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Biodegradation and tissue-reaction in a long-term implantation study of poly(L-lactide)

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Three poly(L-lactides) with different molecular weights were synthesized as solid blocks from the melt. Two of those were ground and samples were produced by injection moulding. Specimens, cubes (2 × 3 × 3 mm) and rods (2 × 3 × 25 mm), were machined out of the samples, yielding the amorphous parts. Specimens of the crystalline parts were directly machined out of the third block. All were implanted into the paravertebral muscle of 70 rats to explore the biodegradation of poly(L-lactide) *in vivo* and the tissue changes at the implantation site. The rats were sacrificed after 1 to 116 weeks and the implants recovered. Histological sections of the cubes including the surrounding tissue were prepared by the cutting–grinding technique according to Donath. The three different materials were incorporated well, forming a collagenous layer. The crystalline poly(L-lactide) (M_{vis} 429 000) remained almost stable in form and structure over the whole observation period. No signs of inflammation or foreign-body reaction were observed. The amorphous poly(L-lactide) of higher molecular weight (M_{vis} 203 000) degraded nearly completely, whereas the amorphous poly(L-lactide) of lower molecular weight (M_{vis} 120 000) was totally resorbed. After about 8 weeks both injection-moulded materials degraded progressively, subsequently accompanied by a mild to moderate foreign-body reaction. The degradation in the inner part of the implants proceeded faster than in the cortex. The final biodegradation appeared accompanied by a resorptive histiocytic inflammation. The degradation rate and velocity of the amorphous poly(L-lactides) did not overtax the absorption capacity of the surrounding tissue. These properties of biodegradation seem to meet the requirements for a biodegradable material in osteosynthesis.

1. Introduction

Over the last two decades there has been considerable development and subsequent application of degradable biomaterials. While biodegradable polymers have been used for surgical sutures (Vicryl®, Dexon®, PDS®) for many years, the application in osteosynthesis is still discussed and subject of ongoing research.

Meanwhile poly(L-lactide), the polymer of the physiological substance lactic acid, is of increasing interest as biodegradable material in osteosynthesis. Poly(L-lactide) tested so far shows hydrolytic degradation in an aqueous environment such as body fluids which initially seems independent of enzymatic or cellular activity of the body [1, 2]. The final hydrolytic degradation product of poly(L-lactide) is lactic acid which is a normal intermediate of the carbohydrate metabolism and becoming ultimately metabolized to carbon dioxide and water. The route of elimination was shown in principle 25 years ago by Kulkarni *et al.* [3]. By implantation of carbon-14-tagged poly(L-lactide) specimens into rats a loss of activity of 14% could be detected over 3 months. As no significant radioactivity was recovered in faeces and urine during this period nor in any of the vital organs at death, they concluded that the degraded polymer had possibly been eliminated through the carbon dioxide in the respiration.

The biocompatibility of poly(L-lactide) is generally accepted as excellent [4, 5]. As long as degradation has not progressed, poly(L-lactide) is usually classified

0951–4530

in the best biocompatibility class for polymers [6]. On the other hand, previous biodegradation studies observed only a partial dissolution of the applied poly(L-lactide) devices [1–4, 7–14], so the biocompatibility and tissue response in the later stages of biodegradation is almost unexplored. Implantation in the paravertebral muscle of rats enabled investigation of the histological tissue changes at the implantation site and the final biodegradation of poly(L-lactide).

2. Materials and methods

Three polymer blocks of poly(L-lactide) were synthesized by ring opening melt polymerization of the cyclic dimer of L-lactic acid using stannous octoate as catalyst. Two of those were ground and samples made by injection moulding. Specimens were machined out of these samples, yielding the amorphous poly(L-lactides) A1 and A2 of this study: A1 and A2 differed in molecular weight. Specimens of poly(L-lactide) C were machined directly out of the third polymer block and were highly crystalline. The analytical data of the starting materials after sterilization with ethylene oxide are summarized in Table I.

The specimens, two larger rods measuring 2 × 3 × 25 mm and one smaller cube measuring 2 × 3 × 3 mm, of each kind of poly(L-lactide) were implanted into the paravertebral muscle of 70 male Cara-rats. The operations were carried out in ketamine-induced anaesthesia and under aseptic conditions.

