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# *In vitro* predegradation at elevated temperatures of poly(lactide)

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In this study *in vitro* predegradation at elevated temperatures, used to obtain an increased degradation rate, was investigated. The *in vitro* degradation was followed by mass loss, molecular weight loss and changes in thermal properties. Two biodegradable polymers, the homopolymer PLLA and a copolymer PLA96 (96% L4%D lactide), were hydrolytically degraded at 90 °C in a phosphate buffered solution. Both polymers, PLLA and PLA96, showed an initial linear degradation rate, but with longer implantation periods the degradation rate decreased and total degradation was best described as an asymptotic. Mass loss of the copolymer PLA96 was twice that of PLLA. The chemical analysis of the *in vitro* predegraded polymers coincided for both the decrease in molecular weight and the thermal properties with physiologically degraded poly(lactide). The results of this study show that although the degradation temperature is well above the glass transition temperature and not comparable to physiological temperatures, there seems to be good correlation between the *in vitro* degraded material and physiologically degraded material. *In vitro* predegradation enables investigation of the entire degradation process of a polymer in a short-term study. Moreover, *in vitro* predegradation allows direct comparison of the degradation rate of various polymers.

## 1. Introduction

Biodegradable materials, such as poly(glycolic) acid (PGA) or poly(L-lactic) acid (PLLA), are widely applied in a number of surgical fields, and many new applications for these biodegradable polymers have been described in recent years [1–7]. However, because of the relatively slow degradation of high molecular weight PLLA, *in vivo* studies of the complete process of degradation and resorption of these polymers have seldom been performed. Christel *et al.* [8] showed that PLLA was still present 4 years after implantation; Suuronen *et al.* [9] reported that in a 35 month follow-up, implanted self-reinforced PLLA screws were still present; and Bergsma *et al.* [10] found that as-polymerized PLLA was still present 5.6 years after implantation. The need for complete *in vivo* degradation studies is emphasized by unexpected reactions in patients during degradation, such as sinus formation in bone with PGA implants or subcutaneous swellings in patients with PLLA implants [11–14].

Considering the above mentioned late complications and the very low degradation rate of high molecular weight PLLA, there is a need for a technique that can accelerate the degradation and resorption of a polymer and provides degradation products that are comparable to long-term physiologically degraded material. Using an accelerated *in vitro* degradation technique enables long-term degradation studies in a relatively short-term experiment. The hydrolytic degradation rate of a bioresorbable polymer such as PLLA is greatly enhanced by extrinsic factors such as the degradation temperature. Accelerated degradation can be obtained by *in vitro* hydrolytic predegradation at temperatures well above physiological temperatures [15–17]. By using this technique the degradation of a polymer can be made to depend mainly on its chemical structure and the degradation temperature. An additional advantage of this predegradation technique is that when the same degradation temperatures are used in a short-term study, comparisons of degradation rate and mass loss of another polymer, such as the copolymer PLA96, can be made with PLLA.

The aim of the present study was to characterize *in vitro* predegradation at elevated temperatures. This technique was used to obtain enhanced *in vitro* degradation of the homopolymer PLLA and the copolymer PLA96. The degradation rates of these polymers were compared in terms of mass loss, molecular weight loss and changes in thermal properties during degradation.

## 2. Materials and methods

In this study poly(L-lactide) (PLLA) and a copolymer of 96%L and 4%D-lactide (PLA96) were used for



