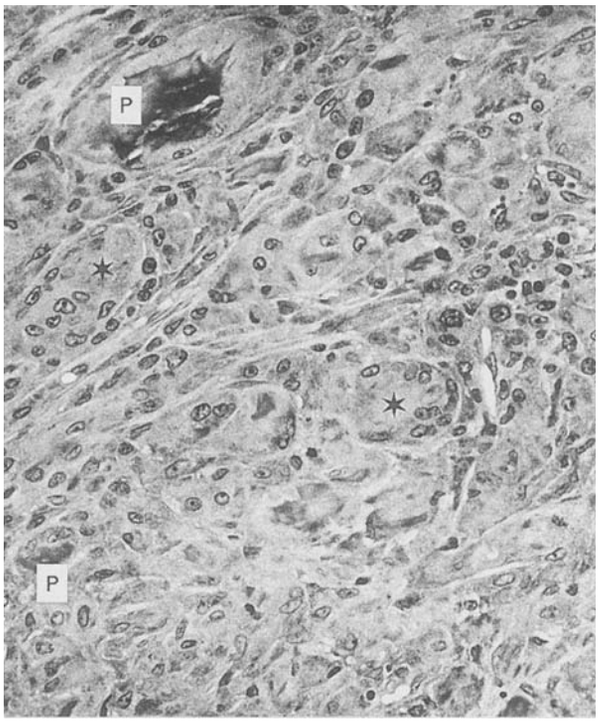

Figure 4 A marked macrophage infiltrate including multinucleated giant cells (asterisks) around a $PLLA_{336}$ implant two weeks after implantation. Note polymer fragments (P) surrounded by macrophages. × 20.

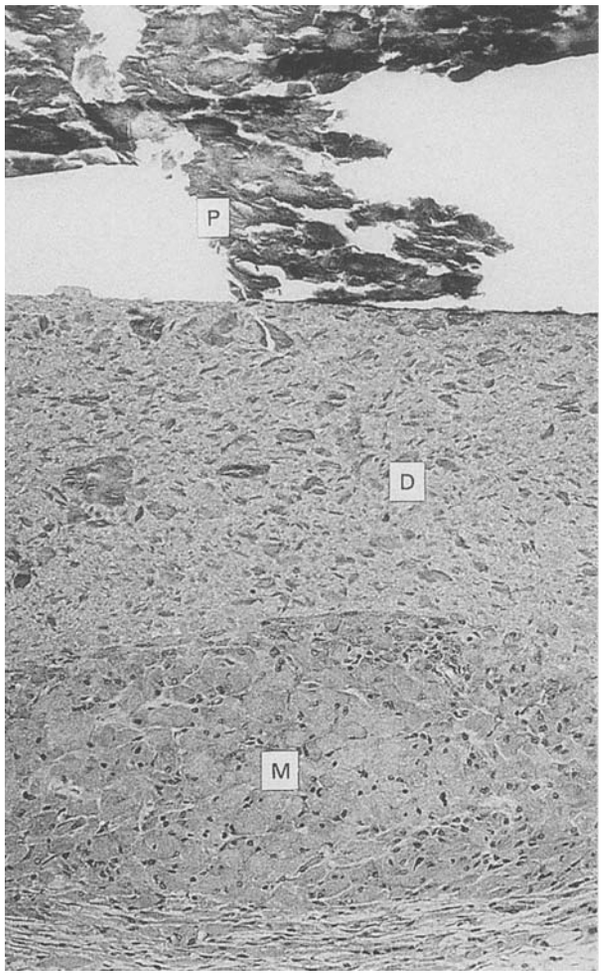

Figure 5 $PLLA_{336}$ implant (P) 12 weeks after implantation with debris zone (D) with polymer fragments and macrophage infiltrate (M) with connective tissue. × 10.

