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Mapping intermediate degradation products of poly(lactic-*co*-glycolic acid) *in vitro*

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Abstract: There is widespread interest in using absorbable polymers, such as poly(lactic-*co*-glycolic acid) (PLGA), as components in the design and manufacture of new-generation drug eluting stents (DES). PLGA undergoes hydrolysis to progressively degrade through intermediate chemical entities to simple organic acids that are ultimately absorbed by the human body. Understanding the composition and structure of these intermediate degradation products is critical not only to elucidate polymer degradation pathways accurately, but also to assess the safety and performance of absorbable cardiovascular implants. However, analytical approaches to determining the intermediate degradation products have yet to be established and evaluated in a standard or regulatory setting. Hence, we developed a methodology using electrospray ionization mass spectrometry to qualitatively and quantitatively describe intermediate degradation products generated *in vitro* from two PLGA formulations commonly used in DES. Furthermore, we assessed the temporal evolution of these degradation products using time-lapse experiments. Our data demonstrated that PLGA degradation products via heterogeneous cleavage of ester bonds are modulated by multiple intrinsic and environmental factors, including polymer chemical composition, degradants solubility in water, and polymer synthesis process. We anticipate the methodologies and outcomes presented in this work will elevate the mechanistic understanding of comprehensive degradation profiles of absorbable polymeric devices, and facilitate the design and regulation of cardiovascular implants by supporting the assessments of the associated biological response to degradation products. © 2017 Wiley Periodicals, Inc. J Biomed Mater Res Part B: Appl Biomater, 106B: 1129–1137, 2018.

Key Words: absorbable polymers, poly(lactic-*co*-glycolic acid), mass spectrometry, degradation products, drug eluting stents

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INTRODUCTION

Drug eluting stents (DES) treat atherosclerosis by structurally opening diseased arteries and releasing an anti-inflammatory drug from the polymer coating, thereby mitigating the extent of in-stent restenosis.[1–3] The new generation of DES either has a completely biodegradable platform or is coated with a biodegradable polymer on a conventional bare metal stent.[4–9] The incorporation of absorbable polymers like poly(lactic-*co*-glycolic acid) (PLGA) not only enable more rapid and complete drug delivery, but also reduce the risk of complications associated with permanent polymeric components of implantable devices, such as the late and very late stent thrombosis. The stent coating consisted of PLGA facilitate to modulate drug release, while the polymer degradation eventually leaves only the bare metal stent behind at the implantation site.[10–15] Though PLGA have been used in varied medical devices, ranging from resorbable sutures to orthopedic implants[16–21] and are generally considered to be biocompatible, the adoption of absorbable polymers into cardiovascular stents calls for specific considerations regarding the circulatory system. Several studies reported inflammatory reactions with poly(lactic acid) (PLA) or poly(glycolic acid) (PGA) implants.[22] Relative higher acute toxicities of degradation products were observed for polyesters like PLA and PGA among six bioabsorbable polymers.[23] An array of biodegradable polymers including PLGA induced marked inflammatory reaction within the coronary artery with subsequent neointimal thickening.[24] These biological responses of absorbable polymers appear to vary with degradation time, which can be attributed to a combination of parent polymer compound, degradation products, and possibly, implant geometry.[24] There is a lack of information in the current standard procedure to properly assess the biocompatibility of absorbable medical devices described in ISO10993. The highly dynamic nature of polymer degradation complicates the biological evaluation of absorbable devices, especially for high-risk cardiovascular implants. Various intermediate degradants of PLGA could present or prevail at the implantation site at different stages of polymer degradation, which potentially complicates biological responses. It is challenging to detect or track the intermediate degradation products and the associated

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TABLE I. Physical Characterization of PLGA Films Prepared for This Study

Polymer	Inherent Viscosity[a]	L[b] Content	M_w[c]	PDI[c]	Mass of Film	Thickness of Film
PLGA 50	0.55–0.75 dL/g	50%	43.6 kDa	1.52	167.2 ± 9.4 mg	111.4 ± 21.9 μm
PLGA 85	0.55–0.75 dL/g	85%	80.4 kDa	1.55	162.8 ± 8.3 mg	117.6 ± 22.3 μm

[a] Data from commercial package.
[b] Lactic acid unit.
[c] Weight average molecular weight and polydispersity (PDI) were determined by SEC in THF vs. polystyrene standards.

biological responses with long term animal studies at multiple time points, as polymer degradation time can range from a few months to several years. The mass loss and molecular weight profiles of PLGA were found to be superimposable between *in vitro* and *in vivo* studies, when *in vitro* studies were properly performed under the physiological conditions.[25] Hence, *in vitro* studies come handy to elucidate degradation kinetics and intermediate degradation products at critical time points, which can be used to evaluate the safety and performance of absorbable cardiovascular implants.

