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Feature Article

Langmuir monolayers as tools to study biodegradable polymer implant materials

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Abstract

Langmuir monolayers provide a fast and elegant route to analyze the degradation behavior of biodegradable polymer materials. In contrast to bulk materials, diffusive transport of reactants and reaction products in the (partially degraded) material can be neglected at the air-water interface, allowing for the study of molecular degradation kinetics in experiments taking less than a day and in some cases just a few minutes, in contrast to experiments with bulk materials that can take years. Several aspects of the biodegradation behavior of polymer materials, such as the interaction with biomolecules and degradation products, are directly observable. Expanding the technique with surface-sensitive instrumental techniques enables evaluating the evolution of the morphology, chemical composition, and the mechanical properties of the degrading material in situ. The potential of the Langmuir monolayer degradation technique as a predictive tool for implant degradation when combined with computational methods is outlined, and related open questions and strategies to overcome these challenges are pointed out.
The challenge of studying polymer degradation

Biodegradable polymer materials enable manufacturing medical implants that degrade into non-toxic fragments that can be metabolized or excreted, providing a temporary support while the body recovers.\[1\] Avoiding surgical implant removal while also preventing any unexpected long-term effects make biodegradable implants a very elegant concept. However, although a variety of biodegradable polymer materials have been around for several decades, the fact that the clinical application is limited to a few select examples, e.g. as suture materials, demonstrates that there are still several issues limiting their applicability. For example, the elastic modulus of copolyesters used as biodegradable suture materials can change drastically within a short period of time, which is difficult to predict.\[2\] Using biodegradable sutures therefore comes at the risk that the required mechanical properties may not be maintained until the healing is completed and hence, non-degradable sutures are still widely used.\[3\] In addition to deterioration of mechanical properties, the products of the chemical reactions leading to implant degradation can cause an undesirable biological response. Polylactide bone fixation devices, while being initially histocompatible and providing sufficient mechanical support for bone repair, are linked to long-term inflammatory and bone resorption response.\[4\] It is assumed that this critical bioresponse is caused by the acidic degradation products,\[4\] which are not released steadily, but in a sudden burst\[5\]. Thus, as long as such important aspects as the evolution of the mechanical properties as well as the fragment release kinetics of biodegrading polymer materials cannot be predicted with the precision that is imperative for medical applications, biodegradable implants will always run into problems in clinical applications. This is demonstrated by the recent example of drug eluting vascular scaffolds based on poly(L-lactide), which showed a three times higher risk of thrombosis compared to non-degradable drug eluting metal stents in a 3 years trial.\[6\] Furthermore, despite the fact that the scaffolds were claimed to degrade within 3 years after implantation, persisting struts were found even 4
years after implantation in certain patients.[6] These findings ultimately forced the manufacturer to halt the commercial production of the scaffold.

Developing an implant to the stage of clinical application is clearly an enormous effort and the consequence of failing in such a late stage is a serious economic setback. A quantitative prediction of the degradation behavior of polymer materials based on comprehensive understanding of the underlying mechanisms deduced from simple and fast measurements would significantly facilitate and enhance the design and engineering of biodegradable implants. By minimizing the risk of failure in a clinical trial, developing biodegradable implants would become much more economically appealing. Moreover, elucidating the mechanisms leading to implant degradation will also assist the development of implants that are not intended to degrade yet still can show degradation \textit{in vivo}.[7]

On the molecular level, a material only has to fulfill two criteria to be considered biodegradable. The smallest fragments generated by the degradation process have to be water-soluble so that the human body can eliminate or metabolize them. Secondly, the chemical reactions leading to bond scission must take place at an appropriate rate in the human body. The most simple of these reactions is the hydrolytic attack by water molecules. Hydrolysis can be catalyzed by protons or hydroxyl anions, but also by natural catalysts such as enzymes. However, other chemical reactions could also lead to bond cleavage \textit{in vivo}, e.g. via radical or oxidative[8] processes.

Naturally, biodegradation of a material starts at its surface. Alteration of surface chemistry due to conversion of certain chemical moieties may result in drastic changes of the physical properties of the surface, which in turn, have an impact on the bioresponse to the material. As an example, the hydrolysis of ester bonds creates carboxyl groups, which are negatively charged in physiological environments, leading to an increased hydrophilicity of the surface. While the surface is degraded, water molecules, acting both as reactant and as reaction medium, diffuse into the material, inducing degradation of the bulk material as well (Fig. 1). However, if the
molecular degradation rate is fast and the water diffusion is slow, the material will be degraded almost exclusively at the surface. This limiting case is called surface erosion. On the other hand, when molecular degradation is much slower than water diffusion, degradation occurs throughout the whole material uniformly, which is called bulk degradation. These limiting cases are idealized concepts and in reality, the extent to which both degradation modes determine the overall degradation of an implant depends on its composition and shape. One has to keep in mind that any bulk degrading material continues to degrade at the surface where the water concentration is much higher than inside the material.