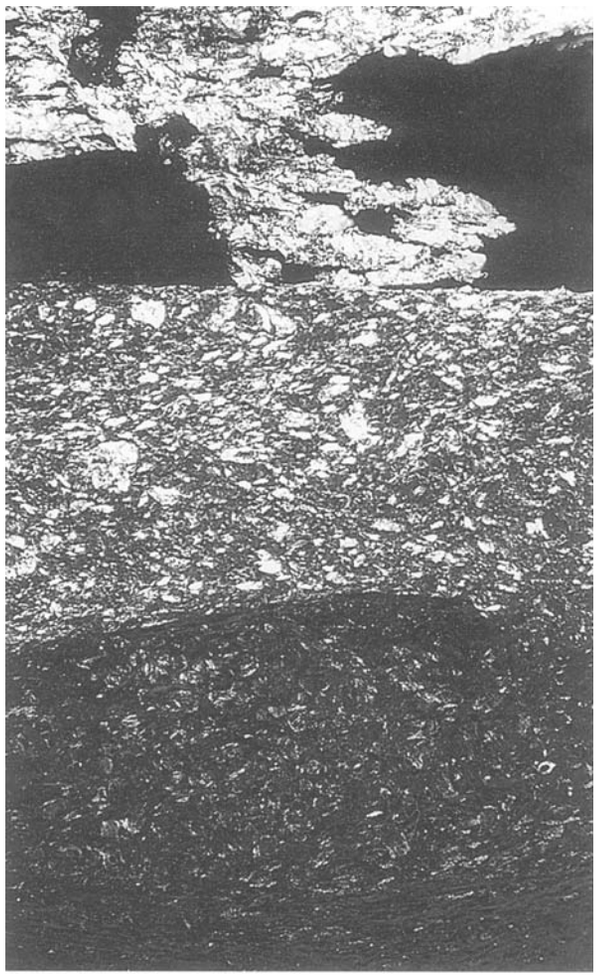

Figure 6 $PLLA_{336}$ implant 12 weeks after implantation (see also Fig. 5). Presence of birefringent material (PLLA) in debris zone and macrophages of infiltrate. × 10.

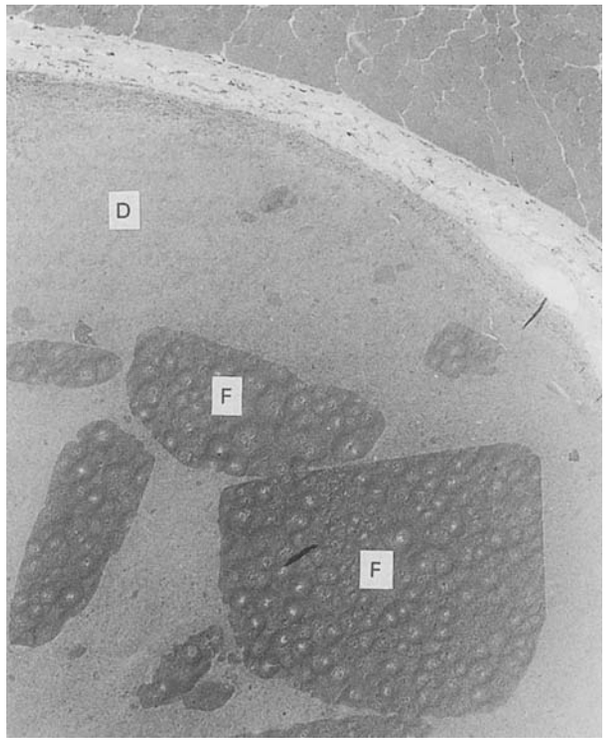

Figure 7 Disintegration of $PLA96_{168}$ disc into fragments (F) with large zones of debris (D) surrounding the implant at 52 weeks after implantation. × 4.

layer surrounding the debris zone consisted mainly of macrophages, some fibroblasts and vascular structures. Macrophages contained variable amounts of internalized polymer material from needle-like structures, regularly in parallel arrangement, to large fragments (Figs 9 and 10). Fibroblasts occasionally showed internalized polymer fragments.