Except for the formation of one abscess postoperative healing of the wounds was uneventful.

From week 1 to 8, every week three rats were sacrificed using ether and the implants recovered. Then three rats were killed after 12, 16, 20, 24, 28 and 32 weeks. One rat was killed and the implants recovered after 36, 40, 44, 48, 52, 56, 60 and 64 weeks. The remaining rats reached week 72, 80, 90, 92, 95, 98, 109, 110 and 116 after implantation.

The larger rods were used for several tests to determine the chemical and mechanical properties. Chemical characterization was carried out by viscosimetry, size exclusion chromatography and differential scanning calorimetry. Bending strength and Young's modulus of elasticity were determined using a universal testing machine.

Tissue specimens including the small cubes were destined for histological examinations (Fig. 1). After fixation in 5% buffered formalin, and dehydration by using increasing glycolmethacrylate concentrations, the specimens were embedded in polymethylmethacrylate. To obtain thin sections, below 10 μm, the embedded specimens were prepared by the cutting–grinding technique according to Donath [15]. Two sections of each specimen were manufactured and stained with Masson–Trichrom–Goldner. The histomorphological changes of the materials and the histological tissue reactions during implantation are presented in this paper.

3. Histological results

3.1. Crystalline poly(L-lactide) C

Light microscopy, especially in polarized light, showed a crystalline structure with round cores and amorphous intercrystalline parts (Fig. 2a).

During the first weeks a tissue layer consisting of thin collagen fibres with numerous fibroblasts and fibrocytes in between was observed around the implants. A mild inflammatory reaction with macrophages and only a few giant cells of foreign-body type, particularly in contact with the poly(L-lactide) surface, were found (Fig. 2b). At about 8 weeks a homogenous connective capsule had developed around the implants with evenly distributed fibrocytes and collagen fibres running parallel to the surface (Fig. 2c). In the following period further maturation of the fibrous layer took place. After 32 weeks the amorphous intercrystalline parts of the outer layer occasionally disappeared. From week 56 onwards this phenomenon was also seen in the inner parts of the implants. Nevertheless the poly(L-lactide) remained stable with regard to form and volume up to the 110th week. Just after 116 weeks at the end of observation time the intercrystalline structure collapsed and the crystalline parts moved together. A slight deformation, particularly at the edges of the cube due to traction of the connective tissue, took place. The implant was surrounded by a thin cell-poor collagenous capsule. A significant degradation of the crystalline structure could not be observed (Fig. 2d).

3.2. Amorphous poly(L-lactide) A1 and A2

Both materials showed amorphous structure in the centre and an outer layer like a cortex of unknown origin varying from 0.12 to 0.24 mm in thickness. In polarized light the cortex looked grainy and had similarities to crystalline structures (Fig. 3a).

The tissue reaction during incorporation of the cubes was comparable to crystalline poly(L-lactide) C. The implants were surrounded by granulation tissue with macrophages and some giant cells of foreign-body type, especially in direct contact with the surface

TABLE I Analytical data of the starting materials

	Crystalline poly(L-lactide) C	Amorphous poly(L-lactide) A1	Amorphous poly(L-lactide) A2
Inherent viscosity η_{inh} (dl g^{-1})	4.90	2.76	1.82
Molecular weight M_{vis}	429 000	203 000	120 000
Crystallinity	73.1%	amorphous	amorphous
Bending strength (N mm^{-2})	118.8	130.3	118.3
Modulus of elasticity (N mm^{-2})	4588.4	3626.6	3579.2

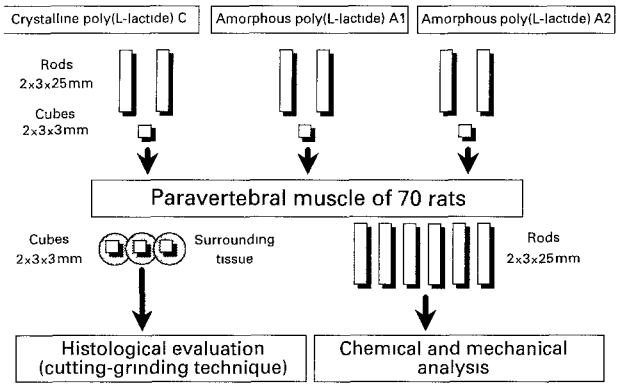

Figure 1 Study procedures.

