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The influence of ph on the molecular degradation mechanism of PLGA

Rainhard Machatschek[1], Burkhard Schulz[1,2], Andreas Lendlein[1,2]*

[1] *Institute of Biomaterial Science, Helmholtz-Zentrum Geesthacht and Berlin-Brandenburg Center for Regenerative Therapies, Kantstraße 55, 14513 Teltow, Germany*
[2] *Institute of Chemistry, University of Potsdam, Karl-Liebknecht-Straße 24-25,14469 Potsdam, Germany*

** Correspondence to: Andreas Lendlein, Institute of Biomaterial Science and Berlin-Brandenburg Center for Regenerative Therapies, Helmholtz-Zentrum Geesthacht, Kantstraße 55, 14513 Teltow, Germany.*
E-Mail: andreas.lendlein@hzg.de

ABSTRACT

Poly[(rac-lactide)-co-glycolide] (PLGA) is used in medicine to provide mechanical support for healing tissue or as matrix for controlled drug release. The properties of this copolymer depend on the evolution of the molecular weight of the material during degradation, which is determined by the kinetics of the cleavage of hydrolysable bonds. The generally accepted description of the degradation of PLGA is a random fragmentation that is autocatalyzed by the accumulation of acidic fragments inside the bulk material. Since mechanistic studies with lactide oligomers have concluded a chain-end scission mechanism and monolayer degradation experiments with polylactide found no accelerated degradation at lower pH, we hypothesize that the impact of acidic fragments on the molecular degradation kinetics of PLGA is overestimated. By means of the Langmuir monolayer degradation technique, the molecular degradation kinetics of PLGA at different pH could be determined. Protons did not catalyze the degradation of PLGA. The molecular mechanism at neutral pH and low pH is a combination of random and chainend-cut events, while the degradation under strongly alkaline conditions is determined by rapid chainend cuts. We suggest that the degradation of bulk PLGA is not catalyzed by the acidic degradation products. Instead, increased concentration of small fragments leads to accelerated mass loss via fast chain-end cut events. In the future, we aim to substantiate the proposed molecular degradation mechanism of PLGA with interfacial rheology.

Introduction

Poly[(*rac*-lactide)-co-glycolide] (PLGA) is an especially widely applied degradable polymer in medicine, with applications ranging from surgical sutures via drug eluting coatings for metal stents [1] to microparticles for drug delivery [2]. In these

applications, the material is either used to provide mechanical support or to release a drug. The performance of the material in medical applications is hence determined by its mechanical properties and by a combination of the diffusion rate constant of the drug in the polymer matrix and the erosion rate of the matrix. Since drug diffusion rate [3] and tensile strength [4] of a polymer material are directly related to the molecular weight of the polymer chains, a prediction of the drug release rate or the tensile strength of the material requires a precise knowledge of the way in which the individual chains are fragmented, i.e. the molecular degradation mechanism. Mechanistic studies with solubilized oligomers and polymer monolayers have suggested that polylactide based materials degrade via a chain-end cut mechanism resulting in a linear decrease of the molecular weight [5-7]. In contrast, most studies on the degradation of bulk PLGA assume a random fragmentation mechanism resulting in exponential decrease of the molecular weight. These studies also presume that the enrichment of small fragments inside the degrading material has a catalytic effect on the local degradation rate since the protons provided by glycolic or lactic acid monomers can catalyze the hydrolysis of ester bonds [8]. One explanation for this contradiction is that the molecular degradation mechanism is not identical to the overall degradation kinetics of the bulk material, which depends on the transport of water and other reactants into the polymer matrix. Reducing the size of the matrix, i.e. working with microparticles, allows for reducing the impact of molecular transport processes on the degradation rate. However, even for these systems, contradicting results were obtained and both exponential [9] and linear [2] decrease of molecular weight were observed. Thus, the challenge in determining the molecular degradation mechanism of PLGA with high precision is to completely avoid mass transport phenomena. We hypothesize that the role of a proton mediated autocatalytic effect on the molecular degradation kinetics of bulk PLGA can be clarified by precisely controlling the proton concentration in the vicinity of degrading PLGA molecules. Therefore, our approach is the application of Langmuir monolayer degradation experiments [10]. Hereby, the molecular degradation kinetics of water-insoluble macromolecules are measured on the air-water interface on a Langmuir trough (Fig.1). In such a monolayer, the proton concentration in the vicinity of the degrading molecules is identical to the proton concentration of the aqueous phase. The transport of reactants and reaction products between the polymer and the aqueous phase is eliminated, and hence, the degradation rate is determined solely by molecular reaction kinetics. To prepare a Langmuir monolayer, a dilute polymer solution in a volatile solvent is spread on the surface of a Langmuir trough in a dropwise fashion. After evaporation of the solvent, the monolayer is compressed to the designated degradation surface pressure π_D. X-ray reflectivity measurements of PLGA Langmuir films have shown that at a surface pressure of $\pi = 4.5\frac{mN}{m}$, the thickness of the film is about 5 Å [11], corresponding to a monolayer. Further compression leads to progressive thickening of the layer. Hence, π_D was chosen as $\pi = 4.5\frac{mN}{m}$. When the ester bonds in the chains are broken, smaller fragments are generated, of which a certain fraction with sizes up to l_{min} are solubilized. The surface pressure is kept constant by compression of the barriers of the Langmuir-trough. The appeal of this isobaric degradation is that a defined areal concentration and organization of molecules in the monolayer is maintained throughout the reaction and that the area of the film is proportional to the number of remaining repeat units. The relevant range of pH for an autocatalytic degradation of PLGA can be inferred from the mass density of the material. Bulk PLGA contains about 10 mol/L of carboxylic acid monomers. Thus, when 10% of the polymer is converted into monomers, the partly degraded polymer may be considered as a 1 molar solution of lactic and glycolic acid, which has a pH of about 2. The lowest pH achievable if all chains are decomposed into monomers would be 1.4. Thus, if autocatalytic effects determine PLGA degradation, we expect faster degradation in the range between pH=1 and pH=3.