The major part of the debris zones consisted of degraded polymer material ranging in size from small needle-like fragments to small and large blunt pieces. In this area also numerous small dense particles,

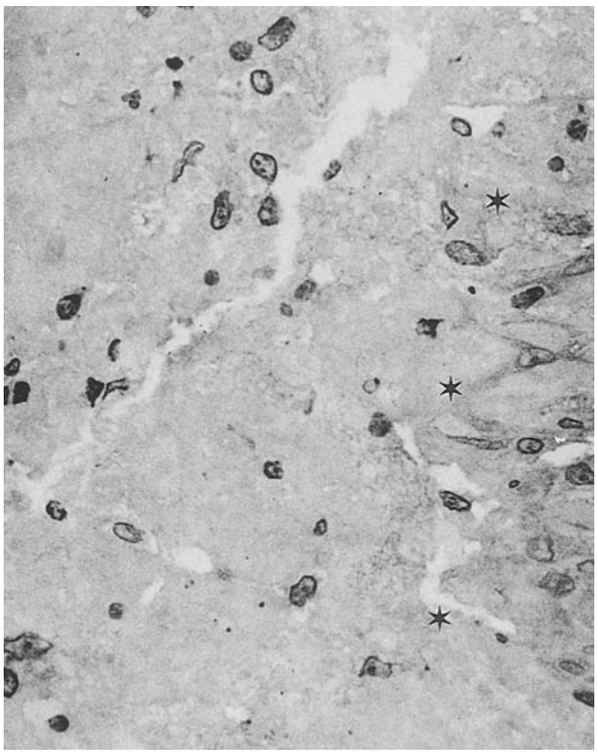

Figure 8 $PLA96_{168}$ implant at 26 weeks after implantation. Interface (asterisks) of macrophage infiltrate and debris zone with remnants of degenerated cells. ×40.

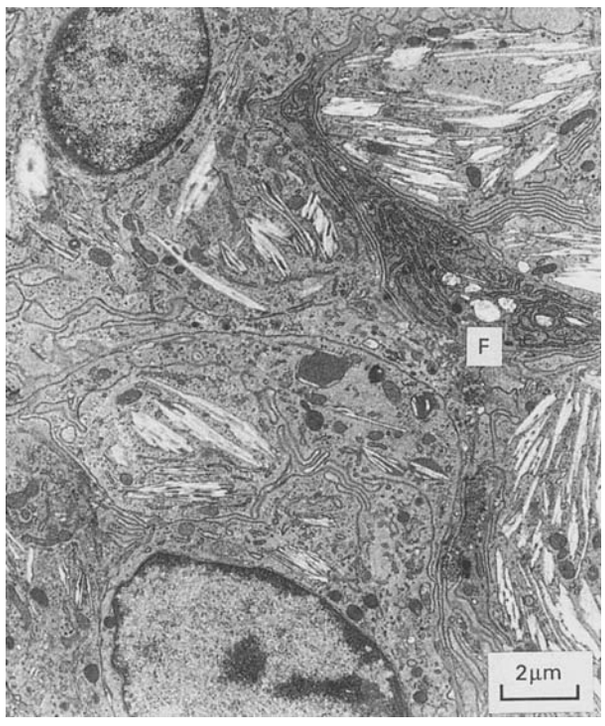

Figure 9 $PLLA_{336}$ implant at 26 weeks after implantation (TEM). Macrophages in the substantial cellular layer lining the debris zone, containing various amounts of phagocytized lamellar PLLA material. The cellular layer also contains fibroblasts (a small compartment is indicated by F). Bar = 2 µm.