(Fig. 3b). Subsequently the number of cells decreased. By week 8 a fibrous layer of homogenous connective tissue had developed around the implants. The fibrous layer matured and became thinner. In contrast to poly(L-lactide) C, the amorphous injection-moulded poly(L-lactides) showed first histological signs of degradation after only 8 weeks, beginning with little ruptures and cracks in the periphery of the implants. After the 16th week fibroblasts and macrophages grew into the larger of these cracks. In the time following they increased in number, extended and were filled with fibrous tissue containing macrophages and giant cells which were seen in direct contact with the poly(L-lactides). Round pores and channels of 1.5–10 μm in diameter developed near the ruptures (Fig. 3c). In later stages the pores and channels dilated and were distributed over the whole internal material. Whereas the centre became porous, the cortex remained stable in structure. From approximately 1 year onwards the implant centres softened more and more. As a result of this inner malleability the original cubic form changed and became rounded due to scar contraction. The large cracks and ruptures extended further and formed resorptive lacunae invading the poly(L-lactide) cubes (Fig. 3d). These contained fibroblasts, macrophages and giant cells of foreign-body type. At this stage single fragments were already separated off. In the further development a partial evacuation, especially of the inner part of the poly(L-lactide) substance, took place. Connective tissue involving macrophages, giant cells and capillary buds permeated the whole implant (Fig. 3e). Starting about 100 weeks after implantation the material gradually disintegrated into several fragments and particles (Fig. 3f). These, encased by connective tissue and