For the idealized case of exclusive surface erosion, a description and prediction is easier than for bulk degradation. Since degradation products are directly solubilized in the surrounding medium, the contribution of diffusional processes in the partly degraded material can be neglected. The average molecular weight of the bulk material remains constant when only the chains at the surface are degraded. While this also means that surface erosion does not affect the elastic modulus of the material as a whole, surface erosion affects the mechanical properties of an implant by altering its geometry. The geometry also determines the degradation rate relative to the remaining mass via the surface to volume ratio. However, for a sheet-like object, the surface area keeps approximately constant during degradation. Such a system is very interesting for controlled drug release systems since it exhibits nearly zero-order release kinetics.[9] In contrast, bulk degradation is characterized by an interplay of diffusional processes[10] and chemical reactions. The local degradation rate depends on the local water concentration, which, in turn, may depend on the local degree of degradation. Fragments which are not generated near the material surface are not directly solubilized. Since diffusion of fragments through the degrading material is slow, fragments enrich inside the material.[11] Therefore, the early stage of bulk degradation is characterized by chain scission and deterioration of mechanical properties with little mass loss while the late stage is characterized by dissolution of a soft material consisting of short chains and fragments.
Figure 1: Schematic representation of the processes characteristic for the early stage (middle) and late stage (left, right) of the biodegradation of polymer materials

The complexity is further increased by the microstructure of polymer materials. Morphological parameters like crystallinity, crystal orientation, porosity, pore size distribution etc. are usually controlled via processing conditions to adjust material properties to specific requirements. However, clearly, these morphological features have an impact on the degradation behavior of the material while also being affected by degradation.\(^{12, 13}\) As an example, pores allow for high water uptake and fast water influx. Degradation at the pore wall leads to growth or merging of pores,\(^{14}\) which may in turn enhance influx and uptake of water. Taking into account the variety of processes that determine the bulk degradation of a macroscopic object, a theoretical prediction is a highly complex problem. Therefore, the only practical method to assess its degradation behavior is to actually degrade it.\(^{15}\) For materials that are typically required to maintain their integrity for months or years, this takes a corresponding amount of time. Moreover, by incubating a polymeric object in a test solution and monitoring its mass and molecular weight over time, only little information about the individual contributions of the different material parameters and even less about the underlying mechanisms of polymer degradation can be extracted. In contrast to degradation experiments with bulk materials, the Langmuir monolayer degradation technique, which we introduce in the following sections,
provides fast and easy access to the molecular mechanisms that determine the biodegradation behavior of polymer materials.

**Methodological approach: Langmuir techniques**

In Langmuir monolayer degradation experiments, ultrathin polymer films at the air-water interface are used to study the degradation behavior of polymer materials. A thin polymer film, when exposed to a degrading medium, is a direct representation of the surface of a degrading polymeric object. Besides degradation kinetics, also the interaction of the biodegrading material with biomolecules can be studied. The kinetics observed in monolayer degradation experiments should more or less directly represent the degradation kinetics at the interface of any bulk material in an aqueous environment. Thus, combining the monolayer degradation kinetics with an adequate estimation of the thickness of the surface-erosing layer can afford a quantitative prediction of the surface erosion kinetics at the surface of a biomaterial. A prime example of a degradation mode proceeding via surface erosion is enzymatic degradation. Typically, enzymes are too bulky to diffuse into a polymer material within reasonable time and hence, degradation of the surface is the main mode of degradation. In addition, enzymatic monolayer degradation experiments also shed light on the interaction between the enzymes and the biomaterial surface. Understanding the interactions between the surface of a degrading biomaterial and biomolecules like enzymes is crucial with respect to the bioresponse to the material. Here, polymer monolayers as surface models have the huge advantage that the number of synthetic polymer molecules and biomolecules is roughly equal. In contrast, studying the same surface processes with a synthetic bulk polymer sample is similar to weighing the ship with and without the captain to determine the captain’s weight. Furthermore, the chemical degradation of the thin film also provides information about the chemical degradation inside a bulk material. Here, however, one has to take into consideration the difference in water concentration. While the water concentration at a material surface is
100%, the water concentration inside of a bulk polymer may range between few percent and almost 100% depending on material and degree of degradation.\cite{18} Hence, when correcting reaction rates for the difference in water concentration, also a quantitative prediction of bulk degradation should be possible based on monolayer degradation experiments. Of course, polymer monolayers have their limitations. Processes relying on mass transport like the diffusion of water into or the diffusion of fragments out of a material do not take place in monolayers. These important aspects of biomaterial degradation have to be evaluated in separate experiments or simulations.\cite{19,20}

**The Langmuir monolayer degradation technique**

A Langmuir film is a monolayer of molecules assembled at the interface between a hydrophilic phase, usually water, and a hydrophobic phase, usually air. The driving force for molecules to form such a film is their amphiphilicity, provided by combining both hydrophilic and hydrophobic moieties in a single molecule. Most common polymers are rather hydrophobic and would form crystals or 3D aggregates when applied to the air-water interface. These polymers cannot be studied with Langmuir monolayer techniques. In contrast, the functional groups that can be cleaved in aqueous environments are hydrophilic and therefore, many biodegradable polymers have sufficient hydrophilicity to form monolayers at the air-water interface. Therefore, the number of hydrophobic moieties per hydrophilic group can be used to estimate, which polymer systems are suitable for Langmuir monolayer degradation experiments. Based
on our own observations, poly(ω-pentadecalactone) (PPDL) is close to the upper limit of hydrophobicity for polyesters.

