Long-term soft tissue reaction to various polylactides and their *in vivo* degradation

P. MAINIL-VARLET, S. GOGOLEWSKI*
Polymer Research, AO/ASIF Research Institute, Clavadelerstrasse, CH-7270 Davos, Switzerland
P. NIEUWENHUIS
Department of Histology and Cell Biology, University of Groningen, Oostersingel 69-1, NL-9713 EZ Groningen, The Netherlands

Cylindrical pins made from poly(L-lactide), poly(L/D-lactide) 95/5% and poly(L/DL-lactide) 95/5% were implanted in the subcutaneous tissue of sheep. The tissue reaction to the implanted materials and their *in vivo* degradation was investigated at 1, 3, 6 and 12 months. The capsule formed around the polylactide implants consisted of fibroblasts, fibrocytes, phagocytes, a few foreign body giant cells and polymorphonuclear cells. For all three polylactides used, the cellular response was most intensive during the first 6 months of implantation and significantly subsided at 1 year. The thickness of the capsule was 200 μm at 1 month, increased to 200–600 μm at 6 months, and decreased to 100 to 200 μm at 1 year, depending on the material used. The tissue reaction was more intense for poly(L/D-lactide) than for poly(L/DL-lactide) and poly(L-lactide). The drop in molecular weight of the implants was highest after 1 month of implantation (70 to 95%). Irrespective of the extensive reduction of the molecular weight at 1 month, none of the polymers used was completely resorbed at 1 year. The most advanced resorption was observed for poly(L/D-lactide). Despite molecular weight reduction, the poly(L-lactide) implants had maintained 70% of their initial bending strength and 95% of their shear strength at 3 months. The poly(L/D-lactide) and poly(L/DL-lactide) had maintained only 26 to 27% of their initial bending strength and 26 to 31% of the initial shear strength, respectively. The crystallinity of all the materials increased after implantation as compared with nonimplanted materials. The overall crystallinity increase and the final crystallinity reached by the materials at 1 year was, however, lowest for poly(L/DL-lactide) as compared with the other two polylactides.

1. Introduction

The interest in medical applications of resorbable polylactides goes back to the early 1970s when they were used in experimental animals for the repair of the orbital floor, as sutures, and for the internal fixation of bone fractures and skin lesions [1–7]. Polylactides were found to be biocompatible. The typical tissue reaction to polylactides may involve fibroblasts, histocytes, lymphocytes, mast cells, foreign body giant cells, macrophages, plasma cells, eosinophils, and lymphoid cells [1–14]. The type of cells present at the implantation site may vary depending on the implant purity (i.e. the presence in the implant of catalyst and monomer residues, particulates, solvents, etc.), the implant mass and geometry, positional stability at the implantation site, ability of the polymer to crystallize, etc. [15]. The *in vivo* resorption rate of polylactides is to a great extent determined by their chemical composition, molecular weight, crystallinity, the degree of chain orientation, the presence of low molecular weight components, leachables and impurities [8, 15]. In the case of plates and screws machined from as-polymerized, microporous poly(L-lactide) with an initial molecular weight of 1 million dalton, highly crystalline polymer debris was found after 5.6 years of implantation [16, 17]. The presence of this debris was claimed to be the reason for subcutaneous swelling and itching 3 years postoperatively [18].

There are no established criteria for selection of a particular polylactide for a specific application. The rate of implant degradation, the intensity of the inflammation process around the implant and the maintenance of mechanical properties *in vivo* for an adequate time should certainly motivate such selection. These factors are to a great extent determined by the chemical structure of the material.

The aim of the present study was to evaluate the soft tissue response to implants of poly(L-lactide), poly(L/D-lactide) 95/5%, poly(L/DL-lactide) 95/5%, and their *in vivo* degradation as assessed from the changes in molecular weight and mechanical properties.

* To whom correspondence should be addressed.

0957–4530 © *1996 Chapman & Hall*

2. Materials and methods

2.1. Implants

Cylindrical pins 3.2×50 mm, weighing 400 mg were produced by injection-moulding from poly(L-lactide) P(L)LA, poly(L/D-lactide) P(L/D)LA 95/5% and poly(L/DL-lactide) P(L/DL)LA 95/5%, purchased from CCA Biochem, Gorinchem, The Netherlands [19]. The pins in double pouches were sterilized with ETO (low temperature cycle), evacuated at 60 °C and 30 Pa for 24 h, and stored in a desiccator prior to use.

2.2. Animals

Twenty-four adult Swiss mountain sheep weighing between 50 and 60 kg were used in the experiment. A few days before the operation the animals were kept in individual pens to get them used to the stable environment. Food was withheld the day before surgery.

2.3. Implantation

The experiment guidelines and animal maintenance were approved by the Swiss Federal Veterinary Commission, Approval No. 5/1993. The animals were premedicated with 0.4 mg Rompung and 2 ml Atropine administered intramuscularly and were anaesthetized with Penthotal, N_2O, O_2 and Halothan. Under sterile conditions, two sagittal incisions were made over the last thoracic vertebrae and two small pockets created in the subcutaneous connective tissue. Hemostasis was achieved with an electrical cautery. Five pins of the same polymer were inserted subcutaneously in each pocket. The wound was rinsed with saline solution and closed in separate layers. No antibiotic preparations were given to the animals at any time during the experiment. Two animals were used per each implant material and implantation period (4, 12, 24 and 52 weeks). Each of these two animals were implanted with pins from one type of polymer only. The sheep were killed by an overdose of Phenobarbital.

2.4. Preparation of explants for testing

For each sheep five explanted pins with the surrounding connective tissue were used for histological evaluation. These were fixed "*en bloc*" in 5% formalin, subsequently dehydrated in a graded series of ethanol and embedded in methylmethacrylate (Fluka 64200, Switzerland). Sections 6 µm thick cut along the longitudinal axis of the implant (Polycut E microtome, Reichert-Jung) were stained with either Giemsa (Fluka, Switzerland) or Hemotoxylin Eosine (Hematoxylin, Merck 15938; Eosin Yellowish, Fluka 45240). The remaining five pins were used for the evaluation of molecular weight, mechanical properties and crystallinity after cleaning off the adherent tissue. Mechanical tests were carried out on wet explants within 5 h after explantation. The implant fragments produced upon mechanical testing were dried to constant weight, stored in a desiccator and subsequently used for the evaluation of molecular weight and crystallinity.

