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Poly(carbonate-ester)s of Dihydroxyacetone and Lactic Acid as Potential Biomaterials

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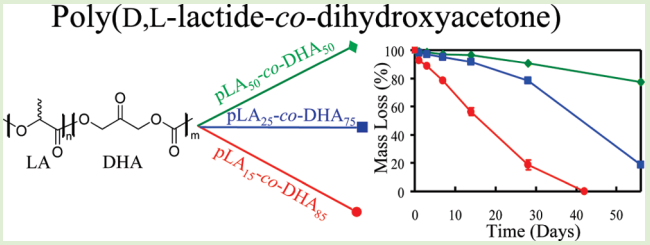
ABSTRACT: The synthesis of new polymeric biomaterials using biocompatible building blocks is important for the advancement of the biomedical field. We report the synthesis of statistically random poly(carbonate-ester)s derived from lactic acid and dihydroxyacetone by ring-opening polymerization. The monomer mole feed ratio and initiator concentration were adjusted to create various copolymer ratios and molecular weights. A dimethoxy acetal protecting group was used to stabilize the dihydroxyacetone and was removed using elemental iodine and acetone at reflux to produce the final poly(lactide-*co*-dihydroxyacetone) copolymers. The characteristics of the copolymers in their protected and deprotected forms were characterized by ^{1}H NMR, ^{13}C NMR, GPC, TGA, and DSC. Hydrolytic degradation of the deprotected copolymers was tracked over an 8-week time frame. The results show that faster degradation occurred with increased carbonate content in the copolymer backbone. The degradation pattern of the copolymers was visualized using SEM and revealed a trend toward surface erosion as the primary mode of degradation.



INTRODUCTION

The creation of new devices and materials with desirable biomedical characteristics, such as biocompatibility and easily tunable physicochemical parameters, has played a key role in the advancement of the biomedical industry. In recent years, the combination of classical engineering principles with polymer chemistry has led to a wide range of materials that influence the manner in which drugs are delivered, tissues are engineered, and surgery is performed.[1−7] The combination of engineering and chemistry, aided by the use of naturally occurring biocompatible monomeric building blocks, has yielded a number of advances in the field of biomedical engineering.

Examples of two naturally occurring monomeric building blocks that have shown to be valuable in the area of polymeric biomaterials are dihydroxyacetone and lactic acid. Dihydroxyacetone (DHA), a glycolytic metabolite, has only in recent years been explored for its use as a polymeric biomaterial. In its natural form, DHA is an intermediate in the conversion of glucose to pyruvate, is used as the active ingredient in sunless tanners, and has been used as a building block for a number of different types of polymers.[8−12]

In contrast with the more recently developed DHA monomer, lactide, a cyclic dilactone of lactic acid (LA), has been used for decades as a robust building block for the creation of a variety of tunable LA-containing polymers via a coordination−insertion controlled ring-opening polymerization.[13−15] One traditional example, poly(lactic acid-*co*-glycolic acid) (PLGA) copolymers, has been one of the most well-characterized and widely used biomaterials because of its low toxicity, easily controllable degradation rates, and ability to be cleared via normal metabolic pathways.[16−20] Previous work by Grinstaff et al. shows the value of polymerizing LA with other cyclic carbonates, such as glycerol-derived monomers, to increase the hydrolytic degradation rate. The characteristics of these new LA-based copolymers for use as scaffolds for delivering drugs has been reported.[21] From this initial work, other poly(carbonate-ester)s have been explored and developed as new biomaterials for various medical applications.[22−24]

The properties imparted to the copolymer from each monomer are the main driving force in exploring the creation of random copolymers formed via ring-opening polymerization of DL-lactide and a protected cyclic carbonate of DHA. Previous studies show that the degradation characteristics enable both LA- and DHA-based polymers to hydrolyze into safe products and are a central motivation to maximize the biocompatibility of the materials.[12,25]

It is the intent of this work to report the creation of poly(carbonate-ester)s derived from DHA and LA and to demonstrate the ease of synthesis for various molar ratios of the two monomers. Additionally, we report a new method through which to deprotect the DHA in a way that is more compatible with polyesters. Previous work using DHA for the synthesis of copolymers exemplified the need to create a protected monomer due to DHA's vulnerability toward nucelophilic addition at the

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C2 carbonyl and its capacity for forming a dimer in solution.[10,26] However, we considered that the previously reported method of acetal deprotection via acid-catalyzed cleavage in the presence of water, while proven to be efficacious for the case of diblock copolymers based on a polycarbonate of DHA and ethylene glycol, may be unsuitable for copolymers containing the more hydrolytically susceptible ester-based copolymer backbone.[27] This motivation led to an alternative deprotection method for the polymeric DHA's protecting group (a dimethoxy acetal) using elemental iodine in the presence of acetone and heat.[28] Additionally, the in vitro degradation characteristics of this new material are reported. Contrary to the behavior normally seen in compressed polyester tablets, specifically those composed of poly(lactic acid), the copolymers of DHA and LA appear to have a primarily surface erosion pattern of degradation rather than a bulk erosion pattern. The tablets also show no sign of a liquid core that would be the result of a faster degradation inside the copolymer than outside, which is the case for tablets composed of poly(lactic acid).[25,29,30] Lastly, contrary to most polycarbonate degradation studies, increasing the carbonate group content in the copolymer backbone increases the rate of degradation.[31−33]

EXPERIMENTAL SECTION

Materials. DHA dimer, *p*-toluene sulfonic acid, stannous octoate ($Sn(Oct)_2$), ethyl chloroformate, iodine, and acetone (CHROMASOLV, for HPLC, ≥99.9%) were purchased from Sigma-Aldrich (St. Louis, MO) and used as received. Triethylamine, tetrahydrofuran (THF), sodium carbonate (Na_2CO_3), dichloromethane (CH_2Cl_2), diethyl ether, and methanol were purchased from VWR (West Chester, PA) and used as received. Trimethyl orthoformate was purchased from Alfa Aesar (Ward Hill, MA) and used as received. DL-Lactide was purchased from VWR (West Chester, PA) and recrystallized in methanol. Phosphate-buffered saline (PBS) without calcium or magnesium was purchased from Bio-Whittaker (Lancaster, MA).

Characterization Methods. ^1H and ^{13}C NMR spectra were recorded on Mercury 300 MHz and Inova 400 MHz spectrometers, respectively. ^1H NMR traces were normalized to the residual DMSO-d_6 solvent peak at $\delta = 2.50$ and ^{13}C NMR was normalized to the DMSO-d_6 residual peak at $\delta = 39.52$. Gel permeation chromatography (GPC) was carried out using SDV columns (Polymer Standard Service) 500A, 50A, and linear M (in series) with a THF mobile phase (1 mL/min) and 20 polystyrene standards ranging from 1000 to 1 000 000 Da (Polymer Standard Service Win GPC software fit to a fifth order polynomial equation) as a reference for the number-average and weight-average molecular weight calculations and UV (Waters 486) and RI (Waters 2410) detection. Thermal gravimetric analysis (TGA) experiments were conducted on a TGA Q500 (TA Instruments) with a heating rate of 10 °C/min and nitrogen flow rate of 50 mL/min. Differential scanning calorimetry (DSC) measurements were performed on DSC Q1000 (TA Instruments) equipment at a heating/cooling rate of 10 °C/min and nitrogen flow rate of 50 mL/min. SEM images were taken with a Leica 440 (5 kV, 600 pA, Leica Microsystems) apparatus on samples sputter-coated with gold−palladium (60−40) using a Denton Vacuum Sputter Coater Desk II. Static contact angle measurements were performed under ambient conditions with a Ramé-Hart contact angle goniometer equipped with a syringe and flat-tipped needle allowing for a minimum droplet size of 10 μL. The probe fluid used was purified water and applied directly to the surface of dry copolymer tablets.