Figure 2: Schematic representation of an enzymatic Langmuir monolayer degradation experiment (top) with the corresponding area vs. time plot (bottom). Insertion of enzymes into the layer in the early stage can cause an expansion of the layer (left). When water-soluble fragments are formed, the layer is compressed. Complementary experimental methods like Brewster angle microscopy and Infrared Reflection Absorption Spectroscopy can be used for further characterization of the layer (middle). Towards the end, most chain segments are converted into water-soluble fragments and the area reduction rate decreases (right).

The thermodynamic state of the polymer monolayer is controlled and examined on a Langmuir trough (Fig.2). This, in its basic version, very simple device, allows for reducing the available area for the film while simultaneously measuring the surface pressure generated by the Langmuir film with a Wilhelmy balance. To study the degradation of a polymer material, a dilute solution of the polymer in a volatile solvent is added to the air-water interface in a drop-wise fashion. After compressing the layer to a packing density resembling the bulk state, degradation is induced or substantially accelerated, e.g. by changing the pH of the water. Since the biodegradable material forms water-soluble degradation products, the concentration of chain segments at the surface decreases during degradation. As the surface pressure is correlated to the areal concentration of chain segments, degradation leads to a decline of the surface pressure. However, usually, the experiment is carried out under isobaric conditions. Therefore, the surface pressure, and hence the areal concentration of chain segments, is kept constant by compressing the film. The great advantage of this isobaric degradation is that a defined areal concentration and organization of molecules in the monolayer is maintained throughout the
the decrease of the surface area is proportional to the number of dissolved chain segments.

Initially, the key points making Langmuir monolayer degradation such a helpful technique were the absence of diffusional transport and the low amount of material required. The first point assures that the measured degradation rates are determined exclusively by the molecular reaction kinetics and the second point leads to short experimental timescales. In a Langmuir monolayer degradation experiment, substantial degradation is typically observed within hours,[21] bringing especially slowly degrading materials into the scope of reasonable experimental timescales.

**Complementary instrumental techniques**

With complementary instrumental techniques (Fig.2), Langmuir films can be used to study the impact of degradation on material morphology, composition and mechanical properties, which are important aspects of biomaterial degradation. Degradation induced morphological changes on the micrometer scale can be studied with Brewster angle microscopy (BAM) [17]. To detect changes at the molecular scale, polarized Infrared Reflection Absorption Spectroscopy (IRRAS) is ideally suited. While this technique is not as sophisticated as Sum Frequency Generation Spectroscopy (SFG), it is much more accessible and very effective for estimating chain orientation,[22, 23] following conversion of chemical groups and detecting changes in crystallinity.[24] Interfacial shear rheology provides access to the evolution of the mechanical properties of the degrading material. Since shear rheology can also be used to study the evolution of the molecular weight of the material, this method provides information about the molecular degradation mechanism as well. Another quantity that can be used to extract information about the molecular degradation mechanism is the surface potential of the layers[25, 26], which can be measured with a corresponding sensor.
Mathematical description of the degradation kinetics of polymer monolayers

When using no complementary instrumental methods, the outcome of an isobaric Langmuir monolayer degradation experiment is a surface area vs. time plot. The amount of information that can be extracted from the curve depends on the complexity of the models applied to analyze the data. Comparing relative degradation rates requires no model at all. This approach was favored by the group of Li et al. for investigating effects like stereocomplexation\cite{27} or packing density\cite{28} on the degradation rate of aliphatic polyesters. A semi quantitative approach was pursued by Kulkarni, Reiche, Lendlein et al. They differed between random chain scission\cite{29} which is the prevalent degradation mode for biodegradable polyesters, and a chain-end cut mechanism, which was postulated for polylactide based polymers.\cite{30} To describe random chain scission, Kulkarni et al. used a model derived from a description of particle size distributions during dynamic fragmentation\cite{31} and assumed that all fragments consisting of less than $n_{\text{min}}$ repeat units are water soluble. The appeal of the model is that it arrives at a rather simple expression for the area $A(t)$ due to random fragmentation:

$$\frac{A_0-A(t)}{A(t)} = A_{\text{corr}} = \frac{\exp(n_{\text{min}} k_{\text{random}} t)}{n_{\text{min}} k_{\text{random}} (t+1)} - 1.$$  \hspace{1cm} (1)