PLGA undergoes hydrolysis reactions via cleavage of cleave ester bonds, eventually giving rise to the monomeric building blocks of the polymer chain—the lactic (L) and glycolic (G) acids.[14] Although PLGA degradation has been well characterized in terms of molecular weight and mass loss, the majority of studies have been solely focused on changes in residual polymers.[26–30] Limited information is available for the soluble intermediate degradation products of PLGA, which transport from polymeric implant to the surrounding tissues. Analytical approaches to determining such intermediate degradation products have yet to be established and evaluated in a standard or regulatory setting. Several standards, such as ISO 13781 and ISO/TS 17137, are under development for absorbable polymers or cardiovascular absorbable implants, but no information is provided on how to analyze intermediate degradation products. Hakkarainen et al. reported the identification of the soluble degradation products of PCL and PLA based on electrospray ionization mass spectrometry (ESI-MS).[31–34] However, results from this study are difficult to extrapolate for *in vivo* degradation, as this *in vitro* study was conducted in water using elevated temperatures that exceeded normal physiological temperature (37°C).

In this study, we systematically evaluated PLGA degradation *in vitro* using physiological conditions with an emphasis on the major intermediate degradation products of PLGA. The degradation studies were conducted under physiologic conditions with two PLGA polymers that were known to be used as coating materials in novel cardiovascular stents.[8,10,11,29,35–37] We developed a methodology using ESI-MS to characterize the intermediate degradation products in the degradation media under normal physiological conditions. The temporal evolution of the degradation products of PLGA polymers were elucidated by time-lapse experiments at multiple critical time points that were selected according to the degradation kinetics. We anticipate the methodologies and outcomes presented here will provide new insights on PLGA degradation and aid future studies to establish degradation profiles for other degradable polymers under *in vitro* conditions, to facilitate the biocompatibility assessments of absorbable cardiovascular implants.

MATERIALS AND METHODS

Chemicals and materials

PLGA containing 50% lactic acid (PLGA 50) and 85% lactic acid (PLGA 85) were purchased from Durect Corp. The properties of these PLGA formulations are listed in Table I. PLGA polymers are selected based on the similar range of intrinsic viscosity. All other chemicals and solvents used in this study were purchased at ACS grade or higher from Fisher Scientific or Sigma Aldrich and used without further processing. Phosphate buffered saline (PBS)was prepared following vendor instructions by dissolving one tablet into 200 mL of deionized water to yield 0.01*M* phosphate buffer with 0.0027*M* KCl and 0.137*M* NaCl, pH 7.4 at 25°C.

Preparation of PLGA films

Preparation of PLGA films via drop-casting was adopted from a procedure described elsewhere.[38] PLGA solutions were prepared by dissolving ~2.8 g of the materials in 10 mL of dichloromethane under gentle shaking for 1 h to facilitate dissolution. Then an aliquot equivalent to ~600 μL of the resulting solution was drop-casted into the bottom of a 20 mL scintillation vial with a micropipette. The solvent was evaporated overnight at room temperature and then dried overnight under vacuum. The resulting film coated the bottom of each vial and weighed ~160 mg (Table I) with a thickness 110–120 μm as measured by a profilometer (Dektak 150, Veeco Instrument). We validated that all resulting PLGA films were amorphous based on differential scanning calorimetry (Q200, TA Instruments).

Hydrolytic degradation *in vitro*

Our *in vitro* experiments for hydrolytic degradation mimicked normal physiological conditions. PBS (10 mL, 0.01*M*, pH 7.4) was added to the sample vials that contained the PLGA films, totaling 4 replicates for each time period. The vials were sealed and incubated at 37°C for predetermined time intervals, including day 3, 7, 14, 21, 28, 35, 42, 49, and 56. After incubation in PBS, each sample was processed via a two-pronged approach, one aimed at preparing the water-insoluble polymeric residue for molecular weight measurement by size exclusion chromatography (SEC), and the other purposed to collect an aliquot for ESI-MS characterization of water-soluble species. For SEC, the solid films were collected and lyophilized. For MS, the remaining buffer solutions were collected and stored frozen at −20°C before analysis. The pH of the aliquot was monitored at predetermined time intervals

using a calibrated pH meter (Accumet AB150, Fisher Scientific). To mimic exposure of the polymer to a constant, pH-buffered medium as expected *in vivo* (for example, by circulating blood), the spent PBS was decanted and fresh PBS was added to each scintillation vial after sampling each week.

Molecular weight measurements

Molecular weight of polymer films was determined using SEC. Briefly, polymer samples were dissolved in tetrahydrofuran (THF), filtered with 0.22 μm PTFE filters, and loaded onto gel permeation columns (models Styragel HR 4 and HR 4E, Waters Corp.) using an autosampler (model 717 plus, Waters). Polymers were separated in THF as the mobile phase supplied at a flow rate of 1 mL/min using an HPLC pump (model 515 HPLC, Waters). Separated polymers were detected based on change in refractive index (refractive index detector model 2414, Waters). Both the number and weight average molecular weights were determined from the SEC curve by calibration against polystyrene standards.