Synthesis of 2,2-Dimethoxypropylene Carbonate (III). This compound was synthesized following a previously published method.[26,27] In brief, DHA dimer (62.5 g, 0.348 mol), trimethylorthoformate (76.1 mL, 0.695 mol), and *p*-toluenesulfonic acid (250 mg) were dissolved with magnetic stirring in methanol (700 mL). After 24 h, sodium carbonate (750 mg) was added, and the reaction mixture was allowed to stir for an additional 24 h, after which the mixture was filtered, the solvent removed in vacuo, and the remaining viscous, brown liquid recrystallized repeatedly from diethyl ether to afford 2,2-dimethoxy-propane-1,3-diol (II) as a white, crystalline material. This compound was then cyclized by a 15 min dropwise addition of triethylamine (27.8 mL, 0.2 mol) in THF (50 mL) to a stirring flask of 2,2-dimethoxy-propane-1,3-diol (14.3 g, 105 mmol) and ethyl chloroformate (19 mL, 0.2 mol) in THF (200 mL) at 0 °C. After the addition of triethylamine was complete, the reaction was allowed to stir at room temperature for an additional 3 h, after which the mixture was filtered and the THF was removed in vacuo. The viscous, yellow product was recrystallized from ethyl ether three times to yield, 2,2-dimethoxypropylene carbonate (III), a white, crystalline powder. Yield and characterization are reported in the aforementioned references.

Synthesis of Poly(DL-lactide-*co*-2,2-dimethoxy-1,3-propylene carbonate) (IV) (Protected pLA_x-*co*-DHA_y). Prior to each reaction, a 5 mL pear-shaped flask with magnetic stir bar was flame-dried and evacuated for 10 min. Varying feed mole ratios of LA and 2,2-dimethoxypropylene carbonate (III), for a combined total of 3 mmol (pLA_{100}, protected pLA_{85}-*co*-DHA_{15}, protected pLA_{75}-*co*-DHA_{25}, protected pLA_{50}-*co*-DHA_{50}, protected pLA_{25}-*co*-DHA_{75}, protected pLA_{15}-*co*-DHA_{85}, $pDHA_{100}$), were added to the reaction vessel and allowed to evacuate for an additional 5 min. The reaction vessel was then sealed and partially immersed in a 130 °C paraffin oil bath for approximately 1−3 min until a melt had formed, at which time the reaction was allowed to stir magnetically. Following this step, 8 μl of $Sn(Oct)_2$ was added immediately using a 20 μL micropipet, and the vessel was evacuated for 10 s to 50 mmHg exactly, then immediately sealed under vacuum. The reaction was allowed to proceed at 130 °C for 1 h with stirring, after which the reaction was cooled to room temperature. The solid, semitransparent copolymer was dissolved in 1 mL of dichloromethane and precipitated by dropwise addition to 60 mL of stirring methanol whereupon the material congealed into a sticky mass which was then isolated by decanting the solvent and dried under vacuum. Reprecipitation was performed twice in the same manner and each time yielded a solid, white copolymer. The yield for each feed ratio ranged from 50 to 80%, and each ratio was synthesized in triplicate. For the in vitro degradation study, the amount of initiator was altered to create consistent molecular weights (M_w ~70 kDa). For the degradation study, the amount varied from 5.1, 6.4, or 12 μL of $Sn(Oct)_2$ for the protected pLA_{50}-*co*-DHA_{50}, protected pLA_{25}-*co*-DHA_{75}, protected pLA_{15}-*co*-DHA_{85}, reactions, respectively. These three ratios were chosen because of their physical characteristics during deprotection. Upon deprotection, the ratios containing ≥50% DHA precipitated out of solution as a fine white powder and thereby were amenable to direct compression into uniform discs. Those with <50% DHA in the backbone remained in solution until precipitation in cold diethyl ether, whereby they became a very tough, solid white mass. Molecular weights obtained by GPC and thermal data are provided in Tables 1 and 2. The ^1H and ^{13}C NMR spectra are similar for each copolymer and are exemplified by the spectrum found for protected pLA_{50}-*co*-DHA_{50} copolymer in Figures 1 and 2, respectively. ^1H NMR (DMSO-d_6) δ: 5.05−5.21 (broad m; LA 1H), 4.12 (s; DHA 2H), 3.18 (s; DHA 3H), 1.43−1.48 (broad; LA 3H). ^{13}C NMR (DMSO-d_6): δ 169 (LA, CO, multiplet), 153 (DHA, O−CO−O, multiplet), 99 (DHA, C), 68−69 and 71 (LA, CH, multiplet), 62 and 60 (DHA, CH2, multiplet), 49 (DHA, OCH3), 16 (LA, CH3). Multiplets and ranges are designated as shown in Figure 2.

Synthesis of Poly(DL-lactide-*co*-2-oxypropylene carbonate) (V) (pLA$_x$-*co*-DHA$_y$). V was prepared via conversion of the dimethoxy acetal to a carbonyl using molecular iodine in acetone. Deacetalization of IV with molecular iodine (1:1 w/w) was carried out in acetone

Table 1. Molecular Weight and Thermal Data of pLA$_x$-*co*-DHA$_y$ Copolymers

		molecular weight (GPC)			protected pLA$_x$-*co*-DHA$_y$			deprotected pLA$_x$-*co*-DHA$_y$	
mole feed LA/DHA	confirmed ratio LA/DHA	M_w (g/mol)	M_n (g/mol)	M_w/M_n	T_d (°C) 50 wt %	T_g (°C)	% deprotected	T_d (°C) 50 wt %	T_g (°C)
0:100	0:100	81000	56400	1.44	246	45	80	273	68
15:85	16:84	75100 ± 700	52500 ± 1 000	1.43 ± 0.05	240 ± 11.40	51 ± 0.90	86	252 ± 1.30	65 ± 1.10
25:75	27:73	62400 ± 900	45500 ± 1 900	1.37 ± 0.04	239 ± 1.30	50 ± 1.00	90	246 ± 4.00	64 ± 1.60
50:50	52:48	51400 ± 4 100	35700 ± 7 200	1.47 ± 0.20	240 ± 3.70	51 ± 0.30	>95	261 ± 5.50	58 ± 0.40
75:25	78:22	50400 ± 3 800	37800 ± 3 200	1.33 ± 0.03	236 ± 2.40	53 ± 0.40	>95	330 ± 2.50	55 ± 0.60
85:15	89:11	47500 ± 7 100	35400 ± 6 900	1.35 ± 0.07	228 ± 5.30	52 ± 1.30	>95	316 ± 27.30	54 ± 0.20
100:0	100:0	37900 ± 1 200	26800 ± 2 700	1.42 ± 0.15	236 ± 5.40	53 ± 0.10			
100:0[a]	100:0							309 ± 37.00	53

[a] This value is based on a pLA$_{100}$ sample subjected to the acetone/I_2 deprotection conditions.