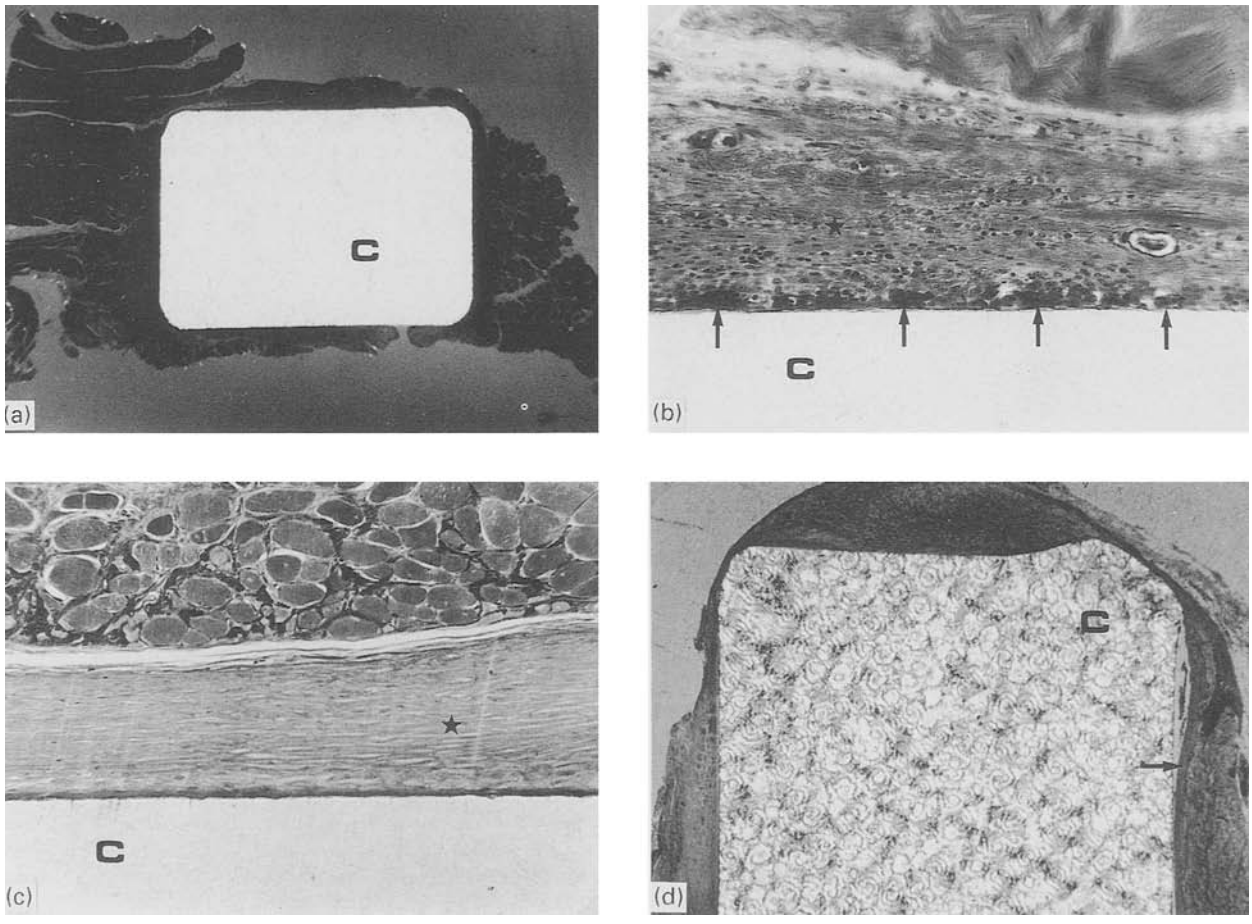

Figure 2 Crystalline poly(L-lactide) C. Histological sections prepared by the cutting–grinding technique. Masson–Trichrom–Goldner stain. (a) 3 weeks after implantation, polarized light, original magnification × 20. Crystalline structure of the implant (C). (b) 3 weeks after implantation, detail of Fig 2a, original magnification × 250. Fresh connective tissue (★) with fibrocytes, macrophages and some giant cells (→) in direct contact to the implant (C). (c) 8 weeks after implantation, original magnification × 250. Collagenous cell-poor connective tissue (★) around the implant (C) (d) 116 weeks after implantation, polarized light, original magnification × 40. Intercrystalline parts have almost disappeared and the structure has collapsed Deformation at the edges of the implant (C). Very thin cell-poor collagenous capsule (→). No significant degradation of the crystalline parts is detectable.