Mass loss analysis

The remaining mass (m_r) of each collected solid PLGA sample was determined after lyophilization. The mass loss was calculated by comparing m_r to the initial weight (m_0) of each film as follows:

$$\text{Mass Loss} = \left(1 - \frac{m_r}{m_0}\right) \times 100\% \quad (1)$$

Mass spectrometry analysis of degradation products

Intermediate degradation products in PBS buffer were characterized via direct ESI-MS. Briefly, the degradation solution fractions (2 mL) collected at different time points were lyophilized, redissolved in 2 mL of acetonitrile, and filtered through PTFE filters (13 mm × 0.22 μm, Fisher) to remove particulate matter from PBS buffer. The filtrate was measured by an ion trap mass spectrometer (model LTQ XL, Thermo) via direct infusion negative ion-mode ESI at a rate of 5 μL/min using −5 kV spray voltage, nitrogen as sheath gas, and the capillary heater set to 175°C. Molecular ions were detected in the range of *m/z* 50–1,200. The structural interpretation of molecular ions was facilitated by collision-induced dissociation in helium gas using collision energy of ~20–45 eV.

Data processing

Primary mass spectrometric data were processed in Xcalibur (version 2.1, Thermo). The ion signal abundance of each oligomer and monomer was normalized to the total intensity of all degradation products in the solution phase. Heat maps were prepared in Origin 8.0 (OriginLab). Error bars represent standard deviation between technical quadruplet measurements.

RESULTS

The goal of this study was to characterize physicochemical processes that govern the mechanism and time-course of degradation for PLGA 50 and PLGA 85. Our experimental strategy addressed processes unfolding during the early time domain, when large PLGA polymers are converted to insoluble oligomers, and later stage, when water-soluble products are generated and further degraded. To this end, we designed a multipronged approach, in which we characterized the physical and chemical properties of both the residual polymer film and the resulting degradation products in degradation medium using complementary analytical technologies. The *in vitro* degradation studies for both PLGA 50 and PLGA 85 were performed using PBS buffer at 37°C to mimic physiological conditions.[26] As a rudimentary mock circulatory system, the PBS buffer was changed weekly after measuring the pH of the degradation media. Each experimental condition (time and PLGA formulation) was assessed in technical quadruplet to improve statistics.

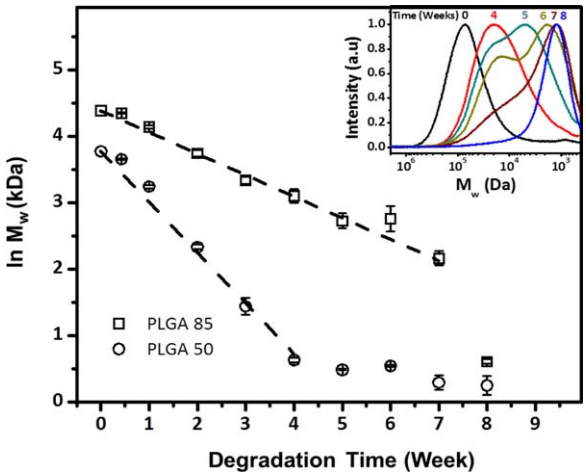

FIGURE 1. Kinetic comparison and evaluation of PLGA degradation profiles for PLGA 50 and PLGA 85 during the first 8 weeks. Inset is SEC profiles for PLGA 85 showing bimodal molecular weight distributions after 5 and 6 weeks.

Degradation kinetics

To compare the degradation kinetics of two PLGA polymers, we characterized the weight average molecular weight (M_w) and mass loss of the polymer films as a function of degradation time (Figures 1 and 2). The degradation profiles of the two polymers showed clear differences in both degradation rate and mass loss results.

The M_w vs. degradation time data for both PLGA 50 and PLGA 85 followed the autocatalytic model,[39,40] allowing us to calculate the rate of degradation for these polymer formulations. As shown in Figure 1, the ln (M_w) as a function of degradation time *(t)* can be used to calculate the degradation rate constant *k,* following the first order reaction of $\ln (M_w^t/M_w^0) = -kt$. Our calculations gave a rate constant of $k = 0.109$ day^{-1} for the degradation of PLGA 50, while PLGA 85 degraded much slower with $k = 0.0461$ day^{-1}. At the end of the degradation, the molecular weights of both polymer films products were close to or below the detection limit of SEC. It is interesting to note that the SEC traces of molecular PLGA 85 suggested a bimodal distribution of