However, to arrive at this simple expression, assumptions and simplifications were made. Firstly, the finite size of polymer chains was not taken into account for fragmentation statistics, which leads to an underestimation of the number of soluble fragments in the early stages of degradation. Secondly, a constant, i.e. time independent reaction rate was assumed. Obviously, the reaction rate cannot be constant, since the scission of polymer chains creates end-groups. Only a certain fraction of these end-groups is attached to fragments that are immediately solubilized, the other end-groups are enriched in the layer, leading to a decreasing concentration of chemical bonds that can be converted. Nevertheless, Eq. 1 could fit the experimental degradation curves of polyesters reasonably well. For the description of degradation proceeding exclusively via the cleavage of the chain-ends, Kulkarni et al. found that the Equation:
describes the experimental isobaric area reduction curves of poly(D,L-lactide) and poly(D,L-lactide-co-glycolide) (PLGA) quite well in the early stages of degradation.\cite{30} A deviation from a linear behavior at later stages was attributed to the complete degradation of smaller chains, resulting in a reduction of the number of reactive chain-ends. However, from Eq. 2, it follows that:

\[
\frac{A_0 - A(t)}{A(t)} = \frac{A_0}{A(t)} - 1 = kt
\]  

(2)

Eq. 3 describes a reaction that is of second order in $A(t)$. Intuitively, for the early stage of the degradation reaction where no chains are dissolved, one would expect a constant number of chain-ends, and hence, a reaction which proceeds at constant rate, i.e. a reaction of zero order in $A(t)$. Here, resolving the contradiction between the postulated mechanism and the observed degradation kinetics is clearly necessary.

A thorough quantitative analysis allowing for determination of reaction rate constants for macromolecules undergoing random fragmentation was developed by Ivanova\cite{32} and Grotzev.\cite{25} They found that for hydrolysis reactions, both catalyzed by enzymes and hydroxyl ions, the bond scission rate decreases exponentially with time, i.e. a first order reaction:

\[
\frac{dN_{bonds}}{dt} = -k \cdot N_{bonds}(t = 0) \cdot \exp(-kt)
\]

(4)

The progress of the reaction is correlated with the fragment size distribution

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

(5)

to calculate the time dependent fraction of bonds that are part of soluble fragments

\[
\beta(a) = \sum_{x=1}^{\min x \omega_x(a)} \frac{n_p}{\sum_{x=1}^{\max x \omega_x(a)}}
\]

(6)

where

\[
\alpha(t) = \frac{N_{broken(t)}}{N_0}
\]

(7)

is the degree of completion of the bond-breaking reaction and $n_p$ is the degree of polymerization. They used a special trough design consisting of two separate compartments, one serving as reservoir while the other served as reaction chamber. This allowed them to stir
the reaction chamber, but forced them to carry out an additional numerical integration of the experimental area reduction weighed by $\beta(\alpha)$.

We infer that this special, so called zero order design, does not pose a real advantage. Accelerating diffusional processes by stirring accelerates local transport of reactants and reaction products at the reaction front and does not reflect most real-world situations. Instead, Eq. 4-7 and a normal Langmuir trough are sufficient to determine bond scission rate constants in Langmuir monolayer degradation experiments. This is exemplified in Fig. 3 which shows an experimental degradation curve of a poly($\varepsilon$-caprolactone) (PCL) oligomer (M$_\text{w} \approx 2500$ g/mol) at room temperature. The corresponding experimental procedure was published previously [33] but for the data represented in Fig. 3, instead of adding enzymes, a subphase adjusted to pH 2.5 by addition of aqueous HCl was used to promote the degradation. The experimental data are new since the hydrolysis of the oligomers was catalyzed by protons instead of enzymes but the curve shape is very similar for both catalysts. In previous publications from our group, the data were represented to fit Eq. 1. Using a more sensible representation reveals the S-shaped area vs. time plot which is typical for a random fragmentation mechanism [34]. The fits in Fig. 3 were generated using Eq. 4-7 and $n_{\text{min}} = 4$ both with variable $n_p$ and $n_p = 25$. 
The fits returned a bond scission rate constant of $k = 1.35 \times 10^{-5}\text{s}^{-1}$ for variable $n_p$ and $k = 1.22 \times 10^{-5}\text{s}^{-1}$ for fixed $n_p$. Thus, the average lifetime of an ester bond at that pH is about 80,000 seconds. The long-term goal of Langmuir monolayer degradation experiments is to predict the degradation kinetics of the corresponding bulk material. In theory, this is possible when the local water concentration inside the bulk material is known. Since the reaction rate is proportional to the water concentration, lifetime estimated from Langmuir monolayers need to be divided by the bulk water concentration to extrapolate to the situation in a bulk material. A typical water concentration for PCL degrading in an aqueous environment would be on the order of 1%\(^\text{[35]}\). Then, the lifetime of an ester bond in bulk PCL as extrapolated from Langmuir monolayer experiments is 100 days at pH 2.5. A recent study found a lifetime of about 55 days for PCL films at very high pH.\(^\text{[35]}\) The comparable values hint that the degradation rate constants determined in monolayers are applicable to bulk materials, but to truly understand how the kinetic data from Langmuir monolayers can be applied to bulk materials, experiments comparing bulk degradation and Langmuir monolayer degradation kinetics over a range of different materials and conditions have to be carried out.
Key insights into polymer degradation based on Langmuir monolayer degradation

