JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY
Article
pubs.acs.org/JACS

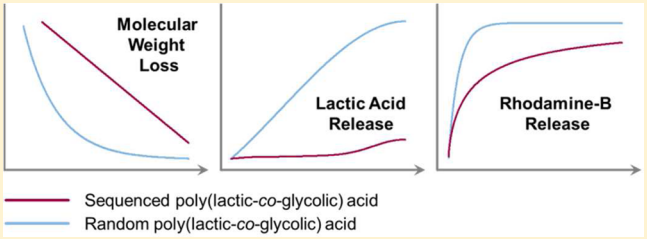
The Effect of Monomer Order on the Hydrolysis of Biodegradable Poly(lactic-*co*-glycolic acid) Repeating Sequence Copolymers

Jian Li,[†] Sam N. Rothstein,[‡,⊥] Steven R. Little,[‡,§,⊥] Harry M. Edenborn,[∥] and Tara Y. Meyer*[,†,⊥]

[†]Department of Chemistry, University of Pittsburgh, Pittsburgh Pennsylvania 15260, United States
[‡]Department of Chemical Engineering, University of Pittsburgh, Pittsburgh Pennsylvania 15261, United States
[§]Department of Bioengineering, Department of Immunology, University of Pittsburgh, Pittsburgh Pennsylvania 15261, United States
[⊥]McGowan Institute for Regenerative Medicine, University of Pittsburgh, Pennsylvania 15219, United States
[∥]National Energy Technology Laboratory, U.S. Department of Energy, 626 Cochrans Mill Road, Pittsburgh, Pennsylvania 15236, United States

Supporting Information

ABSTRACT: The effect of sequence on copolymer properties is rarely studied despite the precedent from Nature that monomer order can create materials of significant diversity. Poly(lactic-*co*-glycolic acid) (PLGA), one of the most important biodegradable copolymers, is widely used in an unsequenced, random form for both drug delivery microparticles and tissue engineering matrices. Sequenced PLGA copolymers have been synthesized and fabricated into microparticles to study how their hydrolysis rates compare to those of random copolymers. Sequenced PLGA microparticles were found to degrade at slower, and often more constant, rates than random copolymers with the same lactic to glycolic acid ratios as demonstrated by molecular weight decrease, lactic acid release, and thermal property analyses. The impact of copolymer sequence on *in vitro* release was studied using PLGA microparticles loaded with model agent rhodamine-B. These assays established that copolymer sequence affects the rate of release and that a more gradual burst release can be achieved using sequenced copolymers compared to a random control.



INTRODUCTION

Copolymers in nature achieve controlled structure, catalytic function, and most relevant to this article, tailored materials properties through the sequential arrangement of a relatively limited set of monomers.[1] Spider silk, which combines strength, elasticity, and adhesive properties, is an excellent example of the power of sequence to regulate material characteristics.[2−4] It has been found that naturally occurring silks consist of crystalline segments, e.g., $(GlyAlaGlyAlaGlySer)_n$, that alternate with amorphous segments bearing amino acids with bulky side groups.[5] The ordered sequences are believed to be key to the formation of antiparallel β-sheets that act as temporary cross-links and lend silk its strength. Synthetic analogues have been prepared using simplified sequences. Rathore and Sogah, for example, reported that multiblock copolymers in which $(Ala)_{4,6}$ or (AlaGlyAlaGly) alternate with short PEG oligomers gave the desired β-sheet structures.[2] Tirrell and co-workers have also prepared related "periodic polypeptides".[6−8] Elastin and collagen are other examples of biopolymer materials whose properties can be traced to specific sequences and which have inspired synthetic analogues.[9,10]

Notwithstanding Nature's lessons on the power of sequence, there are relatively few examples of synthetic copolymers prepared with high degrees of exact sequence control (Figure 1).[11−30] Proteins, such as spider silk, of course are complex in all three of the dimensions of microstructure control: composition, tacticity, and structural sequence. In contrast, most synthetic copolymers are relatively simple in one or more of these dimensions. Even polymers that have well-controlled microstructures, such as syndiotactic poly(lactic acid),[31] do not exhibit a "high" structural complexity overall since they comprise only a single monomer. In the rare cases where structural complexity is high, there is often a dearth of homologous examples from which sequence−property correlations can be generated. Outside of peptide-based materials, the connection between sequence and properties is perhaps the most developed in the study of oligomeric foldamers.[32]

Poly(lactic-*co*-glycolic acid)s (PLGAs) are a class of polymers for which the benefits of sequence control can easily be envisioned. PLGA is the archetypal biodegradable copolymer as it is available from renewable resources, degradable without requiring specific enzymes, and nontoxic both as a polymer and as hydrolyzed monomers.[33,34] Random, nonsequenced PLGA is a key component of FDA-approved microparticle formulations that control drug release as well as degradable surgical sutures and implantable devices that are resorbed by the body.[35] In these applications, the rates of implant resorption and/or drug

Received: July 13, 2012
Published: September 5, 2012

   dx.doi.org/10.1021/ja306866w | J. Am. Chem. Soc. 2012, 134, 16352−16359

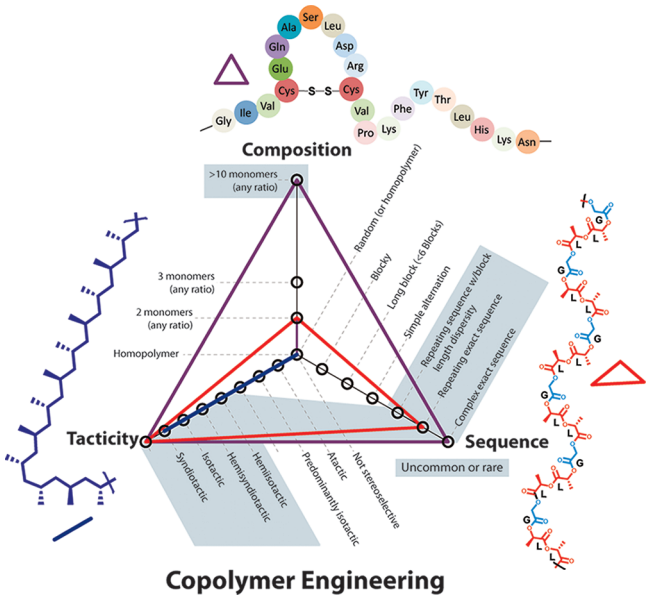


Figure 1. Radar plot of copolymer characteristics: composition (vertical axis), sequence (right axis), and stereochemical control (left axis). Repeating sequence exact tacticity PLGAs (red) exhibit levels of sequence and stereochemical control near to those expressed in proteins (gray). Related synthetic polymers are plotted for comparison.

release both depend heavily on PLGA's degradation rate. Historically, control over PLGA's degradation profile has been afforded by altering the L:G ratio, controlling molecular weight, and/or changing the ratio of racemic to stereopure lactic units.[36] Altering copolymer sequence from random to ordered may provide a new avenue for controlling degradation rate. However, prior to our work, control of L and G sequencing has only been demonstrated by Rebert[37] and Dong et al.,[38−40] who produced the alternating copolymer by a condensation strategy and a ring-opening strategy, respectively.