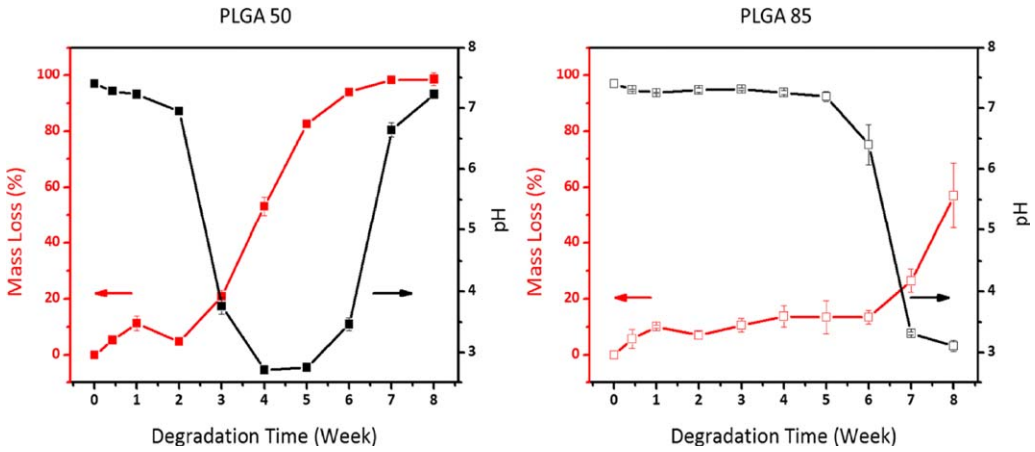

FIGURE 2. Physical changes to PLGA 50 and PLGA 85 during hydrolysis *in vitro*: mass loss in red symbols and lines, pH in black symbols and lines, and arrows to correlate mass loss and pH to corresponding Y-axes. Our time-resolved measurements showed that both polymers followed a similar degradation mechanism but exhibited major differences in the time domain. Error bars are within symbols.

molecular weights at week 5 and 6, which is commonly observed after hydrolysis of polyesters like PLGA due to a heterogeneous degradation process.[26,41,42] The polydispersity index (PDI) for PLGA 85 changed drastically from 1.5 to ~4.0, while PDI for PLGA 50 was at the range of 1.1–1.5 during degradation. By week 8, the high molecular weight peak in SEC spectra dissipated, yielding only one main peak of PLGA at the low molecular weight range, which in turned caused a sudden decrease of molecular weight that was close to the detection limit of SEC.

We also compared the time-course of degradation for films made from PLGA 50 and PLGA 85 by evaluating the mass and pH of degradation media. The results shown in Figure 2 highlight major mechanistic differences in degradation of two PLGA polymers. During the first two weeks, minimal changes in both the film mass loss (<10%) and the pH (constant pH = 7.4) of the media were observed for both polymers. PLGA 50 films then started to exhibit significant mass loss (>20%) at the third week with the media simultaneously acidifying, eventually reaching pH 3. Our experimental design underscores accelerated degradation for PLGA 50, which maintained a highly acidic condition (pH < 4) until the sixth week, even though the PBS buffer was replaced weekly. By weeks 7 and 8, the remaining PLGA 50 films lost ~90% of their original mass, allowing for neutralization upon replenishment of the media. In contrast, the mass loss of PLGA 85 films followed degradation with a significantly different time-course. PLGA 85 films maintained 85% of their original mass during the first 6 weeks of incubation, while the pH of the media changed slightly from 7.4 to 6.5, indicating slower degradation. By the seventh week, polymer mass loss was <50%, and the media became highly acidic with pH < 4. Because lactic acid units are known to hydrolyze slower than glycolic acid units[26,29,43,44] and the polymer backbone of PLGA 85 contains more lactic units than PLGA 50, slower degradation observed for PLGA 85 was in agreement with our expectation. In addition, the higher molecular weights of PLGA 85 also contributed to its slower degradation behavior than PLGA 50.[16]

Intermediate degradation products

In parallel, we qualitatively and quantitatively evaluated oligomers and monomers that were released into the degradation media. To enable the detection of diverse degradation products, we adopted a direct-infusion ESI-MS approach for this portion of the study.[31–34] The mass spectrometric analysis provided a rich set of information on PLGA degradation, both in terms of product identity and relative quantity. For example, Figure 3 identifies oligomers detected from the medium of PLGA 50 after 3 weeks of treatment. For clarity, we limited the data analysis to only singly charged ions that were detected by ESI-MS. A manual analysis of the data revealed >100 unique ions (mass-to-charge, *m/z*, values) in the spectra. Ions that were ascribed to common contaminants arising from laboratory equipment, vials, and mass spectrometric solvents, and so forth, were excluded from this number and disregarded for the remainder of the study. The majority of the detected ions corresponded to PLGA degradation products. For example, the monomeric products $[L + H_2O\text{-}H]^-$ with *m/z* 89.0 and $[G + H_2O\text{-}H]^-$ with *m/z* 76.0 as well as the dimeric products $[LL + H_2O\text{-}H]^-$ with *m/z* 161.0, $[LG + H_2O\text{-}H]^-$ with *m/z* 147.0, and $[GG + H_2O\text{-}H]^-$ with *m/z* 133.0 were readily distinguishable in the mass spectra. Larger oligomers formed entire series corresponding to the formula $[L_mG_n + H_2O\text{-}H]^-$, where "*m*" and "*n*" indicate the number of lactic and glycolic acid blocks, respectively. For example, the ion with *m/z* 407.1 was assigned to the oligomer ion $[L_3G_3 + H_2O\text{-}H]^-$ and the signal with *m/z* 421.1 was ascribed to $[L_4G_2 + H_2O\text{-}H]^-$ (see inset). Furthermore, the ESI-MS data allowed us to calculate the molecular weight of each soluble product generated during hydrolysis according to the following formula:

