



Fundamental insights in PLGA degradation from thin film studies

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ABSTRACT

Poly(lactide-co-glycolide)s are commercially available degradable implant materials, which are typically selected based on specifications given by the manufacturer, one of which is their molecular weight. Here, we address the question whether variations in the chain length and their distribution affect the degradation behavior of Poly[(*rac*-lactide)-co-glycolide]s (PDLLGA). The hydrolysis was studied in ultrathin films at the air-water interface in order to rule out any morphological effects. We found that both for purely hydrolytic degradation as well as under enzymatic catalysis, the molecular weight has very little effect on the overall degradation kinetics of PDLGAs. The quantitative analysis suggested a random scission mechanism. The monolayer experiments showed that an acidic micro-pH does not accelerate the degradation of PDLGAs, in contrast to alkaline conditions. The degradation experiments were combined with interfacial rheology measurements, which showed a drastic decrease of the viscosity at little mass loss. The extrapolated molecular weight behaved similar to the viscosity, dropping to a value near to the solubility limit of PDLGA oligomers before mass loss set in. This observation suggests a solubility controlled degradation of PDLGA. Conclusively, the molecular weight affects the degradation of PDLGA devices mostly in indirect ways, e.g. by determining their morphology and porosity during fabrication. Our study demonstrates the relevance of the presented Langmuir degradation method for the design of controlled release systems.

1. Introduction

Poly(lactide-co-glycolide)s (PDLGAs) are important degradable implant materials and established in clinical applications such as controlled drug release systems and surgical devices [1] [2]. To achieve the best treatment for a given medical condition, it is necessary to adjust and reliably predict its degradation rate. While a precise knowledge of this structure-function relationship is essential, it can only be of help if the structure of PDLGAs is thoroughly characterized. The material specifications given in datasheets provided by different manufacturers may seem similar, while the materials themselves can be drastically different [3]. Typically, PDLGAs are described by four parameters: The molecular weight, the lactide to glycolide ratio, the stereochemistry of the lactide units (both *D*-lactide and *L*-lactide are available) and the chain termination. Many authors also analyze the sequence structure of the polymers, which is determined by the number of lactide-lactide, glycolide-glycolide and glycolide-lactide bonds. For example, in a PDLGA with a molar ratio of 1:1, the sequence can be anything between a strictly alternating copolymer with only glycolide-lactide bonds, and a poly(*rac*-lactide)-*block*-polyglycolide diblock copolymer.

In contrast, PDLGA manufacturers provide no sequence information, and molecular weights are determined from a viscosity measurement, which provides no information about the molecular weight distribution at all. One batch could be highly monodisperse, while the next one, with the same molecular weight as determined by viscosity, could be a bimodal mixture of extremely short and extremely long chains. The question we addressed here, is whether the practice of measuring molecular weight by viscosity can result in variation of the degradation behavior of these materials. The answer to that question can be found in the role of chain length for the degradation kinetics of PDLGAs.

It is often assumed that the termination of the chain-ends has an influence on the degradation rate, with carboxyl groups promoting an autocatalytic effect [4]. If this is true, it follows suit that the degradation time of PDLGAs increases with molecular weight, as has been reported [5]. Two mechanisms for the autocatalytic degradation have been proposed. Each bond cleavage creates a carboxyl group. When the reaction products are not removed from the material, the concentration of acids increases, leading to a decrease of the micro-pH [6,7]. When protons catalyze the hydrolysis of ester bonds, the reaction becomes auto-catalytic. A different hypothesis was based on the analysis of

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polylactide degradation in solution, where the chain-ends catalyzed the hydrolysis of an ester bond in an intramolecular attack, a so called back-biting reaction [8]. In any scenario where chain ends have an autocatalytic effect, the initial molecular weight distribution can influence the degradation rate of PDLLGA, with more polydisperse polymers having a higher number of end-groups than less polydisperse ones [5].

On the other hand, it is known that the molecular weight of e.g. poly[(*rac*-lactide)-*co*-glycolide] with a 1:1 M ratio of lactide and glycolide decreases exponentially during degradation [4]. An exponential decrease of the molecular weight indicates a statistical cleavage of ester bonds along the backbone (random scission) [9]. In such a mechanism, the starting molecular weight of the chains is actually not very important for the overall degradation behavior. The chain-ends affect the overall degradation in two ways: By reducing the number of reactive ester bonds, and by enabling the generation of small, water soluble fragments via single chain cuts, as opposed to several consecutive cuts. Due to the large number of repeat units in a polymer chain, the dilution of reactive intra-chain bonds by chain-ends prior to degradation is rather small. A PLGA chain with a relatively low M_n of 2 kg/mol consists of about 30 repeat units and therefore, only about 7% of all units are forming chain-ends, i.e. 93% of all units are reactive. In a random scission mechanism, the number of end-groups increases steadily, meaning that the hydrolysis rate decreases during degradation. The fact that small fragments can be created by single chain cuts at the chain-ends is relevant only in the very beginning of the degradation reaction, and quickly loses importance due to the exponentially decreasing molecular weight. There are surprisingly few studies in the literature on the impact of molecular weight on the degradation behavior of PDLLGA, especially from objects with controlled morphology like films. One study on PDLLGA films with 75% *rac*-lactide content found that the molecular weight decreased slightly faster for the polymers with higher weight average molecular weight. This finding may be attributed to an oversimplified mathematical treatment of the dependence of the molecular weight on the reaction rate. A better treatment is given in ref. [10], where the reduction of the molecular weight of PDLLGAs with 50% lactide content, originally published in [11], was evaluated. The degradation rates of three PDLLGAs with weight average molecular weight between 8.000 g/mol and 47.000 g/mol were within the experimental error margin from each other. The mass loss rates in ref. [11] appear very similar as well. The higher molecular weight polymers showed a greater lag time before mass loss set in, which was attributed to slower diffusion of water into the macroscopic samples.

The most straightforward way to decide, if the molecular weight affects the degradation kinetics of PDLLGAs, is to compare degradation behavior of a high- and a low molecular weight batch. We selected PDLLGAs with a lactide: glycolide ratio of 1:1, which are known to exhibit the fastest degradation kinetics. The primary pathway for degradation of PDLLGAs *in vivo* is hydrolytic cleavage of ester bonds. It is assumed that glycolide units are more hydrophilic and the hydrolysis of ester bonds between two glycolide units is the fastest. Most PDLLGAs are synthesized from diglycolide. Diglycolide units contain an ester bond between glycolic acid units, making the diglycolide units the weak links of the chain. This explains why sequence controlled PDLLGAs containing no bonds between glycolic acid units undergo slower hydrolysis [12]. Increasing the glycolide content above 75 mol% results in glycolide crystallization, which hinders hydrolytic degradation [5]. For the same reason, a high content of either *D*-lactide or *L*-lactide reduces the degradation rate. The average block length at 75% glycolide content is three glycolide units, and small fluctuations can easily result in block lengths of 5 blocks. This number would be sufficient to form the polyglycolide crystals with a lamellar thickness of about 4 nm that are described in PDLLGA [13]. To avoid any chemi-crystallization effects, which could occur when the material is depleted from glycolide, a PDLLGA with a racemic mixture of *D*- and *L*-lactide units was chosen, referred to as PDLLGA.