We have recently developed a new synthetic route that yields PLGAs of any targeted repeat sequence and have reported the sequence-specific effects on the NMR spectroscopy, solution-phase conformations, and thermal properties of these copolymers.[26] We have similarly explored the effect of sequence using polymers composed of glycolic, lactic and caprolactic units and have prepared PLGA-type copolymers bearing periodically spaced functional side groups.[27,30] Herein, we report in detail on the effects of monomer sequence on the degradation of PLGA copolymer microparticles. As degradation is a property central to drug-delivery applications, we also explore the effect of sequence on the encapsulation and release of a model small-molecule agent from PLGA micro-particles.[41,42]

SYNTHETIC APPROACH TO SEQUENCED COPOLYMERS

Although the preparation of the highly sequenced PLGA copolymers that are the focus of this study has been reported by our group previously,[26] the importance of the microstructure to the degradation studies presented herein necessitates a brief discussion of the synthesis. All of the exactly sequenced polymers in this study were prepared by segmer assembly polymerization (SAP), an approach that entails the step-growth polymerization of exact sequenced segmers (Figure 2). We use the term "segmers" rather than oligomers or macromonomers to emphasize the fact that they are monodisperse units that bear end-groups that allow for subsequent polymerization. Using a SAP strategy, it is possible to encode sequences of modest length—we routinely prepare segmers comprising 2−8 monomers—to produce repeating sequence copolymers (RSCs). Although the SAP approach does not allow for molecular weight control, the reaction conditions employed routinely produce materials with molecular weights of 15−40 kDa. The PLGA RSCs discussed in this paper were produced by the coupling of orthogonally protected lactic and glycolic acids to form segmers, followed by a di-isopropyl carbodiimide (DIC)-mediated condensation polymerization.

Although PLGA copolymers and the homopolymers of lactic acid (PLAs) are more commonly prepared by the ring-opening polymerization (ROP) of lactides and glycolides, the sequence complexity is limited relative to the SAP approach. The ROP strategy is a subset of chain polymerizations that can, under certain conditions, be "programmed" using catalyst design and monomer reactivities to give sequenced microstructures. Indeed, the elegant work by key researchers who have used this approach in preparing polyolefins with controlled tacticities,[12] serves as an inspiration for our interest in probing the role of sequence to a greater depth. Fundamentally,

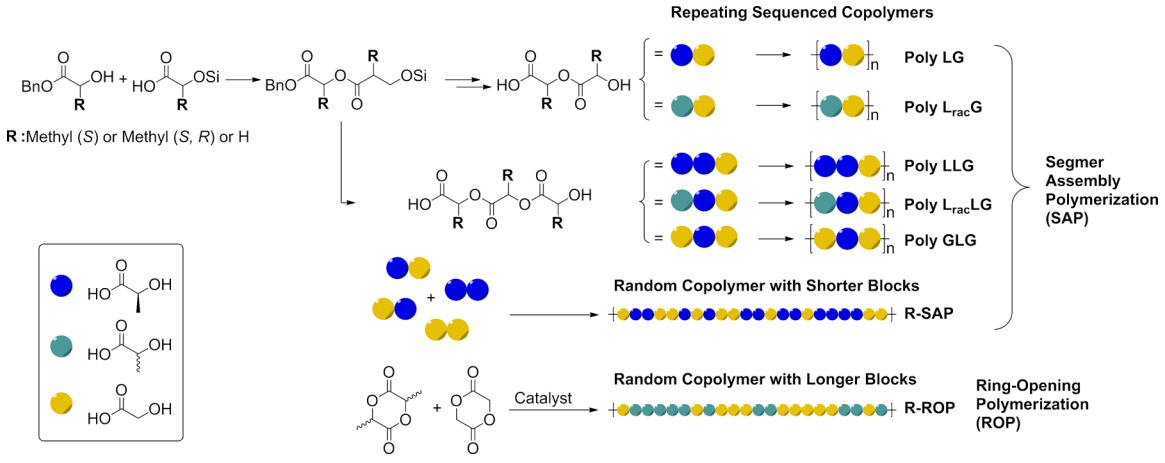


Figure 2. (Top) Segmer assembly polymerization (SAP) used to prepare repeating sequence copolymers and a random copolymer of poly(lactic-*co*-glycolic acid). (Bottom) Ring-opening polymerization (ROP) used to prepare a random copolymer.

dx.doi.org/10.1021/ja306866w | *J. Am. Chem. Soc.* 2012, 134, 16352−16359

however, the "programmed" approach is limited with few exceptions to the alternation of two monomers.[43] In PLA, for example, where there are two lactic stereoisomers, there have been many reports of ROP-prepared polymers with controlled tacticity.[31] However, sequenced PLGAs, with the exception of the simple alternating copolymer,[37,38] cannot be prepared using ROP because no catalytic system exists that can create complex patterns of three monomers. ROP is the preferred method, however, for producing PLGA random copolymers, and nearly all commercially available PLGAs are prepared in this fashion.

Two other approaches, templated synthesis and monomer-by-monomer construction, can produce sequenced copolymers,[13,24] but neither offers the versatility of SAP, which can be applied to a wide variety of monomers and can be scaled up. In nature, of course, the template strategy is used to synthesize biopolymers. Chemists have exploited this mechanism to selectively prepare sequenced materials, both natural and synthetic. To date, however, there are only a few examples of synthetic templates prepared *de novo* for nonbiological monomers.[13,14] Monomer-by-monomer synthesis, which is the basis of commercial peptide synthesizers,[44] can also be used to prepare complex exact sequences. The applicability of this approach to materials is limited, however, because the method does not scale up well and there are practical restrictions on the degree of polymerization that can be achieved due to the iterative nature of the method.

RESULTS

Naming Conventions. The L-lactic unit, *rac*-lactic unit, and glycolic unit are abbreviated as **L**, **L**$_{rac}$, and **G**, respectively. Repeating sequenced PLGA copolymers prepared by SAP are named by listing the order of segmer sequences from the C-side to the O-side preceded by the prefix **poly**. Therefore, **poly L**$_{rac}$**LG** is the polymer prepared from the segmer with a sequence of *rac*-lactic acid, L-lactic acid, and glycolic acid. The random PLGAs are named with prefix **R** followed by the preparation method (SAP or ROP) and the percent of lactic units present. Thus, the **R-SAP 50** is the random PLGA prepared by SAP with 50% **L** units. **R-ROP 50** and **R-ROP 75,** which were used as the controls, are two commercial PLGAs named on the basis of the lactic unit percentage.