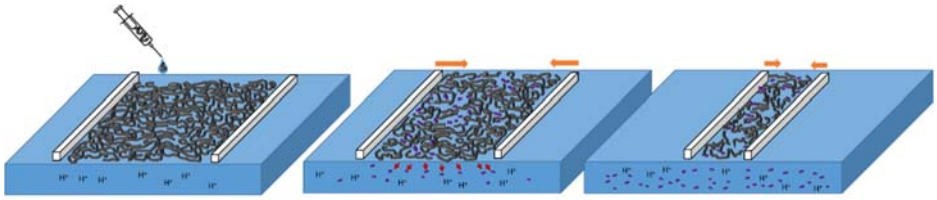

Fig. 1: Schematic representation of a Langmuir monolayer degradation experiment.

Experimental details

For all degradation experiments, $20\mu L$ of a 0.69 mg/mL solution of PLGA (lactide content 68% by weight, $M_n = 8370\,\frac{g}{mol}$ by end-group titration) in chloroform were spread on the surface of a Langmuir-trough (KSV Nima, medium size). The pH of the subphase was adjusted to the desired pH by addition of potassium hydroxide or hydrochloric acid beforehand. Neutral pH was achieved by using a phosphate buffered saline solution (PBS). The degradation surface pressure was $\pi_D = 4.5\ mN/m$. The surface pressure was measured with a Wilhelmy plate made of paper. The trough was equipped with a water level compensation system from KSV NIMA. The experiments were carried out at room temperature. The experimental degradation curves were normalized by the area where $= \pi_D$. The corresponding time was set to zero. The error introduced by the degradation of the film during compression to π_D was negligible for pH<10 because the time required for compression was much smaller than the observed degradation time. For pH=11, the degradation was so rapid that considerable degradation took place during compression to π_D. All experiments were conducted once except for the experiment at pH = 3. The deviations between experiments under identical conditions can be quite large when experimental duration is long. In the case of pH=3, at 50% degradation, the time deviation between experiments was about 40%, which is roughly the deviation between pH=3 and pH=7.4. The experimental degradation curves were fitted using Origin 2016 and a maximum of 400 iteration steps. The initial degree of polymerization was $n_p(t = 0) = 100$ as deduced from M_n.

Results

The experimental degradation curves in buffer solution at pH 7.4 are nearly identical to the experimental degradation curves at pH=1 and pH = 3 (Fig. 2). Clearly, protons are not catalyzing the degradation of PLGA

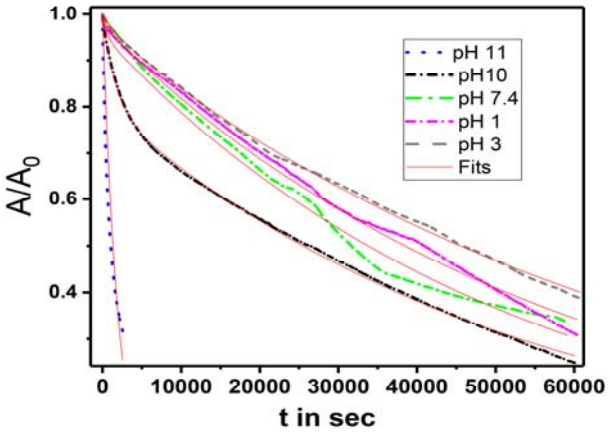

Fig. 2: Experimental area reduction curves (dashed lines) and fits of PLGA degrading at different pH.