$$M_w = M_{w,L} \times m + M_{w,G} \times n + M_{w,end} \quad (2)$$

where $M_{w,L}$ and $M_{w,G}$ are the weight-average mass of the lactic (L) and glycolic (G) acid repeat units, respectively ($M_{w,L} = 72.1$, $M_{w,G} = 58.0$), and $M_{w,end}$ is the weight-average mass of the end group, water ($M_{w,end} = 18.0$). As previously

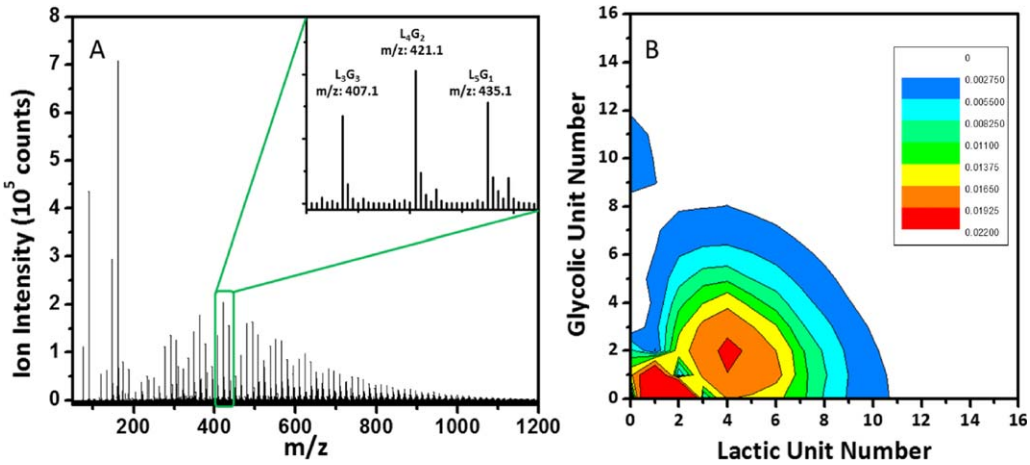

FIGURE 3. Chemical analysis of soluble oligomers produced during hydrolysis of PLGA 50 at week 3. **A**: Monomeric, oligomeric, and polymeric degradation products were identified by ESI-MS. Inset: representative ions are labeled for oligomer composition. **B**: The primary ESI-MS data are represented as a two-dimensional heat map correlating the relative abundance of soluble oligomers containing different numbers of lactic and glycolic acid building blocks.

stated, the values *m* and *n* identify the total number of L and G units in the oligomer, which are in the range 0–16 in our analysis. This information on the chemistry of soluble oligomers and monomers enabled the mechanistic interpretation of hydrolysis-driven PLGA degradation.

The ESI-MS data allowed us to quantify soluble degradation products based on a known correlation between ion signal intensity vs. solution concentration. The relative quantity was obtained by normalizing the peak intensity of each $[L_mG_n\text{-}H]^-$ oligomer by the total intensity of all oligomers in each MS spectrum. To visualize these relative quantities, we generated false-color heat maps [Figure 3(B)] that present the relative intensities of each $[L_mG_n\text{-}H]^-$ oligomer, which the highest relative intensity of oligomer at each time points was set to color red. As monomers and dimers were final degradation products that presented in high concentration, these species were excluded from the contour plot to enable deciphering of the relative quantities of the oligomers. The resulting heat maps provided qualitative and quantitative information on soluble PLGA chemistry.