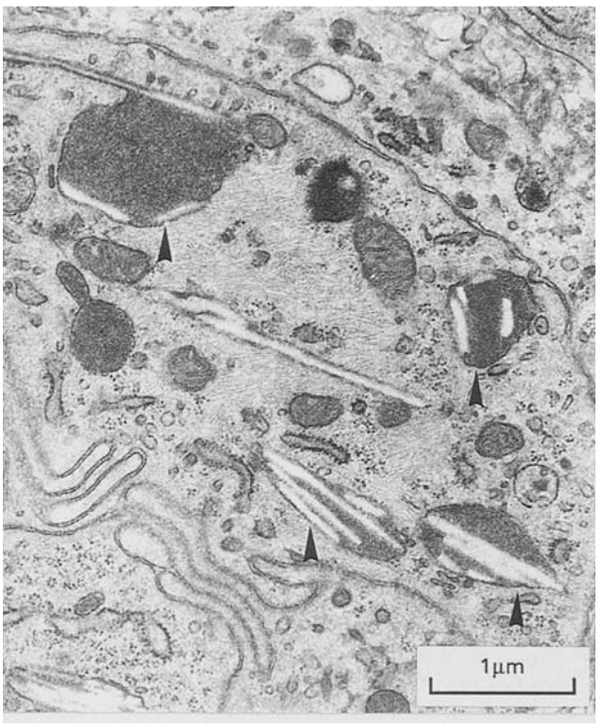

Figure 10 $PLLA_{336}$ implant at 26 weeks after implantation (TEM). Detail of macrophage cytoplasm showing phagocytized PLLA in lysosomes (arrowheads), suggesting that phagosome/lysosome fusion has taken place. Bar = 1 µm.

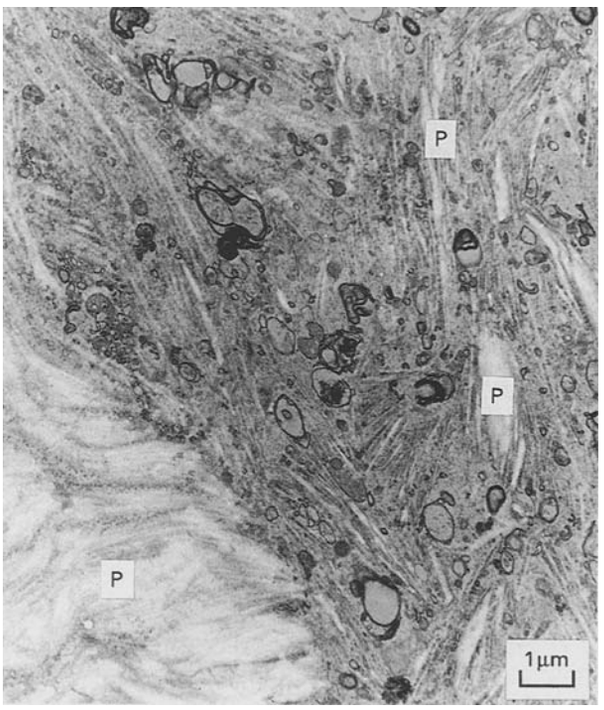

Figure 11 $PLLA_{336}$ implant at 26 weeks after implantation (TEM). Centre of a debris zone showing degraded polymer material fragments (P) ranging from large blunt to small thin lamellar structures, intermingled with numerous small cellular degradation debris. Bar = 1 µm.