2.5. Mechanical tests

The ASTM D790 [20] standard was applied to measure the bending strength, bending modulus (four-point bending) and the shear strength of the pins. The measurements were carried out at 23 ± 1 °C, using an Instron model 4302 testing machine, crosshead speed of 5 mm/min, span of the two lower supports 30 mm, 3:1 span ratio, diameter of the supports 5 mm.

2.6. Thermal analysis

The melting peak temperature and the heat of melting of the samples were measured using a Perkin-Elmer differential scanning calorimeter (UNIX-DSC7) calibrated with indium. The samples, weighing between 2.5 and 5 mg, were scanned under dry, oxygen-free nitrogen at temperatures in the range 20 to 200 °C at a heating rate of 10 °C/min. Five scans were taken from each polymer sample. The relative degree of crystallinity of the material was calculated using the value of the heat of melting of the crystalline regions of poly(L-lactide) $\Delta H_m = 93.1$ J/g [21].

2.7. Molecular weight evaluation

The molecular weight of the polylactides used in the study was assessed by size exclusion chromatography and viscosity measurements. The details of the procedures used have been described elsewhere [19].

3. Results

3.1. Macroscopic findings

In none of the cases studied, was there post-operative infection or clinical signs of inflammation. The P(L)LA pins maintained their initial shape after up to 1 year of implantation and could easily be removed from the surrounding tissue over the whole experimental period. At 1 year the pins being initially transparent became partially opaque and brittle. The pins produced from P(L/D)LA and P(L/DL)LA became opaque after 1 month, but maintained their shape up to 3 months. At 6 months the P(L/D)LA pins became brittle and were fragmented into small blocks. The P(L/DL)LA pins were fractured into small fragments at 1 year.

3.2. Histological analysis

Table I illustrates relative changes in the number of polymorphonuclear cells, capsule thickness and implant appearance with time of implantation. After 1 month of implantation, the P(L)LA pins were surrounded by an approximately 100 µm thick fibrous capsule. This capsule consisted mainly of collagen fibres, fibroblasts, fibrocytes and a capillary network having a typical half-way distribution pattern. Mononuclear cells (macrophages/monocytes) arranged in two or three layers were in direct contact with the surface of the pin (Fig. 1a). At 3 months, the capsule was denser, its thickness and cellularity had slightly increased (some polymorphonuclear cells could still be seen). Layers (mono- and bi-) of mononuclear cells

were in direct contact with the implant (Fig. 1b). At 6 months, the capsule was more matured, the number of polymorphonuclear cells present in the capsule, the capsule thickness, and the layer of monocytes/macrophages in direct contact with the pin had increased (Fig. 1c). The thickness of the capsule at 6 months was around 300 to 400 μm and there was a thicker (3–5 cells) layer of phagocyting cells around the implant. After 1 year of implantation, the inflammatory process subsided. The capsule consisted mainly of mature collagen fibres. The cellularity of the capsule and its thickness decreased. The thickness of the capsule at 1 year was 80–100 μm (Fig. 1d). At each implantation period, the inflammatory process was less intensive at the sharp edges of the pin compared to the other regions (Fig. 2).

TABLE I Relative changes in the number of polymorphonuclear cells, capsule thickness and implant appearance with time of implantation

Implant/ Property	Time of implantation (months) 1	3	6	12
P(L)LA				
RNPC	–	+	+ +	–
CT (μm)	100	200	400	100
EF	–	–	–	–
P(L/D)LA				
RNPC	–	+ + (*)	+ (*)	–
CT (μm)	200	400–600	400–600	400
EF	–	–	+ +	+ + +
P(L/DL)LA				
RNPC	–	+ + (*)	+ + (*)	–
CT (μm)	200	300	500	100–150
EF	–	–	+	+ +

RNPC, relative number of polymorphonuclear cells; CT, capsule thickness; EF, relative extent of fragmentation; (*) a few eosinophils were also present

The pins made from P(L/D)LA were surrounded by a fibrous capsule of 150–200 μm thickness after 1 month of implantation. A capillary network could be seen in the capsule, having a typical halfway vascularization pattern. A layer of 3–4 macrophages/monocytes thick was in direct contact with the pin. There was no polymorphonuclear cells (Fig. 3a). The capsule became thicker (400–600 μm) after 3 months of implantation, and its cellularity increased. The layer of macrophages/monocytes in direct contact with the pin was 4–5 cells thick. Polymorphonuclear cells (eosinophils) were present in the capsule which was more organized, but not completely matured. The number of eosinophils in the capsule seemed to correlate with the number of macrophages (Fig. 3b). After 6 months the pin was less homogenous than at 3 months. Large cracks formed in the material which were filled with macrophages, fibroblasts and blood vessels. Some macrophages in direct contact with the pin surface fused together, and formed giant cells.