The role of enzymes in catalyzing the degradation of PDLLGAs *in vivo* is not clear, predominantly because there is no straightforward way to discern between enzymatic and non-enzymatic degradation *in vivo*. A number of enzymes, amongst them mammalian lipase, is known to degrade PDLLGA [14]. A good indication for participation of enzymes *in vivo* would be a faster mass loss compared to *in vitro* conditions, while the molecular weight loss between the two conditions remains similar. Enzymes are confined to the surface and do not affect the molecular weight of the whole implant and only accelerate mass loss. Such a behavior has been observed for PLLA [15]. Since it is possible that the degradation mechanism under enzymatic catalysis differs from purely hydrolytic degradation, our experiments also included PDLLGA degradation under enzymatic catalysis. Lipases both from fungi and mammals were investigated. Here, the stereochemistry of the lactide-units is highly important. Many enzymes can degrade poly(*L*-lactide), while only few are active against the *D*-form [16]. It seems that this translates to PDLLGA. Many lipases and esterases could not catalyze the degradation of the poly[(*rac*-lactide)-*co*-glycolide] used in our experiments, probably because of the presence of *D*-lactide.

Besides the molecular mechanism, the micro- and nanostructure of PDLLGA devices have a pronounced impact on the degradation kinetics of PDLLGA devices, with micro- and nano-pores facilitating fast transport of water and polymer fragments [3]. The molecular weight can have an impact on the morphology of the device [17], for example by determining the viscosity of the polymer solution droplets in emulsions or during electrospraying.

Analyzing the direct impact of molecular weight on the degradation mechanism of PDLLGAs necessitates to study the hydrolytic degradation of PDLLGA independently of device morphology, which is only possible in solution. While PDLLGAs are not water soluble, the air-water interface is actually a good solvent for the polymer [18]. The Langmuir monolayer degradation technique, as a fast and established tool to study the degradation of polymers [19], is therefore ideally suited to answer the question of the impact of molecular weight on the degradation kinetics of PDLLGAs. Here, we hypothesize that by measuring the degradation rate of Langmuir films formed by PDLLGAs with different molecular weights, we can elucidate the molecular degradation mechanism and thereby the impact of molecular weight on the degradation kinetics.

To study the molecular degradation mechanism of PDLLGAs with the Langmuir technique, a PDLLGA in chloroform solution is applied to the surface of a Langmuir trough in a drop-wise fashion. After evaporation of the solvent, the layer is compressed to the degradation surface pressure π_D , which is commonly chosen in a way that the compressibility modulus $\frac{1}{\kappa} = -A_{\text{trough}} * \frac{d\pi}{dA_{\text{trough}}}$ has a maximum. Choosing π_D in such a way ensures that fluctuations of the surface pressure caused by external perturbations have a minimal impact on the surface area. The degradation of the macromolecules is carried out under isobaric conditions, based on the hypothesis that this results in a constant areal concentration of repeat units. The dissolution of short, water soluble fragments is counterbalanced by compression of the film, and the result of the experiment is a surface area vs. time curve. Here, both enzymatically catalyzed degradation and degradation under standard conditions were examined, based on the working hypothesis that enzymatic catalysis results in a random bond scission while degradation in absence of enzymes results in bond scission at the chain-ends [8,20]. In addition, the impact of pH on the degradation of PDLLGAs was tested, to verify the hypothesized autocatalytic effect of acidic fragments on the ester hydrolysis.

The reduction of the film area in a Langmuir monolayer degradation experiment is the equivalent to mass loss in a bulk degradation experiment. A deeper insight into the degradation mechanism requires a method to simultaneously assess the evolution of the molecular weight: In a chain-end cut mechanism, the molecular weight is expected to decrease in parallel to the film area. In contrast, a random scission

mechanism causes a rapid decrease of the molecular weight before a substantial number of water soluble fragments are formed. Here, we use interfacial rheology as an in situ method to measure the interfacial shear viscosity, which is related to the molecular weight of the degrading macromolecules [21]. In the true monolayer state, where the chain segments were strongly attached to the air-water interface, our interfacial rheology setup could not distinguish between the viscosity of bare water and PDLLGA. One has to keep in mind that entanglements are impossible in two dimensions, and that the interaction between the chain segments is weak or even repulsive under good solvent conditions. However, PDLLGA Langmuir films can be compressed beyond the monolayer state. Then, a certain fraction of the monomers have to leave the interface, and the layer becomes three dimensional (layer thickness on the order of few nm). In such highly compressed layers, entanglements are possible, leading to an increase of the viscosity by several orders of magnitude. Shear rheology was therefore carried out on these highly condensed films. Although such a film does not represent a true monolayer, the results were very conclusive with respect to the molecular degradation mechanism.

2. Experimental

2.1. Materials

Two PDLLGAs were purchased from Sigma-Aldrich (Darmstadt, Germany). A higher molecular weight one (Resomer 504H) and a lower molecular weight one (Resomer 502H). The polymers were synthesized in a way that one chain-end is terminated by an acid group.

The lipases from *Rhizopus oryzae* and from porcine pancreas were purchased from Sigma-Aldrich (Darmstadt, Germany) and used as received. According to the specification, the activity of the protein from porcine pancreas using olive oil was 20.000 U/mg. The activity of the protein from *Rhizopus oryzae* was 10 U/mg using tributyrin.

3. Methods

3.1. Degradation experiments

Degradation experiments were carried out in a „high compression“ Langmuir trough (Biolin Scientific, Espoo, Finland) with a surface area of $A = 550 \text{ cm}^2$ and a “medium area” Langmuir trough (Kibron, Helsinki, Finland) with a surface area of $A = 280 \text{ cm}^2$.

For rheology experiments, the high compression Langmuir trough was used. The area was 530 cm^2 due the bicone with $r = 25.5 \text{ mm}$. The surface pressure was measured with a Wilhelmy plate that was calibrated against air and pure water. The Wilhelmy plate of the high compression trough was made from platinum whilst the Wilhelmy plate of the medium area Langmuir trough was made from an iron-based metal alloy. The polymers were spread from a chloroform solution with $c = 0.5 \text{ mg/mL}$ and the layer was compressed to the degradation surface pressure π_D with a compression rate of 10 mm/min .

Enzymatic and hydrolytic degradation experiments on a phosphate buffered saline (PBS) subphase without rheology were carried out at a constant surface pressure of $\pi_D = 5 \text{ mN/m}$ representing the first maximum of the compressibility modulus. In order to induce enzymatic degradation, a solution of lipase from *Rhizopus oryzae* or lipase from porcine pancreas were injected under the layer. The solution was prepared freshly for each experiment. The lipase from *Rhizopus Oryzae* was dissolved with a protein concentration of $c = 1\text{--}2 \text{ mg/mL}$, corresponding to an activity of $a = 10\text{--}20 \text{ U/mL}$. The activity of the enzyme solution was found to decrease quickly upon storage or freezing. The injected volume was adjusted to achieve a final enzyme activity of $a = 40 \text{ U/mL}$ in the subphase of the Langmuir trough. For the degradation experiment with lipase from porcine pancreas, the protein was dissolved with a protein concentration of 1 mg/mL , corresponding to 20.000 U/mL . The injected volume was adjusted to achieve a final

enzyme activity of $a = 25 \text{ U/mL}$ in the subphase of the Langmuir trough.