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*in vitro* predegradation. Both polymers were as-polymerized in a block according to the method described by Leenslag *et al.* [18]. After purification of the monomers (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-ethylhexanoate as a catalyst. The average molecular weight ($\bar{M}_w$) of the PLLA block and PLA96 block was $880 \times 10^3$ and $1300 \times 10^3$, respectively. The heat of fusion ($\Delta H$) of the PLLA and PLA96 block was, respectively, 60 $Jg^{-1}$ and 26 $Jg^{-1}$, the melting temperature ($T_m$) was 187 °C and 152 °C, and the glass transition temperature was 60 °C and 56 °C. From the blocks, 72 PLLA and 72 PLA96 discs with a thickness of 2 mm and diameter 5 mm were machined. All discs were weighed separately, the mean weight of the PLLA discs was 47.8 ± 2 mg and the PLA96 discs 49.8 ± 3.8 mg. All discs were separately immersed in phosphate buffer of pH 7.4 in a glass tube with a permeable cap. Each disc was enveloped by medical grade stainless steel mesh (interstices 40 μm × 40 μm) to facilitate the complete removal and weighing of the degraded discs. Subsequently, the glass tubes with the discs were placed in a temperature-controlled basin containing 15 l of phosphate buffer pH 7.4 at 90 °C. Based on the expected differences in degradation rate between the two polymers, two removal schedules were applied. Six PLLA discs were removed after 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 800, 1000 h, and six PLA96 discs were removed after 25, 50, 75, 100, 125, 150, 200, 250, 300, 350, 400, 450 h. All discs were dried to constant weight and weighed on a Mettler. The discs were subsequently subjected to chemical analysis.

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 THF relative to polystyrene standards. Thermal properties were evaluated by differential scanning calorimetry (DSC) on a Perkin Elmer DSC-7. PLLA and PLA96 samples of weight 5–10 mg were measured at a heating range of 10 °C $min^{-1}$ to determine the heat of fusion, the melting temperature and the glass transition temperature.

## 3. Results

The *in vitro* mass loss of the PLLA discs with time is represented in Table I and Fig. 1. In the first period of degradation, mass loss seemed to be almost linear with time. After this, the rate of mass loss decreased significantly and appeared to be asymptotic. The best curve fit of 0.982 for PLLA (Fig. 1) can be obtained when mass loss is described by the logarithmic equation $Y_t = (1 - e^{-t/\tau}) \times 100\%$ ($Y_t$ = percentage mass loss, $t$ = degradation time, $\tau$ = half life/ln 2). After 1000 hours of predegradation, total degradation was still not accomplished; macroscopically there were still some remnants of the PLLA discs but weighing was no longer possible. The decrease in the weight average molecular weight ($\bar{M}_w$) and the number average molecular weight ($\bar{M}_n$) of the PLLA samples with degradation time is presented in Fig. 2. The decrease in molecular weight started almost immediately and was very rapid in the first 100 h of degradation. With longer degradation periods the $\bar{M}_w$ remained constant at 1000. Initially there was an increase in the ratio $\bar{M}_w/\bar{M}_n$ but with longer degradation periods the polydispersity remained relatively constant with values between one and two. Fig. 3 shows the change in heat of fusion with degradation time. In contrast to the molecular weight, the heat of fusion of the PLLA samples showed a steep increase from 65 $Jg^{-1}$ to 83 $Jg^{-1}$ after 50 h of degradation and finally stabilized at around 96 $Jg^{-1}$. The melting temperature $T_m$ of the

TABLE I Mass loss as a function of degradation time, at 90 °C in phosphate buffer

| Time (h) | Mass loss (%) PLLA | PLA96 |
|---|---|---|
| 25 | – | 5.8 ± 0.9 |
| 37.5 | – | 22.5 ± 1 |
| 50 | 6 ± 0.7 | 30.2 ± 0.9 |
| 75 | 10.3 ± 0.5 | 35.2 ± 0.9 |
| 100 | 13.7 ± 1 | 39.6 ± 1.2 |
| 125 | 17.4 ± 1.3 | 44.9 ± 1 |
| 150 | 19.3 ± 1 | 48.9 ± 0.8 |
| 200 | 30.8 ± 2.4 | 62.2 ± 1.6 |
| 250 | 36.4 ± 0.9 | 71.1 ± 0.8 |
| 300 | 42.1 ± 1.4 | 78 ± 0.9 |
| 350 | 48.2 ± 2.8 | 82.9 ± 4 |
| 400 | 56.1 ± 2.1 | 88.5 ± 2.6 |
| 450 | 59.3 ± 1.9 | 92.4 ± 1 |
| 500 | 62.2 ± 4 | > 98 |
| 800 | 80.8 ± 2.8 | – |
| 1000 | > 90 | – |