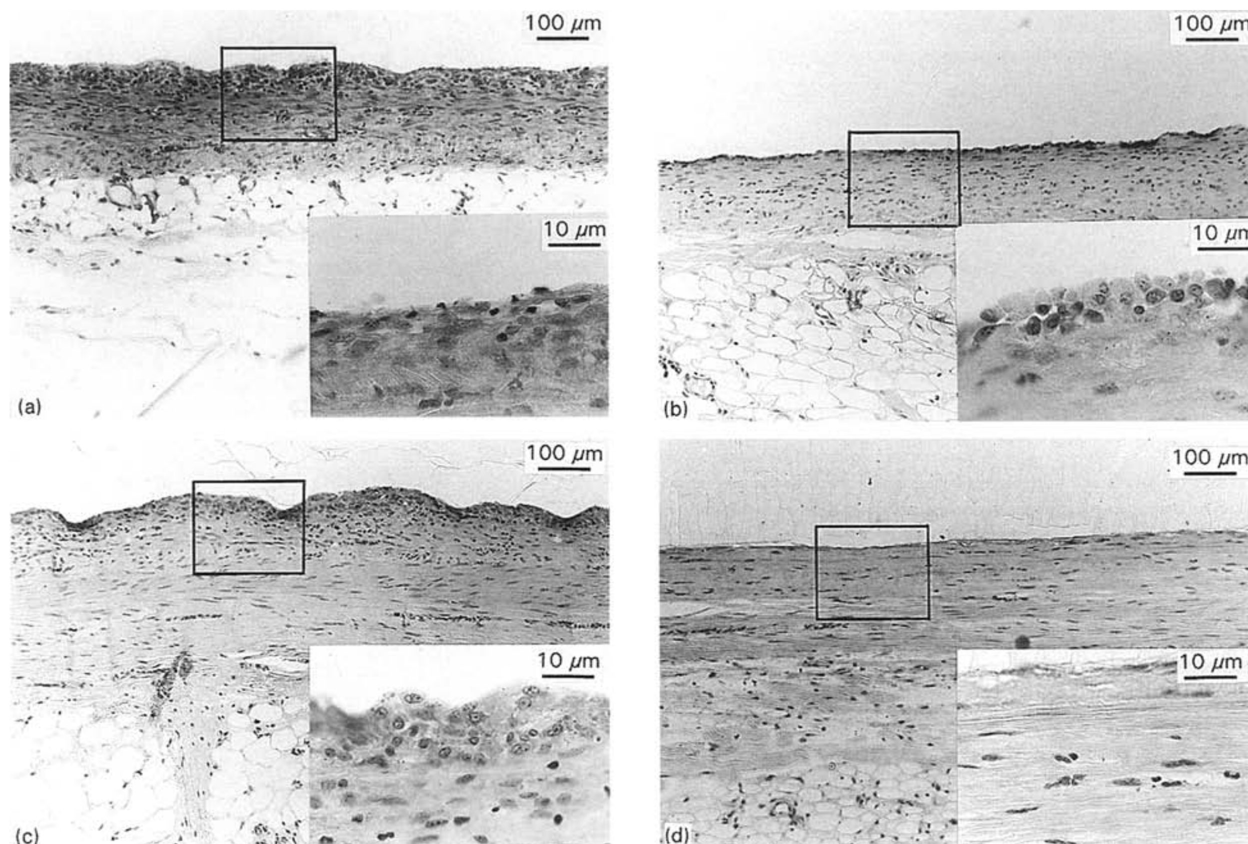

Figure 1 Typical tissue reaction to P(L)LA implant. (a) 1 month. The fibrous capsule surrounding the implant showing the foreign body reaction with fibroblastic and macrophage/monocytes activity. (b) 3 months The number of macrophages/monocytes in contact with the implant is increasing. (c) 6 months. The thickness of the capsule reached a maximum, no changes in the cellularity (d) The capsule consists mainly of mature collagen fibres with some fibrocytes The thickness of the capsule decreased. Note the absence of macrophages or signs of polymer degradation. The inserts show higher magnification of the areas marked with rectangles.

A surface erosion process was clearly visible. The polymer fragments were separated from the pin surface and surrounded by giant cells. The capsule cellularity decreased and its maturation increased (Fig. 3c). After 1 year of implantation the polymorphs disappeared. Few mast cells were present in the capsule. Massive staining of the polymer was observed. There were more large cracks and progressive implant fragmentation. These fragments were surrounded by foamy macrophages, giant cells and fibroblasts which were totally enclosed by a connective tissue layer. Outside that layer, signs of tissue reaction were not observed (Fig. 3d).

After 1 month of implantation the P(L/DL)LA pins were surrounded by a 200 μm thick capsule similar to that of the capsule surrounding the P(L)LA pins at 1 month (Fig. 4a). After 3 months, the capsule thickness increased (300 μm). A 5–6 cell thick layer of macrophages/monocytes was in direct contact with the implant. Numerous eosinophils were present in the capsule (Fig. 4b). At 6 months, the thickening of the capsule had progressed. The capsule was more mature. The thickness of the macrophage/monocyte layer remained the same as at 3 months. The implant began to crack, and the cracks were discretely infiltrated with giant cells, which also seemed to engulf and digest the polymer. The number of giant cells decreased (Fig. 4c). After 1 year, the pins were frequently fragmented, each fragment encapsulated by a fibrous tissue. The thickness and the cellularity of the capsule was significantly decreased as compared with the 6 month implantation period (Fig. 4d). A few giant cells could still be seen in direct contact with the implant. The fragmentation of the implant was more pronounced in its centre than at the surface. Macrophages and giant cells were present at the polymer surface and in the cracks and voids (Fig. 5).

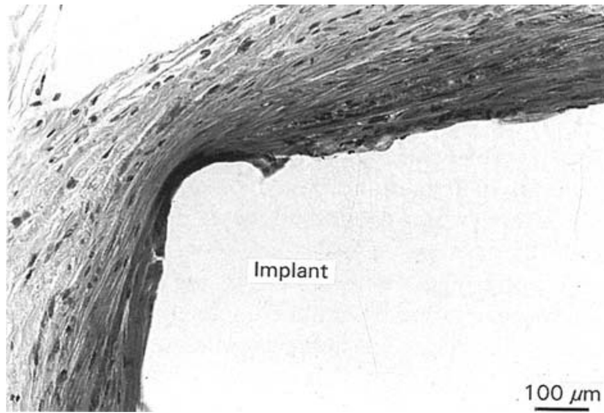

Figure 2 P(L)LA pins 3 months after implantation. Minimal cell reaction at the edges.

