Polymer Chemistry

COMMUNICATION

View Article Online
View Journal

Cite this: DOI: 10.1039/c9py00891h

Received 18th June 2019,
Accepted 10th August 2019

DOI: 10.1039/c9py00891h

rsc.li/polymers

Consequences of isolated critical monomer sequence errors for the hydrolysis behaviors of sequenced degradable polyesters†

Jamie A. Nowalk,[a] Jordan H. Swisher[a] and Tara Y. Meyer *[a,b]

Despite the known sensitivity to sequence mutations of biological polymers, little is known about the effects of errors in sequenced synthetic copolymers. The degradation behaviors of copolyesters, for example, are known to depend on monomer-by-monomer order, yet the contribution of isolated monomer substitutions on hydrolysis behaviors has not been studied. We have developed a synthetic method in which precise quantities of a critical sequence error are doped into a sequenced polyester and studied how hydrolysis behaviors are affected by this distinct and potent sequence-error. The degradation rate proved tolerant to substitutions up to 1% of the monomers but accelerated significantly when the error population was larger.

Although single monomer substitution errors in natural biomacromolecules are known to affect function, the challenges inherent in the synthesis of sequence-controlled polymers (SCPs) have inhibited the development of a parallel understanding of how small populations of monomer sequence errors affect the properties and performance in non-biological polymers.[1,2] In addition to characterizing the negative consequences of error introduction, such studies could also broaden the range of function for polymers prepared from a given library of monomers through deliberate error doping with a property-dominating monomer or segment.

Although uncommon, there have been some relevant reports of small populations of a sequence alteration affecting properties. The most prevalent studies typically involve solution-phase properties, particularly polymer folding,[3–6] aggregation,[7–14] and molecular recognition.[15–18] In the bulk phase, this phenomenon is even less well studied but there are some notable examples including the work of Winey and co-workers who determined that small alterations in side chain spacing can affect morphological order in ionomers,[19–22] Jannasch and co-workers who described the sensitivity of proton conductivity to small deviations in monomer spacing,[23] and Segalman and co-workers who described the dependence of surface structure and hydration of polypeptoids on the positioning of discrete sequences within a chain (Fig. 1).[24]

Our group, which has been active in the synthesis and characterization of SCPs, has established that a wide range of hydrolysis behaviors of poly(lactic-*co*-glycolic acid)s (PLGAs) are highly sequence-dependent, including degradation rates, guest molecule release, internal pH, and water uptake of devices made from this material.[25–27] We have not, however, probed the effects of isolated and cumulating errors on the behavior of these materials. It is important to note that in bioengineering, bioresorbable polyesters such as PLGAs are rich in application, serving as drug delivery vehicles, cell scaffolds,

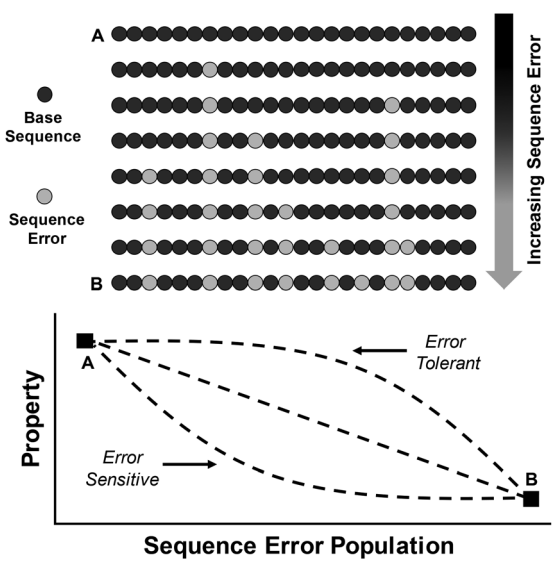

Fig. 1 Sequence tolerance pathways as a function of errors in a sequence-controlled polymer.