The time-course evolution of degradation was evaluated using time-lapse heat maps. Major intermediate degradation products for the PLGA formulations are shown in Figure 4. The critical time points to characterize the intermediate degradation products were selected in accordance with the major mass loss of PLGA films, when large amounts of water-soluble degradation products were generated and migrated out of polymer matrices. Because the mass loss of PLGA 50 and PLGA 85 polymers was initiated at different time points (see Figure 2), only the major time points of active phase for degradation are captured and discussed here. The critical degradation period was between weeks 3–5 for PLGA 50 and weeks 6–8 for PLGA 85, during which each PLGA film dramatically lost 60–90% of its original mass. The corresponding heat maps of speciation revealed notable differences between the prevailing mechanisms of hydrolysis (Figure 4). In the case of PLGA 50, the major degradation products formed two islands in the heat map, one centered at the production/degradation of the oligomer L_4G_2 and the other on G_{10}. By the next week, degradation promoted the formation of an "L-rich" domain encompassing L_4G_2, L_4G_3, L_5G_2, and L_5G_3 as well as a prominent "G-rich" domain with oligomers centered on G_{13}. The higher solubility of longer chain glycolic oligomers as compared with lactic oligomers suggests glycolic units are more hydrophilic and, therefore, more soluble in water than lactic units. This observation is in good agreement with previous findings as discussed earlier.[29,45] As the degradation proceeded, the amounts of oligomers in both L and G regions increased as indicated by expansion in the corresponding contour areas. In contrast, PLGA 85 followed a different degradation pathway. While no products were detectable during the first 5 weeks, significant mass loss occurred by the sixth week. Although the mass loss of PLGA 85 films was only about 15% by week 6, we found that soluble oligomers were present in both L and G regions. The oligomers with 2–10 units were the major degradation products in the media. Although long-chain oligomers containing 8–14 glycolic acid units were detectably formed at the early stage of degradation, their abundance was lower than in the case of PLGA 50. The amount of these soluble degradation products of PLGA 85 also started to decrease in PBS buffer from the seventh week. At this stage, the major degradation products present were mostly oligomers with long-chain lactic components coupled with short glycolic units, $L_{4\text{-}6}G_n$ ($n = 0$–2), and G blocks of varying lengths from G_{10-14}. As the degradation approached 8 weeks, a small but detectable amount of L_mG_5 and L_mG_9 oligomers were produced. The most abundant oligomers in the media were L_{6-9}, $L_{10}G_n$, and $L_{11}G_n$ ($n = 0$–2), suggesting that the residual, insoluble polymer chains of PLGA 85 could be composed of almost 100% lactic units toward the end of degradation.

DISCUSSION

PLGA copolymers degrade following a bulk erosion mechanism in aqueous media, as the polymer undergoes a prolonged