For hydrolytic degradation on PBS subphase, the layer was compressed to a surface pressure of 5 mN/m and the surface pressure was held constant. For hydrolytic degradation under acidic conditions, the layer was compressed and held at a surface pressure of 5 mN/m on a Milli-Q water subphase ($\text{pH} = 6$). After 1400 min , the pH of the subphase was adjusted to 2.2 by injection of 1 mL of ultrapure 37 wt\% HCl (Merck, Darmstadt, Germany) into the subphase with $V = 450 \text{ mL}$.

For hydrolytic degradation under alkaline conditions ($\text{pH} = 10.2$ and $\text{pH} = 11$) at 5 mN/m , the pH of the subphase was adjusted beforehand to $\text{pH} = 10.2$ or $\text{pH} = 11$ by addition of KOH ($c = 25 \text{ mg/mL}$; Merck, Darmstadt, Germany; analysis grade). The medium size trough was used for these experiments. The trough was enclosed in a box and all holes and slits sealed with tape. The box was purged with a constant stream of argon to avoid the dissolution of carbon dioxide which decreases the pH . The pH of the subphase was monitored with a pH electrode and remained within 0.2 of the starting value.

The degradation experiment at $\text{pH} = 11$ with simultaneous interfacial rheology was carried out on the high compression trough. This setup could not be enclosed to seal out carbon dioxide. Within 3 h , the pH decreased from 10 to 8 (see Fig. S1 in Suppl. Mat.). All degradation experiments except the two high pH experiments in the medium area trough were carried out using a water levelling tool from Biolin Scientific, which is able to keep the water level constant for at least two weeks.

3.2. Surface activity

The surface activity of the enzymes was determined in a “medium” size Langmuir trough ($A = 243 \text{ cm}^2$) from Biolin scientific using a platinum Wilhelmy plate. The surface tension of the air-water interface was monitored while increasing the enzyme concentration in a step wise fashion by injection of an enzyme solution. For the lipase from *Rhizopus Oryzae*, the decrease in surface tension was below 0.2 mN/m in the concentration regime that was used for the degradation experiments. The lipase from porcine pancreas was much more surface active. Up to a protein concentration of $1.25 \text{ }\mu\text{g/mL}$, the decrease in surface tension was below 0.4 mN/m . A further increase in concentration resulted in a drastic lowering in surface tension. A higher concentration could not be used for degradation experiments.

3.3. Rheology experiments

Rheology was carried out with a bicone-geometry on an MCR 502 Rheometer (Anton-Paar, Graz, Austria). The bicone had a radius of $r = 25.5 \text{ mm}$. The angle of the tip of the bicone was 166.8° . Measurements were carried out at controlled strain of 1% (assuming an edge-wall distance of 3.5 mm) and an oscillation frequency of $\omega = 2 \text{ rad/s}$. In degradation experiments, we did not use a ring around the bicone, because it was not possible to achieve an equal enzyme distribution between the subphase outside and inside the ring. The diameter of the slit was set to $100 \text{ }\mu\text{m}$ when calculating the interfacial moduli using the algorithm from the rheocompass software (Anton Paar).

The enzymatic degradation experiment was carried out on a high compression trough with a PBS subphase. The spread volume was 2.5 times higher than for experiments without rheology because the degradation surface pressure was higher, requiring a lower mean molecular area. The viscosity of the layer could be distinguished from the viscosity of water when the final, steep increase of the surface pressure was reached, i.e. when $\pi \geq 11 \text{ mN/m}$ at $\text{pH} 7.4$. Enzymatic degradation was carried out at $\pi_D = 11 \text{ mN/m}$. When reaching that surface pressure and holding it constant, the surface area decreased, while the elastic moduli increased continuously (see Suppl. Mat. Fig. S4). Presumably, the reason for the continuous area reduction is a progressive thickening

of the layer [22]. The enzymes were injected after 60 min, so that the final enzymatic activity was $a = 40$ mU/mL. To study hydrolytic degradation in combination with rheology, the film was prepared at 11 mN/m as described above and withheld for 60 min. The compression was paused, and a total of 2.5 mL of aqueous KOH (1 mol/L) was injected under the layer to achieve a pH of 11. This high pH was necessary to achieve a situation where the area reduction due to degradation was much faster than the area reduction due to layer thickening. After 10 min, the surface pressure started to increase. The target surface pressure was set to 14 mN/m and compression was reactivated, resulting in a film compression of about 10%. The moduli increased for another 10 min. The starting point of the degradation curve was therefore set to 80 min. An algorithm implemented in the Rheocompass software (Anton-Paar, Graz, Austria) was used to convert the measured bulk properties into interfacial values.

3.4. Data evaluation

Degradation data were processed with Origin 2018. Curve fitting was carried out with a Levenberg Marquardt with a maximum of 200 iteration steps and a fit function tolerance of 10^{-14} . NMR data were analyzed with MestreNova.

3.5. Characterization of the copolymers

For Resomer 502, ^1H NMR analysis (Ascend 700 MHz, Bruker, Karlsruhe, Germany) yielded a molar lactide: glycolide ratio between 1: 1 and 1: 0.98, depending on which proton signals were analyzed. For Resomer 504, the lactide: glycolide ratio was between 1: 1 and 1: 1.03 (See Suppl. Mat. Fig. S2). In other words, no polymer contained less than 49 or > 51 mol% lactide or glycolide. For both Resomer polymers, the glycolide protons showed a relatively sharp peak at $\delta = 4.73$ ppm with little splitting. This indicates the presence of glycolide blocks [23]. For a strictly alternating copolymer, one would expect a completely split signal with zero intensity at $\delta = 4.73$ ppm [24].

The sequence structure was further analyzed by ^{13}C NMR to determine the average glycolide and lactide block lengths. (See Suppl. Mat. S3). For Resomer 502H, both block lengths were almost 2 (related to single lactide or glycolide). A value of 2 is expected for a random distribution of monomers. The probability that the two monomers next to one glycolide unit are also glycolide units is 0.5 when there is no aggregation of glycolide units. Then, the average block length is 2. For Resomer 504H, the block lengths were slightly higher (up to 2.7), but still close to the value expected when there is no clustering of comonomers. The main error in the analysis arises from the overlapping of the individual signals, which necessitated a peak fitting. When integrating the signals by visual estimation of the bandwidth, one obtains block lengths up to 3, but never below 2. The former values should be considered the maximum and minimum block lengths that could be deduced from our NMR measurements. The molecular weight was determined by multi-detector GPC using a universal calibration with polystyrene standards. The detectors were a dual detector combining a viscometer and right angle light angle scattering as well as a refractive index detector. The high-molecular weight PDLGA had an M_n of 23 ± 2.3 kg/mol and a M_w of 100 ± 10 kg/mol, while the lower molecular weight PDLGA had a molecular weight of 1.7 ± 0.17 kg/mol and a M_w of 19 ± 1.9 kg/mol. The error of 10% represents the maximum error between the calibration curve and an individual standard. The abnormally high polydispersity of the lower molecular weight PDLGA was caused by a bimodal molecular weight distribution. Presumably, an extremely low molecular weight fraction was mixed with a higher one to achieve a viscosity that was within specifications.