To determine the molecular degradation mechanism of PLGA, the degradation curves are analyzed with a model that allows for both random scission and chain-end scission (Fig.3).

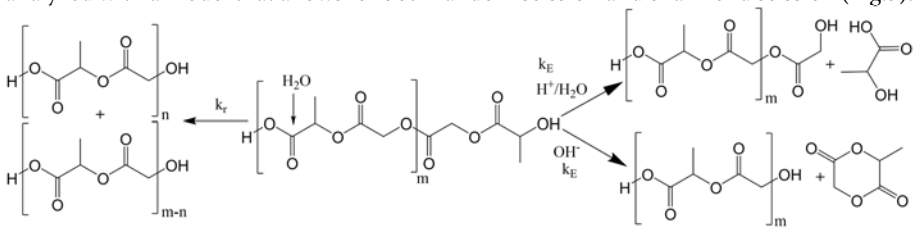

Fig. 3: Schematic representation of the random and chain-end scission of PLGA. Proton catalyzed chain-end cuts are expected to result in α.hydroxyacid fragments while hydroxyl catalyzed chain-end cuts produce cyclic diesters via backbiting reactions [7]. Here, $\boldsymbol{k_r}$ is the rate constant for random scission while $\boldsymbol{k_E}$ is the rate constant of chain-end scission

The ester groups in PLGA can react with water to form a pair of carboxylic acid and alcohol. The reaction can be catalyzed by protons or hydroxyl ions.

$$RCOOR' + H_2O + cat \rightarrow RCOOH + HOR' + cat$$

$$\frac{d\,RCOOR'}{dt} = k_{hydr}[H_2O][cat]RCOOR'$$

The probability for a successful reaction depends on the concentration of water and protons or hydroxyl ions in the vicinity of the reactive ester bonds. In Langmuir films, the aqueous subphase acts as quasi infinitely large reservoir for water. Our Langmuir trough contains 200 mL of water, meaning that a complete dissociation of 15 μg PLGA into monomers would lead to a concentration of $1 * 10^{-6} mol/L$ carboxylic acid. Thus, in the

pH range between 5 and 9, the degradation of the monolayer can have a noticeable effect on the pH. Since we work with a buffer at neutral pH, the concentration of the catalyst can be assumed as constant as well.

$$\frac{d\,RCOOR'}{dt} = k_r RCOOR'$$

In contrast to random scission events, the number of chain-end scission events is proportional to the number of chain-ends. Thus, the reaction rate is proportional to the number of chains with a rate constant k_E. The reaction mechanism for chain-end cuts is assumed to depend on pH [7] (see Fig. 3).

$$\frac{d\,RCOOR'}{dt} = k_E N_{chains}$$

To achieve an expression for the number of repeat units $RU(t)$ in the film, we consider several processes leading to fragment generation and dissolution: Small fragments are generated via chain-end cuts [5] with a rate constant k_E as well as via hydrolysis of ester bonds that is distributed randomly within the chains with a rate constant k_r. Fragments generated via random scission are dissolved if their size does not exceed l_{min}. Here, we assume that l_{min} = 6, based on the analysis of water soluble fragments in the early stage of PLGA degradation by Li et al [12]. In addition, we introduce a deactivation mechanism for chain-ends. A deactivated chain-end does not undergo further chain-end cuts. We assume that the rate of chain-end deactivation is proportional to the number of chain-ends with a rate constant k_{deac}. For random scission, all bonds in the chain are broken with same probability. Since chain-fragments are generated at both ends, the fraction of newly formed fragments that are directly dissolved is given by $2 * \frac{l_{min}}{n_p}$. Similarly, when a bond is broken randomly, on average $\sum_{i=1}^{l_{min}} \frac{2i}{n_p} = \frac{l_{min}(l_{min}+1)}{n_p}$ repeat units are dissolved. With $n_p(t) = \frac{RU(t)}{N(t)}$, we can derive rate equations for $N(t)$ and $RU(t)$:

$$\frac{dRU}{dt} = -2k_E * N - l_{min}(l_{min}+1) * k_r * \frac{RU}{n_p} = -[2k_E + l_{min}(l_{min}+1) * k_r] * N \quad (1)$$

$$\frac{dN}{dt} = k_r * RU - 2 * k_r * l_{min} * \frac{RU}{n_p} - k_{deac}N = k_r * RU - [2 * k_r * l_{min} + k_{deac}]N \quad (2)$$