For polyesters, which are probably the most extensively researched class of biodegradable polymer implant materials, enzymatic degradation is expected to take place at the material surface. There is a general agreement that enzymatic degradation of polyesters proceeds via a random fragmentation mechanism which is characterized by an S-shaped degradation curve as shown in Fig. 3 and can be described by either Eq. 1 or Eq. 4-7. One noteworthy exception is poly(3-hydroxybutyrate) (PHB), which shows a degradation curve resembling a straight line, in analogy to the zero order kinetics that are observed during thermal degradation of the polymer.[36]

A detailed quantitative model for the analysis of enzymatic degradation has been developed by combining surface adsorption, Michaelis-Menten kinetics and statistical fragment size distribution. For polylactide degraded by cutinase, the inverse of the observed, effective reaction rate constant was proportional to the inverse of enzyme concentration.[34] The Langmuir monolayer proved to be an exceptional tool to gain insights into the interaction between the enzymes and the polymer chains. It was clearly shown that enzymes require a minimum packing density of the polymeric substrate to become active. Here, a lower threshold surface pressure was found for proteinase K degrading poly(L-lactide) (but not poly(D-lactide)) than for PHB which was degraded by an extracellular depolymerase.[28] This was attributed to the greater hydrophilicity of poly(L-lactide) compared to PHB. Besides packing density, the impact of temperature and molecular weight on the random degradation of PCL has also been studied.[29] An inverse proportionality between bond scission rate constant and molecular weight was found by fitting the degradation curve with Eq. 1. Using the same analysis, a doubling of the bond scission rate constant was found when going from room temperature to 37°C. This dependence, which is below van’t Hoff’s rule, underlines the importance to carry
out degradation experiments at physiological temperature, since extrapolating from other
temperatures may give misleading results. To further elucidate the mechanism of enzymatic
degradation of polyesters, oligo(ε-caprolactone) was end-capped with phenylboronic acid. It
was hypothesized that these end-caps can mediate a specific binding interaction with the lipase
from *Pseudomonas cepacia*.\[33\] Neither crystallization nor degradation rate in the late stage
were affected by the end-caps. However, in the early stage, degradation was significantly
retarded, and enzyme insertion was hypothesized due the observation of an increase of the film
area under isobaric conditions. Insertion of enzymes into polymer films was also found in other
studies when degradation was either slow\[32\] or inactive enzymes were used.\[26\] This suggests
that enzymes are also able to penetrate into the surface layer of bulk materials, with the
thickness of the eroding layer expanding when degradation is slowed down. This hypothesis is
supported by the observation of a porous surface layer during enzymatic degradation of certain
materials.\[37\]

Considering that polylactide based implants have already made it to clinical application, it is
not surprising that polylactide based polymers and copolymers have been the interest of many
Langmuir monolayer degradation studies. Lactide based polymers are known to show mainly
bulk degradation and hence, it is important to study the degradation behavior of polylactides in
the absence of enzymes. Here, it could be clearly shown that chain organization has a drastic
effect on degradation kinetics. A significantly slower degradation was found for poly(L-lactide)
compared to poly(D,L-lactide) at basic pH, which was attributed to the ability of the
homopolymer to form ordered, tightly packed structures in contrast to the random
copolymer.\[38\] Consequently, blending poly(L-lactide) with poly(D-lactide) resulted in further
decrease of the degradation rate due to the formation of a very stable stereocomplex between
the enantiomeric forms.\[27, 38\] Another interesting result is the observation of an enhanced
degradation rate of polylactides blended with a slowly degrading compound. The effect was
greatest for a 50% mixture of both compounds.\[39\] By carefully adjusting the pH of the
subphase, it was demonstrated that the sensitive region for the hydrolysis of polylactides is at about pH=10.4, with the reaction proceeding nearly 10 times faster at pH=10.5 than at pH=10.3, while negligible degradation was observed at pH = 7 and also at low pH of 3.5.[40] This raises questions considering the catalytic species during the often-cited autocatalytic degradation of polylactides in bulk. The small chain fragments generated inside of a bulk material have a slow diffusion velocity and hence are enriching inside the bulk polymer. For polylactides, it is assumed that the lactic acid monomers have a catalytic effect on the chain scission by lowering the pH inside the material, leading to an autocatalytic degradation.[41] Indeed, in Langmuir films, where the small fragments are readily solubilized, an autocatalytic degradation behavior is not observed. Instead, the degradation curves show a reaction rate that is steadily decreasing. This observation led to the hypothesis of a chain-end scission mechanism for polylactide and polylactide based copolymers.[30] However, since Eq. 2 was used to analyze the area reduction curves of poly(D,L-lactide) and poly((D,L-lactide)-co-glycolide)(PLGA), it is unclear whether their degradation curves are truly supporting a chain-end cut mechanism. Here, clearly, some theoretical work is required. Nevertheless, a chain-end cut mechanism is also supported by the linear reduction of the molecular weight during hydrolysis of polylactide microparticles.[42] Thus, there is a marked difference between the molecular degradation mechanism observed on the nano- and microscale and the degradation observed in macroscopic systems. Nevertheless, an exclusive chain-end scission mechanism is not supported by Langmuir-degradation experiments that were assisted by surface potential measurements.[43] For the purely hydrolytic degradation of PLGA, it was found that the accumulation of charged species in the layer did not match a pure chain-end scission mechanism. Similarly, while the observed reaction rate constants were higher for shorter chains, a linear dependence as expected for exclusive chain-end scission was not observed. Probably, also the generation of new chain-ends by random bond scission needs to be included into the mechanistic description. Nevertheless, a clear trend was observed concerning the glycolic acid content of the copolymer, with higher glycolic acid
content resulting in higher rate constants both in enzymatic \cite{26} and in purely hydrolytic degradation. \cite{43} In another effort to further understand the mechanism of PLGA degradation, partly degraded PLGA was studied at the air-water interface. \cite{44} Therefore, PLGA bulk samples were incubated in ultrapure water at 70°C and samples of both water soluble and insoluble degradation products were taken after different degradation intervals. The water-soluble fragments still showed surface activity, which decreased with degradation time due to decreasing fragment size. The same was true for the insoluble degradation products, for which also the hydrolytic degradation was investigated. Here, the lower the initial molecular weight, the faster the degradation. Since lower molecular weight fractions have a higher concentration of chain-ends, the importance of chain-ends for the degradation of PLGA is confirmed. Finally, by injecting water-soluble PLGA fragments below a monolayer of the original PLGA, it could be shown that the fragments altered the hydrolytic degradation of PLGA.