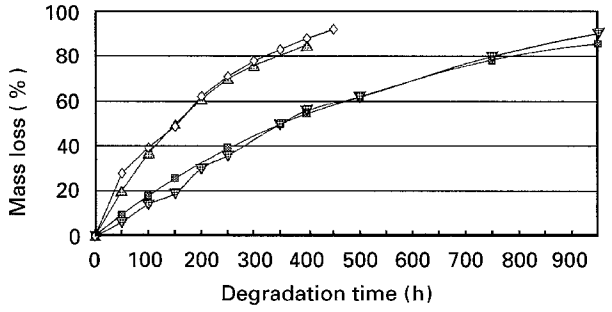


*Figure 1 In vitro* mass loss and calculated mass loss of PLLA (▽; ▦) and PLA96 (◇; △).

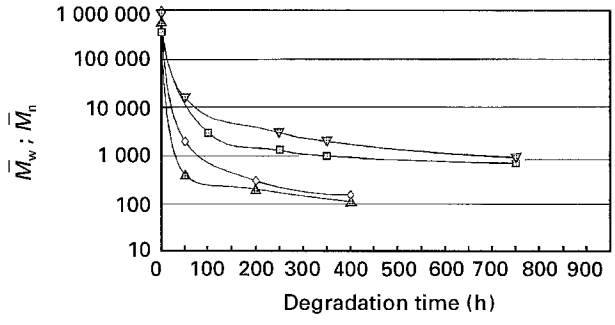


*Figure 2 In vitro* molecular weight loss of PLLA ($\bar{M}_w$ ▽; $\bar{M}_n$ ▦) and PLA96 ($\bar{M}_w$ ◇; $\bar{M}_n$ △).

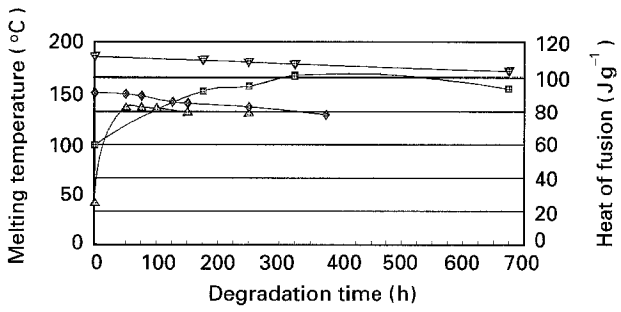


*Figure 3* Thermal properties of *in vitro* predegraded PLLA ($T_m$ ▽; d$H$ ▦) and PLA96 ($T_m$ ◈; d$H$ ◬).

PLLA samples decreased with degradation time and is also presented in Fig. 3. $T_m$ decreased from 187 °C to 170 °C after 800 h of degradation.

The mass loss of the discs of as-polymerized copolymer PLA96 is presented in Table I and in Fig. 1. In the period between 25 and 50 h the PLA96 discs showed a rapid mass loss, and after 150 h 50% of the initial mass remained. With longer degradation periods the rate of mass loss decreased and tended to be asymptotic. For PLA96 a curve fit of 0.97 (Fig. 1) for *in vitro* mass loss at 90 °C can be obtained when using the equation $Y_t = (1 - e^{-t/\tau}) \times 100\%$ ($Y_t$ = percentage mass loss, $t$ = degradation time, $\tau$ = half life/ln 2). Initial mass loss of the copolymer PLA96 was a factor three higher than PLLA over the same period; with longer implantation periods this factor decreased to two. In contrast with the homopolymer PLLA, total *in vitro* degradation was seen after 500 h. For PLA96 the molecular weight showed a steep decline in the first 70 h of degradation; with longer degradation periods the molecular weight levelled off and remained constant (Fig. 2). The polydispersity showed an initial increase but after 100 h of degradation remained stable around one. The melting temperature and glass transition temperature are shown in Fig. 3 and showed a gradual regression. The heat of fusion showed a sharp increase in the first 70 h of degradation and levelled off at around 83 $Jg^{-1}$.

