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The impact of monomer sequence and stereochemistry on the swelling and erosion of biodegradable poly(lactic-*co*-glycolic acid) matrices

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ABSTRACT

Monomer sequence is demonstrated to be a primary factor in determining the hydrolytic degradation profile of poly(lactic-*co*-glycolic acid)s (PLGAs). Although many approaches have been used to tune the degradation of PLGAs, little effort has been expended in exploring the sequence-control strategy exploited by nature in biopolymers. Cylindrical matrices and films prepared from a series of sequenced and random PLGAs were subjected to hydrolysis in a pH 7.4 buffer at 37 °C. Swelling ranged from 107% for the random racemic PLGA with a 50:50 ratio of lactic (L) to glycolic (G) units to 6% for the sequenced alternating copolymer poly LG. Erosion followed an inverse trend with the random 50:50 PLGA showing an erosion half-life of 3–4 weeks while poly LG required ca. >10 weeks. Stereosequence was found to play a large role in determining swelling and erosion; stereopure analogs swelled less and were slower to lose mass. Molecular weight loss followed similar trends and increases in dispersity correlated with the onset of significant swelling. The relative proportion of rapidly cleavable G-G linkages relative to G-L/L-G (moderate) and L-L (slow) correlates strongly with the degree of swelling observed and the rate of erosion. The dramatic sequence-dependent variation in swelling, in the absence of a parallel hydrophilicity trend, suggest that osmotic pressure, driven by the differential accumulation of degradation products, plays an important role.

1. Introduction

We have discovered that sequence can be used to control the degradation behavior of poly(lactic-*co*-glycolic acid)s (PLGAs). Random PLGAs have been utilized extensively in controlled drug delivery systems and clinical applications due to their biocompatibility, biodegradability, and regulatory acceptance [1]. Target applications include controlled release devices for anticancer agents [2–4], surgical sutures and screws [5,6], and porous scaffolds for tissue regeneration [7]. Despite the number of literature reports on the utility of PLGAs for bioengineering, however, there are currently only 15 FDA-approved PLA/PLGA-based drug products on the US market [8]. We hypothesize that one contributing factor to the poor translation into application is the relatively narrow range of performance of the random copolymer PLGAs during degradation, a deficiency that has been addressed by others with a variety of strategies including adjusting molecular weight, controlling ratio of lactic (L) and glycolic (G) units; tuning average L and G block lengths, adding comonomers and chemical additives, and through the design and configuration of devices [9,10].

Our approach to expanding the accessible range of degradation

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profiles is to move beyond random copolymers through the control the sequence of the monomers, an approach which in biological polymers has been shown to provide diverse structure and function from a limited monomer set. The potential for impact of this approach is further supported by the recent promising reports of sequence control in other synthetic copolymers [11–19] and by intriguing studies by Sarasua et al., that link properties with statistical variations in chain microstructure for selected biopolyesters [20–25].

To probe the relationship between monomer order and properties, we have developed a synthetic route that yields PLGAs with repeating sequences [26]. For example, we have prepared **poly LG**, which consists of an exact repeat of the LG dimer for the length of the polymer, i.e., $(LG)_n$. Similarly, we have been able to prepare a variety of polymers bearing periodic repeats of varying lengths, e.g., $(LLG)_n$, $(GLLG)_n$, etc. Using NMR spectroscopy, which is uniquely powerful for the characterization of this particular class of polymer due to solution phase conformational preferences, the periodic structure of these polymers has been confirmed. Moreover, sequence errors are easily detected and can be quantified [26]. The ability to both synthesize exact sequences and verify them enables, for the first time, a thorough exploration of structure and function in PLGAs.

In initial properties studies of this new class of periodic PLGAs [27,28] we have found a surprisingly strong correlation between sequence and properties. In microparticles prepared from these copolymers, we observed a correlation of sequence with molecular weight loss, lactic acid release, and thermal properties. In general, the sequenced PLGAs exhibited a slower and more gradual loss of molecular weight and a longer preservation of morphology, including T_g, than the random analogs. Differences were also observed between sequences, both structural- and stereoisomers. The release of an encapsulated guest molecule, rhodamine B, was also studied and found to depend directly on monomer order; the simple alternating copolymer **poly LG** released the guest more slowly and gradually than did the random PLGA control.

Based on these results, we hypothesize that sequenced PLGAs may have positive implications for bioengineering applications where prolonged delivery times and/or structural integrity are particularly important. For example, a longer release time would be beneficial for long-lasting intraocular implants. These implants, which are designed to replace eye drop regimens, which have low patient compliance, allow for continuous drug release [29]. We also expect that the slower degradation of the sequenced PLGAs will minimize the accumulation of pH-lowering acidic by-products compared to random PLGAs. This behavior could provide a protective effect for tissues such the retina which is known to be particularly sensitive to non-physiological pH [30]. The improved maintenance of morphology for the sequenced PLGAs could also offer advantages for certain applications, e.g., the repair of craniofacial bony defects, which require longer term mechanical strength during the delivery of critical osteogenic growth factors [31].

Swelling and erosion are deeply relevant to these application goals and their study should also lend insight into any sequence-based differences in the underlying mechanism of erosion. It has been previously reported and substantiated by numerous studies that matrices of random PLGAs below certain dimensions degrade by bulk erosion [32,33]. Observable behaviors associated with this mechanism include, in most cases, significant swelling and a rapid loss of mass after an initial latent period. At the other end of the continuum lie materials that decompose by surface erosion. These materials, e.g., polyanhydrides, degrade without significant water uptake and mass loss is gradual as the inner layers only begin to degrade as the outer layers are sequentially hydrolyzed [34–38]. This distinction is particularly important for the use of PLGA in drug delivery, as it is expected and has been observed that the release profile of encapsulated drugs depends on swelling and erosion [39]. Herein, we examine the swelling and erosion of sequenced PLGAs to determine, in part, whether their degradation behavior aligns more closely with a bulk or surface mechanism.

2. Materials and methods

2.1. Materials

Periodic PLGA copolymers were prepared as previously described [26,40]. Poly(D,L-lactide-*co*-glycolide) with a 50:50 ratio of lactic to glycolic acid-derived units and carboxylate end groups (**PDLGA-50**) and poly(D,L-lactide-*co*-glycolide) with a 65:35 ratio of lactic to glycolic acid-derived units and carboxylate end groups (**PDLGA-65**) were obtained from Durect Corporation (Birmingham, AL) as a pelletized solid. Prior to use, the polymers were dissolved in methylene chloride (CH_2Cl_2) and precipitated in methanol to yield off white amorphous solids. Poly(L-lactide-*co*-glycolide) with a 50:50 ratio of lactic to glycolic acid-derived units and carboxylate end groups (**PLLGA-50**), was obtained from Changchun SinoBiomaterials Co. Ltd. (Changchun, China) as a fibrous white solid and was used as provided. Phosphate buffered saline (PBS, pH = 7.4, 10 mM) was purchased from Life Technologies (Carlsbad, CA).