[a]*Department of Chemistry, University of Pittsburgh, Pittsburgh, Pennsylvania, 15260, USA. E-mail: tmeyer@pitt.edu*
[b]*McGowan Institute for Regenerative Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania, 15219, USA*

†Electronic supplementary information (ESI) available. See DOI: 10.1039/c9py00891h

degradable sutures, and osteofixation devices. The wide range of potential applications for this class of polymer inspires our interest in using sequence to tune properties.[28–34]

For the current studies, which are designed to probe the role of sequence errors, we have employed entropy-driven ring-opening metathesis polymerization (ED-ROMP) for the synthesis of the sequenced materials. ED-ROMP, which involves the ring-opening of an unstrained macromonomer that includes a sequenced segment, offers key advantages over our usual approach of coupling preformed linear segments using a step-growth polymerization.[35,36] These advantages include molecular weight control, reproducibility, dispersity minimization, and scalability.[37] Although ED-ROMP polymers necessarily include linker segments in addition to the section bearing the degradable ester sequence, we judge the trade-off to be warranted as molecular weight control is crucial to characterizing often subtle property changes connected with introduction of errors.

We focus in this study on the introduction of G–G linkage errors into an otherwise alternating segment of L and G units because in our prior studies on PLGAs, the most dramatic deviation in behaviors were observed when the hydrolysis of the random-sequence PLGA and the alternating sequence, **Poly LG**, were compared. Additionally, a more recent degradation study, which compared copolymers prepared *via* ED-ROMP, suggested that only a small degree of short-range monomer scrambling of an alternating LG sequence was enough to affect hydrolysis behaviors.[38] It became apparent that glycolic–glycolic (G–G) linkages were a key factor in determining the hydrolytic profile of sequenced PLGAs and similar copolymers. With these results, we endeavour to further quantify this sequence dependence by preparing polymers with varying degrees of precise glycolic acid monomer substitution errors and tracking hydrolysis. We address this question by preparing copolymers that consist of a repeating unit bearing a metathesis-active unit (M), a syringic acid unit (Sy), and a PLGA pentamer. Without the incorporation of Sy, the M unit significantly lowers the Tg of these materials to fall below the biologically-dictated temperature (37 °C) for future applications.[37,39] To introduce errors, we vary the proportion of macromonomers that include a perfectly sequenced LGLGL segment and ones that include the dopant LGGGL segment, *i.e.*, the errormer.

The macrocyclic monomers (Fig. 2) were prepared using previously reported methods that include sequential ester couplings, orthogonal protections, and deprotections (Schemes S1–S3†).[35,36] Benzyl (Bn) protected oligomer, Bn-SyLM, was coupled to either silyl-protected LGLGL-Si or LGGGL-Si (Si = TBDPS). Sequential benzyl and silyl deprotections gave the respective open-chain hydroxy-acid oligomers. These linear oligomers were subsequently ring-closed under dilute lactonization conditions to yield the cyclic macromonomers. Prior to polymerization, the cyclic macromonomers were combined in dichloromethane to prepare mixtures containing 0, 2.5, 5, 10, and 20 mol% of LGGGL segments. The mixtures were subjected to ED-ROMP with 1.5 mol% Grubbs second generation catalyst. The errormer mole percentages correspond to an average of 0, 3.2, 6.3, 12.6,

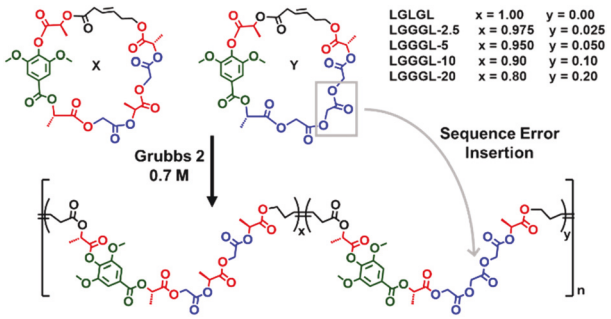

Fig. 2 Polymerization of mixtures of macrocycles to prepare a sequenced polyester with varying errormer dopant.