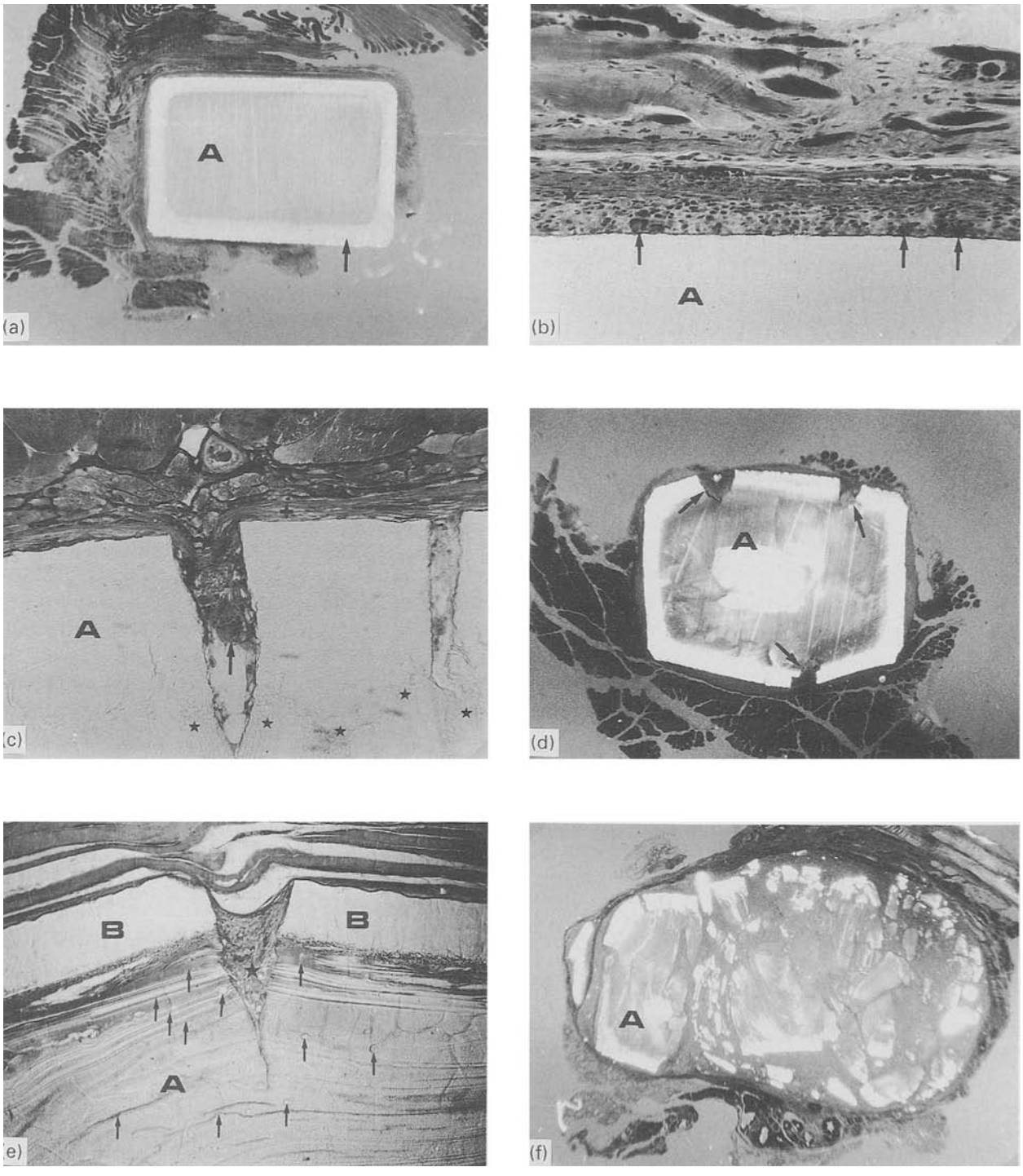

Figure 3 Amorphous poly(L-lactide) A1 and A2. Histological sections prepared by the cutting–grinding technique. Masson–Trichrom–Goldner stain. (a) 3 weeks after implantation, polarized light, original magnification × 20, poly(L-lactide) A2. Amorphous structure in the inner part of the implant (A) and a cortex (→) of unknown origin. (b) 3 weeks after implantation, detail of Fig. 3a, original magnification × 250. Fresh connective tissue (★) with fibrocytes, macrophages and some giant cells (→) in direct contact to the implant (A) identical to poly(L-lactide) C after the same time (see Fig. 2b). (c) 28 weeks after implantation, original magnification × 250, poly(L-lactide) A1. Cracks in the periphery of the implant (A) with fibrous tissue containing macrophages and giant cells (→). Pores and channels (★) have developed near the cracks. Thin cell-poor collagenous capsule (+) (d) 52 weeks after implantation, polarized light, original magnification × 20, poly(L-lactide) A2. The implant (A) has rounded. Deep cracks and lacunae (→) with resorptive tissue are detectable. (e) 90 weeks after implantation, polarized light, original magnification × 100, poly(L-lactide) A1. A partial evacuation of the inner part (A) of the implant appears. The cortex (B) seems unchanged. Fibrous tissue containing macrophages, giant cells and capillary buds (★) permeate the implant. Pores and channels (→) are clearly visible. (f) 98 weeks after implantation, polarized light, original magnification × 20, poly(L-lactide) A1. The implant (A) has disintegrated. (g) 98 weeks after implantation, detail of Fig. 3f, original magnification × 250. Remaining fragments (★) are encased by foamy macrophages (→) and fibrous tissue. (h) 116 weeks after implantation, polarized light, original magnification × 20, poly(L-lactide) A1. The implant is for the most part degraded and resorbed. Fragments of the cortical structure (★) are more stable than the inner part of the implant. A connective capsule (→) encloses the resorptive tissue including the remaining fragments. (i) 116 weeks after implantation, detail of Fig. 3h, original magnification × 250. Pronounced resorptive inflammation. Remaining fragments (+) are surrounded by numerous foamy macrophages and giant cells (★). Tiny drop-shaped particles (→) have separated from cortical fragment (B) and are resorbed by the tissue. (j) 116 weeks after implantation, original magnification × 100, poly(L-lactide) A2. The implant has been totally resorbed. Only a resorptive sinus (★) with single macrophages and giant cells (→) in the surrounding tissue is detectable. The resorptive inflammation has receded nearly totally.