3.6. Error considerations

The random errors of a Langmuir monolayer degradation experiment with accurate water level compensation are extremely small when compared to other methods to study the degradation of macromolecules. The outcome of the measurement is surface area, and the error is determined by fluctuations in surface pressure and the precision of area measurement: $\Delta A_{tot} = \left[\left[\frac{d\pi}{dA} \right]_{\pi_{degr}}^{-1} \right] * \Delta\pi + \Delta A_{trough}$. In the following, the calculation of the error is exemplified for the high compression trough: The surface pressure term is obtained from the slope of the compression isotherm at the degradation surface pressure. At 5 mN/m, we find that: $\left[\frac{d\pi}{dA} \right]_{\pi_{degr}} = \frac{0.033 \text{ mN/m}}{\text{cm}^2}$. The fluctuations of the surface pressure during the degradation experiments was $\Delta\pi = 0.005$ mN/m. The precision of the area recording of our high compression trough was $\Delta A_{trough} = 0.01$ cm².

Altogether, for degradation at $\pi_{degr} = \frac{5 \text{ mN}}{\text{m}}$, we find that: $\Delta A_{tot} \sim 0.16$ cm². Typically, during degradation experiments, the area reduced from about 250 cm² to 50 cm², meaning the relative error was between 0.06% and 0.3%. We also note that for degradation experiments at high surface pressure, where the isotherm is very steep, $\left[\frac{d\pi}{dA} \right]_{\pi_{degr}}^{-1}$ and the associated error are both smaller. Noticeable differences between experiments with identical conditions hint at systematic errors like incorrect adjustment of pH or batch variations of enzyme formulations.

4. Results

4.1. Monolayer studies

The compression isotherms of the low molecular weight PDLGA (1.7 k) and the high molecular weight PDLGA (23k) were nearly identical (Fig. 1). This weak dependence is often observed for polyesters [25,26]. The observation that the shortening of chains does not affect the surface pressure at a given areal concentration of repeat units (RU) inspires the approach to conduct degradation experiments at constant surface pressure and assume a constant areal concentration of RUs during the reaction. There is a slight difference at surface pressures exceeding 11 mN/m, which is the regime where the chains leave the

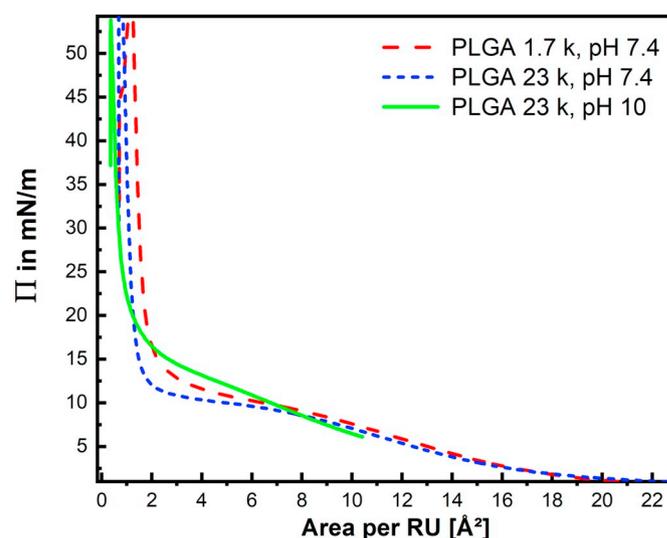


Fig. 1. Compression isotherms of the two PDLGAs on PBS buffer and of the higher molecular weight PDLGA on a subphase with pH = 10. The area per repeat unit was calculated by averaging the molecular weight of glycolic acid and lactic acid repeat units. Compression rate was 1 cm/min. The duration of one experiment was ca. 35 min. (For interpretation of the colour in the figure legend, the reader is referred to the web version of this article.)

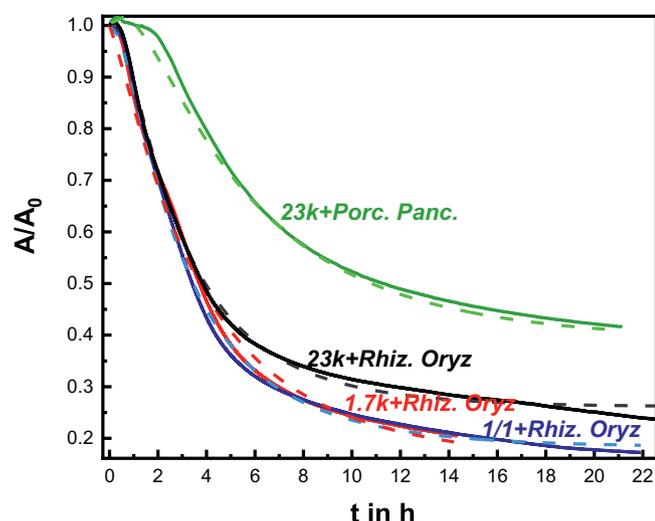


Fig. 2. Enzymatically catalyzed degradation of different molecular weight PDLLGAs on a subphase containing PBS buffer. The 23 k, the 1.7 k and a 1/1 mixture of both PDLLGAs were degraded in a subphase containing lipase from *Rhizopus oryzae* at $a = 40$ mU/mL. The 23 k PDLLGA was also degraded on a subphase containing 25 U of lipase from porcine pancreas. Each degradation curve was fitted with a combination of Eq. 6 and 7. The solid lines are the experimental curves, dashed lines the fits to the experimental curves in corresponding colors. See Suppl. Mat. Table S1 for fit parameters.

interface and form thicker layers. The impact of pH on the compression isotherms was tested because experiments with accelerated degradation at pH = 10.2 and pH = 11 were carried out. Increasing the pH to 10 increases the surface pressures in the densely compressed layers, and the conditions for accelerated degradation experiments were adjusted accordingly. While the pH of the subphase decreased during the experiment, the final pH was about 9.7.

For most (co)polyesters, enzymatic catalysis was found to lead to a random chain fragmentation [19]. Therefore, enzymatically catalyzed degradation of PDLLGA with lipase from *Rhizopus oryzae* was used as a positive control and to simulate a degradation via random bond scission. In addition to showing good activity against PDLLGAs [14], these enzymes fulfilled the requirement of having negligible surface activity at the required concentrations. In addition, lipase from porcine pancreas was used to simulate the enzymatic degradation of PDLLGA in mammals. As expected for a random scission mechanism, the degradation behavior was nearly independent of the molecular weight of the investigated PDLLGAs (Fig. 2). The impact of the molecular weight distribution was assessed by forming a 1/1 mixture of the 1.7 k and the 23 k PDLLGAs, to achieve a monolayer with a broad molecular weight distribution.