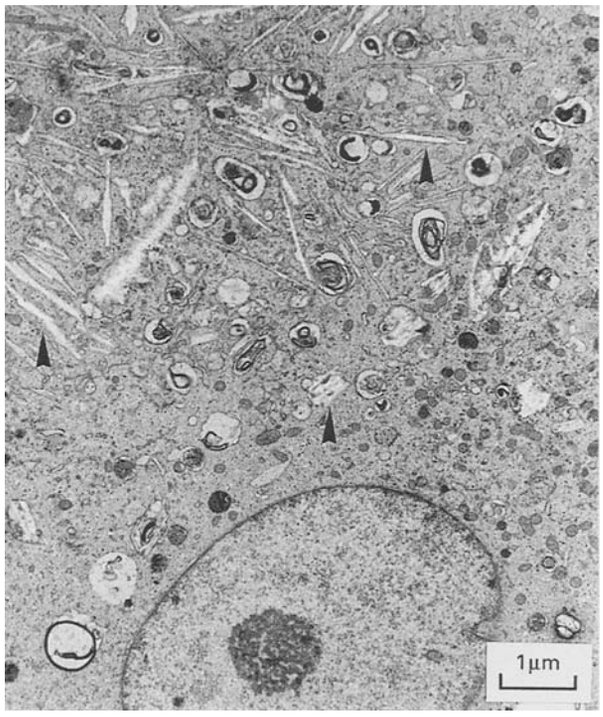

Figure 12 $PLLA_{336}$ implant at 26 weeks after implantation (TEM). Part of the cytoplasm of macrophage in the cellular layer lining the debris zone, showing phagocytized PLLA material (arrowheads) but also autophagic vacuoles and residual bodies, indicative for cell degeneration. Bar = 1 µm.

residual bodies and other cytoplasmic components derived from degenerated cells were observed (Fig. 11). At the boundary between the debris zone and the cellular layer, but also deeper in the cellular layer, macrophage cells and cell compartments were present showing signs of degeneration, with residual (myeloid) bodies (Fig. 12). With progressive degeneration plasma membranes were no longer observed, leaving the intracytoplasmic remnants and phagocytized polymer materials free.

4. Discussion

In most *in vivo* degradation and biocompatibility studies of high molecular weight PLLA the tissue response and polymer degradation are followed in experiments varying from months up to three years, but in none of these studies late or complete degradation was achieved [4, 6, 9]. Still, these polylactides are considered to be fully biodegradable and clinical use was started based on the results of these experiments [6, 16–19].

In the present study *in vitro* predegradation was used to simulate long-term physiologically degraded implants. It is rather difficult to define the exact *in vitro* predegradation period needed to study the final degradation phase of a polymer in an *in vivo* experiment. In the present study we have used predegraded $PLLA_{336}$ with chemical characteristics that were comparable with PLLA material obtained three years postoperatively from patients that were threaded with PLLA bone plates and screws for zygomatic fractures [11, 12]. PLA96 discs were predegraded for 168 h which provided, based on the mass loss, discs that were comparable with $PLLA_{336}$ implants. For a good comparison between PLLA and PLA96, part of the PLLA discs were also predegraded for 168 h.

The chemical analysis of the predegraded discs showed a very low molecular weight with a normal polydispersity when compared with physiologically degraded material [11, 12]. There was an increase in the heat of fusion for all discs indicating that the crystallinity had also increased. The melting temperature had decreased with degradation indicating that the crystalline domains become less perfect or smaller [20]. The heat of fusion and the melting temperature of the PLA96 however, remains much lower compared with the $PLLA_{168}$ and the $PLLA_{336}$. The lower crystallinity and greater imperfection caused by the incorporation of 4% D-lactide has a substantial influence on the resorption rate.

Histopathologic examination showed that the local tissue response to the non-degraded polylactides and the PE discs was very mild during the entire implantation period. The histologic reaction towards the non-degraded PLLA and PLA96 discs consisted of a minor local reaction, which remained almost constant from 12 weeks onwards. This mild tissue reaction is in good accordance with literature, many studies reported only a minimal inflammatory response to the PE control discs, but also to the PLLA and the copolymer PLA96 [17–19, 21]. The slightly elevated histologic response to the PLA96, is probably due to the sterilization procedure. Steam sterilization of moisture labile biodegradable polymers will influence the mechanical and chemical properties, and can be considered as a kind of pre-degradation [22]. During sterilization the chemical properties will be significantly altered, which can explain the early cracking and particle formation and the accompanying increased cellular influx around the implant.

In contrast with the non-degraded PLLA and PLA96 discs, the $PLLA_{168}$ showed a moderate, and the $PLLA_{336}$ and $PLA96_{168}$ showed a marked reaction including degradation, disintegration, and cellular infiltration with longer implantation periods. Although there is a clear tendency for these reactions to increase in time, our results show that the characteristic features of each implant are already present during the first eight to 12 weeks after implantation. Thus the differences between non-degraded and predegraded material could already be established in that period.