Synthesis. A series of PLGA RSCs and one random copolymer, **R-SAP**, were prepared as described previously using the SAP methodology (Figure 2).[26,42,45] Characterization data for these polymers and two commercially purchased random copolymers prepared using a ROP method, **R-ROP 50** and **R-ROP 75**, are summarized in Table 1. Molecular weights were determined by size exclusion chromatography (SEC) in THF and are reported relative to polystyrene standards. As the data analysis in the current study primarily involves comparing the relative molecular weights of samples of the same polymer, absolute molecular weight data were not required. We note, however, that a previous analysis of absolute molecular weight for this class of RSC PLGAs suggests that the SEC molecular weights represent an overestimate of the true molecular weight, and that the correlation depends heavily on sequence.[26]

Table 1. PLGA Copolymer Properties

	M_n (kDa)[a]	M_w (kDa)	PDI	L:G
R-ROP 50	32	43	1.3	1:1
R-SAP 50	31	40	1.3	1:1
R-ROP 75	55	66	1.2	3:1
poly LG (26 k)	26	35	1.3	1:1
poly LG (16 k)	16	25	1.6	1:1
poly L$_{rac}$G	49	103	2.1	1:1
poly LLG	33	54	1.6	2:1
poly L$_{rac}$LG	35	46	1.3	2:1
poly GLG	16	22	1.3	1:2

[a]Molecular weights determined by SEC in THF vs polystyrene standards.

Hydrolysis Profiles. To explore the dependence of hydrolytic degradation behavior on the sequence of PLGA copolymers, a series of sequenced PLGAs and three random PLGAs were selected for an *in vitro* hydrolytic degradation study. As it is common to use PLGA microparticles as hosts for drug delivery and many hydrolysis studies have been conducted on the random PLGA copolymers formulated thusly,[46] we chose in these initial studies to monitor the hydrolysis behavior of the PLGA RSCs using this widely practiced protocol. The copolymers were formulated into microparticles with sizes ranging from 2 to 5 μm using a standard emulsion method (See Table S1, Supporting Information [SI], for particle characterization).[47] This narrow particle size range was purposely targeted to minimize the impact of size-dependent autocatalysis on PLGA degradation.[48,49] Microparticles of each polymer were divided into multiple parallel reaction vessels and suspended in a phosphate buffer (pH 7.4, 37 °C). The supernatant liquid in each was exchanged every two days and retained for analysis of lactic acid content (*vide supra*). The contents of individual reaction vessels were harvested periodically, over the course of 8 weeks, and analyzed by SEC. Selected samples were also characterized by differential scanning calorimetry (DSC).

The molecular weight profiles for all polymers in this study, normalized relative to the original M_n for each sample, are plotted in Figure 3 (See SI, Figure S2, for non-normalized data). Before examining individual trends, it is important to note that, for the majority of the samples, there was an initial molecular weight loss that is dramatic relative to the midcycle degradation behavior. This initial loss appeared to correlate primarily with the "wetting" of the freeze-dried particles during their first few days in the buffer solution. In this initial phase the shedding of surface coatings that are not well adhered and/or rapid cleavage of surface bonds that are particularly accessible is expected.[50] Consistent with this analysis is the fact that initial rapid loss of weight was seen for both the sequenced and random copolymers. The degree of weight loss in this initial phase appeared to depend in a complex fashion on sequence, polymer molecular weight, and particle size. For polymers that have either very rapid or very slow hydrolysis rates, this effect was masked or minimized, respectively.

The rate of hydrolysis, after the initial weight loss, did not seem to depend significantly on the initial molecular weight. In Figure 3a the hydrolysis profiles of all samples with a 1:1 L:G are plotted. For **poly LG(26k)**, it can be seen that, after an initial weight loss of 35%, the rate of weight loss decreased and remained nearly constant to the end of the experiment. **Poly LG(16k)** exhibited a very similar profile: an initial weight loss of 20% followed by more gradual decrease as a function of time. While there is a difference in the relative weight losses at the beginning, the rate after the initial loss appeared to be relatively independent of the starting molecular weight.

The *most important trend* that can be observed in the hydrolysis studies is that the sequenced copolymers degrade more slowly and at a more constant rate relative to the random

dx.doi.org/10.1021/ja306866w | J. Am. Chem. Soc. 2012, 134, 16352−16359

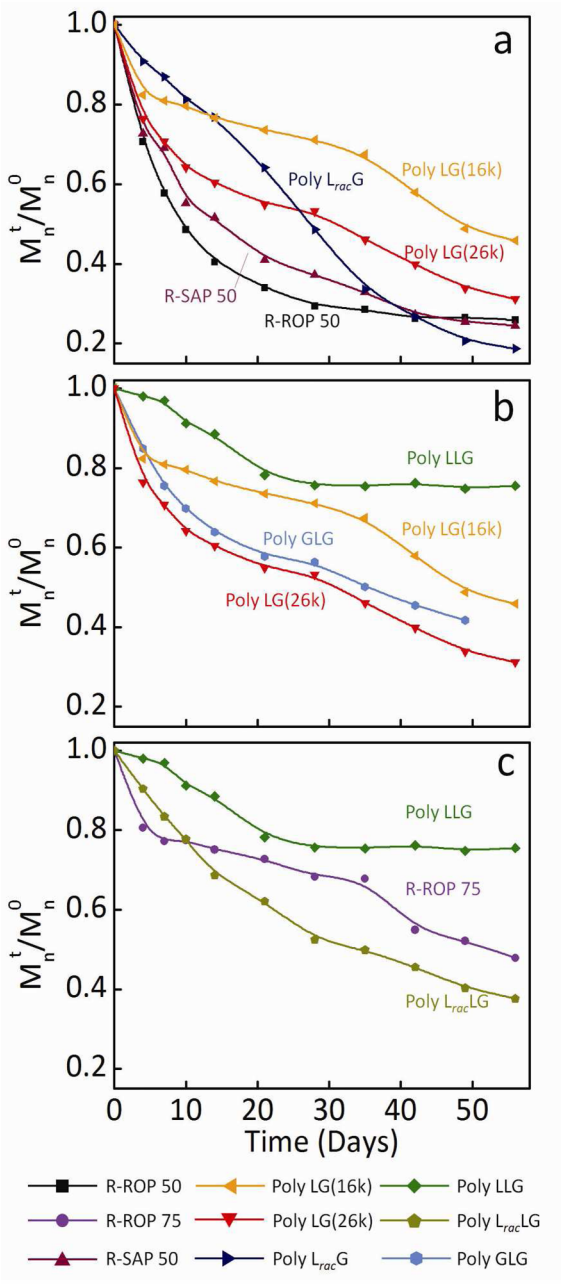


Figure 3. Molecular weight loss as a function of hydrolytic degradation time for the repeating sequenced and random sequenced PLGAs: (a) comparison of all polymers with a 50:50 LG ratio; (b) comparison of polymers with varying L:G ratios; (c) comparison of LLG polymers with varying stereochemistries.

copolymers with the same L:G composition. The 1:1 L:G random copolymers, **R-ROP 50** and **R-SAP 50**, both have exponential weight loss profiles, as shown previously.[51] Interestingly, the **R-ROP 50** (M_n half-life =10 days) copolymer degraded more quickly than the **R-SAP 50** copolymer (M_n half-life =14 days). This difference can be attributed to both the lack of controlled stereochemistry of the racemic **R-ROP 50** copolymer and the differences in microstructure since the **R-ROP 50** copolymer, which was prepared by ring-opening of a mixture of lactide and glycolide monomers, has shorter G and L blocks than the **R-SAP 50** copolymer.[42,52] The relatively high rate of hydrolysis made it impossible to document the presence or absence of an initial rapid degradation period.