Tailoring the properties of polymer materials to meet specific requirements requires more complex molecular architectures than homopolymers and statistical copolymers. Of course, it is expected that the molecular architecture has a pronounced impact on the degradation behavior of a material. An example are star shaped molecules that can be used to generate polymer networks and are claimed to be superior over linear molecules for drug delivery. \cite{45} Here, it was found that star shaped PCL molecules exhibited a much slower enzymatic degradation at physiological conditions than linear oligomers. \cite{46} Shape-memory polymers, which offer the opportunity to introduce implants with complex geometries through minimal incisions, are often block-copolymers comprised of “hard” and “soft” blocks. The hard blocks determine the permanent, complex shape of the implant, while the soft segments allow for fixing the implant in a temporary compressed state that can be erased by an external stimulus. The impact of the nature of the hard segment on the degradation behavior of shape memory polymers was demonstrated by comparing the enzymatic degradation of copolyesterurethanes containing PCL as soft segment and either poly(p-dioxanone) (PPDO) or PPDL as hard segment. \cite{17} Chains
containing the hydrophobic PPDL hard segments degraded several times slower than chains containing the hydrophilic PPDO segments. In both cases, enzymes were only degrading PCL segments, but in the case of PPDL, fragments attached to insoluble hydrophobic blocks were insoluble, so the generation of soluble fragments required at least two consecutive chain cuts. In contrast, short PCL fragments attached to hydrophilic PPDO are soluble after one chain cut.

Unsurprisingly, the degradation rate of the PCL segments decreased with increasing PPDL content. For very high PPDL content, degradation became so slow that insertion of enzymes into the monolayer could be observed as an expansion of the layer under isobaric conditions. Furthermore, by means of BAM, it was shown that the enzymes drastically altered the morphology of the hydrophobic PPDL layer upon insertion.

To construct complex molecular architectures like block-copolymers, often smaller building blocks are joined using urethane linkers. While it is frequently assumed that these linkers have no influence on the overall material properties due to their relatively low concentration, they were found to drastically hinder the enzymatic degradation of PCL segments.[46] This was attributed to the strong attractive intramolecular interaction mediated by these linkers, in agreement with higher packing densities when these linkers were present in PCL chains. Consequently, the linkers had a pronounced impact on the crystallization induced 2D->3D transition of PCL chains.[47] The 3D crystal morphology of PCL chains containing urethane linkers was markedly different from crystals formed by the homopolymer. Furthermore, compression-expansion cycles showed that both crystal formation and dissolution were significantly retarded by the linkers. Interestingly, no effect on the packing behavior and water content in Langmuir films was found when PLGA chains were segmented with urethane linkers. However, slower relaxation was observed.[48] The difference between PCL and PLGA may be explained by the different water content of the films. Since the PLGA monolayers contained a lot of water, the extent of hydrogen bonding between linkers was reduced,[49] especially when compared to PCL crystals. Similarly, due to the already high water content of non-segmented
PLGA Langmuir films, adding urethane linkers did not lead to an increase in the water content. In contrast, since the initial water content of bulk PLGA samples is low, water uptake and hydrolytic degradation are promoted by urethane linkers in PLGA films with millimeter thickness.[42]