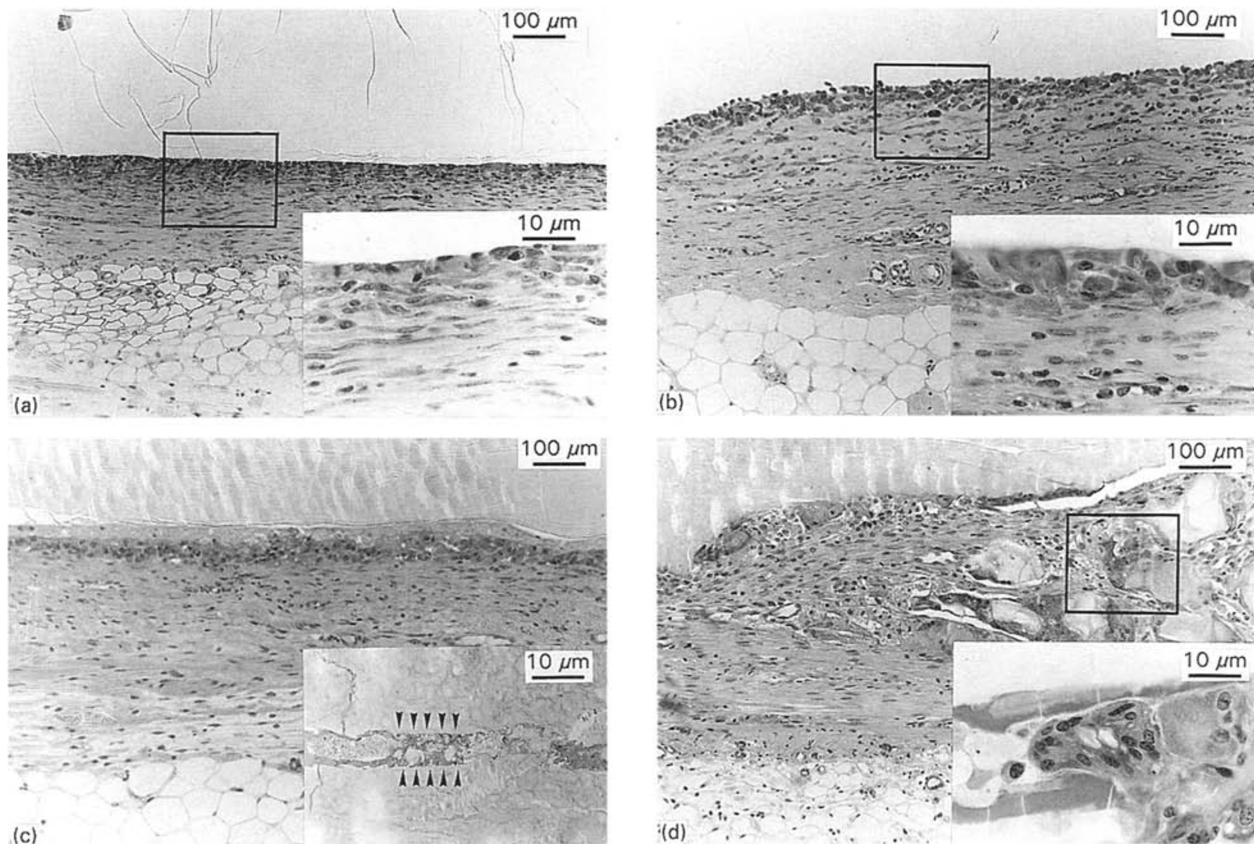

Figure 3 Typical tissue reaction to P(L/D)LA implants. (a) 1 month. Inflammatory cells consist of fibroblasts and macrophages/monocytes. The capsule has a typical half way vascularisation pattern. (b) 3 months. The capsule increased in thickness and vascularity. A layer of 4–5 macrophages/monocytes surrounds the implant with numerous eosinophils. (c) 6 months The pins are cracked The cracks have been infiltrated by macrophages and fibroblasts. (d) 12 months. The pins are disintegrated into small fragments surrounded by macrophages, giant cells and fibroblasts. The inflammatory response is well contained within a fibrous capsule. The inserts show higher magnification of the areas marked with rectangles.

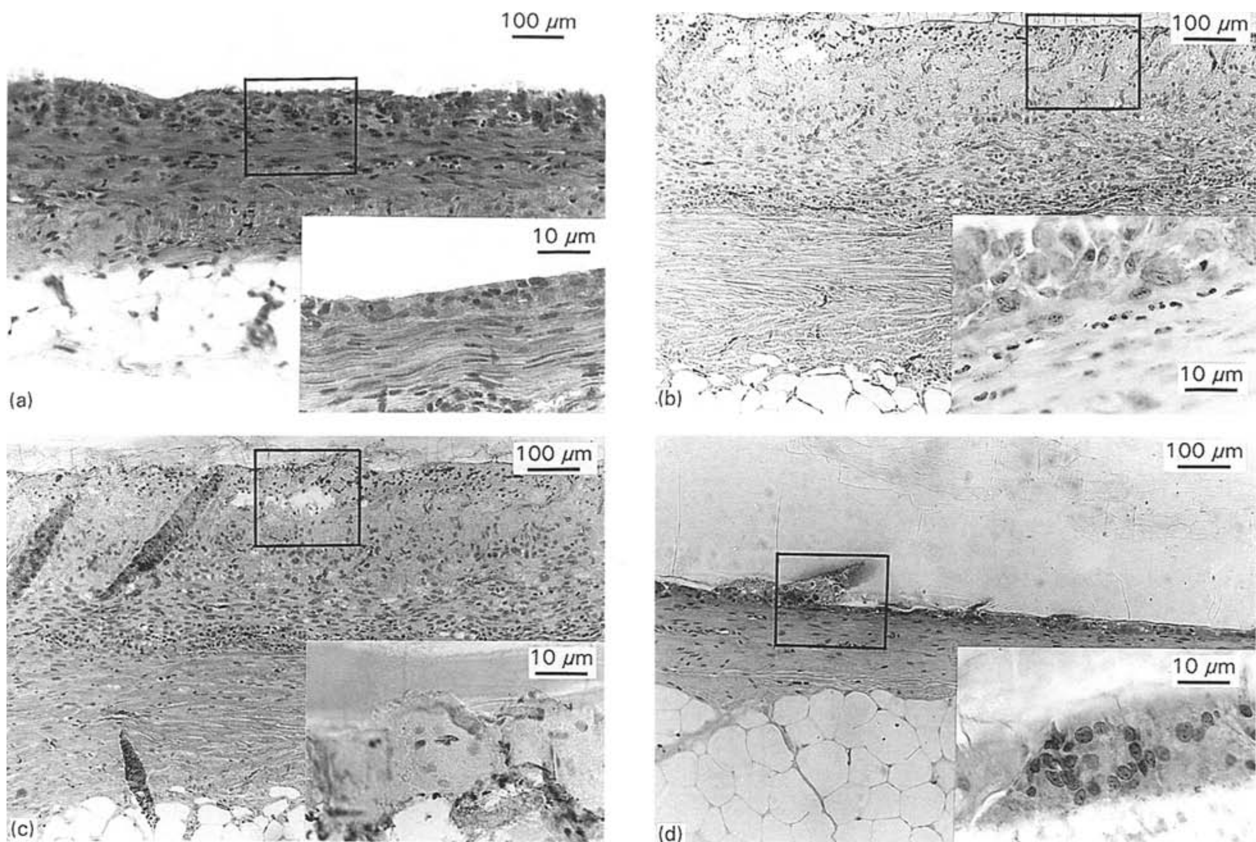

Figure 4 Typical tissue reaction to P(L/DL)LA implants. (a) 1 month. A few macrophages/monocytes are present at the implant-tissue interface. (b) 3 months. The cellular activity and the capsule thickness increased No change in the cell type except for a few eosinophils. (c) 6 months. The capsule has matured, the implant is surrounded by a layer of macrophages/monocytes (5 to 6 cells thick). (d) 12 months The capsule thickness decreased. The inflammatory process is less intense. The tissue–material interface mainly consists of macrophages and giant cells. The inserts show higher magnification of the areas marked with rectangles.