The distinctive differences in degradation between the sequenced and random copolymers can also be observed in the shapes of the SEC traces from which the MW data were extracted. As seen in Figure 4, for example, the molecular

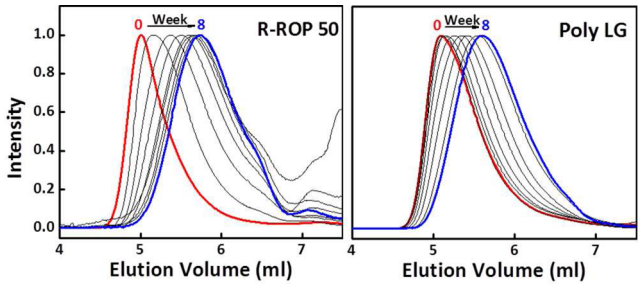


Figure 4. SEC traces for random copolymer (left) and alternating sequence PLGA copolymers (right) with 1:1 ratios of lactic and glycolic acids.

weight profile of the random copolymer broadened and became distinctly polymodal over the course of the degradation, while the profile of the sequenced **poly LG** exhibited only a small amount of broadening. All sequenced copolymers exhibited a similar homogeneity in their evolving SEC traces.

We also examined the relationship of degradation rate to the ratio of L:G in the polymers. It is well-established for random copolymers that the degradation rate depends on the L:G ratio; high lactic unit content leads to slower hydrolysis rates.[53] Examining the subset of hydrolysis profiles plotted in Figure 3b, it can be seen that for the random controls, **R-ROP 50** and **R-ROP 75**, this trend held. Their M_n half-lives were 10 and 56 days, respectively. The sequenced copolymers also conform to this trend: **poly GLG** > **poly LG(16k)** > **poly LLG**. The comparison of the **poly GLG** to **poly LG(16k)** is used because the **poly GLG** sample studied had a similarly modest molecular weight.

Finally, the importance of stereosequence can be seen in the hydrolysis behavior of the sequenced copolymers. Hydrolysis was significantly faster for the racemic analogues of the 1:1 and 2:1 L:G polymers, **poly L_{rac}G** and **poly L_{rac}LG**, relative to their stereopure analogues (a and c of Figure 3).

Lactic Acid Release. The degradation rate of the PLGA microparticles was also studied by monitoring the release of lactic acid over time. The sequence dependence of the degradation can be clearly seen in these data. Monitoring of the monomer release yielded a degradation profile that is complementary to that obtained by the analysis of the molecular weights as discussed in the previous section. The release of L-lactic acid into the buffer solution was assayed using an enzymatic method.[54,55] It should be emphasized that the assay reports only monomer and is unresponsive to oligomeric species.

Cumulative release of lactic acid over time for both the sequenced and random copolymers is plotted in Figure 5. Consistent with the observed rapid degradation of molecular weight discussed above, **R-ROP 50** exhibited the most rapid release of lactic acid. All other samples were significantly slower, including the other 1:1 L:G random copolymer, **R-SAP 50**, and all except the **R-ROP 50** exhibited a profile characterized by an initial quick release of a small amount of the lactic monomer followed by an extended period during which little free lactic

dx.doi.org/10.1021/ja306866w | J. Am. Chem. Soc. 2012, 134, 16352−16359

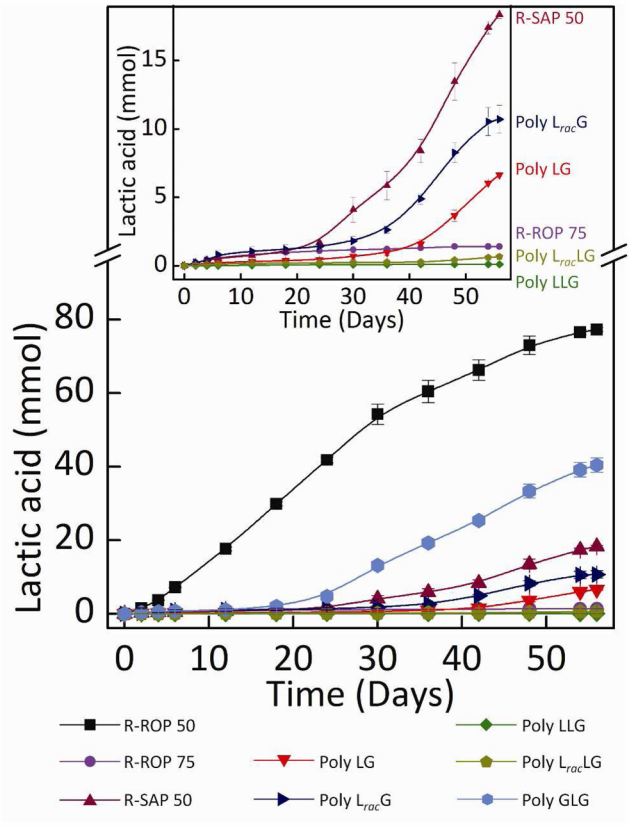


Figure 5. Lactic acid release rate as a function of hydrolytic degradation time. Inset shows the magnified plots of PLGAs with lower lactic acid release rates.