2.2. Characterization

Molecular weights and dispersities were acquired on a Waters GPC system (THF, 0.5 mL/min) with Jordi 500 Å, 1000 Å, and 10000 Å divinylbenzene (DVB) columns and refractive index detector (Waters) which was calibrated relative to polystyrene standards. Thermal properties of all polymers were obtained using TA Instruments Q200 DSC. Standard data were collected with a heating and cooling rate of 10 °C/min. Melting transitions (T_m) were collected from the first heating cycle and glass transition temperatures (T_g) were collected during the second heating cycle. Inflection points of glass transition temperatures are reported. All samples were prepared by drop-casting (CH_2Cl_2) into DSC pans followed by vacuum drying for 24 h and annealing at 85 °C for 3 h. The ^1H and ^{13}C NMR spectra were obtained in $CDCl_3$ using a 500 MHz Bruker spectrometer at 293 K and calibrated to the residual solvent peak at δ 7.26 ppm (^1H) and δ 77.00 ppm (^{13}C). Matrix assisted laser desorption/ionization time-of-flight (MALDI-ToF-MS) spectra were obtained on a Voyager-DE PRO instrument with a 337 nm N_2 laser and 25 kV accelerating voltage. The mass spectra of the polymers were obtained in reflector mode. The matrix consisted of *trans*-2-[3-(4-*tert*-butylphenyl)-2-methyl-2-propenylidene]malonitrile (DCTB) and sodium trifluoroacetate as the cationization agent.

2.3. Preparation of cylindrical-shaped pellets

Cylindrical-shaped pellets of all PLGAs were prepared by heated compression molding in a custom press. The colorless polymer (20–30 mg) was loaded into the press which was warmed to 85–95 °C and compressed with a 1200–1400 lb load for 10 min using a Carver press (Hydraulic unit model #3912; Wabash, IN). The press and sample were then re-heated for 10 min in an oven at 85 °C, and compressed again for 5 min under the same temperature and load. The resulting pellets were opaque or translucent depending on the polymer used and had dimensions of 3 × 3 mm (swelling) or 3 × 1.5 mm (erosion), corresponding to a weight of ~26 mg and ~15 mg, respectively.

2.3.1. In vitro swelling of sequenced and random PLGAs

Two samples of each polymer (26 mg each) were placed in separate Eppendorf tubes containing 2 mL of PBS. All tubes were incubated at 37 °C on a rotating mixer (8 rpm). Samples were removed every 2 d for the first 10 d, then weekly depending on the degree of swelling. Both **PDLGA-50** and **PLLGA-50** were removed every 2 d until sample degradation progressed to the point that the remaining material could not be handled or weighed. Each sample was blotted dry for 30 s and weighed on an analytical balance ($\pm 1.0 \times 10^{-4}$ g) until a stable reading was displayed for 15 s. The phosphate buffer was replaced each week for the entirety of the study.

2.3.2. In vitro erosion of sequenced and random PLGAs

Ten samples of each polymer (15 mg each) were placed in separate Eppendorf tubes containing 2 mL of PBS. Samples of each polymer were then divided into 5 groups of 2 samples, each to determine the mass loss at 5 different time intervals. All tubes were incubated at 37 °C on a rotating mixer (8 rpm). The tubes were refilled with fresh PBS (2 mL) every week. At each time point, duplicate samples of each polymer were collected, blotted dry for 15 s, flash-frozen in liquid N_2, then lyophilized for 2 d. After freeze-drying, the pellets were weighed on an analytical balance ($\pm 1.0 \times 10^{-4}$ g). Masses were recorded once a stable reading was displayed for 15 s.

2.4. Water contact angle experiments

Water contact angle measurements were recorded using a VCA optima XE video contact angle system at 24 °C and 42–48% relative humidity. A droplet with a volume of 1 μL was formed at the end of the needle and then lowered carefully until contact was made with the sample. The needle was withdrawn immediately so that the droplet was left on the sample surface. An image of the droplet was acquired with a charge-coupled device (CCD) camera 5 s after contact with the surface of the film. The static contact angle was calculated automatically by the VCA software. Approximately 45 s was required to complete the whole measurement process. Each measurement was repeated 5× per sample at different locations.

Films of all PLGAs were prepared using a drawdown coating method. Each of the copolymer samples was dissolved in dichloromethane at a concentration of 250 mg/mL. A 20–30 μm film was deposited on a glass microscope slide using a 180–200 μL aliquot of the polymer solution. The resulting film was dried in an oven at 70 °C for 1 h and stored in a vacuum desiccator until used. Samples were exposed to PBS at 23 °C and the hydrated contact angle was monitored over an 8 d time period. After the hydrated water contact angle was recorded, the films were flash-frozen in liquid N_2, lyophilized for 3 d, and the lyophilized film contact angle was determined using the same method.

3. Results

3.1. Naming conventions and characterization of PLGA copolymers

The L-lactic unit, racemic lactic unit, and glycolic unit are abbreviated as **L**, $\mathbf{L}_{rac}$, and **G**, respectively. The periodic copolymers utilized in this study were prepared using segmer assembly polymerization (SAP). In this method, we prepare well-defined oligomers and polymerize them using condensation conditions that have been optimized to preclude sequence scrambling by transesterification (Scheme S1). The resulting copolymers are termed periodic copolymers because they consist of a nearly perfect repetition of the input segmer. As such, a segmer consisting of a lactic and glycolic acid-derived unit, would be termed **LG** and the polymer would be named **poly LG** (Scheme 1). The SAP method was also used to prepare a random copolymer, **R-SAP**, by condensation of a 1:1:1:1 ratio of the segmers **LG**, **GL**, **LL** and **GG**. The random copolymers, **PLLGA-50**, **PDLGA-50** and **PDLGA-65**, prepared by ring-opening polymerization (ROP) of lactide and glycolide, were purchased. In this case, the **PLLGA-50** and **PDLGA-50** are the stereopure and racemic versions of the copolymers with a 50:50 L:G-ratio and **PDLGA-65** is the racemic derivative of a copolymer with a 65:35 L:G-ratio. Sequenced copolymers prepared by SAP range in molecular weight from 18 to 30 kDa and are comparable to purchased random PLGA controls (Table 1, Fig. S4). All polymers exhibited T_gs in the range of 44–49 °C (Table 1, Fig. S5).