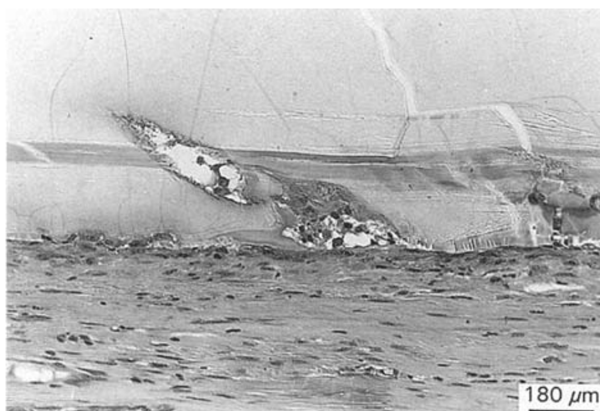

Figure 5 Macrophages and giant cells were clearly involved in the fragmentation process of the polymer surface in the cracks and voids.

3.3. Molecular weight changes

Table II and Fig. 6 illustrate the changes in the viscosity-average molecular weight (M_v) of the poly(L-lactide), poly(L/D-lactide) and poly(L/DL-lactide pins implanted for various periods.

For poly(L-lactide), there was a 72% decrease of M_v at 4 weeks. After that period the decrease of M_v was rather slow, in the range 5–10% of the initial molecular weight per implantation interval. At 1 year, the molecular weight of the samples was only 2% of the initial value. For poly(L/D-lactide) and poly(L/DL-lactide) samples, there was about a 94% and 91% drop of M_v at 4 weeks of implantation, respectively. After that period the decrease of M_v was more abrupt for poly(L/D-lactide) than for poly(L/DL-lactide). At 1 year of implantation the M_v for both polymers reached similar values of 2000–3000 dalton.

3.4. Crystallinity and melting temperature

Fig. 7 shows the changes in the crystallinity and melting temperature of the pins with time of implantation. The crystallinity of poly(L-Lactide) samples increased over the first 12 weeks from 60 to 65%, and subsequently decreased gradually until 1 year to 60% (Fig. 7a). For poly(L/D-lactide) the crystallinity increased continuously from 30 to 60% at 1 year (Fig. 7b), and for poly(L/DL-lactide) from 33 to 44% at 4 weeks, and after that period remained practically constant until the end of the experiment (Fig. 7c). The melting temperature of poly(L-lactide) and poly(L/DL-lactide) decreased slightly over the whole implantation period, while for poly(L/D-lactide) the drop in melting temperature was more significant.

3.5. Mechanical properties

Figs. 8, 9 and 10 show the changes in the mechanical properties of polylactides with time of implantation. For poly(L-lactide) the bending strength of the

TABLE II Changes in molecular weight of polylactides after implantation

Polymer	M_v	M_z	M_w	M_n	M_w/M_n
P(L)LA					
Raw material	431 000	–	–	–	–
Injection-moulded not sterilized	55 500	112 000	58 500	27 700	2.1
Injection-moulded sterilized	56 800	114 500	60 100	28 300	2.1
4 weeks	14 600	48 600	17 150	4 100	4.2
12 weeks	8 500	37 200	10 900	1 500	7.4
24 weeks	1 800	6 300	2 400	300	7.9
52 weeks	1 700	8 500	2 400	300	7.6
P(L/D)LA					
Raw material	770 000	–	–	–	–
Injection-moulded not sterilized	98 700	110 300	56 800	24 800	2.2
Injection-moulded sterilized	99 500	115 000	57 800	25 100	2.3
4 weeks	6 000	10 400	3 500	500	6 9
12 weeks	3 000	6 000	1 700	300	6.9
24 weeks	2 900	5 800	1 800	300	7.1
52 weeks	2 800	5 800	1 600	300	6.6
P(L/DL)LA					
Raw material	707 800				
Injection-moulded not sterilized	76 200	93 900	47 000	20 200	2.3
Injection-moulded sterilized	75 000	95 000	43 700	19 500	2.2
4 weeks	6 700	7 400	3 800	1 200	3.1
12 weeks	6 200	8 000	3 600	800	4.3
24 weeks	2 300	4 400	1 400	195	7.2
52 weeks	2 250	4 200	1 400	160	8.7

Viscosity-average molecular weight values are means of three measurements. Average relative standard deviations (RSD) were $\pm$ 1%. All SEC molecular weight values are means of three measurements. RSD were $M_n \pm 5\%$; $M_z \pm 13\%$; $M_w \pm 9\%$.

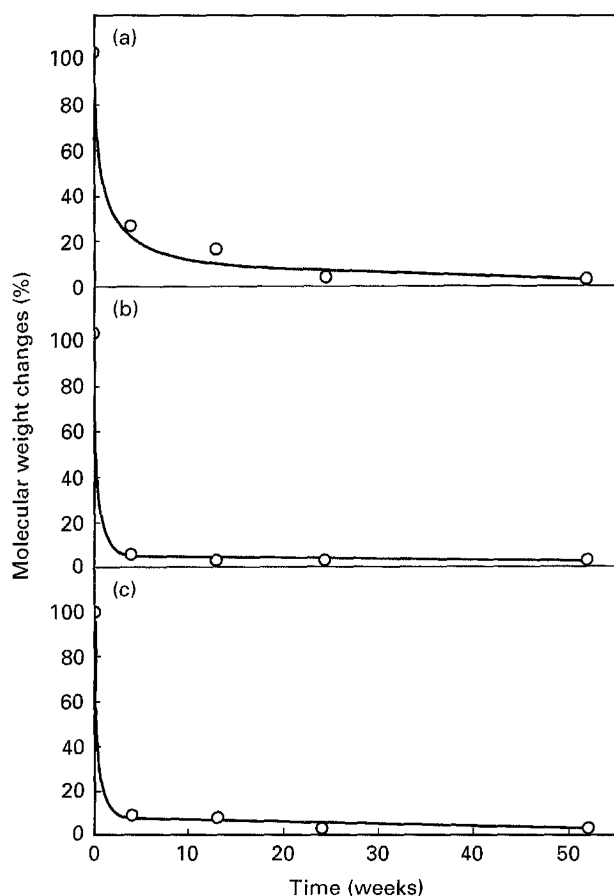

Figure 6 Changes of molecular weight for polylactide implants with time of implantation. (a) P(L)LA, (b) P(L/D)LA; (c) P(L/DL)LA. All data points are means of three measurements. Average relative standard deviations (RSD/S) were $\pm$ 1.0%.