Table 2. Molecular Weight and Thermal Data for the Copolymers Used in the in Vitro Degradation Study

	molecular weight (GPC)			deprotected pLA$_x$-*co*-DHA$_y$		
mole feed LA/DHA	M_w (g/mol)	M_n (g/mol)	M_w/M_n	contact angle (deg)	T_d (°C) 50 wt %	T_g (°C)
15:85	69900 ± 1 200	40500 ± 3 800	1.77 ± 0.10	60.5 ± 0.7	233	58
25:75	71200 ± 2 400	39800 ± 2 600	1.79 ± 0.10	61.5 ± 0.7	234	56
50:50	70000 ± 2 300	43500 ± 2 000	1.66 ± 0.10	61.5 ± 0.7	260	56

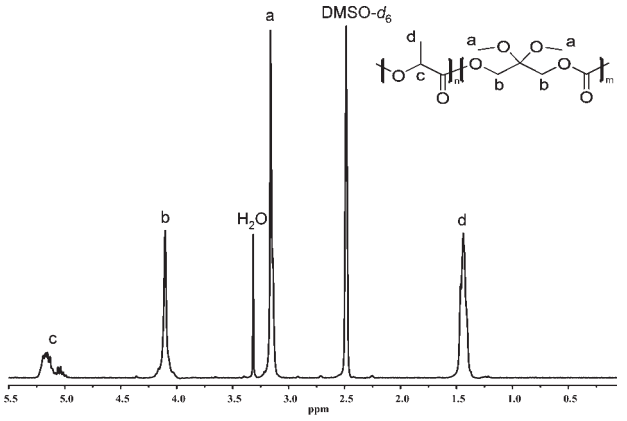


Figure 1. ^{1}H NMR spectrum of protected poly(lactic acid-*co*-2,2-dimethoxy-1,3-propylene carbonate) protected (IV) (pLA$_{50}$-*co*-DHA$_{50}$) in DMSO-d_6.

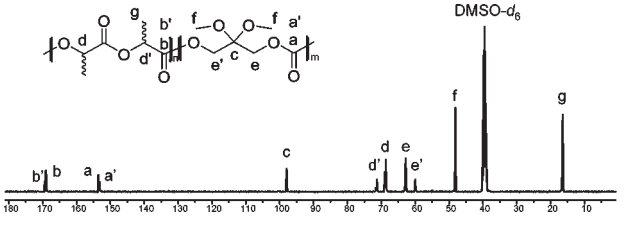


Figure 2. ^{13}C NMR spectrum of protected poly(lactic acid-*co*-2,2-dimethoxy-1,3-propylene carbonate) (IV) protected (pLA$_{50}$-*co*-DHA$_{50}$) in DMSO-d_6. The peaks assigned to a′, b′, c′, and d′ are peak shifts due to the heterogeneous placement of monomers throughout the backbone, for example, a DHA next to an LA or a DHA next to another DHA.

(CHROMASOLV, for HPLC, ≥99.9%). A 25 mL round-bottomed flask was flamed under vacuum before being charged with IV (250 mg) and iodine (250 mg) in acetone (7 mL) and allowed to stir for 2 h partially submerged in a 52 °C paraffin oil bath. For the copolymers containing 50% DHA or more, the deprotected copolymer was too insoluble to remain in solution for the entirety of the set time period. For these copolymers, the insoluble, partially deprotected copolymer was allowed to settle to the bottom of the flask after the 2 h reaction, whereupon the acetone was decanted. The fine powder product was then washed repeatedly with fresh diethyl ether to remove any visible signs of residual iodine before being transferred to a 20 mL scintillation vial. Fresh ether (15 mL) was added, the vial was capped, and the suspension was allowed to stir for an additional 3 days. During this time, the ether was decanted twice daily, and fresh ether was added as needed to remove excess iodine before being dried under vacuum. For the copolymers containing <50% DHA, the deprotected copolymer remained in solution for the set time period. For these copolymers, all but 2 mL of solvent was removed in vacuo, and the copolymer was precipitated by the slow dropwise addition of cold ether to the vigorously swirling flask containing the copolymer/iodine solution. The precipitated copolymer was washed with diethyl ether before dissolution in a minimal amount of dichloromethane and precipitated in stirring methanol (60 mL) three times. The copolymers were washed a final time with diethyl ether, isolated by filtration, and dried under vacuum. The yield for each deprotected copolymer was ∼75%. Thermal data are shown in Tables 1 and 2. Elemental analysis (Intertek QTI Laboratory) of the material shows minimal residual iodine (<0.1%). The ^{1}H NMR spectra are similar for each copolymer and are represented by Figure 4 for the pLA$_{50}$-*co*-DHA$_{50}$ copolymer. Because of low solubility, a ^{13}C NMR analysis of the deprotected copolymer was not possible. ^{1}H NMR (DMSO-d_6) δ: 5.05−5.21 (broad m; LA 1H), 4.99 (broad; DHA 2H), 1.43−1.48 (broad; LA 3H).

Monomer Distribution Analysis. ^{13}C NMR was performed on the protected copolymers using an Inova 400 MHz spectrometer in DMSO-d_6 for a period of 12 h, and the results are provided in Figure 3.

In Vitro Degradation Analysis. The in vitro degradation study was designed to run for 56 days and followed three copolymer monomer ratios, pLA$_{50}$-*co*-DHA$_{50}$, pLA$_{25}$-*co*-DHA$_{75}$, and pLA$_{15}$-*co*-DHA$_{85}$ all with M_w ∼70 000 g/mol. Each ratio was synthesized at least five times,