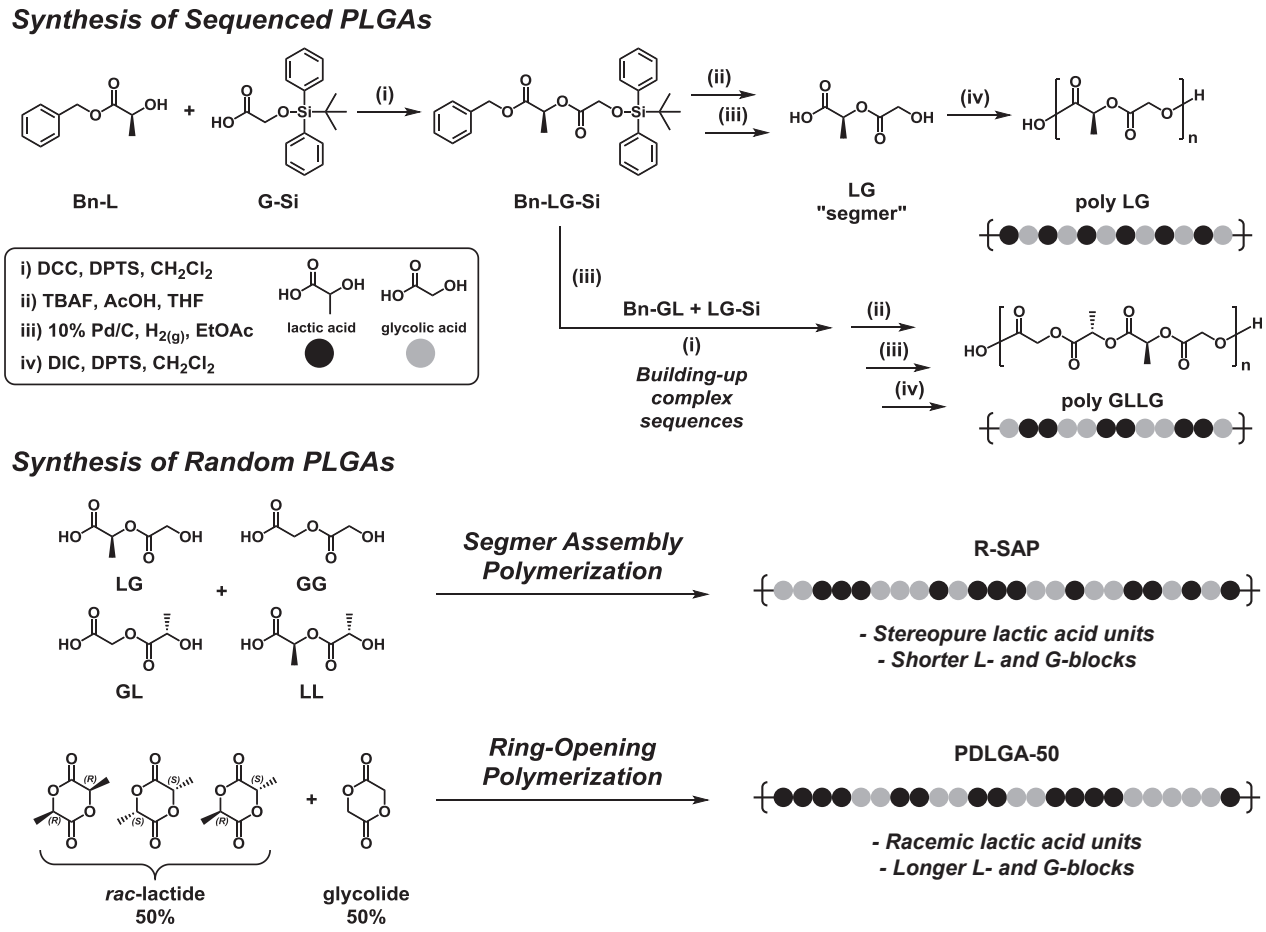
3.2. Characterization

The copolymers were characterized by ^{1}H NMR, ^{13}C NMR, and MALDI-ToF mass spectrometry (Figs. 1 and 2, Fig. S1-2). As we have previously described in detail the assignment of ^{1}H and ^{13}C NMR spectra of these polymers [26], we will comment on only few key features. Most importantly, we note that these sequenced copolymer exhibit unusually sharp and well-resolved ^{1}H NMR peaks, even for high molecular weight samples. These copolymers have strong sequence-based conformational preferences which result in unique and assignable resonances for each variant. The diastereotopic glycolyl protons are particularly useful in the ^{1}H NMR spectrum. The combination of clear sequence based differences in chemical shift, along with high resolution means sequence mistakes are readily identifiable and quantifiable.

MALDI-ToF mass spectra (Fig. S1) of these copolymers also confirms the periodic structure of these copolymers as the chain lengths observed are all multiples of the target sequence. With the exception of **poly GLG** the sequenced copolymers, while not perfect, exhibit very small amounts of error and epimerization. The **poly GLG** sample, as can be seen in both the ^{1}H and MALDI-ToF spectra does contain a slightly higher rate of sequence errors, primarily consisting of the loss/addition of G or LG units. The rate is fairly low, with no more than one error per chain seen for chains in $(GLG)_{10}$ region of the MALDI-ToF spectrum. We believe the error arises, in this case, from the relatively low stability of the GLG segmer from which it is prepared. Again, it should be noted that the assignment of these NMR spectra has been extensively investigated [26]. The ^{1}H NMR spectra of the random copolymers, **PDLGA-50, PLLGA-50** and **R-SAP** were also acquired and the actual L:G unit ratio (Table 1) was calculated by integration of the glycolic methylene and lactic methine resonances.

The ^{13}C NMR spectra of the carbonyl region of the sequenced and random copolymers are particularly interesting to the current discussion as they highlight the dramatic difference in numbers and types of sequence environments (Fig. 2). The random copolymers show a large variety of carbonyl environments, while the sequenced copolymers exhibit only a few. The introduction of stereosequences also increases the complexity of this region, even for the periodic copolymer **poly $\mathbf{L}_{rac}$G**.

As **R-SAP** had been prepared from the dimers LL, GG, LG, and GL, we expect that the average L and G block lengths are most probably shorter than those in commercial **PDLGA-50** and **PLLGA-50** produced from the ROP of lactide and glycolide. We were not, surprisingly, able to confirm this hypothesis spectroscopically because we found that the ^{13}C NMR resonances of the periodic copolymers produced for this study and others overlapped in a way that precludes a simple interpretation of this region for random mixtures (Fig. S3 for an example). We note that the prior assignments of resonances that have been proposed based on ROP copolymer syntheses [41–44] cannot be applied in the case of **R-SAP** because those analyses were based on the predicted absence of resonances



Scheme 1. Synthesis of periodic PLGA copolymers using segmer assembly polymerization (SAP) method along with random analogs synthesized by SAP and ring-opening polymerization (ROP).

Table 1
PLGA characterization data.

Polymer	M_n[a] (kDa)	M_w[a] (kDa)	Đ[a]	T_g (°C)[b]	Ratio L:G[c]
Poly LG	23.5	30.0	1.3	44	50:50
Poly $L_{rac}G$	30.8	44.4	1.4	49	50:50
Poly GLG	21.6	28.3	1.3	45	34:66
Poly GLLG	18.7	25.1	1.3	45	50:50
R-SAP	24.1	33.3	1.4	46	44:56
PDLGA-50	30.7	38.4	1.3	48	51:49
PLLGA-50	23.9	38.8	1.6	47	54:46
PDLGA-65	28.3	39.2	1.4	47	65:35

[a] Determined by size exclusion chromatography in THF relative to polystyrene standards.
[b] Obtained in the second heating cycle at 10 °C/min.
[c] Results based on ^{1}H NMR spectroscopy and presented as the ratio of the lactic (L) and glycolic (G) units.

for certain sequences that are not forbidden in the R-SAP case. That being said, it is clear from the observed differences in the glycolyl carbonyl region of **PLLGA-50** and **R-SAP**, that there are significant microstructural differences. These differences should, based on our sequence hypothesis, be reflected in the properties.

3.3. In vitro swelling of sequenced and random PLGAs

PLGA performance has been examined in a variety of constructs including microparticles [39,45], films [46,47] and solid matrices in a variety of geometries [33,48]. For the current studies, which focus on bulk properties rather than drug release, we have chosen to fabricate the polymers into macroscale cylindrical matrices to increase their relevance to larger implantable devices, e.g., screws and plates. To determine dependence of the uptake of water on sequence, swelling studies were performed on cylindrical pellets (3 × 3 mm), two per polymer, prepared using heated compression molding. For random PLGA, these matrices would be expected to degrade by a bulk hydrolysis mechanism since they are thinner in all dimensions than the critical thickness of 7.4 cm that has been identified as the transition point between bulk and surface erosion for poly(α-hydroxy ester)s [33]. Data for cylindrical constructs similar to those used in this study, prepared using random PLGAs, were reported by von Bukersroda et al., in their efforts to develop improved osteosynthetic devices [49–51].