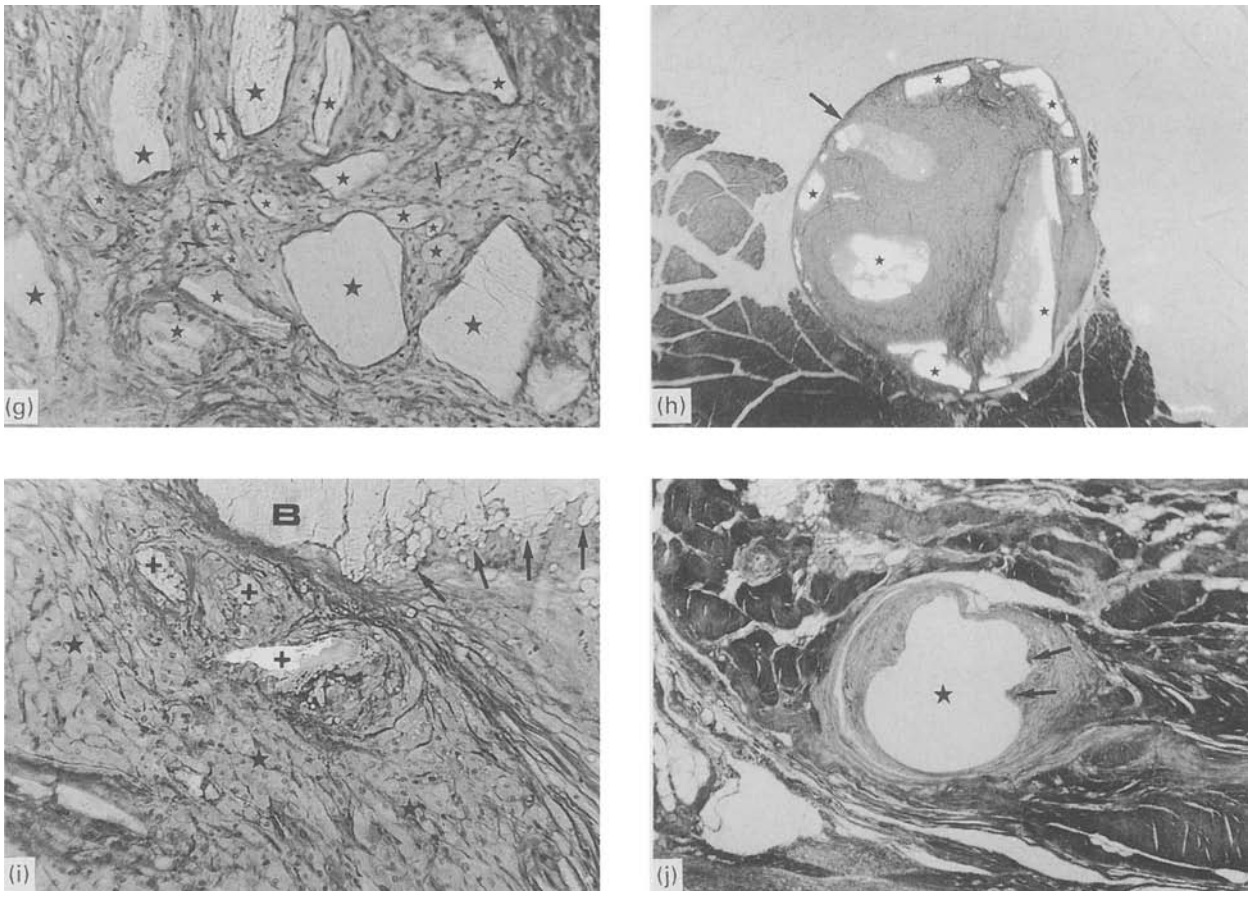

Figure 3 Continued.

foamy macrophages, were resorbed in a histiocytic inflammation (Fig. 3g).

After 116 weeks the amorphous poly(L-lactide) of higher molecular weight A1 was for the most part degraded (Fig. 3h). Only small fragments originating from the cortical structure were detectable. The former inner part had almost disappeared, absorbed by the tissue. The remaining material was surrounded by numerous foamy macrophages and giant cells indicating a pronounced resorptive inflammation. Tiny drop-shaped particles had separated from the cortical fragments and were mainly endocytosed by phagocytic cells (Fig. 3i). The resorptive tissue, including the remaining poly(L-lactide), was totally enclosed by a connective capsule. An irritation of the adjacent tissue was not observed.