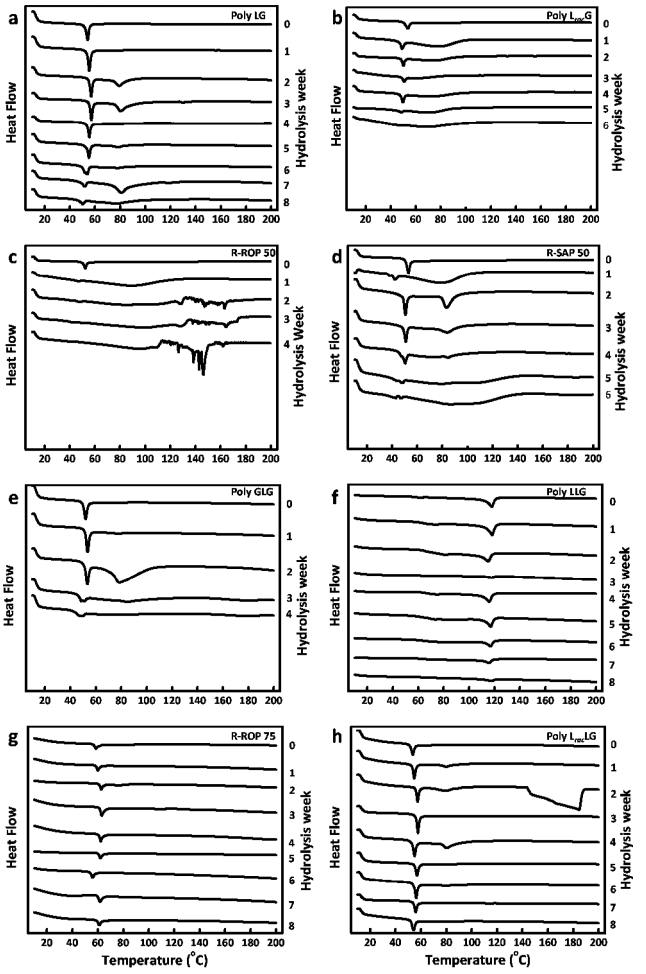


Figure 6. DSC thermograms of PLGAs in the hydrolysis study. (a) **Poly LG**; (b) **Poly L$_{rac}$G**; (c) **R-ROP 50**; (d) **R-SAP 50**; (e) **Poly GLG**; (f) **Poly LLG**; (g) **R-ROP** 75; (h) **Poly L$_{rac}$LG** (large feature at >160 °C week 2 is not related to the sample). For rapidly degrading samples, DSCs could not be obtained for the entire 8-week-period due to the decrease in material available.

acid release was detected. The initial release is likely related to either the wetting of the particle and subsequent dissolution of lightly trapped monomer or the rapid hydrolysis of very short oligomers on or near the surface of the particle. The low release period that followed is not a dormant period, as we know from the molecular weight data, but rather corresponds to a time when the polymers were partly hydrolyzed to oligomers that do not register in the enzymatic assay. Only in the later stages of hydrolysis would these oligomers be expected to degrade to monomeric lactic acid.

Significant sequence-dependent trends were observed in these data, and most corresponded well with those observed in the molecular weight studies. In particular, for the 1:1 LG polymers the release rate followed the following trend: **R-ROP 50** ≫ **R-SAP 50** > **poly LG** (Figure 5, see inset for expansion of the profiles for the more slowly degrading polymers). The random copolymer, **R-ROP 50**, was much faster than the less blocky **R-SAP 50**, and both were faster than the stereopure alternating **poly LG**. There was also a pronounced dependence of lactic acid release rate on L:G ratio, with a trend, **poly GLG** ≫ **poly LG** > **poly LLG**, that runs contrary to what would be expected based on the total L content (given that this is an assay for L). The differences in total lactic acid release are significant over the time period studied: 40 mmol from **poly GLG** vs 0.1 mmol for **poly LLG**. Finally, the rate of release of lactic acid was faster for the racemic sequenced copolymers **poly L$_{rac}$G** > **poly LG** and **poly L$_{rac}$LG** > **poly LLG.**

Thermal Properties. As there is a complex but important relationship between the thermal properties of PLGAs and their degradation and release behaviors,[56] DSC thermograms for selected sequenced and random PLGAs were acquired (Figure 6). The first heating cycle is reported to reflect the *in situ* thermal properties of the microparticles after hydrolysis (although it is understood that the T_g's of the immersed particles would be lower).[57] The DSCs of the polymers that have a 1:1 L:G ratio, showed a single phase transition, $T_g \cong 50$ °C, at the beginning of the experiment. The most dramatic difference can be seen in the comparison of the thermograms for **poly LG(26k)** and **R-ROP 50**. The random sample degraded so quickly that reliable DSC data could not be acquired after week 4, while those of **poly LG(26k)** continued to exhibit clear transitions. There was also a distinctive difference in the transitions exhibited. The DSC trace for **poly LG(26k)** appeared nearly the same in week 8 as it did prior to hydrolysis. The T_g shifted slightly to lower temperature and broadened, consistent with the drop in molecular weight, and there was a new broad peak at ~80 °C that is likely due to the melting of small amounts of crystalline oligomers.[42] In contrast, a clear T_g for **R-ROP 50** was no longer visible by the end of week 1, and the DSC traces were dominated by multiple melting transitions ranging from 80 to 160 °C that have been shown in prior studies to be due to crystallized oligomers with a high lactic acid content.[48]

dx.doi.org/10.1021/ja306866w | J. Am. Chem. Soc. 2012, 134, 16352−16359

Poly $L_{rac}G$, although faster to degrade, followed the same pattern as **poly LG**, exhibiting a clean but slightly shifted T_g until the samples could no longer by analyzed due to low molecular weight. The other random copolymer, **R-SAP 50** exhibited a behavior intermediate between the **poly LG** and **R-ROP 50** polymers as would be expected from its less blocky nature. The T_g of the bulk remained distinct, but a broad melting transition which is lower in temperature than that of **R-ROP 50** dominated after week 5. **Poly GLG**, which has a slightly lower T_g of ~52 °C, exhibited the same uniformity of degradation as that seen for the sequenced LG polymers.

The DSCs for the higher L:G content polymers showed that the RSC **poly LLG** was semicrystalline with a T_g of 60 °C and T_m of 118 °C. **Poly L_{rac}LG** and **R-ROP 75**, in contrast, exhibited only T_g transitions of 53 and 58 °C, respectively. None of these polymers changed significantly over the time period of the experiment, however, since their degradation is quite slow.

Rhodamine-B Release. As one of the primary applications of PLGA is drug delivery,[41] we have examined the effect of sequence on the release rate for microparticles loaded with rhodamine-B (RhB), a low-molecular weight hydrophilic dye used as a model in *in vitro* drug-delivery studies because of its water solubility and characteristic absorbance at 556 nm.[58] RhB was incorporated into microparticles via a double emulsion method.[47] RhB loading was estimated by dissolving a weighed portion of each sample in a known volume of acetonitrile and comparing the UV−vis absorption with a calibration curve created from solutions of RhB of known concentration. This method gave more precise and reproducible results than a low pH digestion of the particles in water[59] as we observed that the absorption intensity of acidic RhB solutions decreased rapidly with time. The polymers selected for this study were **poly LG**, **poly $L_{rac}G$**, and **R-ROP 50** (Table 2, top section). These polymers had comparable but higher molecular weights than those used in the hydrolysis studies but were consistent relative to each other.