The samples were exposed to buffer at physiological temperature and pH and their hydrated mass was recorded over an eight-week time period. The size and shape of the hydrated pellets at each time point were documented photographically (Fig. 3). The swelling % of the duplicate samples was calculated according to Eq. (1) where m_0 is the initial sample mass and m_t is the mass of the hydrated pellet at time t (Fig. 4).

$$\text{swelling } \% = \frac{m_t - m_0}{m_0} \times 100\% \quad (1)$$

In examining the swelling data, it can be seen that upon immersion there was an initial increase in swelling of ~2% for all polymers during the first two days. The pellet size and morphology

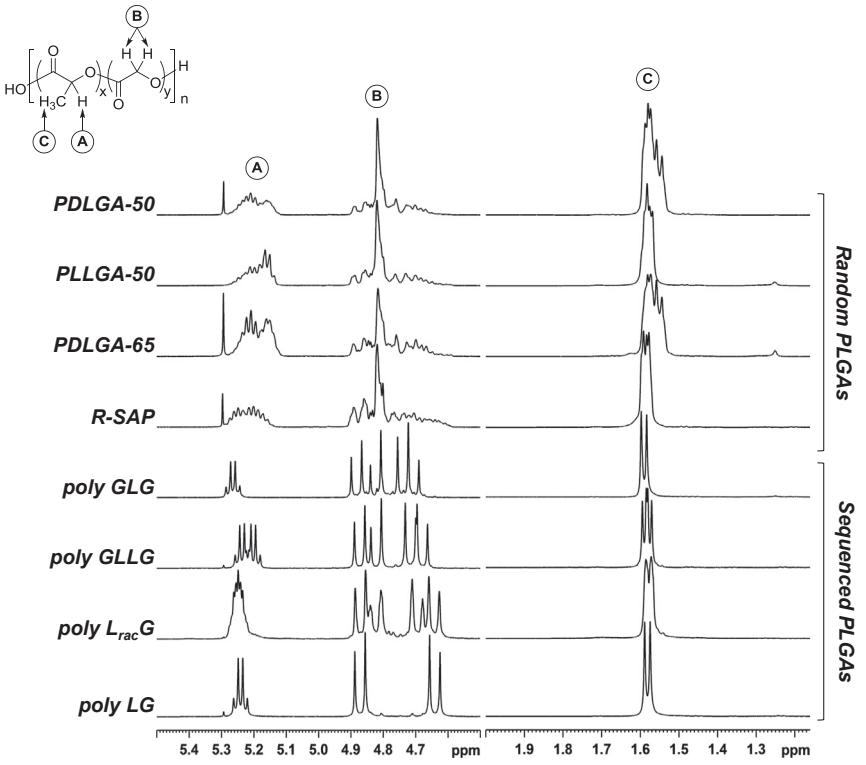


Fig. 1. ^{1}H NMR (500 MHz) spectra of sequenced and random PLGAs (δ 5.5–4.5 and 2.0–1.2 ppm). Labels corresponding to the locations of methine (A), methylene (B), and methyl (C) proton chemical shifts for poly(lactic-*co*-glycolic acid) are included for reference.

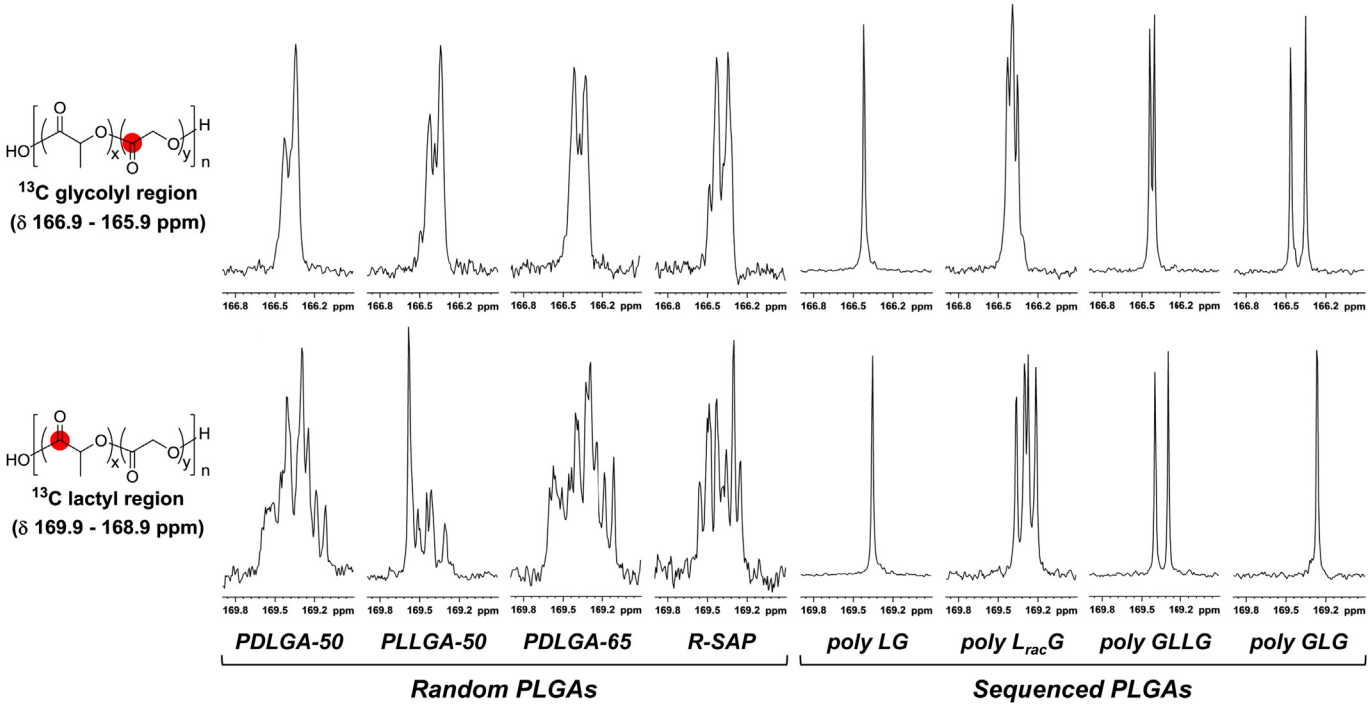


Fig. 2. ^{13}C NMR (500 MHz) spectra of glycolyl (top) and lactyl (bottom) carbonyl regions of sequenced and random PLGAs.

remained constant during this time period but gradually changed over the first week depending on the sequence of the PLGA. The onset of swelling for both random PLGAs, **PDLGA-50** and **PLLGA-50**, occurred during the first week. Interestingly, the morphology of **PDLGA-50** changed during this time period and **PLLGA-50** remained unchanged.