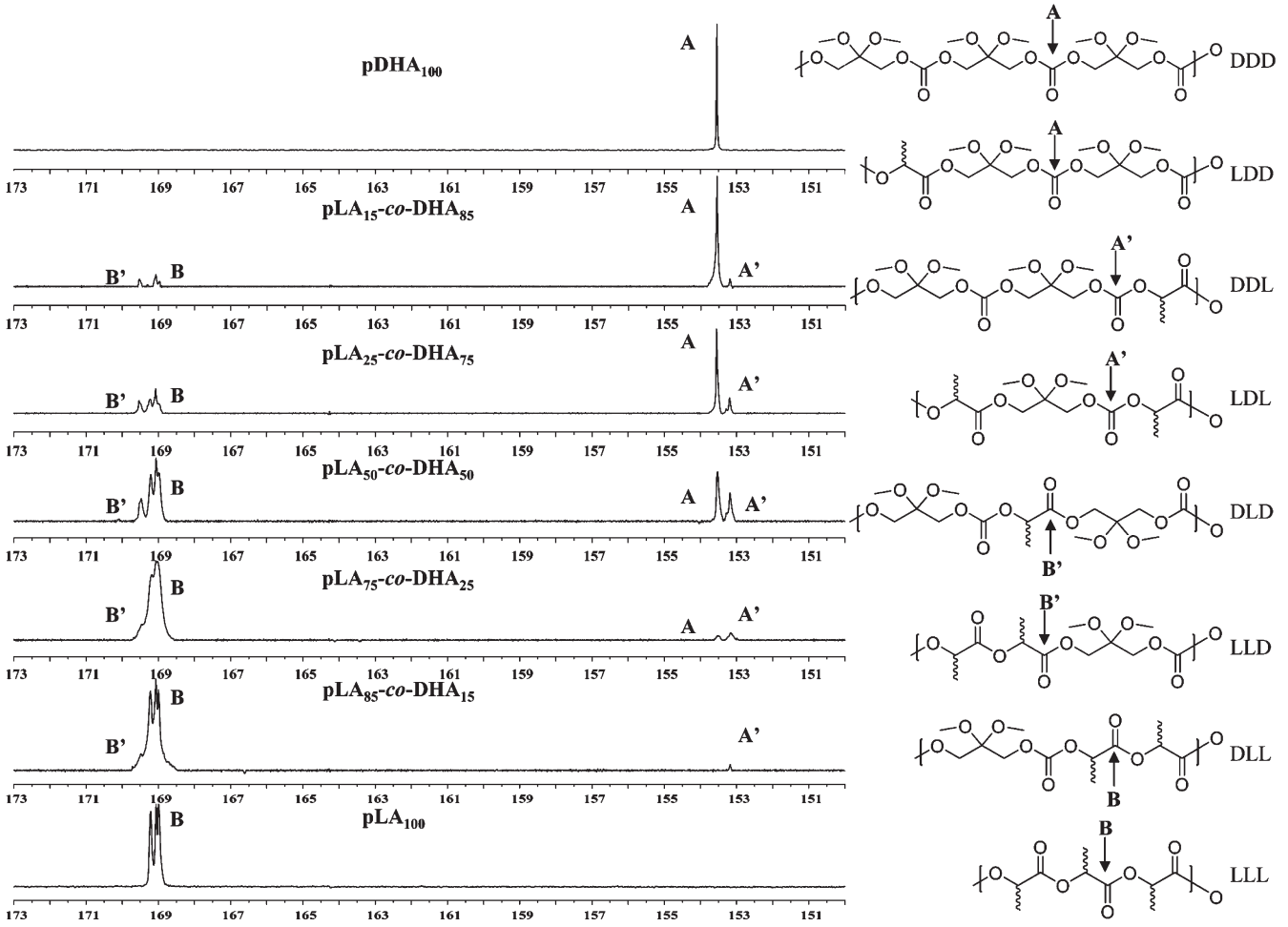


Figure 3. ^{13}C NMR spectra for protected copolymers of $pDHA_{100}$, pLA_{15}-*co*-DHA_{85}, pLA_{25}-*co*-DHA_{75}, pLA_{50}-*co*-DHA_{50}, pLA_{75}-*co*-DHA_{25}, pLA_{85}-*co*-DHA_{15}, and pLA_{100} in DMSO-d_6. The chemical structures of the eight possible triads along with their respective carbonyls noted have been included.

and the fine, white powders were combined before being sized through a 250 μm sieve. Tablets were formed using direct mechanical compression to 1000 psi for 10 s on 20 mg of copolymer at room temperature. The final cylindrical tablets had dimensions of approximately 5.32 ± 0.02 mm × 0.84 ± 0.02 mm (diameter × thickness), as measured by digital calipers. Each tablet was placed in 1× PBS buffer solution (1.5 mL, pH 7.4) and incubated with rotation (N = 3) at 37 °C. Every 3 days the buffer solutions were exchanged, and a control was used to ensure a constant pH. At predetermined time points, the samples were removed from the buffer solution, rinsed with DI water, dried by KimWipe, and lyophilized for 3 days. The mass loss and new tablet geometry were then recorded. The percent mass loss was calculated using the following expression

$$M_{\text{loss}}(\%) = \left(\frac{m_0 - m_f}{m_0}\right) \times 100 \qquad (1)$$

where m_0 refers to the initial mass and m_f refers to the final mass at a specific time point.

Morphological Characterization. Scanning electron microscopy (SEM) was used to determine the morphology of the surface and the cross-section of each tablet at each time point (Leica 440). In brief, each tablet was placed on a small metal stub and sputter coated with gold−palladium (60−40) to a thickness of 10 nm using a Denton Vacuum Sputter Coater Desk II. For the cross-sectional view, each pellet was cut in half with a razor blade, immersed in liquid nitrogen and fractured in half again by hand before sputter coating.

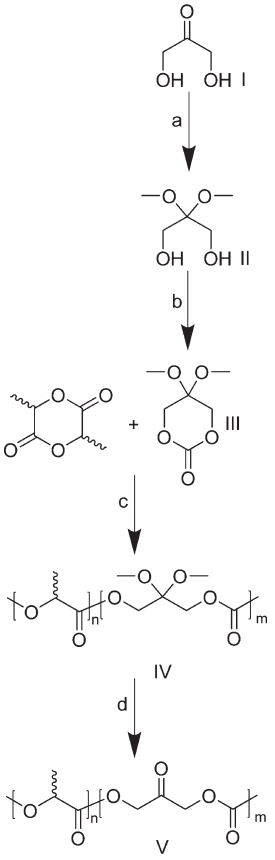
Surface Characterization. Static contact angle measurements were used to explore the surface of the three copolymer ratios used in the hydrolytic degradation study. Manual addition of a droplet of water to the top surface of each copolymer tablet allowed the contact angle to be measured. This experiment was repeated for each ratio to confirm the validity of the reading.

RESULTS AND DISCUSSION

Synthesis of Poly(DL-lactide-*co*-2-oxypropylene carbonate). The synthetic strategy employed to create copolymers of lactide and DHA is shown in Scheme 1. The DHA monomer was prepared based on earlier work using a dimethoxy acetal protecting group to prevent the DHA monomer from forming a dihemiacetal dimer.[26,27] The protected DHA was then converted to a six-membered, cyclic carbonate ring to be used for ring-opening polymerization (III).[26] Stannous octoate ($Sn(Oct)_2$) was used as the coordination catalyst because of its efficacy to promote ring-opening polymerizations of both the cyclic lactide and DHA monomers.[12,14,26,27]

Five monomer ratios of the pLA_x-*co*-DHA_y (pLA_{85}-*co*-DHA_{15}, pLA_{75}-*co*-DHA_{25}, pLA_{50}-*co*-DHA_{50}, pLA_{25}-*co*-DHA_{75}, pLA_{15}-*co*-

Scheme 1. Synthetic Route to Poly(DL-lactide-*co*-2-oxypropylene carbonate) (V)[a]



[a] (a) Trimethyl orthoformate, *p*-toluene sulfonic acid, methanol, 24 h; sodium carbonate, 24 h. (b) Ethyl chloroformate, triethylamine, tetrahydrofuran, 3 h. (c) Stannous octoate, vacuum, 130 °C, 1 h. (d) Iodine, acetone, reflux, 2 h.