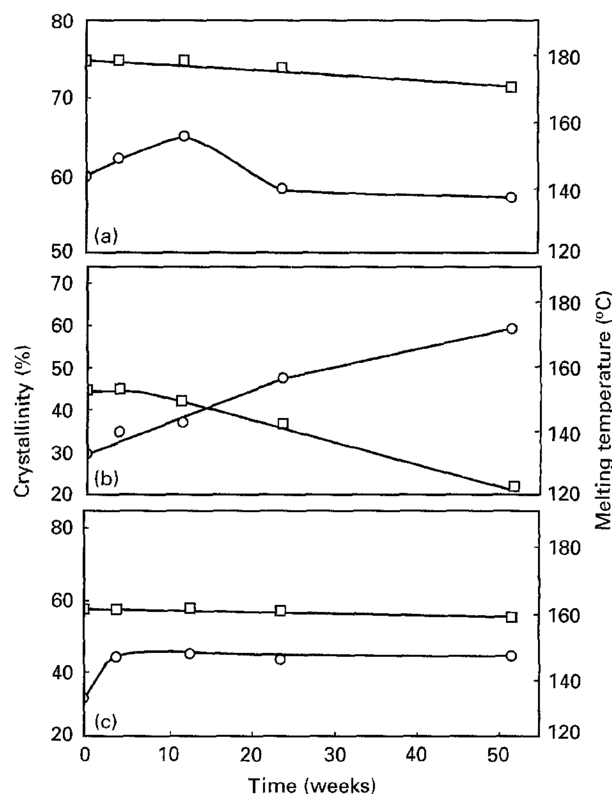

Figure 7 The effect of implantation time on crystallinity (○) and melting peak temperature (□) of polylactides. (a) P(L)LA; (b) P(L/D)LA; (c) P(L/DL)LA. All data points are means of five measurements. RSD's were $\pm$ 5.3% crystallinity, 0.8% melting peak temperature.

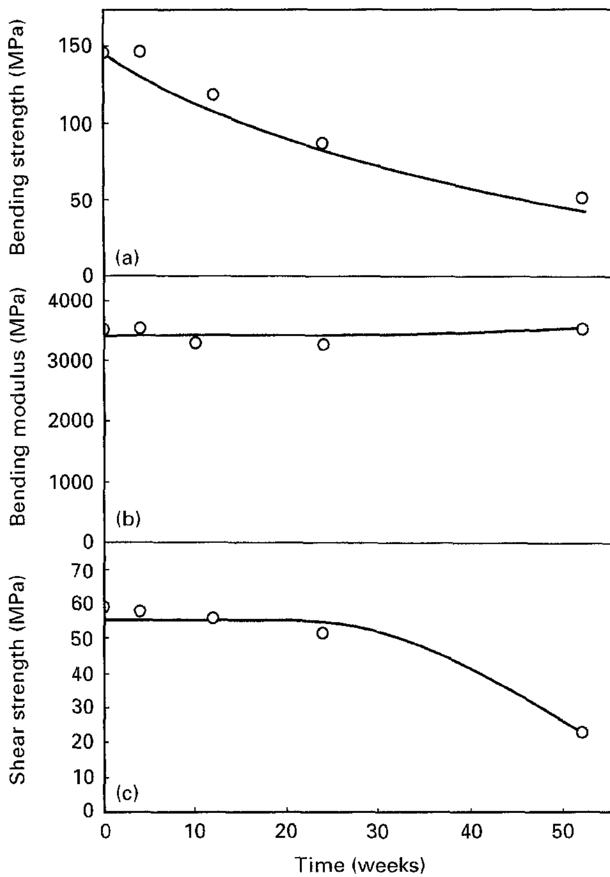

Figure 8 The effect of implantation on: (a) bending strength; (b) bending modulus, and (c) shear strength of P(L)LA samples. All data points are means of five measurements. RSDs were $\pm$ 10% bending strength, 12% bending modulus, 8% shear strength.

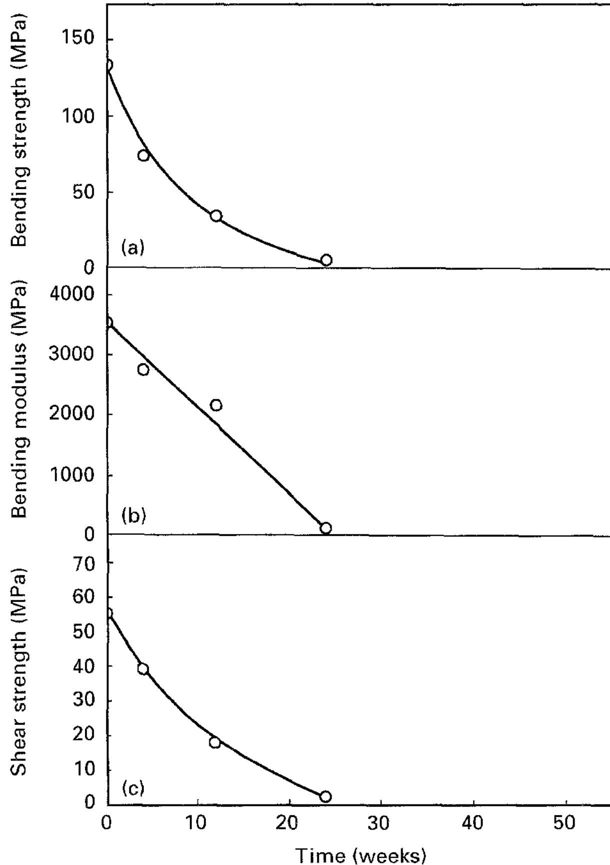

Figure 9 The effect of implantation on: (a) bending strength; (b) bending modulus; and (c) shear strength of P(L/D)LA samples. All data points are means of five measurements RSDs were $\pm$ 13% bending strength, 12% bending modulus, 8% shear strength