During the second week, swelling increased from 5% to 42% for **PDLGA-50**; significant swelling and altered shape morphology was also observed. The stereopure random analog **PLLGA-50** slowly increased in water content from 6% to 11% while retaining its

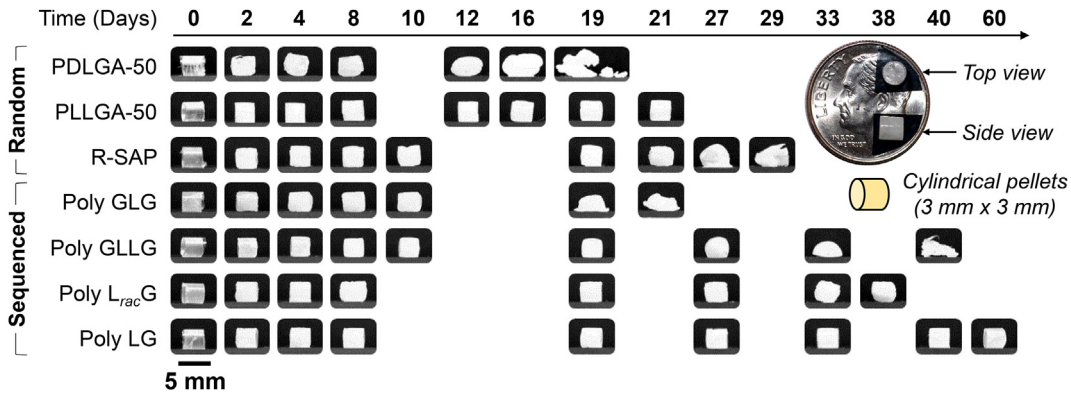


Fig. 3. Side-view appearance of water-swollen cylindrical pellets as a function of hydrolysis time.

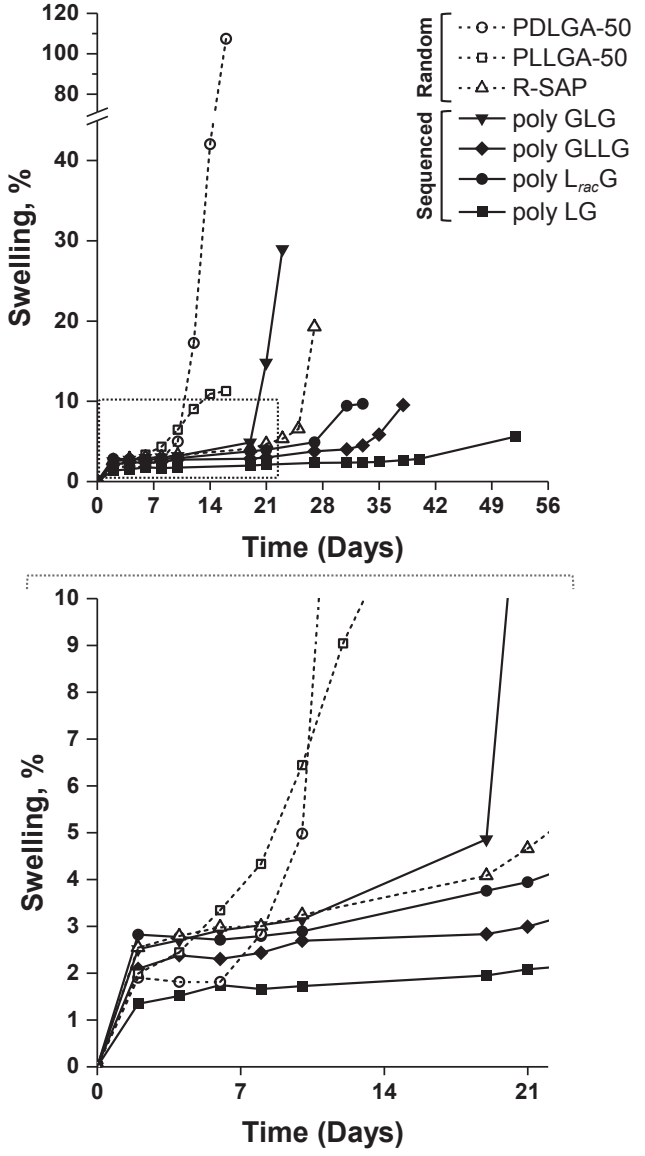


Fig. 4. Swelling profiles of sequenced and random PLGAs over 8 weeks (top); enlargement of the first 3-week time period (bottom). Open symbols represent random copolymer controls and closed symbols represent sequenced copolymers.

structural integrity. The swelling of sequenced PLGAs remained unchanged during this time period with only **R-SAP**, the random PLGA analog prepared by SAP, exhibiting slight changes in morphology. After three weeks of immersion, the influence of sequence and stereochemistry was more pronounced. The water content of **PDLGA-50** reached its maximum of 107% and significant sample fracturing was observed. The loss of integrity precluded any further measurements. **PLLGA-50** exhibited nominal visual swelling and had a maximum of 11% prior to sample failure. No significant changes in swelling were observed for **poly LG**, **poly L$_{rac}$G** and **poly GLLG**, 2%, 4% and 3%, respectively. Noticeable changes in morphology were observed in **poly GLG** (15%) and **R-SAP** (5%) which were followed by a continual increase in swelling resulting in rupture after 23 days for **poly GLG** (30%) and 27 days for **R-SAP** (20%). In the time period of week four to five, the morphology of **poly GLLG** and **poly L$_{rac}$G** begins to change and is accompanied by an increase in swelling, 5–10% and 4–6%, respectively, with each sample failing at a maximum swelling of 10%. Throughout this time period, **poly LG** exhibited minimal changes in swelling and morphology; at 52 days swelling was only 6%.

3.4. In vitro erosion of sequenced and random PLGAs

To understand the erosion behavior of sequenced and random PLGAs, mass loss experiments were conducted in parallel to the swelling studies. Cylindrical pellets with a height of 1.5 mm and width of 3 mm, prepared by heated compression molding were used. These studies were performed under physiological pH and temperature and mass loss data were obtained on the lyophilized pellets at selected time points over 19 weeks. The size and shape of the lyophilized pellets were recorded photographically and are reported in (Fig. 5) and erosion profiles of sequenced and random PLGAs are shown in (Fig. 6). The erosion is reported as % mass loss which was calculated using Eq. (2) where m_0 is the initial sample mass and m_t is the mass of the lyophilized pellet at time t.

$$\%mass\ loss = \frac{m_0 - m_t}{m_0} \times 100\% \quad (2)$$

The mass loss profiles for periodic PLGAs were found to be dramatically different than random PLGAs for a variety of sequences. All samples began with an initial period of stability. After this initial period, the degradation proceeded at a rate that depended on L:G ratio, structural sequence and stereochemistry. For the random PLGAs, **PDLGA-50** and **PLLGA-50**, the onset of erosion occurred at two weeks. The 1:1 LG ratio polymer, **PDLGA-50**, then lost mass rapidly over the next three weeks before losing structural integrity. The random stereopure analog, **PLLGA-50**, retained its structural integrity after the onset of erosion for an

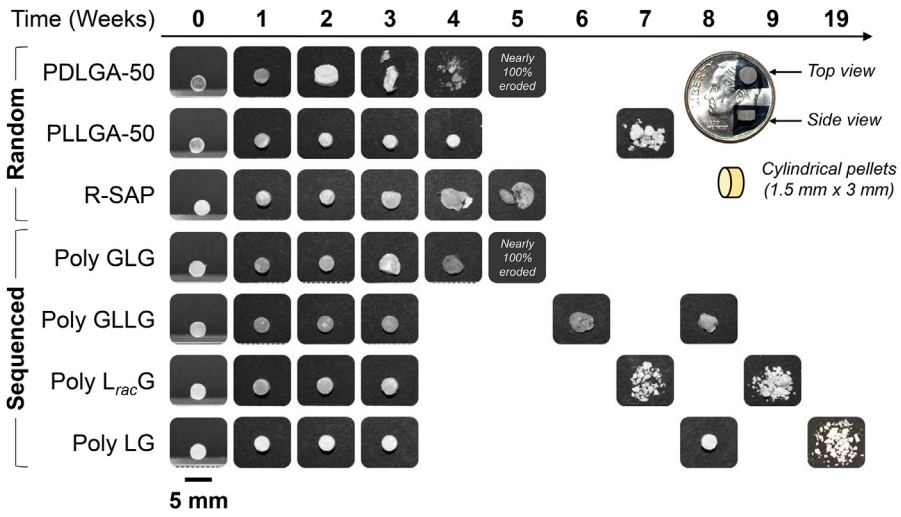


Fig. 5. Top-view appearance of eroded cylindrical pellets, after lyophilization, as a function of hydrolysis time.