DHA_{85}) were synthesized by adjusting the feed ratio of the two monomers while maintaining a constant injection volume of 8 μl of $Sn(Oct)_2$. As a basis for head-to-head comparisons, LA and DHA homopolymers (pLA_{100} and $pDHA_{100}$) were also synthesized. For the three ratios of copolymers that were investigated in the in vitro degradation study (pLA_{50}-*co*-DHA_{50}, pLA_{25}-*co*-DHA_{75}, pLA_{15}-*co*-DHA_{85}), a constant molecular weight was maintained by varying the amount of $Sn(Oct)_2$, as described in the Experimental section. Because of the higher melting temperature of the LA monomer as compared with the protected DHA monomer, all molar ratios were synthesized by a melt injection of the $Sn(Oct)_2$ at 130 °C to ensure the creation of a homogeneous monomer mixture. The mole fractions of each monomer in the copolymers were confirmed via ^{1}H NMR analysis of the $-CH_3$ species present in the LA (δ = 1.43−1.48) and the protected DHA (δ = 3.18), which is shown in Figure 1, and the average values reported in Table 1. GPC analysis of the protected copolymers with different feed ratios was performed, and the relative molecular weight, shown in Table 1, shows that a direct relationship exists between increasing DHA content and M_w for each copolymer. In addition, the GPC traces, which show a single peak, helped to substantiate the creation of the copolymers rather than two populations of homopolymers.

To elucidate further the distribution of the monomers in the copolymers, ^{13}C NMR was employed to first verify the structure, seen in Figure 2, and then to determine the monomer sequence based on the carbon resonances, which can be seen in Figure 3. ^{13}C NMR spectra provided the means to determine whether the copolymer is random, block, or alternating depending on how each monomer interacts with its neighboring species.[21,34−37] In the case of a statistically random copolymer, 2^3 triads can be theoretically formed, and these can be used to understand the molecular architecture of the copolymer. For the DL-lactide (l) and the protected DHA (d) copolymers, the eight possible triads that can be formed are as follows: LLL, LLD, DLL, DLD, LDL, LDD, DDL, and DDD. ^{13}C spectra of the homopolymers provided the basis for the LLL and DDD homopolymer resonances and thus the position of the subunits throughout the backbone. The pLA_{100} spectrum indicated that the resonance for the central carbonyl carbon in LLL is a trio of peaks that lies between 169.0 and 169.21 ppm. The multiple resonances seen for pLA_{100} were attributed to the creation of a mixture of atactic and isotactic regions along the polymer backbone and are due to a number of influencing factors. These factors include, but are not limited to, the polymerization kinetics, lactide feed composition, the favoring of meso dyads in L,L or D,D dimeric repeat units when D,L lactide is used, the extent of conversion, and the possibility of transesterification when the reaction conditions have a temperature set point >120 °C.[35−38] The protected $pDHA_{100}$ spectrum showed the DDD resonance at 153.52 ppm for the carbon associated with its carbonate bond. The determination of the two extra shifts associated with the carbonate bond's carbon seen in the copolymer spectra was determined by the gradual superposition of the spectra toward the homopolymers as the molar feed ratios changed, as shown in Figure 3. Using this information and preliminary predictive ^{13}C NMR modeling software, the new peaks observed were assigned to the two XLD triads (LLD, DLD) and two XDL triads (DDL, LDL) with signals located at 169.47 and 153.18 ppm, respectively.[39] This information supports the conclusion that the synthesized polymers are random copolymers of the LA and DHA monomers.

The removal of the dimethoxy acetal group to obtain the final copolymer, (V), was conducted utilizing a method different from the one previously reported by our group for the protected DHA derived monomer.[10,12,27] To eliminate the possibility of ester hydrolysis from the reaction conditions present in a trifluoroacetic acid−water deprotection, a deprotection catalyzed by molecular iodine in acetone was investigated.[28] With this method, the degree of deprotection was directly correlated to the molar ratio of LA and DHA within the copolymer. This relationship was found to be caused by the poor solubility of the deprotected DHA monomer in acetone. At higher concentrations of DHA in the copolymer (≥50%), the deprotection reached a solubility limit prior to complete deprotection. When a significant amount of the protected DHA within the copolymer backbone had been deprotected, the LA and remaining protected DHA were no longer able to keep the copolymer soluble in solution, leading to its precipitation. The degree of deprotection for these ratios, including $pDHA_{100}$, ranged from 80 to >95% and was determined via ^{1}H NMR. An example of the deprotected copolymer ^{1}H NMR spectrum is shown in Figure 4. Interestingly, it was noticed that even though the pLA_{50}-*co*-DHA_{50} ratio copolymers came out of solution prior to the 2 h time point, their degrees of deprotection were all >95%. In contrast, at lower

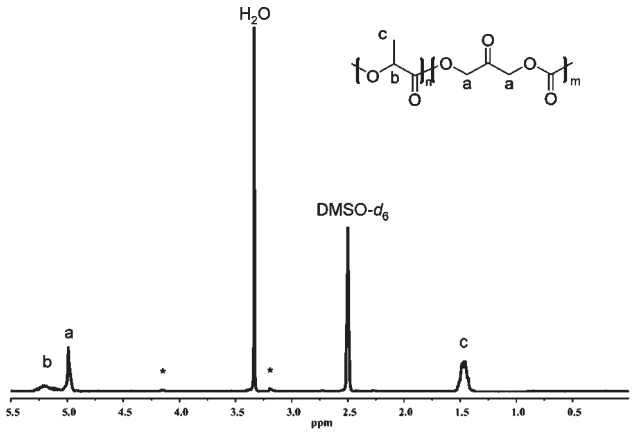


Figure 4. ^{1}H NMR spectrum of deprotected poly(lactic acid-*co*-2-oxypropylene carbonate) (V) (pLA_{50}-*co*-DHA_{50}) in DMSO-d_6. (*) denotes residual protected DHA peaks.

concentrations of DHA (<50%), all of the copolymers remained soluble in the acetone, and the degrees of deprotection were all found to be >95%. We anticipate that total deprotection was not achieved over this 2 h time scale because of the presence of trace amounts of water in the acetone, which has been reported to affect the rate and degree of deprotection.[28]

Thermal analysis of the materials was conducted to characterize the influence of each ratio of monomers on the copolymer characteristics. TGA revealed that protected $pDHA_{100}$ had a T_d of ~246 °C, whereas LA_{100} showed a T_d of ~237 °C. The T_d for all of the protected copolymers was consistent within this temperature range, and the data are presented in Table 1. Also shown in Table 1 are the T_d values for the deprotected copolymers. Upon deprotection, the T_d of $pDHA_{100}$ was elevated to 273 °C, and a new trend was observed. As the ratio of LA increased within the deprotected copolymers, the observed T_d also increased to values greater than pLA_{100}. This phenomenon may be attributed to the solubility difference of the copolymers with varying pLA_x-*co*-DHA_y ratios. Because of the insolubility of the copolymer with ≥50% DHA, these copolymers precipitate out of solution upon deprotection as a fine powder. In contrast, the copolymers that contain more LA remain soluble throughout the deprotection and precipitate in cold ether as a sticky mass. Using pLA_{100} as a control under the same deprotection conditions, elemental analysis found a very small residual amount of iodine in all of the deprotected copolymers (<0.1%), which may be the cause for the increased T_d found in the deprotected pLA_{100}, pLA_{75}-*co*-DHA_{25} and pLA_{85}-*co*-DHA_{15} samples.