Table 2. PLGA Polymer Microparticle Properties

polymer	M_n (kDa)	PDI[a]	RhB (mg)	loading[b] (mg × 10^{-4} per mg)	loading efficiency (%)
LG-RhB1	37.2	1.4	0.2	1.9	19
LG-RhB2	37.2	1.2	0.6	2.2	7.5
LG-RhB3	37.2	1.4	1.0	3.4	5.9
R-ROP 50-RhB1	32.0	1.3	0.2	4.0	40
LG-RhB4	37.2	1.4	1.0	2.7	5.3
L_{rac}G-RhB	38.2	1.4	1.0	2.8	5.6
R-ROP 50-RhB2	32.0	1.3	0.1	2.9	60
R-ROP 50-RhB3	32.0	1.3	1.0	5.9	11

[a]Molecular weights and polydispersity indices determined by SEC in THF vs polystyrene standards. [b]Based on 200 mg polymer sample size, calculated by the mass of RhB loaded per 1 mg of microparticles.

To study the relative loading capacities of the sequenced copolymer and plan for subsequent *in vitro* release studies, the **poly LG** particles were prepared with different initial RhB concentrations. Specifically, the concentration of the RhB solution was adjusted from 0.2 to 1.0 mg/mL. The release of RhB from the resulting particles was analyzed over a period of 18 days and the data are plotted in Figure 7a. These data were derived from a set of samples prepared and handled under identical conditions to ensure that any effect from photobleaching was systematic and did not affect the following comparisons. The random copolymer reached its maximum cumulative release of approximately 90% after only 9 days while all of the sequenced samples released their payload at a lower rate. Of particular interest, however, was the dramatic difference in loading efficiency for the two types of polymers. When identical amounts of RhB were used in the particle formulation/loading procedure (0.2 mg), the random copolymer particles encapsulate 40% of RhB whereas the alternating copolymer **poly LG-RhB1** exhibited a loading efficiency of 19%. Much larger concentrations of RhB were necessary to attain loadings of the dye into **poly LG** that were comparable to those achieved in the random copolymer.

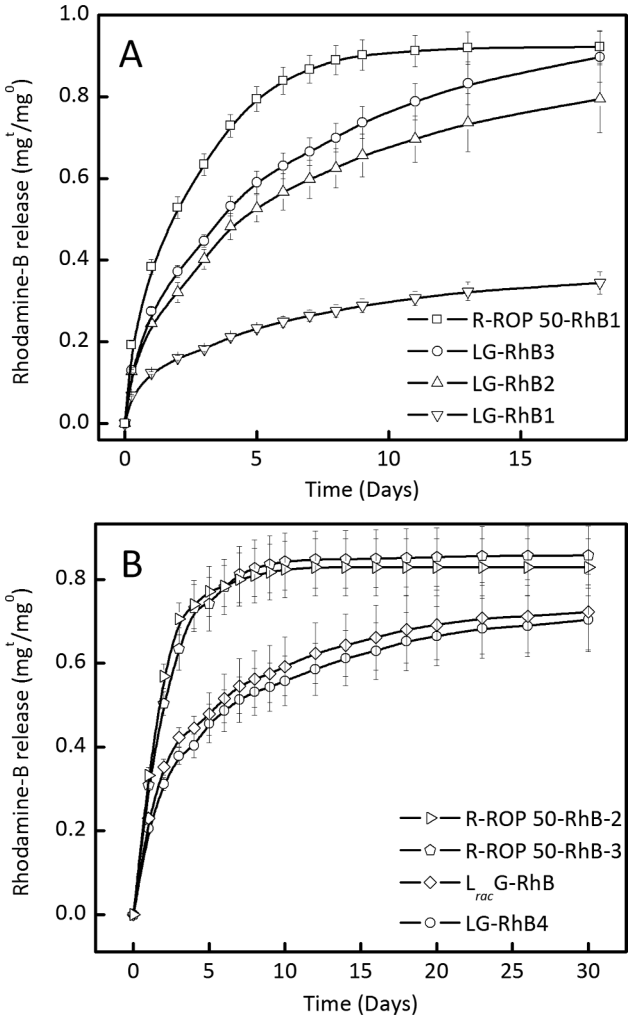


Figure 7. Release of RhB from PLGA microparticles immersed in a pH 7.4 buffer at 37 °C: (A) 18-day study focusing on particle loading; (B) 30-day study focusing on sequence dependence of release. Error bar showing standard deviation is obtained from four replicates.

On the basis of the data collected from the loading efficiency experiments, microparticles of both sequenced and random copolymers with the same RhB loading were produced. A new study of the release rates was conducted, and the results are plotted In Figure 7b. **Poly LG**, **poly $L_{rac}G$**, and the random copolymer, **R-ROP 50-RhB2**, all have similar loadings while **R-ROP 50-RhB3** was prepared with a much higher RhB load.

The key result from this study is that RhB release was significantly more gradual for both of the sequenced

dx.doi.org/10.1021/ja306866w | J. Am. Chem. Soc. 2012, 134, 16352−16359

copolymers when compared to the random copolymers. This trend held despite significant differences in the **poly $L_{rac}G$** and **poly LG** degradation rates between days 0 and 20 (*vide supra*). Additionally, random copolymers gave nearly the same release profile despite the differences in loading, in contrast to the behavior of **poly LG** in Figure 7a. Along with the differences in loading efficiency (Table 2), these results suggest that **RhB** release from sequenced copolymer particles depends not only on hydrolysis rate, but also on other factors.

DISCUSSION

The introduction of sequence control to the PLGA system changes the hydrolysis pattern significantly relative to random analogues. Both molecular weight loss and lactic acid release measurements establish that the sequenced copolymers degrade at a steady rate which contrasts with the rapid, exponential profile exhibited by the random copolymers with similar L:G ratios. The differences are likely attributable to the homogeneity of the sequenced copolymers. It has been observed by others who have studied the degradation of random PLGA copolymers that hydrolysis of the more sterically accessible glycolic units is rapid relative to lactic-rich blocks.[60] In Figure 8

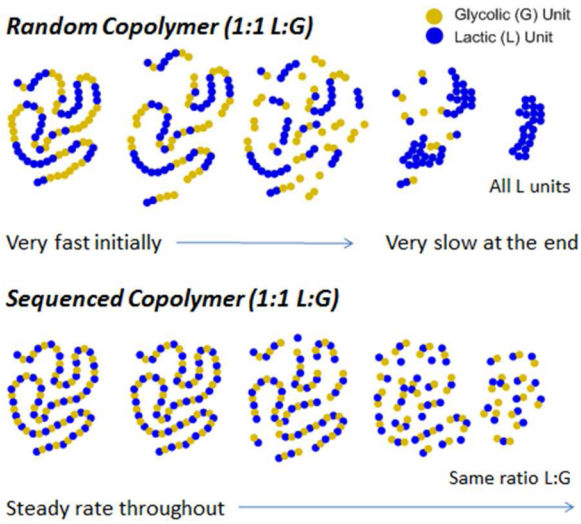


Figure 8. Proposed difference in hydrolysis pattern for random and sequenced PLGA copolymers with the same L:G ratio.

a conceptual comparison is made between the simple alternating copolymer **poly LG** and **R-ROP 50**. The sequenced copolymer should break down evenly as hydrolysis proceeds, while the random copolymer can be selectively attacked in such a way that slowly degrading lactic oligomers are left to crystallize. Both the SEC and DSC data for the 1:1 copolymers are consistent with this model.