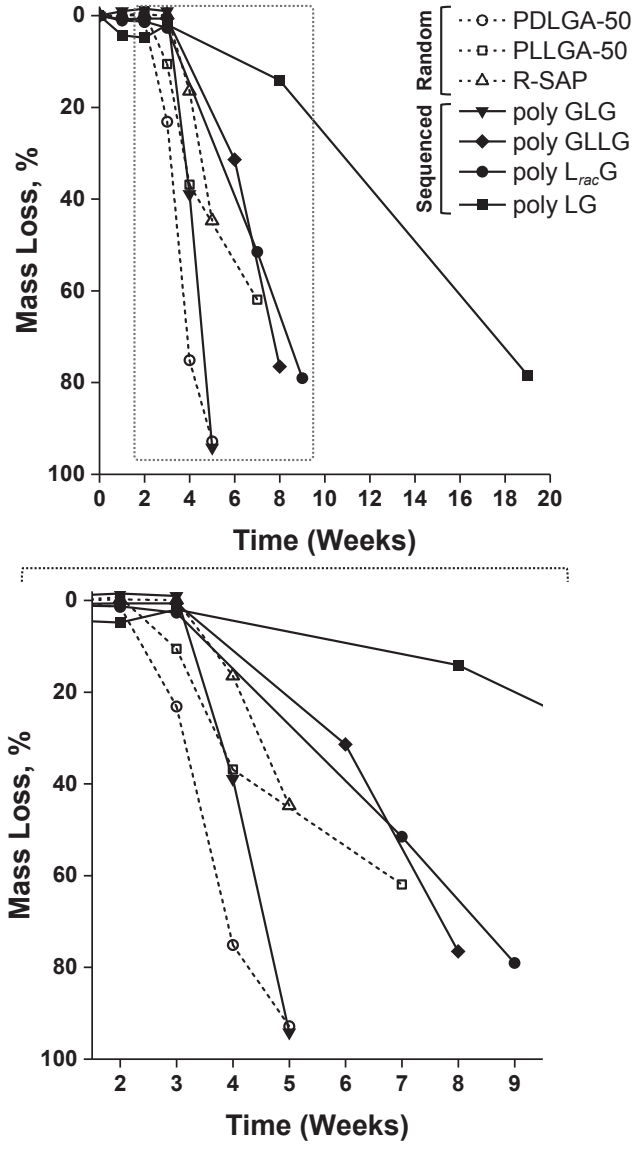


Fig. 6. Erosion profiles of sequenced and random PLGAs over 19 weeks (top); enlargement of weeks 2–9 (bottom). Open symbols represent random copolymer controls and closed symbols represent sequenced copolymers.

additional two weeks but lost its structural integrity at week seven, at a 62% mass loss. Interestingly, for **PLLGA-50**, the erosion rate plateaued between weeks four and seven.

The sequenced PLGAs, with the exception of **poly LG**, and the random analog prepared by SAP exhibited an onset of erosion during week three. Interestingly, both **poly GLG** and **R-SAP** lost their structural integrity at week three and had rapid stages of mass loss throughout weeks four and five. During these time periods, **poly GLG** lost 39% of its mass at week four and an additional 55% by week five. **R-SAP** at the same time points lost 16% and an additional 28%. Mass loss for **poly GLLG** and **poly L$_{rac}$G** was more gradual. Despite having the same onset of erosion, **poly GLLG** and **poly L$_{rac}$G** retained their structural integrity over weeks four and five but failed at weeks six and seven, respectively. During the time period of weeks three to six for **poly GLLG**, only 31% eroded and an additional 45% eroded after eight weeks. For **poly L$_{rac}$G**, 49% of the sample was lost between weeks three and seven and an additional 27% after nine weeks. The erosion profile of **poly LG** was more linear and structural integrity was maintained over a longer time period; no pronounced onset of erosion was evident over the time period studied. The structural integrity of **poly LG** was retained for eight weeks with only a 14% mass loss. At the end of the study, **poly LG** had lost 78% of the sample's initial mass.

3.5. Correlated trends in swelling and erosion

When the swelling and erosion behaviors of copolymers are compared, there is a clear inverse relationship. Those polymers that exhibited a higher degree of swelling were also observed to erode more quickly (Fig. 7). Another important observation that can be made by examining these trends is that both the random **PDLGA-50** and **poly LG** are outliers amongst their analogs. **PDLGA-50** swelled by a factor of 3× more than any other sample. On the other end of the spectrum is the alternating copolymer **poly LG** which erodes 2× more slowly than any other PLGA examined, including other sequences. The dependence of both processes on stereochemistry can also be seen, with the racemic versions exhibiting more swelling and shorter erosion half-lives than their stereopure analogs. Finally, it is interesting to note that **poly GLG** which would be expected, based only on the ratio of L:G units, to degrade the quickest, has a half-life that is slightly longer than the random **PDLGA-50**.

Although the large and repeatable differences in behavior

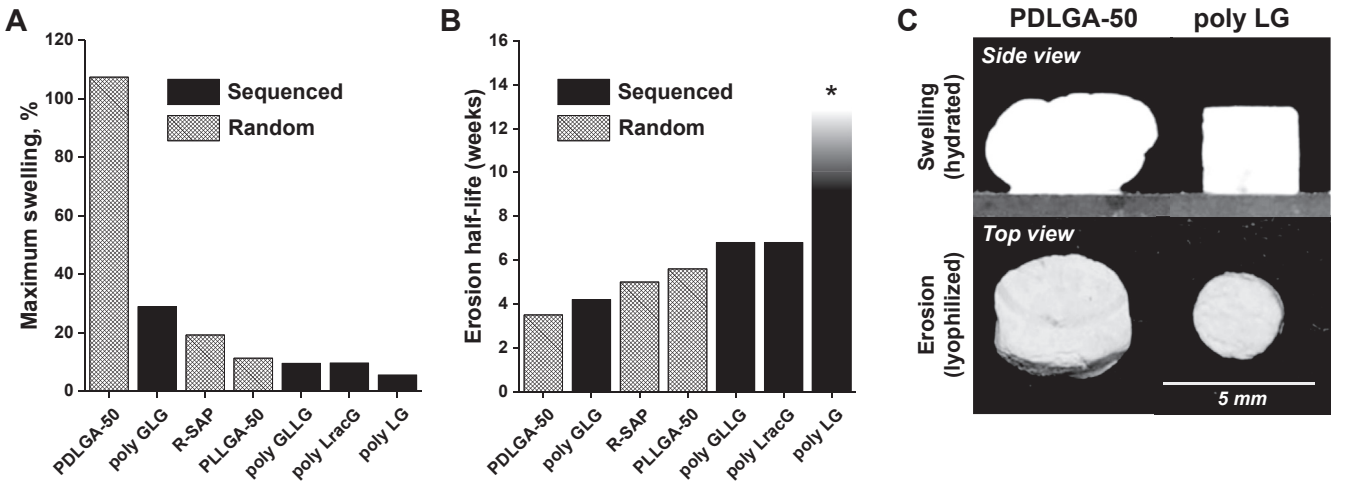


Fig. 7. Maximum swelling % (A) and erosion half-life (B) of sequenced and random PLGAs. *Erosion half-life is approximately 10–14 weeks. Enlarged views (C) of cylindrical constructs of **PDLGA-50** and **poly LG** in week 2 of degradation highlighting the dramatic sequence-based differences in swelling and erosion behavior.