Further challenges and perspectives

So far, the Langmuir-degradation technique has been applied solely to the group of hydrolytically degradable polyesters. However, many materials for medical applications are actually not intended to degrade, e.g. poly(methylmethacrylate) used for bone cement or polyurethanes used in catheters. Exploring the degradation behavior of a material that is considered biostable under physiological conditions with tests based on bulk samples takes considerable amounts of time. Here, the generally much shorter timeframes required for monolayer degradation experiments may prove a real advantage. However, for materials that are not intended to degrade, hydrolysis may not be the main pathway leading to biodegradation. For example, oxidative species were shown to attack polyurethanes in vivo.[8] Of course, for materials that are not intended to degrade, one cannot presume that all fragments are water-soluble and therefore, surface-sensitive spectroscopic techniques such as IRRAS and SFG may be required to detect the conversion of bonds. While these two powerful techniques are widely used to characterize Langmuir films, so far, they have not been applied in conjunction with Langmuir monolayer degradation experiments. We believe that by means of these techniques, Langmuir monolayers have the potential to enhance the understanding of the interplay between degradation and changes in morphology. A common example for such a relation is the often-observed increase in crystallinity when semicrystalline polymers are subjected to degradation. Both selective degradation of amorphous chains[50] and enhanced chain mobility due to chain scission[51] can contribute to this effect. Moreover, by preparing and degrading Langmuir films with different crystallinities, it should be possible to determine the degradation rate of both
crystalline and amorphous chains. Since the alteration of mechanical properties is a crucial aspect for degrading implant materials, it is important to understand and predict these changes leading to embrittlement of the material.

An experimental challenge associated with slowly degrading materials is the evaporation of water from the Langmuir trough. Lowering of the water level due to evaporation causes a reduction of the surface pressure as detected by the Wilhelmy plate. A linear decrease of the surface pressure reading due to water evaporation does not necessarily result in a linear area reduction. The compression required to compensate a given reduction of surface pressure depends on the slope of the isotherm according to \( \Delta A = \left(\frac{d\pi(A)}{dA}\right)^{-1} \Delta \pi \). While compressing the film to compensate for evaporation, the \( \pi(A) \) isotherm is scanned and \( \Delta A \) varies accordingly. \( \pi_0 \) should be chosen where the compressibility modulus has a broad maximum to minimize the effect. In this way, it is possible to conduct experiments lasting several hours without severe impact from water evaporation. For example, on a “medium” sized Langmuir trough (\( A \approx 200 \text{ cm}^2 \)) with a depth of 5 mm at room temperature, the surface pressure decreases by about 0.04 mN per 1000 s. During the experiment shown in Fig. 2, the surface pressure would have decreased by 2 mN/m, which is still within the broad compressibility modulus maximum of PCL \(^{52}\) and therefore, only a small and almost linear \( \Delta A(t) \) would have been added to the degradation curve. For experiments at physiological temperature, evaporation rates are of course much higher and such a broad maximum of the compressibility modulus does not exist for every polymer system. Increasing relative humidity to reduce the evaporation rate is not advisable because high humidity promotes condensation which in turn enhances contamination. Keeping the water level constant by monitoring the water level and replacing evaporated water is preferable. Self-made water level compensation systems have been used in the past.\(^{30}\) Recently, a level compensating system has become commercially available.
The bioresponse to materials is a field where the Langmuir monolayer degradation technique has the potential to bypass time consuming bulk experiments. When biodegradation alters the surface chemistry of a material, an impact on the bioresponse to the material is expected. This is especially relevant for shape-memory materials consisting of blocks displaying different degradation rates. Here, leaching of the fast degrading component leads to severe changes of the material composition. On a Langmuir trough, monolayers with a precisely defined degree of degradation can be prepared conveniently and rapidly. After Langmuir-Schäfer transfer to solid substrates, the bioresponse to materials prepared on a Langmuir trough can be investigated, e.g. in cell culture.[53] Another aspect related to the bioresponse to a biodegradable material is the nature of the fragments generated during the degradation process. Analyzing the subphase after or during a Langmuir monolayer degradation experiment using ultrasensitive mass-spectroscopy will provide a better understanding of the degradation mechanism and the degradation products. Here, the tiny amounts of material required to prepare a monolayer create a real challenge, as typical fragment concentrations are expected to be in the range of 0.1 \( \mu g/mL \).