## 4. Discussion

In an *in vivo* study with as-polymerized PLLA, Bos *et al.* [19] observed in the period between 20 and 80 weeks a linear mass loss, with a maximum mass loss of 14% at 80 weeks. Pistner *et al.* [20] also described an almost linear mass loss with as-polymerized PLLA implants *in vivo*, with, after 72 weeks of implantation, a mass loss of 16.4%. These findings coincide with the initially rapid linear degradation pattern of both the PLLA and PLA96 in this *in vitro* study. This relatively rapid initial mass loss could be explained by the presence of polymer impurities as suggested by Zhang *et al.* [21]. They suggest that the polymer purity is the most critical factor affecting the initial hydrolytic degradation. Polymers with a relatively low purity, such as the as-polymerized polymers used in this study that contained residual monomer and residual catalyst, showed an initially rapid degradation and then slowed down. Besides purity, another possibility is that the initial degradation predominantly occurs in the amorphous zones, from where degradation products can diffuse through channels of the microporous structure. The amorphous phase of the polymers is believed to degrade and dissolve much more rapidly that the crystalline phase. This would also explain the enhanced degradation of the copolymer PLA96.

Total *in vitro* mass loss of the predegraded as-polymerized polymers in this study was best described by a logarithmic function. The mass loss of both PLLA and PLA96 at 90 °C was best described by the equation $Y_t = (1 - e^{-t/\tau}) \times 100\%$ ($Y_t$ = percentage mass loss, $t$ = degradation time, $\tau$ = half life/ln 2). This equation can perhaps also be used to calculate the degradation at 37 °C. However, it is not certain whether calculations made for the degradation at 90 °C can be used to predict degradation rates at temperatures below the glass transition temperature since the degradation characteristics may be different in the glass or rubber state. To avoid this problem, data obtained from measurements below the glass transition temperature should be used. Bos *et al.* [20] measured *in vivo* mass loss of PLLA at up to 80 weeks of implantation. Based on this relatively short-term measurement, using the same logarithmic equation as for the *in vitro* degradation, a prediction of *in vivo* degradation can be calculated (Fig. 4). These calculations for *in vivo* mass loss indicate that total degradation will not be accomplished within 950 weeks. It is rather difficult to verify these calculations and assess the validity, but in a trephined screw hole of a patient that had been implanted for 5.6 years (290 weeks) and even 8 years (420 weeks), the screws were still clearly visible in their original shape indicating that *in vivo* mass loss was restricted. The same assessment can be made for PLA96: Cordewener *et al.* [25] followed *in vivo* mass loss of the copolymer PLA96 up to 55 weeks with a mass loss of 28%. Again the entire mass loss process with implantation time can be calculated (Fig. 4). The calculated total degradation time of PLA96 is at least 400 weeks. From these calculations it can be concluded that the incorporation of 4% D-lactide enhances the degradation rate by a factor of two. It can be concluded that although these calculations may not be very accurate, they give a good indication of the degradation process when implanted in patients.

Molecular weight loss of the PLLA discs started almost immediately and showed a rapid decrease

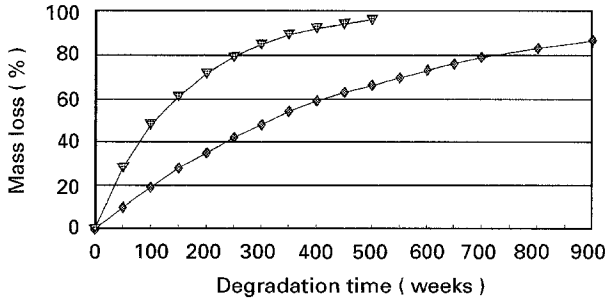


*Figure 4* Calculated physiological mass loss of PLLA (◈) and PLA96 (▽).