A quantitative description of the dissolution of a monolayer of macromolecules degrading via random fragmentation is given by Eq. 1 [27]. The film area A , starting from A_0 at $t = 0$, decreases due to dissolution of water soluble fragments.

$$\beta_x(\alpha) = \frac{\sum_{x=1}^{l_{min}} x \omega_x(\alpha)}{n_p} = 1 - \frac{A}{A_0} \quad (1)$$

with

$$\omega_x(\alpha) = \alpha(1 - \alpha)^{x-1} [2 + (n_p - 1 - x)\alpha] \quad (2)$$

here, l_{min} is the size of the largest water soluble fragment, n_p is the initial degree of polymerization and α is the fraction of broken ester bonds. The decrease of the number of intact ester bonds B can be calculated according to:

$$\frac{dB}{dt} = -k_{reac} * a_{enz} * B \quad (3)$$

In Eq. 3, k_{reac} is the reaction rate constant and a_{enz} the activity of enzymes in the subphase. Ideally, enzymes should be treated as catalysts that are not converted in the reaction, meaning that their activity is constant. However, the lipase from *Rhizopus oryzae* was rather fragile, losing most of its activity when stored at 4 °C in PBS for 24 h. The degradation curves published in ref. [14] suggest that the activity of lipases decreases according to first order kinetics when incubated with PDLLGA. This is incorporated into the model by Eq. 4:

$$\frac{dB}{dt} = -k_{reac} * a_{enz,0} * e^{-l * t} * B \quad (4)$$

here, l is the rate constant for the first order decrease of enzymatic activity. The solution of Eq. 4 is given by:

$$B(t) = B_0 * e^{\frac{k}{l} a_{enz,0} (e^{-l * t} - 1)} \quad (5)$$

$$\alpha = \frac{B(0) - B(t)}{B(0)} = 1 - e^{\frac{k}{l} C_{enz,0} (e^{-l * t} - 1)} \quad (6)$$

Fig. 2 shows the fitted degradation curves obtained by combining Eq. 1, 2 and 6, where l_{min} was set to 12, and the degree of polymerization n_p to the number of repeat units per chain according to M_n . We chose l_{min} in such a way because examinations of the PDLLGA degradation products [28] have shown that the water soluble fraction contains fragments consisting of 10 and more repeat units.

When a pair of chain-ends generated by bond scission has a higher foot-print on the air-water interface than an intrachain-bond, there is an expansive effect on the layer. This phenomenon can be added to model using Eq. 7 [29], where C' is the ratio between the footprint of a pair of end-groups and an intrachain-bond:

$$1 - \frac{A}{A_0} = \sum_{x=1}^{l_{min}} \frac{(x + C') \omega_x(\alpha)}{n_p} - C' \alpha \quad (7)$$

The shape of the degradation curves was similar for the lipases from *Rhizopus Oryzae* and porcine pancreas. The rate constant was about 2 times lower for the lipase from porcine pancreas, but the protein concentration in mg/mL was more than three times lower. This suggests that, per mg of protein, the lipase formulation from porcine pancreas had a higher activity towards PDLLGA. Fig. 3 presents a comparison between the experimentally observed impact of molecular weight (Fig. 3A) and the model (Fig. 3B) for the lipase from *Rhizopus Oryzae*. According to the models, the only impact of molecular weight on area reduction is a delay of the area reduction with increasing molecular weight. This is confirmed by the experiment. There is a short additional delay between experiment and simulation because the enzymes need a certain time to distribute in the Langmuir trough and reach the layer. For the lipase from porcine pancreas, the delay extends further. These enzymes have a certain surface activity and adsorb to the interface, meaning that area reduction is delayed until degradation is faster than enzyme adsorption. This was not the case for the lipase from *Rhizopus oryzae*. These proteins had very little surface activity at the applied concentration, and no trace amide groups could be detected with PM-IRRAS spectroscopy during degradation of the 23 k PDLLGA (see suppl. Mat. Fig. S5).

The catalytic activity of the lipase from porcine pancreas suggests that enzymes could play a role in accelerating the degradation of PDLLGAs in vivo. The contribution depends on the concentration of these enzymes in the vicinity of the device. Since enzymes are confined to the surface of the device, a reduction of the molecular weight of PDLLGA devices in vivo cannot be attributed to enzymatic catalysis. This process is determined by hydrolytic bulk degradation, starting at physiological pH. The pH can decrease over time due to accumulation of acidic fragments. The impact of starting molecular weight on this bulk degradation process in the absence of catalyst is shown in Fig. 4. At room temperature, such monolayer degradation experiments takes several days, which is only feasible when using a levelling tool. To

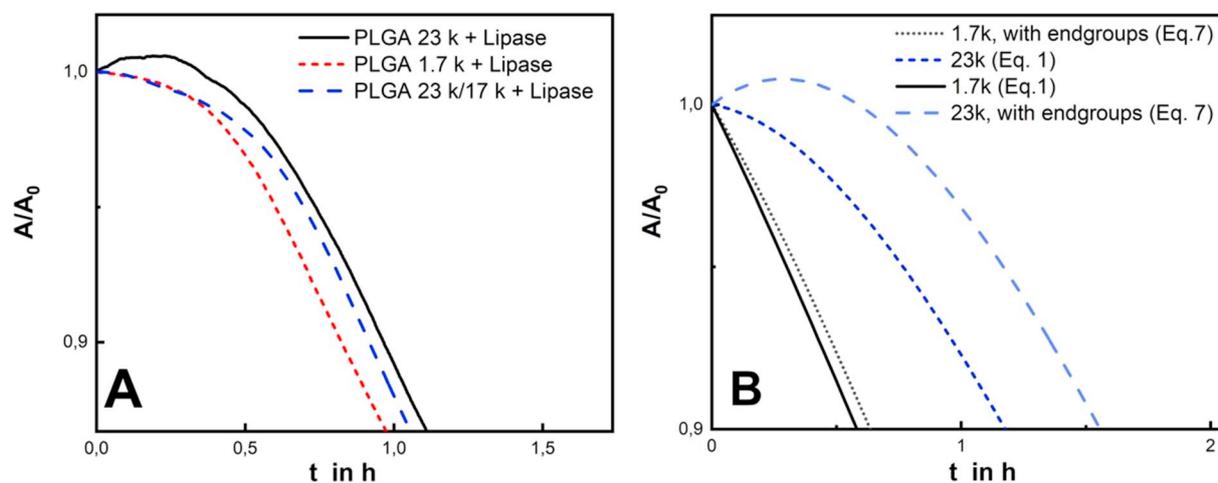


Fig. 3. A: Magnified representation of the early stage of the random scission process of PDLLGAs under enzymatic catalysis. B: Simulation of the impact of molecular weight in the early stage of degradation of PDLLGAs using Eq. 1, 2 and 6 with identical rate constants and the corresponding molecular weights ($n_p = 25$ for 1.7 k and $n_p = 350$ for 23 k). For the simulation with end-groups (Eq. 7), C' was set to 2.5. (For interpretation of the colour in the figure legend, the reader is referred to the web version of this article.)