25.2 substitution errors per chain, where the average degree of polymerization is 530 (50 kDa). The five copolymers are named by their error content, *e.g.*, **LGLGL** contains only the base alternating sequence and **LGGGL-5** contains 5 mol% errormer. Due to the unsymmetric design of the macrocyclic monomers, the resulting copolymers contain statistical head-tail disorder at each olefin connection as evidenced by the olefin region in the ^{1}H NMR spectra. Importantly, however, the NMR data confirm that none of the other monomer linkages were affected by the polymerization which means that there are no errors outside of those deliberately introduced.[37]

Typically, the degree of sequence fidelity of PLGA-like copolymers can be quantified in the 4.5–5.0 ppm region in ^{1}H NMR spectra, where the signal of the incredibly sensitive, diastereotopic G-methylene protons appears (Fig. 3). Additionally, the ^{13}C spectra showed a gradual decrease in the sharpness and intensity of the central L carbonyl carbon in the LGLGL segment (Fig. S4†). MALDI-TOF mass spectra of the copolymers were consistent with the degree of error incorporation targeted. The fact that the lower molecular weight dimers showed a clear statistical distribution of errors supports this conclusion. Although it is challenging to use mass spectrometry for quantification, in this case the dimeric species are sufficiently similar in composition that their representation in the spectrum should correlate well with the representation in the original sample. Extrapolation of these mass data, paired with the ^{1}H NMR spectra, indicated that the error dopant was randomly and quantitatively incorporated during the polymerizations. It is worth noting that the statistical distribution of head-to-tail, head-to-head, and tail-to-tail couplings of the macromonomers are present in all samples and are not considered "errors" for the purposes of this study.

For the degradation studies, thick polymer films of 7.5 mm diameter were prepared by solution casting 28 μL of a 100 mg mL^{-1} solution onto a circular aluminium base. The solution was air dried for three hours followed by further drying in a vacuum chamber for 72 h. Each film was easily removed from the base prior to hydrolysis. In our prior work involving the determination of bulk phase sequence/property relationships with limited sample mass, we found that solution casting of thick polymer films was both efficient and reproducible.[39]

Polymer	M_n (kDa)	M_w (kDa)	Đ
LGLGL	54	66	1.22
LGGGL-2.5	54	66	1.22
LGGGL-5	50	61	1.23
LGGGL-10	50	61	1.24
LGGGL-20	49	60	1.24

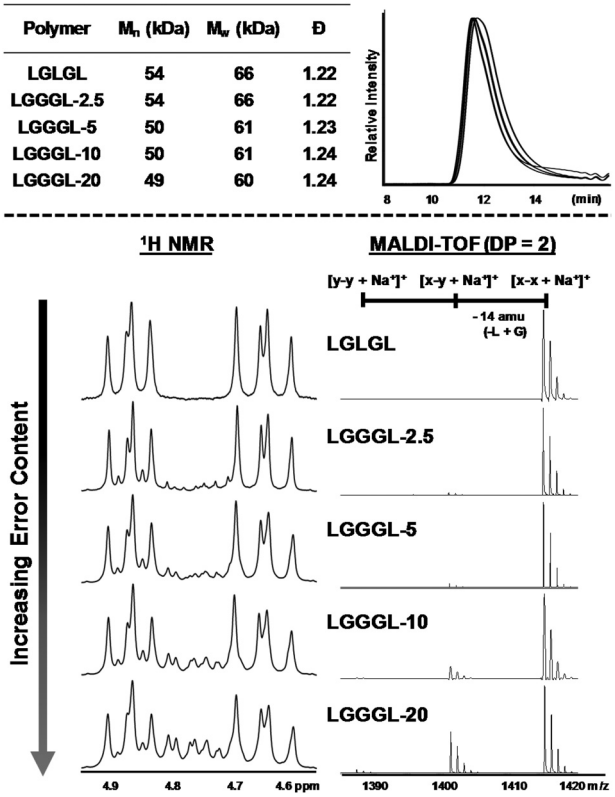

Fig. 3 (Top) Molecular weight data of the five copolymers prepared from entropy-driven ring-opening metathesis polymerization. (Bottom) Sequence characterization of the five copolymers, including ^{1}H NMR spectral region of the diastereotopic G methylene protons and MALDI-TOF spectra.