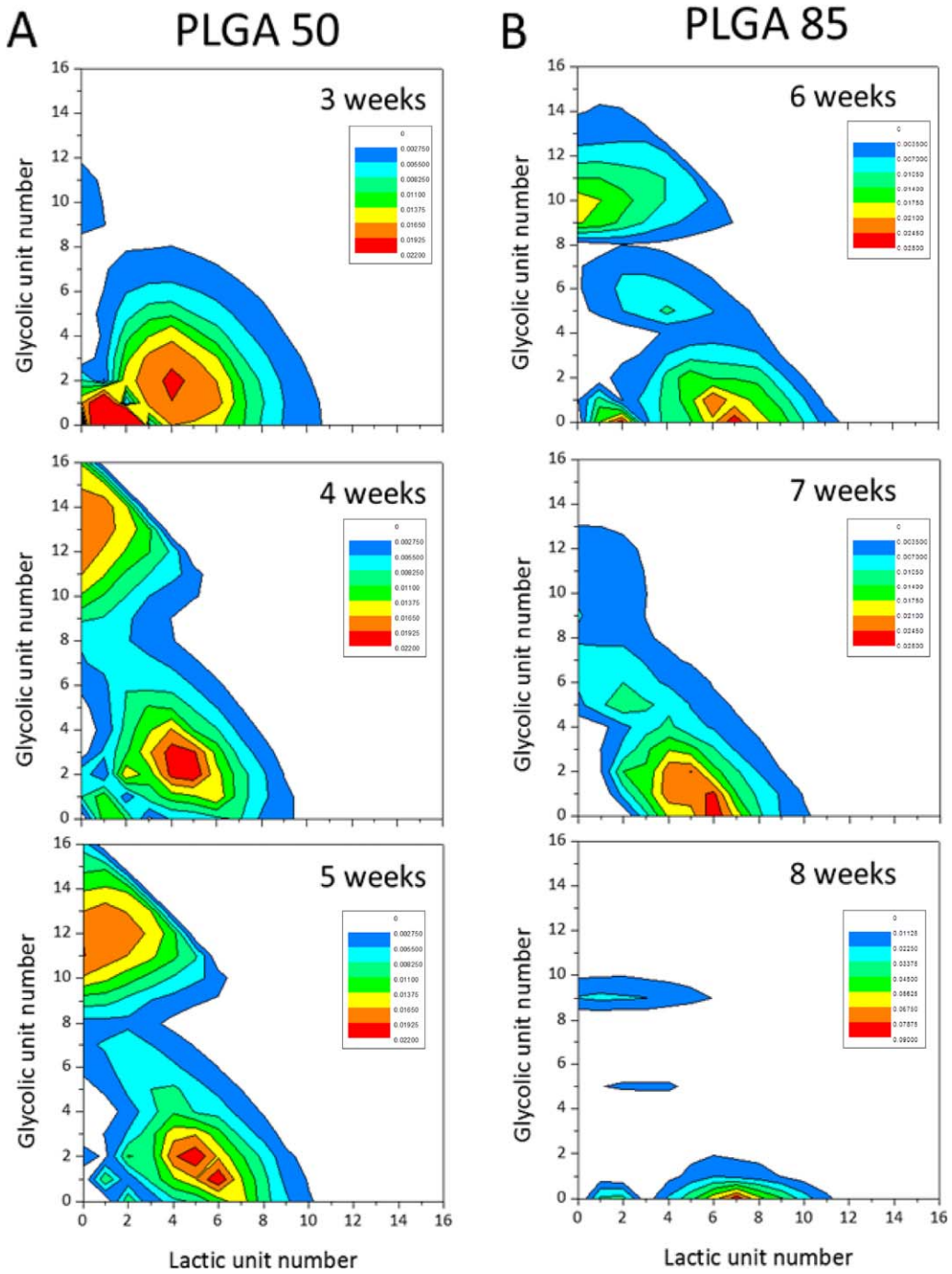

FIGURE 4. Differences in the time-course of degradation for PLGA 50 and PLGA 80 on their individual critical degradation time period: A: PLGA 50 (weeks 3–5); and B: PLGA 85 (weeks 6–8). The heat maps identify the formation of major species at these degradation time points. To aid visibility, monomeric and dimeric products are excluded from the plots.

period of reactive hydrolysis followed by sudden mass loss.[26,41,46,47] We established a multi-pronged approach in a holistic evaluation of PLGA degradation under experimental conditions that mimic normal physiological environments encountered *in vivo* by DES. The major degradation products for each PLGA were characterized by characteristic time points leading to major mass loss (see Figure 2), which were between week 3 and 5 for PLGA 50 and week 6 to 8 for PLGA 85. As summarized in Table II, the molecular weight range of the major degradation products—other than monomers and dimers—was found ~400–1,000 Da, which is well within the detection range of MS at *m/z* 50–1,200. The major degradants for PLGA 50 were L_4G_2 oligomers at the initial stage. As degradation proceeded, the long-chain oligomers G_{12-14} and eventually the short-chain oligomer L_7 became the predominant species in the media at the later stages of degradation.

TABLE II. Major degradation products of PLGA 50 and PLGA 85 from individual critical degradation time points[a]

Time (week)		L[b] rich oligomers				G[c] rich oligomers				
						PLGA 50				
Third	Oligomer	L_4G_2								
	MW[d]	422								
Fourth	Oligomer	L_5G_1	L_4G_3	L_5G_3		G_{12}	G_{13}	G_{14}	L_1G_{13}	L_1G_{14}
	MW	436	480	552		714	772	830	844	902
Fifth	Oligomer	L_6G_1	L_5G_2			G_{11}	G_{12}	L_1G_{12}	L_2G_{12}	L_3G_{12}
	MW	508	494			656	714	786	858	930
						PLGA 85				
Sixth	Oligomer	L_6G_1	L_7			G_{10}				
	MW	508	522			598				
Seventh	Oligomer	L_6	L_5G_1	L_6G_1	L_5G_2					
	MW	450	436	508	494					
Eighth	Oligomer	L_6	L_7	L_8						
	MW	450	522	594						

[a] Only degradants from critical degradation time were selected because during these periods the polymer film lost most of their mass. The critical degradation time for PLGA 50 is 3–5 weeks and for PLGA 85 is 6–8 weeks.
[b] Lactic acid unit
[c] Glycolic acid unit.
[d] Molecular weight (Da).

However, in the case of PLGA 85, the major degradants were the oligomers G_{10} and L_7 at the initial stage, followed by the production of lactic rich oligomers $L_{6\text{-}8}$.

Mapping the major intermediate degradation products provided a comprehensive understanding of the degradation behavior of PLGA. Several important factors have been reported to affect polyester degradation behavior, which are: (i) structure of polymer chains such as chemical structure, molecular weight, and molecular weight distribution; (ii) thermal/mechanic properties related to polymer conformation and configurations, including glass transition temperature (T_g), melting temperature (T_m), and crystallinity; and (iii) polymer surface properties, such as hydrophilicity or hydrophobicity.[26–30,32,34,41,44,50] As the target polymers in our study (PLGA 50 and PLGA 85) were amorphous polymers with comparable intrinsic viscosities, polymeric chemical structure played a critical role in determining degradation behavior. PLGA polymer chains are composed of lactic and glycolic units linked together with different kinds of ester bonds, including G-G, L-G/G-L, and L-L combinations. Numerous studies have demonstrated that ester bonds of PLGA polymer chains are hydrolyzed at different rates after water penetration due to the difference in hydrophilicity of lactic and glycolic units.[38] Our observations verified that G-G bonds are the first to be cleaved during hydrolytic degradation, followed by the ester bonds L-G or G-L. The ester bonds L-L degrade the slowest. As shown in Figure 4, PLGA with 50% glycolic components completes degradation several weeks earlier than PLGA 85. The most abundant degradants for both PLGA 85 and PLGA 50 were L_mG_n ($m = 4$–6 and $n = 1$–3), while oligomers with long lactic chains came to prominence only toward the end of hydrolysis.