between sequences suggest that the sequence is retained during the degradation process, esters are known to undergo transesterification in the solid state under some conditions. Indeed the synthesis of random PLGA has been reported by the thermolysis of PLA with PGA [41]. To probe the possibility of transesterification during hydrolysis in these studies, we collected ^{1}H NMR spectra of the simple alternating stereopure **poly LG** as a function of degradation (Fig. S6). As the NMR spectra for these polymers is exquisitely sensitive to changes in sequence and stereochemistry, any changes would be evident [26]. Over 35 days, however, despite evidence of some degradation to form oligomers, there was no observed transesterification nor epimerization of the **poly LG** structure (Fig. S7).

3.6. Changes in molecular weight and distribution

The molecular weight profiles for all polymers in this study, normalized relative to the original M_w and M_n, along with the dispersity data are plotted in Fig. 8. The molecular weight loss profiles for random PLGAs, **PDLGA-50** and **PLLGA-50**, decreased rapidly with time which is typical of 50:50 ratio random PLGAs [52]. In contrast, **R-SAP**, **poly GLG** and **poly L$_{rac}$G** did not exhibit significant molecular weight loss until after 7 d. **Poly LG** and **poly GLLG** retain their initial molecular weights for 3 weeks.

Polymer chain dispersity ($Đ$) was also found to depend on monomer order and stereochemistry. **PDLGA-50**, **R-SAP**, **poly L$_{rac}$G** and **poly GLG** exhibit a sharp increase in dispersity by week 3. During this time period **PLLGA-50** also increases but at a slower rate. The dispersities of **poly LG** and **poly GLLG** remain constant over 35 d, and increase gradually over the following 4 weeks.

3.7. Surface water contact angle

To determine if there were significant differences in the inherent hydrophilicity of the sequenced and random PLGAs, the surface contact angles of selected samples, **PDLGA-50**, **PDLGA-65** and **poly LG**, were measured after exposure to hydrolyzing conditions. Thick films (20–30 μm) were submerged in PBS buffer at 23 °C and the hydrated contact angle was monitored over an 8 day time period. The samples were subsequently dried by lyophilization and the dry film contact angles were recorded (Fig. 9).

The initial water contact angle for all samples was relatively similar, 76.5 ± 0.5° and agrees with previously reported measurements [53,54]. There were no significant changes in the hydrated film contact angles over an 8 day time period, with the average contact angles for **PDLGA-50**, **PDLGA-65**, and **poly LG** being 63°, 66° and 65°, respectively (Fig. S8). The lyophilized film water contact angles, however, do show initial differences after 1 day of exposure to physiological pH and temperature (Fig. S9). The initial contact angle of **PDLGA-50** decreases from 77° to 63°, which then remains unchanged at around 62–63° for the remaining 8 days of exposure. In contrast, the lyophilized contact angles for **PDLGA-65** and **poly LG** do not change significantly after 1 day of exposure. The change in the lyophilized contact angle for **PDLGA-50** and **poly LG** over 8 days was minimal with the overall averages being 72° and 71°, respectively. The differences observed between sequences in the lyophilized angles appears to correlate with the amount of film degradation—the slower degrading samples maintain their surface hydrophobicity longer.

4. Discussion

The ultimate goal of this work and that of many researchers in bioengineering is to be able to control the degradation of implantable/injectable polymers. While the architecture, i.e., size, shape, etc., of particular matrices contributes significantly to their behavior [55,56], the chemical composition of the material from which they are made is ultimately responsible for determining the degradation mechanism, profile, release rate of encapsulated drugs, and mechanical properties. The most widely adopted strategy for changing polymer composition, and thereby tuning properties, is to add new or modify the current monomers. While this approach works well for materials that will not be employed in biomedical applications, the use of new monomers in a biomaterial presents significant challenges as all of the degradation products must be non-toxic and clearable.

Our approach to composition control, which avoids the introduction of new chemical entities, is to adopt nature's own solution to this problem: use the same monomers but change the order. Despite the obvious nature of this idea, the synthetic challenges in making sequenced copolymers have long inhibited the exploration of this strategy. There are only isolated, nearly anecdotal, studies relating bulk properties to sequence outside of amino and nucleic acid polymers [11–14]. We have focused our research on PLGA due to the ubiquity of its use and because the poor match of PLGA properties with those required for particular applications has been

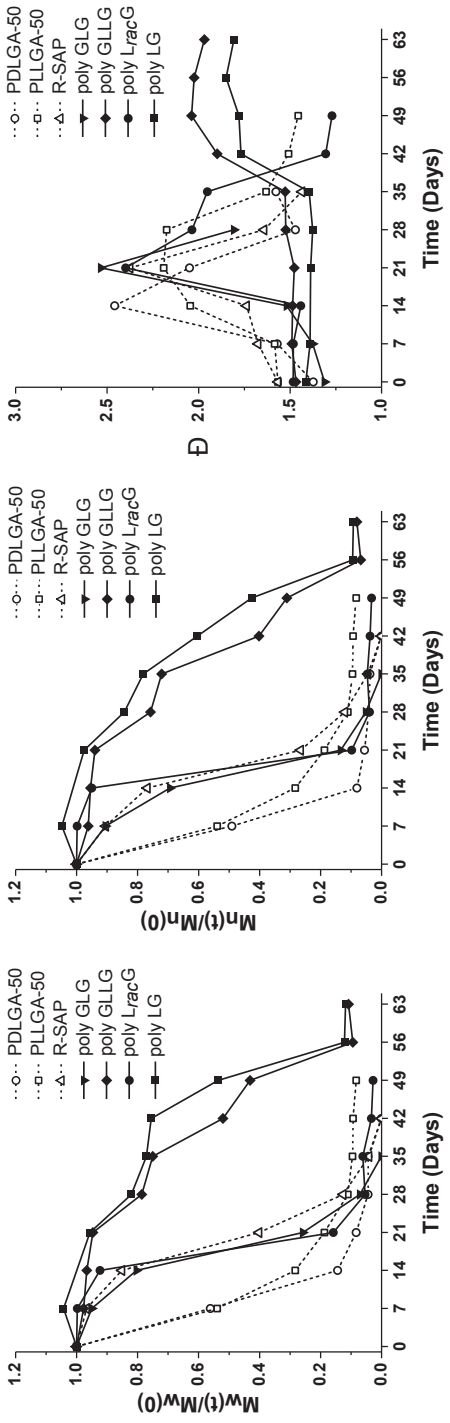


Fig. 8. Weight average molecular weight (left); number average molecular weight (center); dispersity (right) of sequenced and random PLGAs as a function of time. Open symbols represent random copolymer controls and closed symbols represent sequenced copolymers.

cited as the justification for the synthesis of a multitude of polymers based on alternate monomers [56–58]. It was our hypothesis, one now supported by the results herein, that sequence control may offer an alternate approach to adjusting PLGA properties to those required for particular applications.