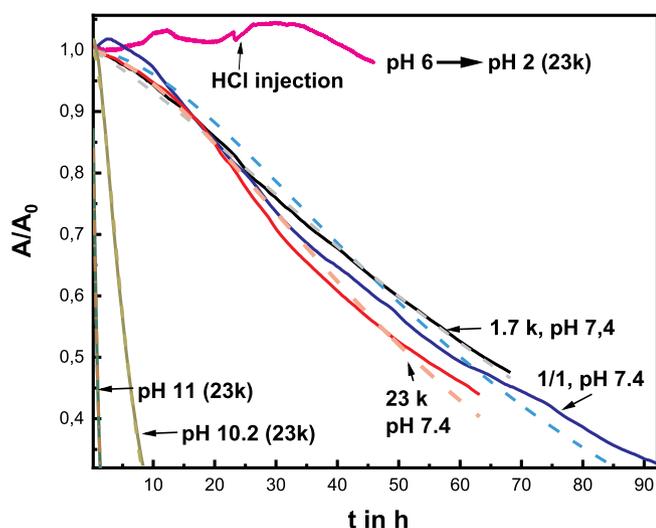


Fig. 4. Hydrolytic degradation of different PDLLGAs at pH = 7.4 and of the higher molecular weight PDLPGA at pH = 2, pH = 10.2 and pH = 11. The solid lines are the experimental curves, dashed lines the fits to the experimental curves in corresponding colors. The fits were calculated using Eqs. 2, 7 and 10 for the case of pH 7.4. For the case of pH > 10, the fit was calculated according to Eq. 3 from ref. [20]. See Suppl. Mat. Table S2 for fit parameters.

mimic the acid-catalyzed, autocatalytic degradation, PDLPGA 23 k was kept on a Milli-Q-water subphase for about one day to achieve a certain degree of degradation, and then, the pH was lowered to 2 by injection of hydrochloric acid. This had a negligible effect on the monolayer. Already, lowering the pH from 7.4 (PBS) to 6 (Milli-Q water) drastically retarded degradation. Lowering the pH to 2 might have increased the degradation rate compared to pH = 6, but the degradation was still much slower compared to pH = 7.4. This observation supports the hypothesis that for the degradation of PDLLGAs, hydroxyl ions are more effective catalysts than protons. The hypothesis was confirmed by degradation experiments at pH = 10.2 and 11. The polymer was spread on a subphase that was adjusted to the high pH beforehand. At pH = 11, the surface pressure started to decrease immediately after spreading. Under these alkaline conditions, degradation of PDLLGAs is so fast that the polymers were already degrading during compression to π_D .

Since the high- and low molecular weight polymer as well as their

mixture degrade at nearly identical rates, a chain-end degradation mechanism is highly unlikely. The analysis of the degradation of PDLPGA at pH = 7.4 is based on the same random scission model as enzymatic degradation. The solution of Eq. 4 in the case of purely hydrolytic degradation is simpler, because the concentration of the catalytically active hydroxyl ions is constant in PBS buffer. The degree of bond dissociation is therefore given by a pseudo first order reaction [30]

$$\frac{dB}{dt} = -k_{\text{reac}} * C(\text{OH}^-) * B = -k' * B \quad (8)$$

$$B(t) = B(0) * e^{-k'*t} \quad (9)$$

$$\alpha = \frac{B(0) - B(t)}{B(0)} = 1 - e^{-k'*t} \quad (10)$$

As in the case of enzymatic degradation, the fit curves based on the random scission model agree well with the experimental data at pH = 7.4. The experiments were carried out over 3 to 4 days, and the experimental degradation curves have a somewhat undulating shape. A Langmuir monolayer is still a relatively sensitive system, and changes in air humidity, air temperature and flow as well as sun exposure during day-night cycles account for some of the deviations between experiment and simulation.

At elevated pH, the degradation behavior does not match with a random scission mechanism. There is no initial lag-time followed by increasing dissolution velocity. Instead, the initially very high dissolution rate decreases continuously. Such a behavior suggests that single chain cuts are sufficient to produce small fragments. This is only the case if these chain cuts take place near the chain-end. The preferential chain-end degradation model [20], which was introduced to analyze the degradation kinetics of oligo[*rac*-lactide-*co*-glycolide] diols, is perfectly suited to describe the degradation of PDLPGA at pH > 10. While it is not yet clear how the evolution of the mechanical properties of a Langmuir monolayer during degradation can be extrapolated to the mechanical properties of a bulk material, previous studies have obtained quantitative results for the interdependence between interfacial viscosity and molecular weight [21]. With the aim of correlating the calculated molecular weight with the experimentally determined interfacial viscosity, in situ interfacial rheology was used to further elucidate the degradation mechanism of PDLLGAs. Our rheometer, equipped with a bicone for interfacial rheology, was not able to distinguish between the bare air-water interface and the PDLPGA layer at 5 mN/m. We had to compress the layer to at least 11 mN/m to register