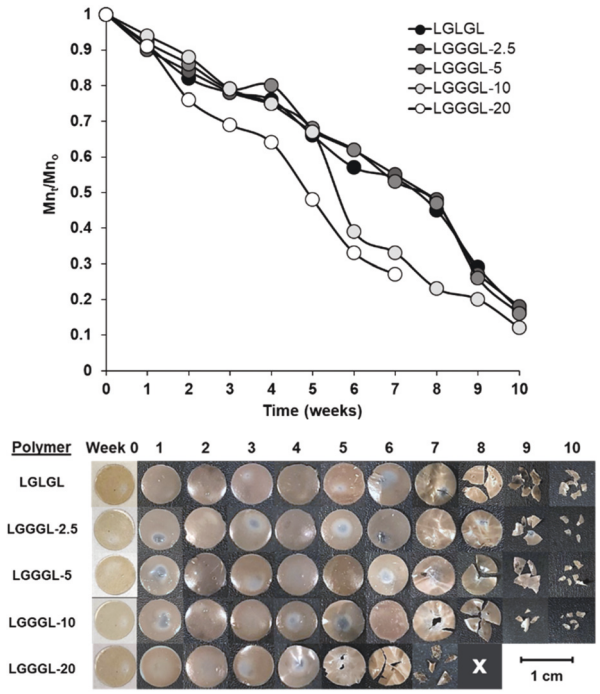

Fig. 4 (Top) Molecular weight loss during hydrolysis. (Bottom) Photographs of films at given time points during hydrolysis. X denotes particles too small to photograph.

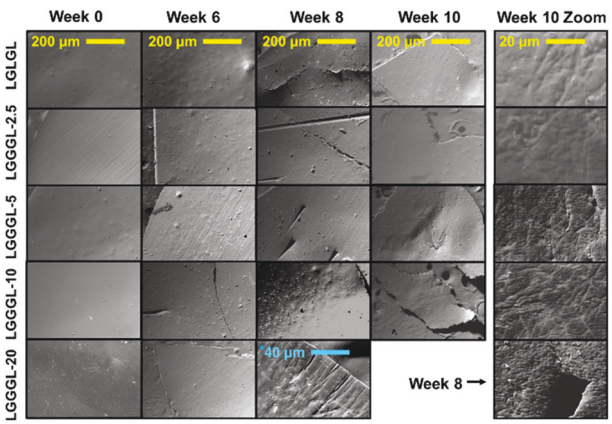

Fig. 5 Scanning electron microscopy images of thick films at varying time points in hydrolysis.

Each film, 70 μm thick and weighing ~3 mg, was placed in a dram vial with 2 mL of 10× phosphate buffer solution (PBS, pH = 7.4) and placed in an incubator at 37 °C on a rotating platform (8 rpm). Each week for ten weeks, three films of each sample were removed for SEC analysis and the reported molecular weights are an average of these three samples. The copolymers degraded uniformly with extremely little variation in molecular weights in a given set of three (range of values less than 0.04, smaller than symbols used in Fig. 4). Over time, the copolymers **LGLGL**, **LGGGL-2.5**, and **LGGGL-5** behave similarly. **LGGGL-10** degraded identically to the others until week 7; thereafter, a more rapid degradation was observed. **LGGGL-20** degraded more rapidly beginning after week 2.

When significant degradation occurred, the films became brittle and fractured into pieces (Fig. 4). It became difficult to remove the films from vials without damaging them. The extent of this fracturing seemed to depend on error content. For **LGGGL-10** and **LGGGL-20**, the film masses and particle size decreased quickly after initial fracturing. Fractures were also observed in the SEM images of each film. Additionally, high magnification images (1000×) showed an increase in surface roughness as more error was introduced (Fig. 5, Fig. S2–3†). It should be noted that the week 8 samples for **LGGGL-20** were essentially powder. They did not photograph well macroscopically, and SEM data could only be acquired at high magnification.

To interpret these results, we take into consideration that the pattern of polyester chain cleavage can dramatically affect the rate of molecular weight loss. If significant intrachain scission occurs, molecular weight drops rapidly. When the primary cleavage mechanism is end-chain scission, however, molecular weight decreases gradually as short segments near chain ends are cleaved and eliminated. We previously observed that the alternating LG sequence is resistant to significant amounts of intrachain scission.[26] Consistently, we found that

G–G linkages increased the prevalence of intrachain scission events that lead to an accelerated molecular weight loss.[40–42] In the current samples we must, therefore, hypothesize that each G–G linkage error increases the chance of MW-decreasing intrachain scission.