Swelling and erosion are both intrinsically involved in degradation [33]. While our data conclusively demonstrate that both behaviors depend on sequence, the reason for the correlation is not immediately apparent. One possible explanation, especially given the dramatic differences in swelling, is that the properties correspond to sequence-based variations in hydrophilicity. Although not a true measure of intrinsic hydrophilicity, the fact that the surface contact angle of **PDLGA-50**, which necessarily possesses runs of L and G, is nearly the same the simple alternating **poly LG**, suggests that sequence-based differences in the interaction with water are minimal. More convincingly, we find that the water uptake in the first 3 days of the swelling experiment was nearly the same for all samples. These results suggest that the differences seen are not a function of the initial interaction with water but rather come about as a result of chain degradation processes.

The molecular weight loss profile matches the trends that we reported previously [27,28] and tracks well with the swelling and erosion behaviors. The ROP-random copolymers degrade more quickly than either the **R-SAP** or sequenced copolymers. The dispersity trends are also interesting as the dispersity increase correlates in time with the onset of significant swelling in all of the polymers.

Stereosequence also plays a key role in degradation. Both stereopure **PLLGA-50** and **poly LG** retain their structural integrity over a slightly longer time period than their racemic analogs, **PDLGA-50** and **poly L*rac*G**, respectively. While it is known that crystallinity plays a role in the differences between the random copolymers [59,60], the sequenced copolymers do not appear to crystallize to a significant extent, even during degradation [27]. A fuller understanding of this phenomenon will require further study outside the scope of the current investigation.

Overall, we hypothesize that the differences in hydrolysis profile, both in swelling and erosion, are due primarily to the variation in kinetic rates of cleavage of the L-L, L-G, G-L and G-G linkages (Fig. 10). This rate difference has long been established in the

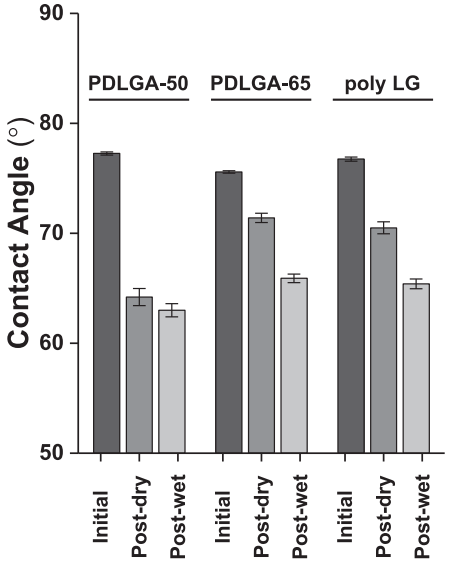


Fig. 9. Average water contact angles from unexposed films (initial) and films exposed to physiological conditions over 8 days measured in their hydrated (post-wet) and lyophilized (post-dry) states. Error bars represent the standard error of the mean.

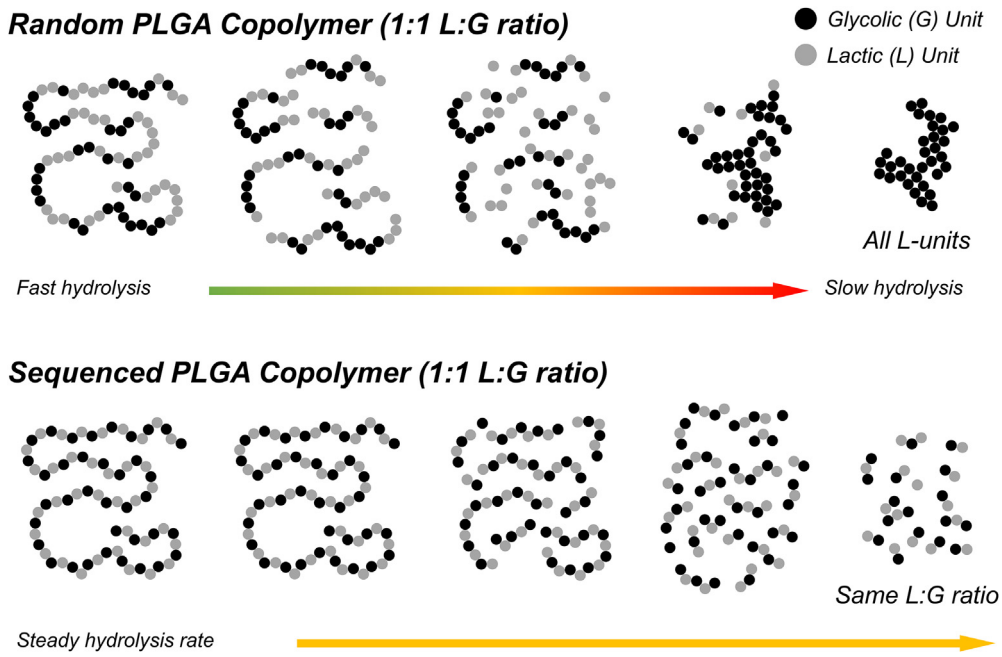


Fig. 10. Differences in the rates of hydrolysis for random and sequenced PLGA copolymers with the same L:G ratio.

random copolymer literature and has been used to explain, for example, the increase in L to G ratio during the hydrolysis period [45,59,61,62]. The observed molecular weight behavior is consistent as **PDLGA-50**, which has the full spectrum of linkages, exhibited a faster drop in molecular weight as a function of hydrolysis time than did the isomeric **poly LG**, which comprises only L-G and G-L linkages. The dispersity behavior of these copolymers also correlates as rapid cleavage of G-G linkages would be expected to result in earlier increases in polymer chain dispersity.

In the current study, the variation in cleavage rates would be expected to produce significant differences in species population within the pellets which we propose leads to the observed differences in degradation profile. The rate of chain cleavage and concomitant generation of acidic oligomers and monomers would be expected to correlate with the frequency of G-G linkages within the chain—a larger percentage of G-G linkages should lead to higher local concentrations of hydrolytic products within the construct. Although the higher local concentrations of acidic species would be expected to autocatalytically enhance interior degradation and polymer erosion [63–65], this effect alone does not explain the swelling behavior. In addition, we propose that osmotic pressure may play an important role in this case because the degradation products are likely produced more rapidly than they are released [66–68]. Under these circumstances a concentration gradient could drive the osmotic uptake of water to give swelling that correlates with the number of entrapped products [33]. While swelling should eventually facilitate clearance, thereby relieving osmotic pressure, the constructs appear to lose both structural integrity and morphological stability before such an equilibrium is reached. This hypothesis is consistent with the observation that pellets with identical compositions but higher numbers of G-G linkages degrade much faster and exhibit dramatic morphological changes.

5. Conclusions

We have demonstrated that swelling and erosion, which are both key properties related to potential applications of PLGAs, depend dramatically on the sequence of the L and G monomers. Specifically, as the number of G-G linkages is decreased, the degree of swelling is diminished and the erosion is slowed. This dependence on sequence allows for the tuning of the hydrolytic profile without additives or other comonomers. Future work will focus on both improving our understanding of the dependence of hydrolysis on sequence and on studies that test the *in vivo* performance of these materials.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.biomaterials.2016.11.037.

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