The amorphous poly(L-lactide) of lower molecular weight A2 was totally degraded and absorbed by the tissue. At the end of the observation time only tiny resorptive sinuses without poly(L-lactide) could be found. A nearly complete involution of the resorptive inflammation was noticed. Only single macrophages and giant cells were seen in the surrounding connective tissue (Fig. 3j).

4. Discussion and conclusions

The three different poly(L-lactides) were incorporated well, which confirms the excellent biocompatibility of this material. Up to the 8th week a homogenous connective tissue maturated in direct contact with all implants.

As long as the degradation of the crystalline poly(L-lactide) C was at a low rate neither foreign-body reaction nor inflammatory signs develop. It remained stable in form and structure for more than 2 years and a very slow, hardly detectable degradation process was observed. Therefore, and in accordance with our results showing persistence of this material for more than 5 years [16], it might not be favourable for application in biodegradable osteosynthesis. Only a slight degradation, predominantly in the amorphous regions, took place, leaving behind crystalline domains which appeared to be very resistant to degradation. Consequently the overall crystallinity increased during the degradation time which was confirmed by the findings of the differential scanning calorimetry [17].

The tissue reaction changes when biodegradation has progressed. Detectable signs of degradation started about 8 weeks after implantation within the amorphous poly(L-lactides). Subsequently the tissue reaction transformed into a mild to moderate foreign-body reaction containing resorptive cells as well as fibrous tissue which invaded the implants to an increasing extent. During these stages the degradation proceeded faster in the inner parts which softened and became pliable. In agreement with previous *in vitro* studies it seems that the hydrolytic degradation products near the surface could dissolve more easily than

those located inside the implants. Therefore the concentration of carboxylic endgroups increased in the centre and autocatalysed the degradation process [18]. These findings are in good agreement with a newly published *in vivo* degradation study of poly(DL-lactide) observing also a faster degradation in the centre of amorphous specimens than at the surface because of the autocatalytic effect [19]. The pores and channels could be indicators for a localized softening during the autocatalysed degradation process. During ongoing degradation the pores and channels dilated and joined together. If connection to the exterior was made, the content consisting of degradation products diffused and was absorbed by the tissue. A partial dissolution of the material was detectable.

After about 100 weeks, when complete disintegration of the implants took place, final biodegradation occurred. The degradation products, however, have to be removed by cells. The prerequisite is the presence of tissue suitable for absorption, which is found in the form of histiocytic resorptive inflammation tissue observed around the remaining fragments. A higher rate of degradation and elimination during the final stage could be observed which may be due to an interaction with this tissue. The increased fluid transfer related to inflammatory response may well explain an increase of the hydrolysis rate. Whereas the role of enzymes during initial degradation is under discussion [8, 20, 21], enzymatic influence in the later stages is more readily accepted [21–23]. Presumably, enzymes released by inflammatory and resorptive cells have an additional effect and were able to accelerate the biodegradation. Finally, not only the liquid degradation products but also tiny particles were phagocytosed by histiocytic resorptive cells. Apparently they were digested by intracellular enzymatic processes before elimination through the metabolism. The fragments originating from the cortex degraded at a reduced rate. To date it has not been possible to clearly identify the origin of this amorphous structure, which was already detectable before implantation was performed. Either it resulted from the machining process after injection-moulding or from the sterilization procedure.

As mentioned above the rate of biodegradation is an important aspect for the use of biodegradable materials in osteosynthesis. Not only a too slow, but also a too rapid degradation process is unsuitable: the capacity for absorption might be overtaxed and the inflammation may elicit unfavourable effects. A total or nearly total degradation of the amorphous poly(L-lactides) during an adequate tissue response within 2 years meets the requirements for osteosynthesis and seems to be recommendable.

Probably because of the higher molecular weight it was not possible to observe the final liquidation of poly(L-lactide) A1 in the observation period, whereas the amorphous poly(L-lactide) of lower molecular weight A2 was totally resorbed. Altogether no other differences in biodegradation between both amorphous poly(L-lactides) were detectable.

Further studies in progress regarding bony implantation following osteosynthesis will show whether the remaining tissue at the implantation site differentiates into bone.

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