In conclusion, we have embedded varying quantities of a glycolic acid monomer sequence error to disrupt a primarily alternating base sequence within degradable polyesters and subjected the polymers to hydrolysis. Hydrolysis rates and surface features were monitored over time. Molecular weight loss was largely unaffected up to the incorporation of 10 mol% cyclic macro monomer error, which translates to an average of 6.3 monomer sequence errors per chain of ~530 monomers or approximately 1%. Above 10 mol%, the degradation accelerates, indicating that the errors are becoming more dominant in controlling hydrolysis patterns.

We anticipate that the knowledge gained from the current study can aid in the engineering of PLGA-type polymers with specific properties. One approach would be to exploit potent error dopants to tune one property with a minimal impact on another. We could, for example, tune degradation times by adding a small number of G–G linkage errors without dramatically affecting other properties like swelling or loading capacities. We are continuing our investigations into semi-sequencing techniques to manipulate behaviors.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

The authors would like to acknowledge the University of Pittsburgh and the National Science Foundation for financial support – NSF CHE-1709144 and CHE-1625002 for MALDI-ToF instrumentation.

Notes and references

1 *Sequence-Controlled Polymers*, ed. J. F. Lutz, Wiley-VCH Verlag GmbH & Co. KGaA, 2017.
2 K. L. George and W. S. Horne, Foldamer Tertiary Structure through Sequence-Guided Protein Backbone Alteration, *Acc. Chem. Res.*, 2018, **51**(5), 1220–1228.
3 B. V. K. J. Schmidt, N. Fechler, J. Falkenhagen and J.-F. Lutz, Controlled folding of synthetic polymer chains through the formation of positionable covalent bridges, *Nat. Chem.*, 2011, **3**(3), 234–238.
4 A. S. Knight, E. Y. Zhou, M. B. Francis and R. N. Zuckermann, Sequence Programmable Peptoid Polymers for Diverse Materials Applications, *Adv. Mater.*, 2015, **27**(38), 5665–5691.
5 M. Luo, J. R. Brown, R. A. Remy, D. M. Scott, M. E. Mackay, L. M. Hall and T. H. Epps, Determination of Interfacial Mixing in Tapered Block Polymer Thin Films: Experimental and Theoretical Investigations, *Macromolecules*, 2016, **49**(14), 5213–5222.
6 J. P. Cole, A. M. Hanlon, K. J. Rodriguez and E. B. Berda, Protein-like structure and activity in synthetic polymers, *J. Polym. Sci., Part A: Polym. Chem.*, 2017, **55**(2), 191–206.
7 J. A. Crapster, I. A. Guzei and H. E. Blackwell, A Peptoid Ribbon Secondary Structure, *Angew. Chem., Int. Ed.*, 2013, **52**(19), 5079–5084.
8 M. L. Hebert, D. S. Shah, P. Blake, J. P. Turner and S. L. Servoss, Tunable peptoid microspheres: effects of side chain chemistry and sequence, *Org. Biomol. Chem.*, 2013, **11**(27), 4459–4464.
9 J. R. McDaniel, I. Weitzhandler, S. Prevost, K. B. Vargo, M.-S. Appavou, D. A. Hammer, M. Gradzielski and A. Chilkoti, Noncanonical Self-Assembly of Highly Asymmetric Genetically Encoded Polypeptide Amphiphiles into Cylindrical Micelles, *Nano Lett.*, 2014, **14**(11), 6590–6598.
10 H.-M. Shin, C.-M. Kang, M.-H. Yoon and J. Seo, Peptoid helicity modulation: precise control of peptoid secondary structures via position-specific placement of chiral monomers, *Chem. Commun.*, 2014, **50**(34), 4465–4468.
11 Y. Chen, T. Liu, G. Xu, J. Zhang, X. Zhai, J. Yuan and Y. Tan, Aggregation behavior of X-shaped branched block copolymers at the air/water interface: effect of block sequence and temperature, *Colloid Polym. Sci.*, 2015, **293**(1), 97–107.
12 X. Xu, G. Shan and P. Pan, Amphiphilic quasi-block copolymers and their self-assembled nanoparticles via thermally induced interfacial absorption in miniemulsion polymerization, *RSC Adv.*, 2015, **5**(62), 50118–50125.
13 C.-L. Chen, R. N. Zuckermann and J. J. DeYoreo, Surface-Directed Assembly of Sequence-Defined Synthetic Polymers into Networks of Hexagonally Patterned Nanoribbons with Controlled Functionalities, *ACS Nano*, 2016, **10**(5), 5314–5320.
14 P. J. M. Stals, C.-Y. Cheng, L. van Beek, A. C. Wauters, A. R. A. Palmans, S. Han and E. W. Meijer, Surface water retardation around single-chain polymeric nanoparticles: critical for catalytic function?, *Chem. Sci.*, 2016, **7**(3), 2011–2015.
15 Z. Zhu, C. J. Cardin, Y. Gan, C. A. Murray, A. J. P. White, D. J. Williams and H. M. Colquhoun, Conformational Modulation of Sequence Recognition in Synthetic Macromolecules, *J. Am. Chem. Soc.*, 2011, **133**(48), 19442–19447.
16 K. Yoshimatsu, B. K. Lesel, Y. Yonamine, J. M. Beierle, K. J. Shea and Y. Hoshino, Temperature-Responsive "Catch-and-Release" of Proteins using Multifunctional Polymer Nanoparticles, *Angew. Chem., Int. Ed.*, 2012, **51**(10), 2405–2408.
17 J. Romulus and M. Weck, Single-Chain Polymer Self-Assembly Using Complementary Hydrogen Bonding Units, *Macromol. Rapid Commun.*, 2013, **34**(19), 1518–1523.
18 C. S. Mahon and D. A. Fulton, Mimicking nature with synthetic macromolecules capable of recognition, *Nat. Chem.*, 2014, **6**(8), 665–672.