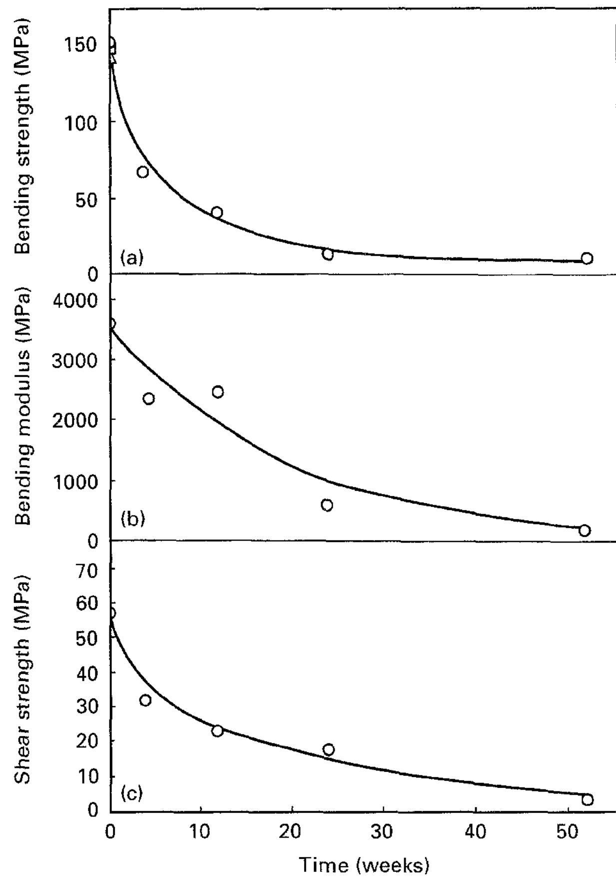

Figure 10 The effect of implantation on: (a) bending strength; (b) bending modulus; and (c) shear strength of P(L/DL)LA samples. All data points are means of five measurements. RSDs were $\pm$ 12% bending strength, 12% bending modulus, 10% shear strength.

samples remained constant during the first 3 months of implantation, decreased from the initial value of 141 MPa to 87 MPa at 6 months, and to 52 MPa at 1 year. The bending moduli did not change over the whole implantation period. The shear strength of the implants remained practically constant over the 6 month period, and at 1 year, dropped from the initial value of 57 MPa to about 20 MPa. For poly(L/D-lactide) and poly(L/DL-lactide) there was a significant 20 to 40% loss of mechanical properties during the first 4 weeks of implantation. The reduction of the shear strengths, bending strengths and moduli was lower for poly(L-lactide) than for poly(L/DL-lactide) and poly(L/D-lactide) implants. At 6 months of implantation, it was not possible to measure the mechanical properties of the P(L/D)LA implants as they were extensively fragmented.

4. Discussion

The inflammatory response to biomaterials is determined by their composition, purity, shape of the implant and its surface properties, implantation site, positional stability at the implantation site, porosity, chemical stability, etc. [1–8, 15, 22–26]. In the case of resorbable polymers, the same factors determine to a great extent the material degradability in the tissues and hence, also the type and intensity of the inflammatory response. The tissue reaction to the polylactides used in the present study was manifested by the presence of fibroblasts, fibrocytes, macrophages,

foreign body giant cells, and polymorphonuclear cells. The number of cells around the implants was relatively higher in the case of the poly(L/D-lactide) than poly(L-lactide) and poly(L/DL-lactide) which is not surprising since this polymer degraded the fastest of the three materials used. It has been appreciated for some time that the cellular response to degradable implants is more intense for fast degrading polymers than for slow degrading polymers [8, 27]. At 12 months the number of inflammatory cells seemed to decrease for all the materials under investigation.

It has been found that the capsule surrounding the implants produced from poly(L/D-lactide) and poly(L/DL-lactide) contained numerous eosinophils. Eosinophils are usually an indication of an allergic response to the materials, but they have also been detected in a variety of inflammatory and fibrotic disorders [28]. Eosinophils are able to secrete an eosinophil cationic protein (ECP) which activates fibroblasts. This is manifested in the increased amount of connective tissue [29]. The presence of eosinophils in the capsule around the polylactide implants may therefore be a normal feature of the regular inflammatory process. Eosinophils have previously been observed with other nondegradable and degradable polymers [30, 31].

In general, the cellular response to implants in connective tissue is usually more intense at the implant edges [32–34] than at its smooth surfaces. Surprisingly, in the present study the cellular reaction at the sharp edges was less intense than in the smooth implant areas. A similar reaction was previously reported for implants of PTFE and P(L)LA [35].

The crystallinity changes of the polylactide implants observed in the present study were similar to those reported previously for poly(L-lactide), poly(D-lactide) and copolymers of the D and L lactides [8]. As might be expected, the crystallinity of the copolymers used was lower than that of the homopolymer, and the overall increase of crystallinity upon implantation was also lower for copolymers. Interestingly, of the three polymers used, the increase of crystallinity in the course of implantation was lowest for poly(L/DL-lactide) (from 30 to maximum 45% at the late stage of degradation). It has been reported that the late inflammatory process observed at 6 years in patients implanted with ultra-high molecular weight poly(L-lactide) screws and plates can be associated with the highly crystalline debris formed in the late stages of the implants degradation [18]. Hence, this feature of the poly(L/DL-lactide) might be exploited in the designing of resorbable implants with a low degree of crystallinity. Due to the presence of the small amount of crystalline phase such implants will have improved mechanical properties as compared with fully amorphous materials, while the unwanted inflammatory process caused by the highly crystalline debris can be significantly diminished.

5. Conclusions

The tissue capsule formed around all three polylactides used in the present study consisted of fibroblasts, fibrocytes, phagocytes, a few foreign body giant cells and polymorphonuclear cells. The cellular response was most intense during the first 6 months of implantation and significantly subsided at 1 year. The thickness of the capsule was 200 μm at 1 month increased to 200–600 μm at 6 months, and decreased to 100–200 μm at 1 year depending on the material used. The tissue reaction was more intense for the poly(L/D-lactide) than for poly(L/DL-lactide) and poly(L-lactide). At 1 year of implantation none of the polymers used was completely resorbed, although the most advanced resorption was observed for poly(L/D-lactide).

Despite the molecular weight reduction, the poly(L-lactide) implants maintained 70% of the initial bending strength and 95% of the shear strength at 3 months. The poly(L/D-lactide) and poly(L/DL-lactide) showed a significant 20 to 40% loss of mechanical properties during the first 4 weeks of implantation. At 6 months of implantation, it was not possible to measure the mechanical properties of the P(L/D)LA implants as they were extensively fragmented.