Solubility is another important factor that governs the evolution of PLGA degradation products. As shown in our experiment (Table II), the upper limit of molecular weights for the water-soluble oligomers L_mG_n is < 600 Da, except for oligomers with extremely long glycolic blocks. Oligomers with longer glycolic blocks (G_{10-14}) demonstrated excellent solubility in the aqueous media. These long glycolic chains were ubiquitous through the entire process of degradation for PLGA 50 (see Table II). Even for PLGA 85, which contains fewer glycolic units, oligomers with 10 glycolic units are still the major degradants at the early stage of degradation. Low levels of oligomers with 6–8 lactic units were detected only by the end of degradation. Oligomer solubility as an important contributor to degradation was noted in our results on the molecular weight of residual PLGA 85. During the fifth and sixth week of degradation, a bimodal distribution became evident in the molecular weight information deduced from SEC spectra (Figure 2). A possible explanation is that oligomers with long lactic chains, L_m ($m > 10$), have low solubility in PBS buffer, leaving them trapped inside the residual polymer. These long-chain L_m oligomers were then further hydrolyzed to the short-chain products, $L_{4\text{-}6}$, which were able to migrate out of the polymer films.[28,43]

Finally, our observations revealed that PLGA degradation is also associated with the process by which these polyesters are synthesized. Random copolymers like PLGA 50 and PLGA 85 are usually synthesized or manufactured by ring-opening polymerization (ROP) with a predetermined lactide-to-glycolide ratio, so as to achieve a randomly sequenced distribution of repeating L and G units in the final PLGA chain.[49–53] The lengths and combinations of L and G blocks are determined by not only their mixing ratio, but also their competitive reactivity to form ester bonds. Based on a higher reactivity ratio between G and L units, $r_G/r_L = 10$,[51] both glycolic and lactic monomers prefer to react with glycolic monomers. Furthermore, two glycolic monomers are known react faster than two lactic monomers.[41,43] It follows that longer G blocks and shorter L blocks are anticipated in PLGA polymer chains produced by ROP, which in turn, yield oligomers with long glycolic chains (for example, G_{10-14}) and short lactic units (for example, L_{4-8}) from PLGA 50. For PLGA 85, oligomers enriched in G units only occurred at the early stage of

degradation, as its polymer chains were majorly composed of longer lactic blocks. The predominant degradation products of PLGA 85 were oligomers with short lactic chains, L_{6-8}, and molecular weights below 600 Da. The solubility of lactic oligomers is anticipated to become the dominating factor at the late term of degradation when most of the glycolic units have been degraded and further depleted from the polymer matrix after changing the degradation media.

CONCLUSIONS

We investigated the degradation mechanism of PLGA with two model polymers, PLGA 50 and PLGA 85, which are commonly used as components in absorbable DES. Our focus was to establish comprehensive degradation profiles for residual polymers and soluble degradants for PLGA by using complementary analytical technologies. We have successfully mapped out the temporal evolution of major degradation products for both PLGA 50 and PLGA 85 based on ESI-MS analysis. The data presented here demonstrate that PLGA polymers with different chemical structures (varying L/G ratios) give rise to degradation products with dissimilar compositions at various time points during hydrolysis. Specifically, we found that the major degradants for PLGA 50 were long-chain oligomers, G_{12-14}, which were not observed for PLGA 85 at any time points. Besides polymer chemical structure, we also found degradant solubility and the route of polymer synthesis to contribute to the mechanism of PLGA degradation. We envision that the dual analytical strategy for characterizing residual polymers and soluble degradation, as shown in this work, is applicable to broad categories of biodegradable polymers used in absorbable implants. The adoption of the protocols developed here could further facilitate the assessment of key factors that impact polymer degradation profiles, which in turn will help to assess associated biological response to these degradants. Knowledge of the identity and quantity of such intermediate degradation products and related a toxicological risk measurements will support biocompatibility assessments of implantable devices containing biodegradable polymers from various degradation stages.

A remaining question is how the tissue environment impacts polymer degradation. At the implantation site, the rate of hydrolysis of common degradable polymers may be sensitive to the local tissue concentration of oligomeric breakdown products. Thus, transport rates of degradation products away from the DES surface may significantly impact the degradation process. Our previous work showed that variations in the chemical characteristics of atherosclerotic plaque can significantly alter the release rate and distribution of drug following DES implantation.[54] The experiments detailed in this work combined together with our previous model of artificial plaque opens the possibility to quantifying the impact of plaque chemistry on the uptake of these oligomeric species, addressing an important aspect of the safety and effectiveness of DES that incorporate polymer species that undergo hydrolysis *in vivo*.

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DISCLAIMER

The mention of commercial products, their source, or their use in connection with the material reported herein is not to be construed as either an actual or implied endorsement of the US Food and Drug Administration and the Department of Health and Human Services. Certain commercial equipment, instruments, or materials are identified in this article to adequately specify the experimental procedure.

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