Some predegraded implants clearly showed an increase in volume, this increase of implant plus capsule could be ascribed only partly to the granulomatous reaction surrounding the implant. In addition, large zones of degraded polymer with mostly acellular debris, were observed around the implants. Between the seemingly intact implant and the debris zone a rather sharp boundary was present. A similar sharp boundary between PLLA bone plates and surrounding

polymer debris was observed in a cross-section of material explanted from patients implanted with PLLA bone plates and screws in the zygomatic region [13]. These polymer fragments and debris were surrounded by a granulomatous reaction which resembled the swollen $PLLA_{336}$ and $PLA96_{168}$ discs. Although in the present study predegradation was used to simulate long term physiological degradation, the results i.e. the histopathological reaction, suggests that the degradation and swelling occurs according to a similar mechanism as seen in patients.

Light microscopically, the debris zones contained only a minor amount of cells or were virtually acellular. As TEM examination revealed the presence of subcellular structures in these debris zones, it remains a question whether such zones truly only consist of polymer fragments or also of debris from degenerated macrophages. It is possible that the environment in the debris is cytotoxic for cells. Lactic acid can cause a decrease of pH resulting in lysis and the egestion of the internalized particles. In the literature lactic acidosis has been described to be a major mechanism promoting cell damage or death [23, 24]. The debris may cause a continuous attraction of macrophages that may again phagocytize the PLLA particles and thus repeating the intracellular cycle.

The mechanism of the debris formation is not fully understood. A possibility might be that mechanical damage to the degraded discs causes fragmentation and particle formation which can lead to debris formation and swelling of the implant. In a study with intraosseously used polyglycolide pins, Böstman *et al.* [8] observed osteolytic areas which seemed to be related with the formation of degradation products and debris formation. They also suggest that this debris would be very hydrophillic which causes an increased osmotic pressure and thereby osteolytic changes. In the present study polylactide was used but perhaps a similar mechanism may account for the subcutaneous swellings. The formation of a PLLA debris probably depends on the degradation stage, fragmentation, and the hydrophilicity of these fragments. Non-degraded PLLA is a less hydrophilic polymer when compared with polyglycolide, but during degradation the hydrophilicity of the low molecular weight degradation products increases. This increase can be explained by the fact that these low molecular weight particles have polar and free carboxylic endgroups. At some point PLLA debris will be formed that can cause an osmotic pressure within the enveloping fibrous capsule prompting an increase in volume if the resistance of the surrounding tissue does not exceed the osmotic pressure. Our results show that in contrast to $PLLA_{336}$ the $PLLA_{168}$ discs did not show debris formation and swelling. So, it is likely that a certain level of degradation is needed to result in debris formation followed by osmotic swelling within the fibrous capsule. Whether there is an increase in osmotic pressure during degradation of PLLA can perhaps be determined using *in vitro* osmometry as described by Hodson *et al.* [25] to measure the swelling power.

The degradation pattern and the formation of particles and debris and the histological reaction towards the $PLA96_{168}$ implants was comparable to the histological reaction to the $PLLA_{336}$ discs. The $PLA96_{168}$ discs did show debris formation and after 52 weeks of implantation a significant increase in volume was detected. Based on these results it can be predicted that PLA96 can induce a swelling similar to the PLLA implants. The results of this study suggest that clinically detectable swellings can be expected when PLA96 is used as bone plates and screws for the fixation of zygomatic fractures in patients. It can therefore be concluded that although the degradation of PLA96 is enhanced, as-polymerized PLA96 should not be used as a new osteosynthesis material.

In conclusion the results of this study show that *in vitro* pre-degradation in combination with *in vivo* implantation is a good method to simulate long-term physiological (clinical) degradation. However, the implantation of a polymer with a certain predegradation time does not exclude the possibility of a swelling when used in a clinical setting. The level of degradation obtained in the predegradation process is, in view of our results, likely to be of more importance than the predegradation period. By varying the pre-degradation periods the simulated implantation period can be increased thereby decreasing the risk of missing essential stages in the polymer degradation and accompanying tissue response.

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