DSC was conducted to distinguish further the thermal characteristics of each copolymer. A glass-transition temperature, T_g, was observed for each copolymer, whereas a melting temperature, T_m, was not observed for any of the copolymers within the set temperature range of −60 to 140 °C. The lack of a T_m also supports the conclusion that the LA in the copolymer backbone has little stereospecificity due to the random distribution of the D- and L-chiral carbons from the DL-lactide monomer and the inclusion of DHA in the backbone that may disrupt the configuration of chain segments needed to form the crystalline lattice.[40] The T_g of the protected $pDHA_{100}$ was ~45 °C, whereas the T_g of pLA_{100} was ~53 °C. All of the T_g values for the protected copolymers were between 49 and 53 °C. However, deprotected $pDHA_{100}$ showed an increased T_g at ~68 °C. The increase in T_g

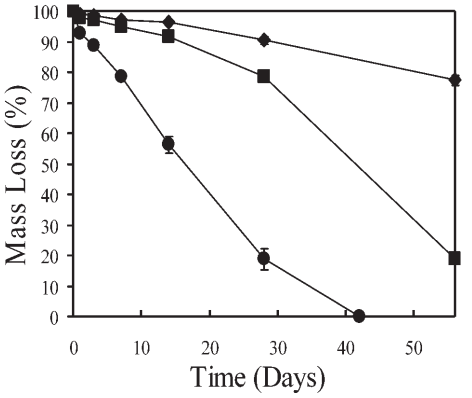


Figure 5. Mass loss as a function of degradation time for LA and DHA copolymers: (◆) pLA_{50}-*co*-DHA_{50}, (■) pLA_{25}-*co*-DHA_{75}, and (●) pLA_{15}-*co*-DHA_{85}.

observed for the deprotected DHA homopolymer was also observed for each deprotected copolymer. A trend was observed toward an increasing T_g as the DHA content within the copolymers increased, as shown in Table 1. The trend of an increased T_g as the molecular weight increased was expected because of the reported relationship that exists between T_g and molecular weight.[41]

An interesting feature of the pLA_x-*co*-DHA_y copolymers is their variable solubility as the DHA content within the copolymers changes. Previous work on DHA homopolymers showed that characterization was a challenge.[27] The introduction of 50% or more LA monomer within the random copolymer allowed more facile characterization of the copolymers. Copolymers with higher LA monomer ratios were soluble in common organic solvents such as acetone, dimethyl sulfoxide, and dichloromethane. This solubility allowed the I_2/acetone deprotection method to be much more reliable and effective than the previously reported TFA-based methods because it avoids the potential acid-catalyzed hydrolysis of the copolymer backbone, which can lead to a reduced molecular weight.

In Vitro Degradation of Poly(DL-lactide-*co*-2-oxypropylene carbonate). To begin to evaluate hydrolysis of the copolymer, an 8 week in vitro degradation study was performed. Because of the physical form of the >50% LA deprotected copolymers when precipitated, the pLA_{75}-*co*-DHA_{25} and the pLA_{85}-*co*-DHA_{15} copolymers were unsuitable candidates for the degradation study. Following deprotection, the precipitated pLA_{75}-*co*-DHA_{25} and the pLA_{85}-*co*-DHA_{15} became very tough, solid materials that were not amenable to grinding into a powder. However, the ratios of pLA_x-*co*-DHA_y with ≤50% LA had a fine powdery physical form upon deprotection, making them much more suitable for a study of this nature. Three copolymers with monomer ratios were synthesized: pLA_{50}-*co*-DHA_{50}, pLA_{25}-*co*-DHA_{75}, and pLA_{15}-*co*-DHA_{85} by varying the amount of initiator to obtain copolymers with a M_w ~70 kDa and slightly higher molecular weight distribution of ~1.7 to 1.8 than the previous materials (Table 2). Because of the insolubility of the deprotected copolymers in common GPC mobile phase solvents, molecular weight values to track the degradation of the copolymer backbone versus time were unable to be obtained. Thermal characterization of these higher molecular weight copolymers showed slightly altered values for T_d and T_g due to the change in molecular weight and polydispersity. The glass-transition temperature data observed for the 70 kDa in vitro degradation

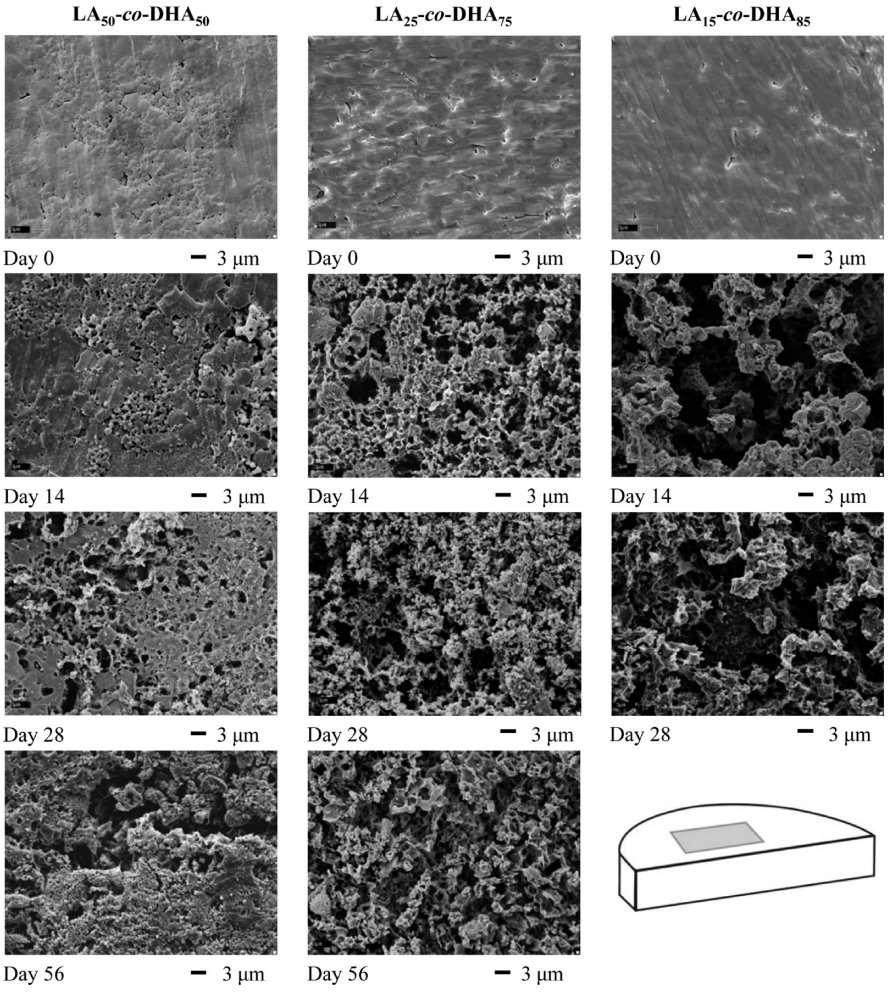


Figure 6. SEM of the top surface of pLA_x-*co*-DHA_y copolymers taken at select time points (magnification = 5000×). The day 56 image for pLA_{15}-*co*-DHA_{85} does not exist because of complete degradation prior to this time point.

copolymers are shown in Table 2. The decrease in T_g as compared with the T_g of the copolymers shown in Table 1 is most likely attributed to the increase in the polydispersity, where the molecular weight range increases the number of lower molecular weight polymers in the final material that can act as plasticizers.[42] Static contact angle measurements revealed that the surface of each copolymer ratio, while tending to be more hydrophilic in nature, did not vary significantly among the three different ratios.