The efficiency of biodegradable polymers in fulfilling their designated task strongly depends on their degradation profile. Quite often, the function of a polymeric material is to provide mechanical support. The mechanical properties of a polymeric material are strongly affected by the degradation process.[54] In analogy to the mechanical properties of partly degraded bulk samples, also the mechanical properties of partly degraded Langmuir films can be analyzed. However, the dilational elastic modulus of a polymeric Langmuir film, which can be measured easily by inducing barrier oscillations, is insensitive to molecular degradation. It was clearly shown that at any given surface pressure, the elastic modulus of a PCL Langmuir film is independent of molecular weight.[52] The factors determining elastic response differ fundamentally between two-dimensional and three dimensional polymer materials. At the point of highest compressibility modulus, the packing density of PCL chains in a Langmuir
monolayer is similar to the packing density of chains in PCL crystal planes.\cite{47} For the hypothetic graphene 3D material, the Young’s modulus was derived by dividing the Young’s modulus of the 2D material by the distance between the sheets in graphite.\cite{55} When doing the same extrapolation with the maximum compressibility modulus of PCL Langmuir monolayers and the distance between crystal planes in PCL (ca. 2.5 Å), one arrives at 56 MPa, which is about 8 times smaller than the Young’s modulus of bulk PCL. Probably, even at high packing density, a PCL monolayer behaves more like a semidilute solution than a bulk material. One has to keep in mind that overlapping and entanglement are not possible in two dimensions. Therefore, interfacial shear rheology may prove superior to dilational rheology when it comes to detecting alterations of the monolayer mechanical properties. However, extrapolating monolayer mechanics to bulk properties will still require a substantial theoretical contribution. Additional theoretical work is also required to enhance the models used to analyze the area reduction curves. As can be seen in Fig.3, the equations derived by Ivanova and Grotzev already provide an excellent description of homopolymers undergoing random fragmentation. They should therefore serve as starting point to describe the fragmentation of more complex molecules, e.g. block copolymers, star shaped polymers or end-capped macromolecules. Furthermore, refining their equations to account for the insertion of enzymes and the different areal requirements of chemical bonds before and after hydrolysis may provide an even better description of the area reduction curves. In contrast, a model describing chain-end scission needs to be developed from the ground up. The area reduction curves of polylactides are clearly neither described by random fragmentation nor by a zero order process. A treatment combining chain-end scission, random scission and chain dissolution may deliver a more appropriate description of reality. While it has not been done in the literature cited above, we note that any appropriate model of the fragmentation and dissolution kinetics of a polymer monolayer also affords a prediction of the evolution of the average molecular weight of the chains.
When combining the kinetic models and data derived from Langmuir monolayer experiments with water uptake measurements, a first approximation of the time a material persists under given conditions can be obtained. However, to assist the development of biodegradable implants, a predictive tool that can calculate such important aspects as the evolution of mechanical properties and the fragment release kinetics on the device scale is required. Due to the local variation of material parameters in a real world object and the multitude of involved processes at different timescales, a description of the degradation of a macroscopic object requires much more extensive calculations than the analysis of Langmuir monolayer degradation curves.

To predict the degradation of medical devices, we suggest a knowledge based approach\cite{20} combining fast and straightforward experiments with multiscale computational simulations. On the molecular scale, bond scission rate constants can be obtained using quantum chemical calculations. Since kinetic parameters can also be determined experimentally in Langmuir monolayer degradation experiments, the calculated reaction rates can be verified experimentally. In fact, the calculation of reaction rate constants can be bypassed using Langmuir monolayer degradation experiments, which have the advantage that the size dependent fragment solubility is directly included in the experimental data.

To simulate the degradation of the material on the micrometer scale, a “digital twin” of the inhomogeneous material is required. Here, we suggest a cellular automaton approach\cite{56} In a three dimensional, discrete lattice (see Fig. 4), every volume element is assigned an initial state, i.e. amorphous, crystalline or pore. The distribution of the different phases in the material can be estimated based on methods for microstructure analysis like X-ray microtomography, scanning electron microscopy and small angle X-ray scattering. To model the diffusion of water through the virtual material, diffusion rate constants need to be known. As the crystalline parts of the polymer can be considered impenetrable for small molecules such as water,\cite{57} only the diffusion coefficients for the amorphous domains are needed. These are sometimes available
from the literature or can be determined using e.g. experimental methods\cite{58, 59} or molecular dynamics simulations.\cite{20} With the water concentration and the reaction rate constants of both amorphous and crystalline domains, the time dependent mass density and average number molecular weight can be calculated for each volume element of the simulation (Fig.4). Then, the local yield strength can be inferred from the molecular weight. To determine fragment release kinetics, their size dependent diffusion rate constants need to be determined. This material property is certainly difficult to access and may require molecular dynamics simulations.

On the macroscopic scale, the behavior of the implant device under load can be described by Finite Element Method (FEM) analysis,\cite{60} where the dynamic material properties of each volume element are determined in the digital twin. Thus, by combining computational models

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure4.png}
\caption{Contribution of Langmuir monolayer degradation experiments to a lifetime prediction model of medical implants. A digital twin of the material is constructed according to a microstructure analysis. The molecular reaction kinetics and rate constants are determined by Langmuir monolayer degradation experiments. The diffusion coefficient of water in the amorphous domains of the polymer is measured in sorption experiments. Combining water transport and molecular reaction kinetics facilitates a calculation the molecular weight and the resulting mechanical properties of the digital twin. These can then be used for an FEM simulation of the real object. FEM simulation figure partly adapted with permission under the terms of the CC BY 4.0 license from \cite{60}. Copyright 2014 PLoS}
\end{figure}
on the microscopic and macroscopic scale with fast and straightforward measurements, a predictive tool for the design of biodegradable implants can be created.

Conclusions

Langmuir monolayers are a versatile tool when it comes to mimicking and understanding the processes happening at the interfaces of polymers used as implant materials. However, so far, they have mainly been used to study the hydrolysis kinetics of biodegradable polyesters. More advanced theoretical models and instrumental techniques will allow for expanding their scope towards other aspects of biodegradation and more complex molecular architectures. Transferring the results from these 2D model systems to bulk polymers can be straightforward in the case of surface eroding materials but will require complementary measurements and computer simulations in the case of bulk degrading materials. A deeper understanding and predictability of polymer degradation in biological environments is not only required with respect to medical biomaterials, but also regarding the growing interest in biodegradable polymer materials to combat environmental pollution.

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Conflict of Interest

The authors declare no conflict of interest.

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