19 E. B. Trigg, B. J. Tiegs, G. W. Coates and K. I. Winey, High Morphological Order in a Nearly Precise Acid-Containing Polymer and Ionomer, *ACS Macro Lett.*, 2017, **6**(9), 947–951.
20 M. E. Seitz, C. D. Chan, K. L. Opper, T. W. Baughman, K. B. Wagener and K. I. Winey, Nanoscale Morphology in Precisely Sequenced Poly(ethylene-co-acrylic acid) Zinc Ionomers, *J. Am. Chem. Soc.*, 2010, **132**(23), 8165–8174.
21 L. R. Middleton, E. B. Trigg, E. Schwartz, K. L. Opper, T. W. Baughman, K. B. Wagener and K. I. Winey, Role of Periodicity and Acid Chemistry on the Morphological Evolution and Strength in Precise Polyethylenes, *Macromolecules*, 2016, **49**(21), 8209–8218.
22 T. W. Baughman, C. D. Chan, K. I. Winey and K. B. Wagener, Synthesis and Morphology of Well-Defined Poly(ethylene-co-acrylic acid) Copolymers, *Macromolecules*, 2007, **40**(18), 6564–6571.
23 X.-F. Li, F. P. V. Paoloni, E. A. Weiber, Z.-H. Jiang and P. Jannasch, Fully Aromatic Ionomers with Precisely Sequenced Sulfonated Moieties for Enhanced Proton Conductivity, *Macromolecules*, 2012, **45**(3), 1447–1459.
24 C. Leng, H. G. Buss, R. A. Segalman and Z. Chen, Surface Structure and Hydration of Sequence-Specific Amphiphilic Polypeptoids for Antifouling/Fouling Release Applications, *Langmuir*, 2015, **31**(34), 9306–9311.
25 J. Li, S. N. Rothstein, S. R. Little, H. M. Edenborn and T. Y. Meyer, The Effect of Monomer Order on the Hydrolysis of Biodegradable Poly(lactic-co-glycolic acid) Repeating Sequence Copolymers, *J. Am. Chem. Soc.*, 2012, **134**(39), 16352–16359.
26 M. A. Washington, D. J. Swiner, K. R. Bell, M. V. Fedorchak, S. R. Little and T. Y. Meyer, The impact of monomer sequence and stereochemistry on the swelling and erosion of biodegradable poly(lactic-co-glycolic acid) matrices, *Biomaterials*, 2017, **117**, 66–76.
27 M. A. Washington, S. C. Balmert, M. V. Fedorchak, S. R. Little, S. C. Watkins and T. Y. Meyer, Monomer sequence in PLGA microparticles: Effects on acidic microclimates and in vivo inflammatory response, *Acta Biomater.*, 2018, **65**, 259–271.
28 J. M. Anderson and M. S. Shive, Biodegradation and biocompatibility of PLA and PLGA microspheres, *Adv. Drug Delivery Rev.*, 1997, **28**(1), 5–24.
29 L. Lu, C. A. Garcia and A. G. Mikos, In vitro degradation of thin poly(DL-lactic-co-glycolic acid) films, *J. Biomed. Mater. Res.*, 1999, **46**(2), 236–244.
30 R. A. Jain, The manufacturing techniques of various drug loaded biodegradable poly(lactide-co-glycolide) (PLGA) devices, *Biomaterials*, 2000, **21**(23), 2475–2490.