Visual inspection of the compressed tablets from each time point, post lyophilization, showed a white exterior and cylindrical shape, with each tablet maintaining its geometry and color over the course of the erosion study. Upon fracture, the interior was the same white color as the exterior, and each sample appeared to have uniform structural integrity throughout the tablet. This result is significant because monomeric DHA can degrade into a dark brown product, suggesting that chemical degradation does not occur within the pellet. Depending on the copolymer composition, the longer time points of 4 and 8 weeks contained samples that were more brittle. However, comparing the interior and exterior of each sample, there were no visual signs of any large difference in mechanical properties, color changes, or morphology. This result is particularly interesting because a number of studies have tracked the in vitro degradation of LA-derived polymers and copolymers and have found significant morphological changes over the course of the experiment. To compare directly with relevant literature, Li et al. tracked the degradation of DL-LA-based polymer tablets formed using direct compression molding at a temperature below its T_g. They observed that amorphous PLA polymers underwent heterogeneous degradation with the rate of degradation on the inside faster than that of the outside.[43,44] This "inside-out" degradation pattern leads to the formation of a hard, white outer shell surrounding a viscous, transparent core that could be seen starting at 3 weeks for 100% LA (M_w = 65 000 kDa). Other studies have investigated similar morphological changes and the autocatalytic effect that causes these polyesters to degrade in this particular pattern.[25,29,30,44]

Compared with the in vitro degradation of polyesters, specifically LA homopolymers, the in vitro degradation of polycarbonates and poly(carbonate-ester)s is quite different. Long-term in vitro degradation studies using high molecular weight polycarbonate homopolymers derived of 1,3-trimethylene carbonate (TMC) show no sign of degradation after 2 years (M_n = 320 kDa), whereas $pTMC_{50}$-*co*-LA_{50} copolymers (M_n = 220 kDa) show mass loss starting around 10 weeks with the onset of degradation occurring later with increasing TMC content.[31] Another study with lower molecular weight homopolymers of TMC (M_n = 114 kDa) showed no mass loss during a 53 week in vitro degradation study, whereas $pTMC_{67}$-*co*-LA_{33} (M_n = 88 kDa) and $pTMC_{50}$-*co*-LA_{50} (M_n = 104 kDa) displayed mass loss at 9

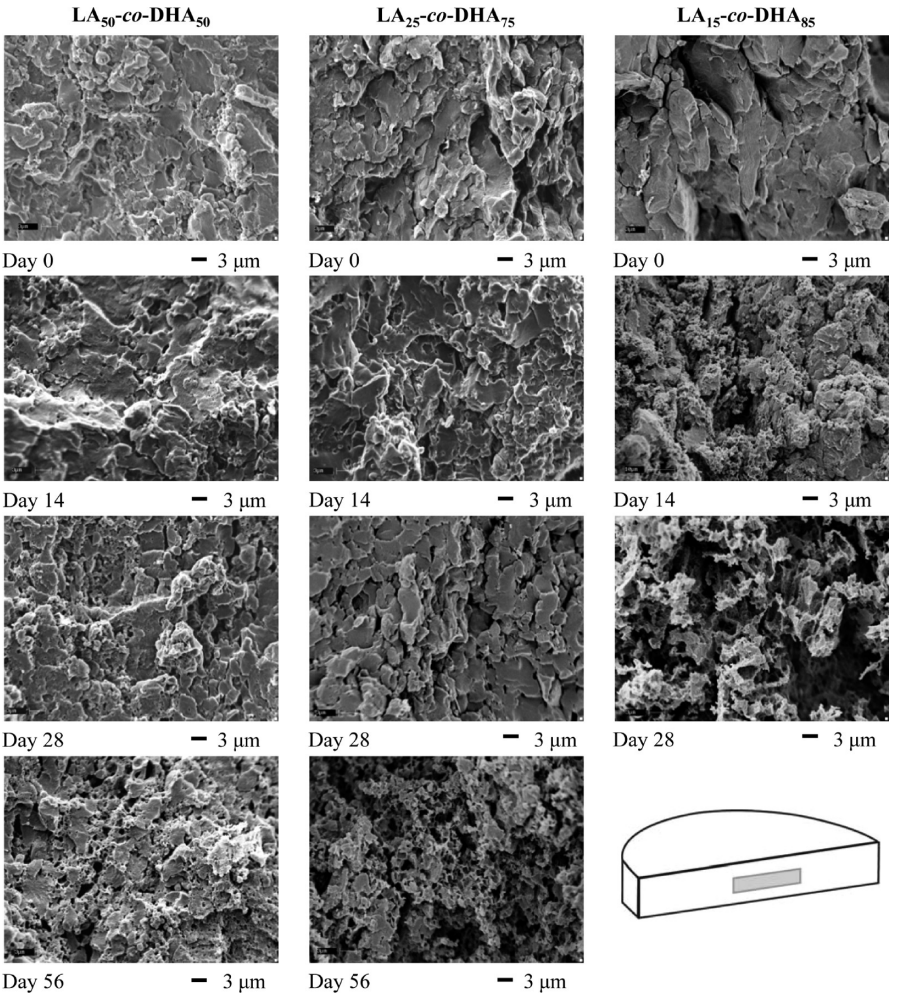


Figure 7. SEM of the cross-sectional surface near the interior of LA_x-*co*-DHA_y copolymers taken at select time points (magnification = 5000×). The day 56 image for pLA_{15}-*co*-DHA_{85} does not exist because of complete degradation prior to this time point.

weeks.[32] Both studies exemplify the trend that TMC- and LA-based poly(carbonate-ester)s have an increasing degradation rate with increasing ester content in their backbone, opposite of the results with pLA_x-*co*-DHA_y copolymers seen in Figure 5. In general, the poly(carbonate-ester) degradation pattern can be attributed to carbonate bonds being less labile to hydrolysis than ester bonds, but for pLA_x-*co*-DHA_y copolymers, we see the opposite.[33] These results, however, are consistent with previous reports of DHA polycarbonates.[26,27] Also of note is that unlike copolymers containing only ester bonds, studies using other poly(carbonate-ester)s composed of glycerol and L-LA showed no appreciable change in physical appearance throughout a 2 week study time period, even though there was a molecular weight decrease of 35%.[21]

To explore further the mechanism of degradation, SEM was used to visualize the erosion pattern on a microscopic level. Images were taken from the top, side, and cross-sectional surfaces to get a better understanding of the copolymer erosion mechanism. The images show that those copolymers with more LA in their backbone took longer to erode during the experimental time frame than those richer in DHA. This visual finding correlates to the mass loss data seen in Figure 5, which also shows that copolymers with more LA in the backbone erode at a much slower rate than those with more DHA in the backbone. The top and side views show a similar erosion pattern for the three ratios, although the erosion was much more accelerated in pLA_{15}-*co*-DHA_{85} than in pLA_{50}-*co*-DHA_{50} and can be seen in Figure 6. Similar to the top and side views, upon comparison of the cross-sectional views, a different degradation pattern was seen among the three ratios and these results are shown in Figures 7 and 8. The materials made of pLA_{50}-*co*-DHA_{50} showed very little morphological change in the center of their cores until 4 weeks, whereupon small pores had begun to form. However, the edges closest to the outside surfaces of the cross-sectional view appear to erode much more rapidly at earlier time points. At the 1 day time point, subtle morphological changes and small pores seem to form near the edge. This area of erosion increased in size with the length of time the tablets were allowed to rotate in the buffer solution, and by day 56, noticeable regions of erosion near the edges can be seen in Figure 9, as compared with the relatively unaltered interior. Tablets made of pLA_{25}-*co*-DHA_{75} showed a similar erosion pattern to those made of pLA_{50}-*co*-DHA_{50}, where the very center of the material did not show signs of degradation until the 4 week time point. At this time, a change in morphology and pores was once again observed. Unlike pLA_{50}-*co*-DHA_{50}, the interior edge of pLA_{25}-*co*-DHA_{75} showed a larger, more distinct region of erosion, and by week 8, the entirety of the material's cross-section showed