The specific characteristics of the degradation profiles for the sequenced copolymers are also of interest. In contrast to the exponential degradation observed for the random copolymers, the sequenced copolymer profiles have a somewhat sigmoidal shape. Based on our experimental observations, we believe that the profile can be attributed, in part, to the following phases of particle degradation. The initial steep rate of hydrolysis can be attributed the "wetting effect" discussed earlier. The middle region, by this hypothesis represents the sequence dependent degradation rate while the final steeper curve is due to particle collapse effects including the solubility of the increasingly short oligomers and the increase in surface area caused by the physical disintegration of the particle. As no simple fitting algorithm captures this complexity, and as there are likely other factors that contribute to the degradation, a more quantitative assignment of rates cannot be made at this time. Additional experiments and modeling studies, which have yet to be undertaken for ordered copolymer systems of this type and which can account for a population of polymer chains,[61] will be required before these degradation profiles can be fully explained and the rates quantified.

The RhB release studies presented suggest that the hydrolysis rate profiles and release rates correlate to some degree. The sequenced copolymers degrade at a slower rate than do random copolymers and also release the encapsulated dye molecule more gradually. This is a promising discovery as many drug delivery applications specifically target a slow release over time. It is clear, however, from the loading capacity studies and early period release data that the slower release rate depends on more than degradation rate. The repeated **LG** sequence may, for example, strengthen electrostatic or hydrophobic interactions between the guest and polymer, which would also slow the rate of release.

It is also of interest to compare the hydrolysis and release behavior of our SAP-produced copolymer with that previously reported by Dong et al. for a ROP-produced alternating copolymer. There are both similarities and differences in the two systems.[62] Although Dong et al. did not directly compare their polymer with a random control when studying hydrolysis rate, the plot of molecular weight vs time for their microparticles shows a nearly linear decrease analogous to our observations. Dong et al. also studied release profiles from their alternating copolymer, although their guest was bovine serum albumin (BSA) which is a large protein, as compared to the small molecule release agent used in the current work. For this part of the study a random copolymer control was used. Similar to our RhB studies, they observed a higher burst release from the random copolymer than from the ROP-alternating copolymer in the first few days. It is difficult to compare the systems beyond this point, however, as the model protein BSA was released very slowly relative to RhB, reaching only 20% completion from the random copolymer within 40 days and less than 10% from the ROP-alternating copolymer. Another difference which is likely related to the particular characteristics of BSA vs RhB, was the fact that they reported a similar (and much higher, > 30%) loading efficiency for both the random and the ROP-alternating copolymer.

CONCLUSIONS

The potential implications of the observed degradation and release behavior of the sequenced PLGAs are clearly relevant to the biomedical applications that employ these materials as (1) simply ordering L:G units in a repeating sequence leads to more sustained release of encapsulated guests as compared with a random copolymer with the same overall composition; (2) a slower loss of molecular weight should lead to longer retention of mechanical properties which is important in stem cell scaffolding applications; and (3) a more homogeneous degradation profile may lead to more uniform erosion or clearance of the polymer matrix, thus preventing the accumulation of extremely slow degrading material, such as lactic acid oligomer crystals, that cause local inflammation long after the function of the PLGA construct is completed.[63] Future studies will probe these questions in greater detail.

dx.doi.org/10.1021/ja306866w | J. Am. Chem. Soc. 2012, 134, 16352−16359

■ ASSOCIATED CONTENT

Supporting Information

Experimental details, microparticle sizes and size distribution, typical scanning electron microscopy image of microparticles, plot of number average molecular weight change as a function of hydrolytic degradation time, and size exclusion chromatography traces of PLGA polymers for each week during hydrolytic degradation. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

tmeyer@pitt.edu

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank Sid Jhunjhunwala for helping with the preparation and characterization of microparticles, Hsiang-Kai Lin and Prof. Michael Trakselis for microcentrifuge access, and Ethel Burse for lactic acid release assays. Funding from NSF (CHE-0809289) is acknowledged.