31 M. Darestani, A. Akbar Entezami, H. Mobedi and M. Abtahi, *Degradation of Poly(D,L-lactide-co-glycolide) 50 : 50 Implant in Aqueous Medium*, 2005, vol. 14.
32 P. Gentile, V. Chiono, I. Carmagnola and V. P. Hatton, An Overview of Poly(lactic-co-glycolic) Acid (PLGA)-Based Biomaterials for Bone Tissue Engineering, *Int. J. Mol. Sci.*, 2014, **15**(3), 3640–3659.
33 D. N. Kapoor, A. Bhatia, R. Kaur, R. Sharma, G. Kaur and S. Dhawan, PLGA: a unique polymer for drug delivery, *Ther. Deliv.*, 2015, **6**(1), 41–58.
34 A. Shibata, M. Machida, N. Kondo and M. Terakawa, Biodegradability of poly(lactic-co-glycolic acid) and poly(l-lactic acid) after deep-ultraviolet femtosecond and nanosecond laser irradiation, *Appl. Phys. A*, 2017, **123**(6), 438.
35 R. M. Stayshich and T. Y. Meyer, New Insights into Poly (lactic-co-glycolic acid) Microstructure: Using Repeating Sequence Copolymers To Decipher Complex NMR and Thermal Behavior, *J. Am. Chem. Soc.*, 2010, **132**(31), 10920–10934.
36 R. M. Weiss, E. M. Jones, D. E. Shafer, R. M. Stayshich and T. Y. Meyer, Synthesis of repeating sequence copolymers of lactic, glycolic, and caprolactic acids, *J. Polym. Sci., Part A: Polym. Chem.*, 2011, **49**(8), 1847–1855.
37 J. A. Nowalk, C. Fang, A. L. Short, R. M. Weiss, J. H. Swisher, P. Liu and T. Y. Meyer, Sequence-Controlled Polymers Through Entropy-Driven Ring-Opening Metathesis Polymerization: Theory, Molecular Weight Control, and Monomer Design, *J. Am. Chem. Soc.*, 2019, **141**, 5741–5752.
38 J. A. Nowalk, J. H. Swisher and T. Y. Meyer, Influence of Short-Range Scrambling of Monomer Order on the Hydrolysis Behaviors of Sequenced Degradable Polyesters, *Macromolecules*, 2019, **141**.
39 J. H. Swisher, J. A. Nowalk and T. Y. Meyer, Property impact of common linker segments in sequence-controlled polyesters, *Polym. Chem.*, 2018.
40 A. Gleadall, J. Pan, M.-A. Kruft and M. Kellomäki, Degradation mechanisms of bioresorbable polyesters. Part 1. Effects of random scission, end scission and autocatalysis, *Acta Biomater.*, 2014, **10**(5), 2223–2232.
41 Y. K. Ho, P. Doshi, H. K. Yeoh and G. C. Ngoh, Modeling chain-end scission using the Fixed Pivot technique, *Chem. Eng. Sci.*, 2014, **116**, 601–610.
42 K. Pahnke, J. Brandt, G. Gryn'ova, C. Y. Lin, O. Altintas, F. G. Schmidt, A. Lederer, M. L. Coote and C. Barner-Kowollik, Entropy-Driven Selectivity for Chain Scission: Where Macromolecules Cleave, *Angew. Chem., Int. Ed.*, 2016, **55**(4), 1514–1518.