within the first 50 h of degradation. After this period the molecular weight continued to decrease at a much lower rate and remained stable at 1000 after 750 h of degradation. In none of the discs at any degradation time could a bimodal degradation of the surface and the centre be observed, as suggested by Li *et al.* [22]. The GPC analysis showed a homologue molecular weight distribution for both the homopolymer PLLA and the copolymer PLA96. Li *et al.* [23] suggested that with amorphous polylactate, but also with a crystalline PLLA [22], there was a bimodal degradation, with more enhanced degradation in the centre compared to the surface of the specimen, which leads to a hollow centred implant. In none of the discs used in the present study did cross-sections reveal a difference between centre and surface. Perhaps the different polymerization techniques (compression moulding versus as-polymerized) could account for the observed difference between the results of our study and the study of Li *et al.* [23].

The decrease in the molecular weight of *in vivo* degraded material showed a very similar pattern, a fast decrease within the first 20 weeks of degradation and a much lower rate afterwards [20]. After 3 years of physiological degradation $\bar{M}_n$ dropped to 5000; after 5.6 years $\bar{M}_n$ (as determined with NMR) was still about 5000 [10, 12]. Rozema *et al.* [10] suggested that a molecular weight for PLLA of about 5000 could be a break-even point for high disintegration. However, in this *in vitro* predegradation study molecular weights of 5000 or even lower do not seem to be related with massive degradation. On the contrary, with longer implantation periods and low molecular weights the rate of mass loss decreased and became asymptotic. This decrease in the rate of mass loss with longer implantation periods is probably related to changes in the thermal properties during degradation.

The melting temperature and the glass transition temperature of the *in vitro* degraded PLLA discs showed a linear regression with time. Rozema *et al.* [10] describe similar observations in a patient study, although after 3 years of implantation the melting temperature had only decreased from 188 °C to 184 °C. The slow decrease in melting temperature indicates that crystalline domains of the degraded PLLA become smaller or less perfect. However, the fast increase in the heat of fusion is a result of both degradation in the amorphous regions and recrystallization with longer implantation times. Physiologically degraded PLLA has a similar degradation pattern to the *in vitro* degraded PLLA; a fast increase of the heat of fusion up to 96 $Jg^{-1}$ [10]. The rather high crystallinity is probably one of the factors that makes PLLA very stable and not very susceptible to hydrolysis. This could imply that, although the molecular weight is very low, due to the high crystallinity total degradation, resorption, and mass loss will be very slow. The initial linear mass loss could be explained by the loss of the amorphous phase, but due to crystallization and the remaining crystalline phase, at some point the resorption rate and the rate of mass loss decrease. Li *et al.* [22] describe similar observations with crystalline PLLA. They suggest that crystalline microdomains formed during degradation are very resistant to further degradation. Both in this *in vitro* study and *in vivo* studies at up to eight years, total degradation and resorption of as-polymerized PLLA was not observed. Perhaps the highly crystalline fragments of low molecular weight are completely stable and are no longer susceptible to hydrolysis. As suggested by Fisher *et al.* [24], copolymerization of L-lactide with 4% D-lactide units will cause a much lower initial crystallinity, 26 $Jg^{-1}$ for PLA96 versus 65 $Jg^{-1}$ for PLLA. Based on the lower crystallinity during degradation of PLA96 (83 $Jg^{-1}$ versus 98 $Jg^{-1}$ for PLLA) and a lower melting temperature, PLA96 forms smaller and less perfect crystalline domains during degradation which results in an enhanced degradation rate expressed as a two times higher rate of mass loss when compared to PLLA. It remains the question whether PLA96 will fully degrade and will be completely resorbed *in vivo*, but total resorption *in vitro* is possible.

The results of this study show that *in vitro* predegradation is a suitable method for the study of the degradation behaviour of a polymer in a short-term experiment. These *in vitro* results can be used to predict, to some extent, *in vivo* degradation, and it can be a good method to compare and evaluate the degradation of different polymers. In future studies *in vitro* degraded polylactide will be used in an *in vivo* implantation study to gain more insight and predict the long-term degradation behaviour and tissue response when used as subcutaneous bone plates or as intraosseous screws.

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