■ REFERENCES

(1) McGrath, K. P., Kaplan, D. L., Eds. *Protein-Based Materials*; Birkhaeuser: Cambridge, 1997.
(2) Rathore, O.; Sogah, D. Y. *J. Am. Chem. Soc.* **2001**, *123*, 5231.
(3) Heim, M.; Roemer, L.; Scheibel, T. *Chem. Soc. Rev.* **2010**, *39*, 156.
(4) Vollrath, F.; Porter, D. *Polymer* **2009**, *50*, 5623.
(5) Kaplan, D., Adams, W. W., Farmer, B., Viney, C., Eds. *Silk Polymers: Materials Science and Biotechnology*; American Chemical Society: Washington, DC, 1994.
(6) Smeenk, J. M.; Otten, M. B. J.; Thies, J.; Tirrell, D. A.; Stunnenberg, H. G.; van Hest, J. C. M. *Angew. Chem., Int. Ed.* **2005**, *44*, 1968.
(7) Panitch, A.; Yamaoka, T.; Fournier, M. J.; Mason, T. L.; Tirrell, D. A. *Macromolecules* **1999**, *32*, 1701.
(8) Krejchi, M. T.; Cooper, S. J.; Deguchi, Y.; Atkins, E. D. T.; Fournier, M. J.; Mason, T. L.; Tirrell, D. A. *Macromolecules* **1997**, *30*, 5012.
(9) MacEwan, S. R.; Chilkoti, A. *Biopolymers* **2010**, *94*, 60.
(10) Woolfson, D. N. *Biopolymers* **2010**, *94*, 118.
(11) Badi, N.; Lutz, J.-F. *Chem. Soc. Rev.* **2009**, *38*, 3383.
(12) Coates, G. W. *Chem. Rev.* **2000**, *100*, 1223.
(13) Hibi, Y.; Ouchi, M.; Sawamoto, M. *Angew. Chem., Int. Ed.* **2011**, *50*, 7434 (S7434/1−S7434/7).
(14) Ida, S.; Ouchi, M.; Sawamoto, M. *J. Am. Chem. Soc.* **2010**, *132*, 14748.
(15) Kramer, J. W.; Treitler, D. S.; Dunn, E. W.; Castro, P. M.; Roisnel, T.; Thomas, C. M.; Coates, G. W. *J. Am. Chem. Soc.* **2009**, *131*, 16042.
(16) Lutz, J.-F. *Polym. Chem* **2010**, *1*, 55.
(17) Lutz, J.-F.; Schmidt, B. V. K. J.; Pfeifer, S. *Macromol. Rapid Commun.* **2011**, *32*, 127.
(18) Minoda, M.; Sawamoto, M.; Higashimura, T. *Macromolecules* **1990**, *23*, 4889.
(19) Ouchi, M.; Badi, N.; Lutz, J.-F.; Sawamoto, M. *Nature Chem.* **2011**, *3*, 917.
(20) Ouchi, M.; Terashima, T.; Sawamoto, M. *Acc. Chem. Res.* **2008**, *41*, 1120.
(21) Pfeifer, S.; Lutz, J.-F. *J. Am. Chem. Soc.* **2007**, *129*, 9542.
(22) Satoh, K.; Matsuda, M.; Nagai, K.; Kamigaito, M. *J. Am. Chem. Soc.* **2010**, *132*, 10003.
(23) Satoh, K.; Ozawa, S.; Mizutani, M.; Nagai, K.; Kamigaito, M. *Nat. Commun.* **2010**, *1*, 1 (S1−S9).
(24) Soeriyadi, A. H.; Boyer, C.; Nyström, F.; Zetterlund, P. B.; Whittaker, M. R. *J. Am. Chem. Soc.* **2011**, *133*, 11128.
(25) Srichan, S.; Oswald, L.; Zamfir, M.; Lutz, J.-F. *Chem. Commun.* **2012**, *48*, 1517.
(26) Stayshich, R. M.; Meyer, T. Y. *J. Am. Chem. Soc.* **2010**, *132*, 10920.
(27) Stayshich, R. M.; Weiss, R. M.; Li, J.; Meyer, T. Y. *Macromol. Rapid Commun.* **2011**, *32*, 220.
(28) Sworen, J. C.; Smith, J. A.; Berg, J. M.; Wagener, K. B. *J. Am. Chem. Soc.* **2004**, *126*, 11238.
(29) Thomas, C. M. *Chem. Soc. Rev.* **2010**, *39*, 165.
(30) Weiss, R. M.; Jones, E. M.; Shafer, D. E.; Stayshich, R. M.; Meyer, T. Y. *J. Polym. Sci., Part A: Polym. Chem.* **2011**, *49*, 1847.
(31) Ovitt, T. M.; Coates, G. W. *J. Am. Chem. Soc.* **1999**, *121*, 4072.
(32) Smaldone, R. A.; Moore, J. S. *Chem.—Eur. J.* **2008**, *14*, 2650.
(33) Anderson, J. M.; Shive, M. S. *Adv. Drug Delivery Rev.* **1997**, *28*, 5.
(34) Athanasiou, K. A.; Niederauer, G. G.; Agrawal, C. M. *Biomaterials* **1996**, *17*, 93.
(35) D'Souza, S. S.; DeLuca, P. P. *Pharm. Res.* **2006**, *23*, 460.
(36) Giteau, A.; Venier-Julienne, M. C.; Aubert-Pouessel, A.; Benoit, J. P. *Int. J. Pharm.* **2008**, *350*, 14.
(37) Rebert, N. W. *Macromolecules* **1994**, *27*, 5533.
(38) Dong, C. M.; Qiu, K. Y.; Gu, Z. W.; Feng, X. D. *J. Polym. Sci., Part A: Polym. Chem.* **2000**, *38*, 4179.
(39) Dong, C. M.; Qiu, K. Y.; Gu, Z. W.; Feng, X. D. *J. Polym. Sci., Part A: Polym. Chem.* **2002**, *40*, 409.
(40) Dong, C. M.; Qiu, K. Y.; Gu, Z. W.; Feng, X. D. *J. Polym. Sci., Part A: Polym. Chem.* **2001**, *39*, 357.
(41) Balmert, S. C.; Little, S. R. *Adv. Mater.* **2012**, *24*, 3757.
(42) Li, J.; Stayshich, R. M.; Meyer, T. Y. *J. Am. Chem. Soc.* **2011**, *133*, 6910.
(43) Dechy-Cabaret, O.; Martin-Vaca, B.; Bourissou, D. *Chem. Rev.* **2004**, *104*, 6147.
(44) Bray, B. L. *Nat. Rev. Drug Discovery* **2003**, *2*, 587.
(45) Stayshich, R. M.; Meyer, T. Y. *J. Polym. Sci., Part A: Polym. Chem.* **2008**, *46*, 4704.
(46) Allen, T. M.; Cullis, P. R. *Science* **2004**, *303*, 1818.
(47) Jain, R. A. *Biomaterials* **2000**, *21*, 2475.
(48) Park, T. G. *Biomaterials* **1995**, *16*, 1123.
(49) Grizzi, I.; Garreau, H.; Li, S.; Vert, M. *Biomaterials* **1995**, *16*, 305.
(50) Dunne, M.; Corrigan, O. I.; Ramtoola, Z. *Biomaterials* **2000**, *21*, 1659.
(51) Siepmann, J.; Elkharraz, K.; Siepmann, F.; Klose, D. *Biomacromolecules* **2005**, *6*, 2312.
(52) Kasperczyk, J. *Polymer* **1996**, *37*, 201.
(53) Li, S. *J. Biomed. Mater. Res.* **1999**, *48*, 342.
(54) Yorita, K.; Janko, K.; Aki, K.; Ghisla, S.; Palfey, B. A.; Massey, V. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 9590.
(55) Takagi, M.; Fukui, Y.; Wakitani, S.; Yoshida, T. *J. Biosci. Bioeng.* **2004**, *98*, 477.
(56) Fredenberg, S.; Wahlgren, M.; Reslow, M.; Axelsson, A. *Int. J. Pharm.* **2011**, *415*, 34.
(57) Badri Viswanathan, S. S. P.; J., K.; Pandil, A. K.; Lele, M. G.; Kulkarni, R. A.; Mashelkar, N. *J. Microencapsulation* **2001**, *18*, 783.
(58) Berkland, C.; King, M.; Cox, A.; Kim, K.; Pack, D. W. *J. Controlled Release* **2002**, *82*, 137.
(59) Jhunjhunwala, S.; Raimondi, G.; Thomson, A. W.; Little, S. R. *J. Controlled Release* **2009**, *133*, 191.
(60) Vey, E.; Rodger, C.; Booth, J.; Claybourn, M.; Miller, A. F.; Saiani, A. *Polym. Degrad. Stab.* **2011**, *96*, 1882.
(61) Antheunis, H.; Van Meer, J. C. D.; De Geus, M.; Kingma, W.; Koning, C. E. *Macromolecules* **2009**, *42*, 2462.
(62) Dong, C.-M.; Guo, Y.-Z.; Qiu, K.-Y.; Gu, Z.-W.; Feng, X.-D. *J. Controlled Release* **2005**, *107*, 53.
(63) Nicolete, R.; dos Santos, D. F.; Faccioli, L. H. *Int. Immunopharmacol.* **2011**, *11*, 1557.