The crystallinity of the materials increased after implantation as compared with reference nonimplanted materials. The crystallinity increase in the course of implantation and the final crystallinity reached by the materials at 1 year was, however, lowest for poly(L/DL-lactide) as compared with the other two polylactides. It can be expected that this polymer, which develops a low amount of crystalline phase, will have better mechanical properties than those of fully amorphous polylactides, yet, the unwanted inflammatory process caused by the highly crystalline debris may be significantly diminished. Hence, it may be a promising candidate for the preparation of resorbable implants.

References

1. D. E CUTRIGHT and E E. HUNSUCK, *J Oral Surg.* **31** (1971) 134.
2. D. E CUTRIGHT. E E. HUNSUCK and J. D. BEASLEY, *ibid.* **29** (1971) 393.
3. D. E CUTRIGHT. J. D. BEASLEY and B PEREZ. *ibid* **32** (1971) 165.
4. D E. CUTRIGHT and E E. HUNSUCK, *ibid.* **33** (1972) 2834
5. J M. BRADY, D E. CUTRIGHT, R. A MILLER, G. C BATTISTONE and E. E. HUNSUCK. *J. Biomed. Mater. Res.* **7** (1973) 155.
6. D. E. CUTRIGHT. B. PEREZ. J D BEASLEY, W J. LARSON and W R POSEY, *J. Oral Surg.* **37** (1974) 142.
7. R K. KULKARNI. K. C. PANI, C NEUMAN and F LEONARD, *Arch Surg.* **93** (1966) 839
8. S. GOGOLEWSKI, M JOVANOVIC, S M PERREN, J G DILLON and M K. HUGHES. *J. Biomed. Mater. Res.* **27** (1993) 1135.
9. A. MAJOLA, S VAINIONPÄÄ, K. VIHTONEN, M MERO. J. VASENIUS. P. TÖRMÄLÄ and P ROKKANEN. *Clin Orthop.* **268** (1991) 260.
10. T. NAKAMURA, S. HITOMI, S. WATANABE, Y. SHIMIZU, K. JAMSHIDI. S -H. HYON and Y YKADA, *J. Biomed. Mater. Res.* **23** (1989) 1115.
11. J M SCHAKENRAAD. P NIEUWENHUIS, M J. HARDONK. I MOLENAAR, J. HELDER, P J DUKSTRA and J FEIJEN. *ibid.* **23** (1989) 1271.
12. R R M BOS, F. R ROZEMA, G BOERING, A J. NIJENHUIS, A J PENNINGS, A. B VERWEY, P. NIEUWENHUIS and H. W. B. JANSEN, *Biomaterials* **12** (1991) 32.

13. Y MATSUSUE, T YAMAMURO, M OKA, Y SHIKINAMI, S.-H. HYON and Y. IKADA, *J. Biomed. Mater. Res.* **26** (1992) 1553.
14. P GALGUT, R PITROLA, I WAITE, C DOYLE and R SMITH *J. Clin Periodontol.* **18** (1991) 581.
15. S GOGOLEWSKI in: "Die Plattenosteosynthese und ihre Konkurrenzverfahren" edited by D Wolter and W. Zimmer (Springer-Verlag, Berlin, 1991) p. 340.
16. M ROHOVSKY, E. CHEN, A CLEMOW and S WEISBRODE, in: "Biodegradable implants in fracture fixation", edited by K. S. Leung, L. K Hung and P. C. Leung (Chinese University of Hong Kong & World Scientific Publ., Hong-Kong/Singapore 1994) p. 125.
17. K H. LAM, J M SCHAKENRAAD, H ESSELBRUGGE, J FEIJEN and P NIEUWENHUIS, *J. Biomed. Mater. Res* **27** (1993) 1569.
18. J E. BERGSMA, W CD. BRUIJN, F R ROZEMA, R R BOS and G. BOERING, *Biomaterials* **16** (1995) 25.
19. S. GOGOLEWSKI, M JOVANOVIC, S. M. PERREN, J G. DILLON and M. K. HUGHES, *Polymer Degrad Stabil* **40** (1993) 313.
20. Test methods for flexural properties of unreinforced and reinforced plastics and electrical insulations material, ASTM D790. *ASTM Standards* **08.01, 410** (1984)
21. E W. FISCHER, H J STERZEL, and G WEGNER, *Kolloid-Z u Z-Polymere* **251** (1973) 980.
22. D BAKKER, C A VAN BLITTERSWIJK, S C HESSELING, J. J GROTE and W. T DAEMS, *Biomaterials* **9** (1988) 14
23. B F MALTAGA, L. P YASENCHAK and T. N SALTHOUSE, *J. Biomed. Mater Res.* **10** (1976) 391
24. R. A WHITE R. W SPROAT, R. S. LAWRENCE and R J NELSON, *Biomaterials* **2** (1981) 171.
25. J M. SCHAKENRAAD, H J BUSSCHER, C R H WILDEVUUR and J ARENDS, *J. Biomed. Mater. Res.* **20** (1986) 773.
26. J E. DAVIES. "Surface characterization of biomaterials" edited by B. D. Ratner (Elsevier, Amsterdam) (1988) p. 219.
27. L GETTER, D E CUTRIGHT, S. N BHASKAR and J K AUGSBURG. *J. Oral Surg.* **30** (1972) 344.
28. E G BASSET, *Biochem. J.* **213** (1983) 769.
29. J. G CLARK, in "The molecular pathology of pulmonary fibrosis", edited by J Vitto and A. J. Pereidi (Marcel Decker, New York, 1987) p. 321.
30. B. ZETTER, N RASMUSSEN and L BROWN. *Laboratory Investigation* **53** (1985) 362.
31. J ADENIS, M LEBOUTET J SALOMON, F DUPRAT and R LOUBET, *Ophtalmologie* **3** (1989) 231.
32. D. F. GIBBONS, H V MENDENHALL, C L V. KAMPEN and E G LAMPRECHT, *Clin. Mater.* **15** (1994) 37
33. E J KAMINSKI, R. J. OGLESKY, N K. WOOD and J SANDRIK, *J. Biomed. Mater Res.* **2** (1968) 81.
34. D L OLEMAN, R. N. KING and J D ANDRADE *J. Mater Sci.* **8** (1974) 199.
35. K LAM, PhD thesis, University of Groningen (1992)

Received 6 February
and accepted 14 March 1996