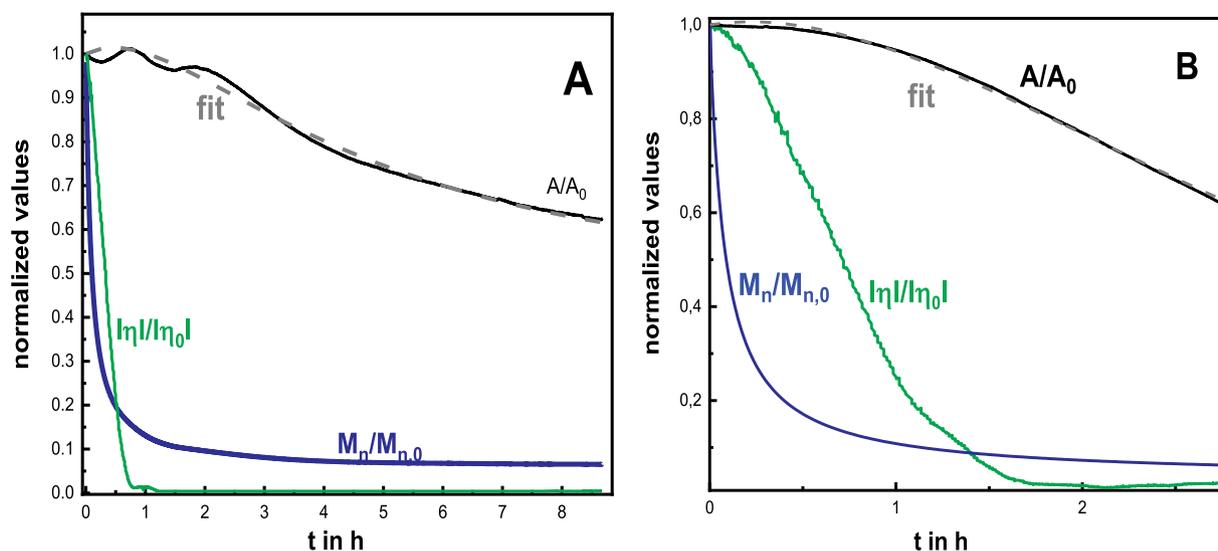


Fig. 5. A: Normalized complex viscosity (green) of the 23 k PDLGA degrading at a surface pressure of 14 mN/m and a pH of 11. The normalized area of the film (black) was fitted with Eq. 2, 6 and 7 (grey, dashed). See Suppl. Mat. Table S3 for fit parameters. The evolution of the normalized molecular weight was deduced from the fit curve according to Eq. 11 (blue). B: Normalized complex viscosity (green) of the 23 k PDLGA degrading at a surface pressure of 11 mN/m under enzymatic catalysis at pH 7.4. The normalized area of the film (black) was fitted with Eq. 2, 6 and 7 (grey, dashed). The evolution of the normalized molecular weight was deduced from the fit curve according to Eq. 11 (blue). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

dynamic interfacial moduli greater than the one of water. Previous investigations have shown that the chains fold up and the layer gradually thickens [31] when the surface pressure exceeds 11 mN/m. This leads to a continuous, albeit steadily slowing area reduction. In order to carry out a degradation experiment in combination with rheological measurements, it is necessary to achieve conditions where degradation is much faster than the area reduction due to thickening of the film. The pH had to be increased to 11 to achieve this situation. It needs to be emphasized that at this pH, degradation was nearly instantaneous when the PDLGAs were in the semi-dilute state. When the pH of the subphase is increased, the surface pressure of PDLGA layers also increases (see Fig. 1). Therefore, after the PDLGA layer was kept at 11 mN/m on a water subphase for 1 h, the pH was increased to 11 by injection of KOH. The area was kept constant until the surface pressure started to increase. Then, the surface pressure was set to 14 mN/m. This led to a slight compression of the layer and a small increase in the interfacial moduli. The point, at which the moduli started to decrease was set as the starting point for the degradation in Fig. 5A. The complex interfacial viscosity decreased almost instantaneously, despite the fact that the surface pressure was kept constant at 14 mN/m. In comparison, the area decreased rather slowly. This observation supports a random scission mechanism, where the molecular weight decreases rapidly, while much more time is needed until a meaningful number of water soluble fragments are formed. The degradation curve could be fitted quite well with Eq. 2, 6 and 7. The reason for using Eq. 6, which assumes a decreasing catalyst concentration, was the fact that the pH of the subphase decreased rapidly, as observed with a pH electrode. This rapid decrease of pH is also observed in a high surface to volume glass vessel without any monolayer, and therefore attributed to the dissolution of carbon dioxide forming carbonic acid. The high pH experiments shown in Fig. 4 were conducted in an enclosed device under an argon atmosphere, but such a setup is not compatible with a rotational rheometer. Nevertheless, for 1.5 h it took the viscosity to drop to the base level, the pH was above 9.5. The fit result was used to calculate the evolution of the molecular weight according to Eq. 11 (see ref. [29] for derivation):

$$\frac{M_n(t)}{M_n(0)} = \frac{n_p(t)}{n_p(0)} = \left[\frac{\frac{A(t)}{A(0)} n_p(0)}{\{\alpha * n_p + 1 - \sum_{x=1}^{l_{min}} \omega_x(\alpha)\}} - C' \left(1 - \frac{C'}{n_{p0}}\right) \right] * \frac{1}{n_p(0) - C' \left(1 - \frac{C'}{n_{p0}}\right)} \quad (11)$$

The interfacial viscosity and the calculated molecular weight decrease on the same time-scale, which further supports the proposed mechanism. However, we were not able to identify a scaling law behavior according to $|\eta| \propto M_n^y$ which has been observed for polymers at interfaces [21].

A similar experiment was carried out for the case of enzymatic degradation (Fig. 5B). The experimental procedure was simpler, because injection of the lipase did not increase the surface pressure. After the layer was held on a PBS subphase at 11 mN/m for 1 h, the enzymes were injected, and the starting point of the degradation was identified with the time when the interfacial moduli started to decrease. Again, the interfacial viscosity decreased on the same timescale as the calculated molecular weight. For both types of degradation, the viscosity decreased by an order of magnitude while there was < 10% of dissolution of the layer. It is therefore strongly implied that the chains degrade via random bond scission both under enzymatic catalysis and at high pH.

5. Discussion

If chain-ends were catalyzing the degradation in a considerable way, one would expect a significantly faster degradation for the 1.7 k PDLGA in comparison to the 23 k PDLGA. This is clearly not observed in Fig. 4. After the initial expansion of the layer in case of the 23 k PDLGA for the reason explained above, the degradation rate is almost identical for both polymers as well as their mixture. Also, if chain-ends were catalyzing the degradation, the reaction rate would have to increase instead of decrease towards the end, provided that occasionally new chain-ends are generated by random bond scission. Since the chain length has little effect on polymers degrading via random scission, it is clear that the molecular weight distribution has a negligible effect as well. In conclusion, all experiments at neutral pH (see below) support a

random scission mechanism, where the effect of the molecular weight on the mass loss kinetics is limited to the initial stage (Fig. 3). Chain cuts in 1.7 kg/mol PDLLGAs containing only about 25 repeat units have a high propensity to produce water soluble fragments when dodecamers are water soluble. Dissolution of the layer starts immediately, whereas the initial degradation is slower in the 23 kg/mol polymer because not all chain-cuts lead to water soluble fragments. The effect is visualized in the simulated degradation curves in Fig. 3B. The areal expansion due to the additional of end-group pairs generated by bond scission also agrees between experiment and simulation. For the higher molecular weight polymer, both simulation using Eq. 7 and experiment show an initial expansion of the layers, whereas this is not observed for the short chain one. The reason is explained above. In case of the short chain PDLLGA, many fragments are immediately dissolved, meaning they generate no additional end-groups. The generation of end-group pairs is over-compensated by the fragment dissolution. For the higher molecular weight polymer, there is a certain time where chain cuts have a low chance to lead to water soluble fragments. In this induction period, a slight layer expansion is observed. We note that expansion is also observed in the absence of enzymes (see Fig. 4) and should therefore not be attributed to adsorption of enzymes to the air-water interface. An exception is the experiment with porcine liver lipase, which was very surface active. In any case, this expansion effect is unique for Langmuir monolayers and has no direct implications for real world materials. However, including layer expansion into the model probably yields in a more accurate reaction rate constant.