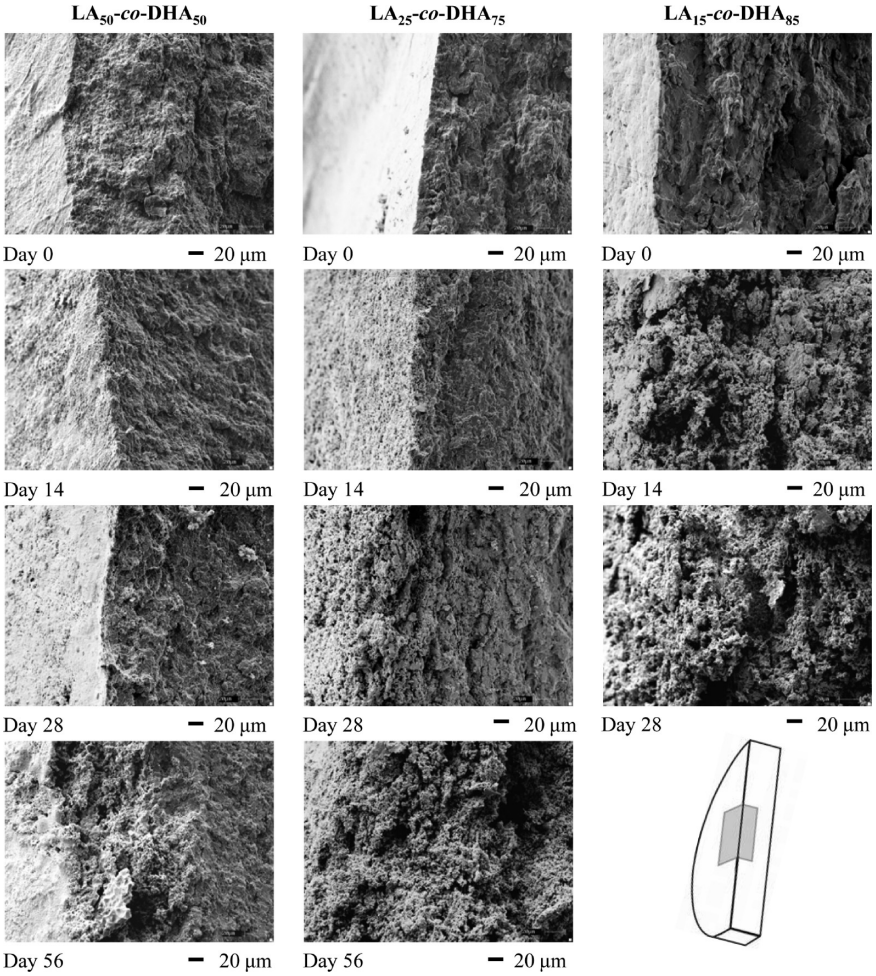


Figure 8. SEM of the cross-sectional surface at the edge of pLA_x-*co*-DHA_y copolymers taken at select time points (magnification = 1000×, tilt = 61°). The day 56 image for pLA15-*co*-DHA85 does not exist due to complete degradation prior to this time point.

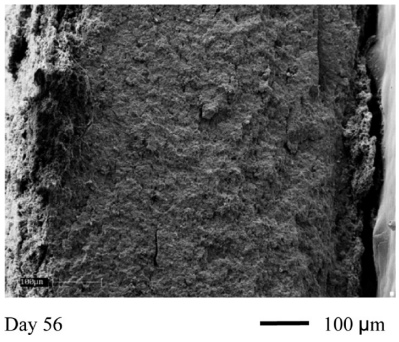


Figure 9. SEM of the entire cross-sectional surface of pLA_{50}-*co*-DHA_{50} copolymers taken at 56 days (magnification = 315×).

morphological changes. In comparison, the copolymers composed of pLA_{15}-*co*-DHA_{85} began to show morphological changes to the center of the interior by 2 weeks. These copolymers also had the largest eroded region near the edges of the interior as compared with the copolymers containing more LA. Also of note is that the pLA_{15}-*co*-DHA_{85} copolymers eroded to completion between weeks 6 and 7, and by week 2, the copolymer's cross-section showed morphological changes throughout the tablet.

The findings indicate that these poly(carbonate-ester)s display both surface and bulk erosion patterns that are unlike the erosion patterns seen with other LA or LA-derived copolymers. The SEM images show that there is an erosion zone near the edges of the sample, and the area of this zone increases during later time points. However, at later times during the study, the very center of the interior does begin to show morphological changes as well. The erosion rate for all three samples does display some linearity, but there is not a constant value throughout the study that would indicate that this material erodes by only surface erosion. The data also do not indicate that this material erodes only by bulk erosion because the geometry of the materials stays consistent and there is no distinct time at which a drastic change in erosion rate occurs, a phenomenon normally seen with LA or LA copolymers. Li et al. reported previously that pure DL-LA polymers (M_w = 65 kDa) showed this extreme rate change at 7 weeks, whereas for the pLA_{50}-*co*-DHA_{50} copolymer there is no extreme change of erosion rate throughout the 8 week study.[43,44] This is a surprising result because extensive work by the Göpferich group to model erosion behavior shows that there is a critical thickness required before a sample can undergo surface erosion.[45,46] This thickness has been theorized to be 7.4 cm for poly(α-hydroxy-acids). Other studies that track the erosion of TMC and LA poly(carbonate-ester)s reported a shift from surface erosion to bulk erosion with a LA content of > 30% in the backbone.[33]

CONCLUSIONS

Random copolymers of cyclic DL-lactide and a 6-membered ring of carbonyl-protected DHA can be synthesized via a ring-opening polymerization. The chemically protected DHA subunits of the copolymer were deprotected with a new method using elemental iodine in acetone. By successfully employing this new deprotection scheme, future DHA based polymers can be reliably created in a more favorable environment (i.e., less hydrolysis) than previously studied.[27] It was found that by copolymerizing DHA with a monomer of higher solubility in common solvents, a better degree of deprotection could be achieved. The intrinsic characteristics of each molar ratio of the LA and DHA copolymers revealed degradation temperatures and glass-transition temperatures above physiological temperature. The copolymer also showed unique degradation and erosion characteristics. It was found that even with LA amounts of 50% within the copolymer backbone, there appeared to be a more surface-like erosion pattern. It was also observed that contrary to previously reported polycarbonate behavior, increasing the amount of polycarbonate content within the copolymer increased the rate of degradation by hydrolysis. These poly(carbonate-ester)s possess very unique and favorable intrinsic characteristics such as the apparent lack of a viscous core formation due to LA build up in the interior of the pellet and the increased degradation rate with copolymers more rich in carbonate bonds.

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