By differentiating (2) with repect to t and inserting (1), we arrive at a second order differential equation for $N(t)$. By inserting the solution for $N(t)$into Eq. 2, we obtain an expression for $RU(t)$. The time dependent area of the Langmuir film is then calculated via normalization:

$$\frac{RU}{RU_0} = \frac{A}{A_0} = \exp\left(-[k_r l + \frac{k_{deac}}{2}] * t\right) * \left[\cos h(at) + \left(\frac{\left[l + \frac{k_{deac}}{2k_r}\right]\left(k_r - [k_r l + \frac{k_{deac}}{2}]\frac{1}{M_0}\right)}{a} + \frac{a}{k_r n_{p_0}}\right) * \sin h(at)\right] \quad (3)$$

$$a = \sqrt{\left[k_r * l + \frac{k_{deac}}{2}\right]^2 - k_r * [2k_E + l(l+1) * k_r]}$$

The fit results for k_r, k_E and k_{deac} are listed in table 1.

Table 1: Rate constants obtained from fitting experimental data with Eq. 3.

pH	k_r (1/s)	k_E (1/s)	k_{deac} (1/s)	k_E/k_{deac}	k_E/k_r
1	3.95E-06±1%	0.00223±50%	0.00103±40%	2.1	564.7
3	2.35E-06±1%	0.00133±40%	4.31E-04±30%	3.08	566
7	3.39E-06±2%	0.00482±60%	0.00171±60%	2.8	1421.8

10	8.36E-07±1%	0.00662±10%	6.07E-04±30%	10.9	7914.8
11	2.36E-06±5%	0.02532±4%	6.82E-04±30%	37.1	10721.9

Discussion

The parameters of the best fits were strongly interdependent. Nevertheless, the ratios of the rate constants reveal clear trends. The ratio of the random scission and chain-end scission rate constants is very high for all pH values, suggesting that chainend scission is the much faster mechanism. The ratio of chain-end scission rate constant and deactivation rate constant (k_E/k_{deac}) reveals that at low and neutral pH, chain-ends undergo only few chain-end cuts before they are deactivated. The limiting process determining the overall degradation rate is the relatively slow formation of new chain-ends. This leads to a peculiar mechanism where for each random chain-cut, a certain number of small water soluble fragments are released. In contrast, when fragments are generated exclusively by random scission, the average fragment size decreases progressively with proceeding degradation. At neutral and acidic pH, the dominant mechanism leading to a decrease of molecular weight is the random fragmentation of the chains, so our measurements support an exponential decrease of the molecular weight. According to k_r, M_n decreases by a factor of e^{-1} in about 3 days. This is similar to the results obtained with PLGA microparticles at 37°C [9]. In suggesting such a complex mechanism of a series of random scission and chain-end scission events, we emphasize that a simpler model cannot explain the experimental results. An exclusive random scission mechanism produces increasing area reduction rates due to progressively decreasing fragment sizes. An exclusive chain-end scission mechanism could explain the area reduction curves observed at neutral and high pH. The continuous reduction of the degradation rate could be attributed to polydispersity, i.e. a continuous dissolution of smaller than average chains that are crossing the solubility limit due to chain-end scission. However, the degradation curve at pH 10 cannot be explained with such a simple model. It shows a more or less sharp crossover from a fast process to a slow process at $\frac{A}{A_0} \approx 0.8$. A bimodal chain length distribution of many small and few long chains could explain such an effect, however, this effect would also be observable at low and neutral pH. One open question is the nature of the deactivation reaction. The dissolution of the chain-ends due to random fragmentation is already included in the model. A polydispersity effect as described above is also unlikely because polydispersity is an intrinsic property of the polymer sample and hence k_E/k_{deac}would have to be independent of pH. We therefore suggest that the deactivation is related to the molecular mechanism of the chain-end cut reaction. For example, hydrolysis of the intermediate product could give different results depending on pH or certain glycolic acid/lactic acid sequences in the statistical copolymer could undergo chain-end cuts much more rapidly than others. Our observation of a degradation that is not accelerated by protons is not in general disagreement with an autocatalytic degradation of PLGA in bulk. However, the species that causes the acceleration are not protons, but the chain-ends themselves.

Conclusions

In Langmuir layers, protons do not accelerate the degradation of PLGA macromolecules, in contrast to very alkaline conditions. By analyzing the molecular degradation curves with a model that takes into account both random fragmentation and chain-end degradation, we found that fragmentation at the chain-ends is much faster than random fragmentation, but counterbalanced by a deactivation of chain-ends at low and neutral pH. To afford a prediction of the degradation kinetics of bulk materials, our kinetic model, which predicts an exponential decrease of the molecular weight, can be combined with calculations and measurements of water uptake in bulk PLGA. Such a combined model could improve the prediction of drug release kinetics and mechanical properties of degrading PLGA based medical devices.

Acknowledgments

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