The findings from Langmuir monolayer degradation experiments are in line with degradation experiments on bulk PDLLGA [11], which found that the molecular weight only affects the initial lag-time. There is compelling evidence that there is an acidic micro-pH inside of degrading PDLLGA devices [6,7]. However, our experiments have shown that this does not lead to accelerated degradation of PDLLGA. These findings are in line with degradation experiments from the literature, where bulk samples were investigated at pH 2 [11]. One could argue that the protons were not diffusing into the bulk samples and therefore no accelerated degradation was found. The absence of proton catalysis in monolayers disqualifies that argument. In contrast, we found a drastically accelerated degradation at pH > 10 when PDLLGA was in the semi-dilute state. The pH-sensitivity of PDLLGA has implications for drug delivery. It has been reported that encapsulation of alkaline drugs, such as those containing secondary or tertiary amine groups, can accelerate the degradation of PDLLGA [32]. We could clearly show that this effect is caused by catalysis at the molecular level, and not by differences in chain packing or mobility. In our work, the catalysis is provided by hydroxyl ions, so nucleophilic amine groups are not essential for the catalytic effect. In contrast, encapsulation of acidic drugs will certainly not accelerate the degradation of the PDLLGA matrix.

At pH > 10, the degradation behavior was more in agreement with a chain-end cut than random scission mechanism. However, when the layer was compressed to 11 mN/m, the degradation mechanism changed to random scission. This raises the question why the high pH degradation mechanism differs between the highly concentrated and the semi-dilute state. Our measurements showed that the viscosity of the polymer layer increased by at least 4 orders of magnitude when going from the semidilute to the melt-like state. This implies that there is enough chain overlap to form entanglements which support a substantial resistance to shear deformation. The chain-end degradation mechanism assumes a backbiting step, where the chain-end carries out an intramolecular attack on another bond. This explains why a chain-end mechanism is only observed in semi-dilute Langmuir films [20] or in polymer solutions [8]. In bulk PDLLGA, the flexibility of the chain-ends is probably too low, especially below the glass transition temperature. The little free volume in a glassy polymer makes such a back-folding event highly unlikely. When comparing the reaction rate of the enzymatic degradation in the semidilute and the melt-like state, we find that the degradation proceeds with almost the same relative velocity

(ca. 40% degradation in 3 h). About 2.5 times more material was applied to the air-water interface in the high surface pressure experiment, so the absolute reaction rate must have been higher by the same factor. Assuming a reaction rate that is proportional to the areal concentration of repeat units, the foot print of a repeat unit at the air-water interface at 11 mN/m would have to be about 5 \AA^2 . A packing density of 5 \AA^2 per repeat unit is probably not achievable in a PDLLGA monolayer, given that the length of a repeat unit is about 3.6 \AA and the distance between two chains in polymer crystals is of the order of 4 \AA or more. However, x-ray reflectivity measurements suggest that at a packing density of 5 \AA^2 per repeat unit, the electron density profile of PDLLGA Langmuir films still resembles the monolayer state [31]. Such a layer is of course not perfectly flat, and the air-water interface has a certain extension where the water concentration decreases from the bulk value to the gas value. The areal concentration of repeat units can get as low as 5 \AA^2 per repeat unit if the chains are stacked in that layer. It is therefore imaginable that at 11 mN/m, where the nominal areal density is about 1.5 \AA^2 and the film thickness several nanometers [31], the areal density of repeat units that can be attacked by enzymes is on the order of 5 \AA^2 per repeat unit.

The molecules used for our experiments did not have a blocky sequence structure. A faster degradation of lactide or glycolide blocks could therefore not be observed both for purely hydrolytic as well as enzymatically catalyzed degradation. Also, to our understanding, a moderate increase in the average block length would not change the degradation behavior of PLGA. One has to keep in mind that fragments containing 12 α -hydroxyacid units are still water soluble. As long as the average block length stays below that value, slowly degrading blocks can be dissolved by cuts in the neighboring fast degrading blocks. Then, the overall degradation is determined by the degradation rate of these weak links. For hydrolytic degradation, those are probably the glycolide units while for enzymatic degradation, the L-lactide units should take that role.

Fig. 5 makes a compelling case for a solubility controlled [33] mass loss of PDLLGAs and bulk degrading polymers in general. This mechanism assumes that the mass loss is determined mainly by the solubility of the material, i.e. the fraction of water soluble fragments. In a bulk material, the delay between mass loss and molecular weight reduction is caused by the slow diffusion of water soluble fragments, which are trapped inside the degrading material. Clearly, dissolution of water soluble fragments in a Langmuir film would be nearly instantaneous. Yet, the molecular weight drops by a factor of 10 before any meaningful mass loss is observed. In other words, the conversion of polymer to sludge is the prerequisite for the generation of water soluble fragments, even in the absence of diffusion. The solubility control of the reaction is directly observable from the sharp decline of the viscosity at negligible mass loss. The calculation of the molecular weight was based on the assumption of having water soluble dodecamers. For a more hydrophobic polymer, the fragmentation would have to go even further before a substantial number of water soluble fragments are even formed, meaning mass loss would be preceded by an even deeper drop in molecular weight and viscosity.

6. Conclusion

By combining Langmuir monolayer degradation experiments with interfacial rheology, multiple effects of polymer degradation can be analyzed in situ and a relatively short time period. The mass loss is registered as area reduction, and the alteration of mechanical stability by a decrease in interfacial moduli or viscosity. The analysis of the mass loss allows for calculating the reduction of the molecular weight as a third effect. All three effects are interlinked and coupled to the degradation mechanism, and a precise understanding of the mechanism is the key to their prediction. Additional degradation effects that could be analyzed in Langmuir monolayer experiments are e.g. the generation of surface charges as well as chemi-crystallization in semicrystalline

polymers. The monolayer degradation experiments have clearly shown that due to the random fragmentation mechanism of PDLGA, the initial molecular weight has a very small impact on the kinetics of mass loss. In a bulk material, molecular weight could also affect material parameters that were not systematically investigated in this study, such as water uptake and chain mobility. However, the calculations show that before substantial mass loss occurs, the molecular weight drops to a value close to the molecular weight of the longest soluble fragment. This leads to an induction period with little mass loss. The initial molecular weight certainly has an influence on the duration of that induction period, which is nonetheless short compared to the time it takes to dissolve the whole material. The total duration of the mass loss period depends mostly on the size of the largest water soluble fragment and the hydrolysis rate of the ester bonds. In a bulk material, there is an additional delay because fragments need a certain time to diffuse to the surrounding medium. We want to emphasize that the sharp molecular weight reduction during negligible mass loss is not unique for PDLGA. This behavior is a consequence of the random scission mechanism and therefore expected for all bulk degrading polymers. Working with quasi 2D polymer solutions at the air-water interface has many advantages when it comes to studying fundamental molecular processes, but this approach cannot account for the variances in the morphology of real world devices. Parameters such as shape, microstructure and porosity of PDLGA devices affect the degradation behavior by determining diffusion pathways. The answer to the question of the impact of molecular weight on the degradation behavior of PDLGA is mainly given by the impact of molecular weight on the morphology of PDLGA devices during their fabrication. For devices with identical morphology made from different molecular weight PDLGA, little difference in mass loss or molecular weight loss behavior should be expected, except for the case of very short oligomers.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at [<https://doi.org/10.1016/j.jconrel.2019.12.044>].

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