Title

Dual responsive polymeric nanoparticles prepared by direct functionalization of polylactic acid-based polymers via graft-from ring opening metathesis polymerization

Journal

Chemical Communications, 52(3)

ISSN

1359-7345

Authors

Veccharelli, Kate M
Tong, Venus K
Young, Jennifer L
et al.

Publication Date

2016-01-11

DOI

10.1039/c5cc07882b

Peer reviewed

ChemComm

COMMUNICATION

View Article Online
View Journal | View Issue

Cite this: *Chem. Commun.*, 2016, 52, 567

Received 20th September 2015,
Accepted 22nd October 2015

DOI: 10.1039/c5cc07882b

www.rsc.org/chemcomm

Dual responsive polymeric nanoparticles prepared by direct functionalization of polylactic acid-based polymers *via* graft-from ring opening metathesis polymerization†

Kate M. Veccharelli, Venus K. Tong, Jennifer L. Young, Jerry Yang* and Nathan C. Gianneschi*

Polylactic acid (PLA) has found widespread use in plastics and in biomedical applications due to its biodegradability into natural benign products. However, PLA-based materials remain limited in usefulness due to difficulty of incorporating functional groups into the polymer backbone. In this paper, we report a strategy for PLA functionalization that establishes the preparation of highly derivatized materials in which ring opening metathesis polymerization (ROMP) is employed as a graft-from polymerization technique utilizing a norbornene-modified handle incorporated into the PLA backbone. As a demonstration of this new synthetic methodology, a PLA-derived nanoparticle bearing imidazole units protected with a photolabile group was prepared. The morphology of this material could be controllably altered in response to exposure of UV light or acidic pH as a stimulus. We anticipate that this graft-from approach to derivatization of PLA could find broad use in the development of modified, biodegradable PLA-based materials.

Polymers with hydrolyzable backbones such as polylactic acid (PLA) have opened new avenues for research and development with their enhanced biodegradability and resulting low toxicity for *in vivo* use.[1] PLA has been utilized heavily as a biodegradable polymer in numerous *in vivo* applications ranging from surgical sutures,[2] surgical implants[3] and drug delivery systems.[4,5] However, the utility of PLA is limited as the polymer backbone is difficult to chemically functionalize. As such, significant effort has been expended to increase functionalization of PLA polymers and to tune its mechanical properties.[6–9]

Current strategies for functionalizing PLA typically involve copolymerization of D,L-lactide with functionalized lactides in the presence of a catalyst and an initiator to generate functionalized PLA copolymers *via* ring opening polymerization (ROP).[10–15] While this technique has led to the development of a range of functionalized PLA polymers, it remains synthetically challenging to produce high molecular weight polymers with a large weight percentage of the added functionality. This is likely due to decreased rates of polymerization resulting from the steric bulk of functionalized lactides, leading to incomplete polymerization of the substituted monomer.[11,16] Therefore, ring substitution has a major impact on the polymerizability of functionalized lactide monomers.[11,16] To generate PLA polymers with a higher degree of functionality, post polymerization modification strategies *via* a graft-to approach have been demonstrated. For example "Click" chemistry allows functionality to be incorporated into the polymer relatively easily since the backbone and the side chains can be prepared separately prior to coupling.[12,15,17–20] However, efforts to increase graft densities are usually limited as a result of steric repulsion between the bulky side chains.[21–24] Another way to prepare functionalized PLA materials is to utilize a graft-through polymerization method. In this scenario, a macromonomer is polymerized *via* polymerizable end groups to create a brush polymer. PLA polymers have been synthesized *via* this method by coupling the PLA polymer to a strained olefin and subsequently polymerizing the olefin, resulting in bottle-brush PLA polymers.[25–28] Utilizing this grafting-through technique is attractive for polymer synthesis because it does not entail orthogonal chemistries for grafting various side chains. However, this polymerization technique can be challenging due to the increased steric hindrance of the propagating polymer chain. As a result, polymerizations can be slow and may not proceed to complete conversion.[21–24]

An alternative technique utilizes a graft-from polymerization strategy to incorporate initiation groups into ROP monomers, such as cyclic esters and carbonates, prior to ROP. This novel technique has been demonstrated in the literature only a handful of times.[29–33] In this method, polymer chains are grown from the polymer as a macroinitiator, with multiple initiation sites located along its backbone. This technique should allow for greater graft densities along the polymer backbone since only small monomers are added to the growing polymer chain, mitigating steric repulsion.[21–24]

Here we report the use of a graft-from polymerization technique to generate chemical functionality onto a PLA backbone

Department of Chemistry and Biochemistry, University of California, San Diego 9500 Gilman Drive, La Jolla, CA 92093, USA.
E-mail: jerryyang@ucsd.edu, ngianneschi@ucsd.edu

† Electronic supplementary information (ESI) available. See DOI: 10.1039/c5cc07882b

via ring opening metathesis polymerization (ROMP). ROMP was chosen as the grafting technique because it can be initiated by catalysts with known tolerance to a range of functional groups and results in polymers with narrow dispersity.[34] In this method, PLA polymers containing ROMP-reactive norbornene handles were first prepared. The norbornene units on this starting polymer were then used to prepare an initiator, which could be readily functionalized by reaction with substituted norbornene monomers. As a demonstration of the utility of this strategy, nanoparticles that change morphology in response to both pH and UV light were prepared.

Preparation of a norbornene-functionalized PLA polymer began with the synthesis of the bifunctional lactide **1**, which was prepared in five steps from commercially available *exo*-5-norbornenecarboxylic acid in 14% overall yield (see ESI,† for experimental details). Norbornene-substituted PLA polymers were then synthesized by copolymerizing lactide with 15 mol% of monomer **1**, utilizing 0.7 mol% of stannous octoate as the catalyst and 4-*tert*-butylbenzyl alcohol as an initiator.[11] The resulting polymers were then characterized by size exclusion chromatography coupled with multiangle light scattering (SEC-MALS) and ^{1}H-NMR to determine the percent conversion of lactides to polymer, the number-average molecular weight (M_n), and the dispersity ($Đ$ or M_w/M_n) of the copolymers (Table 1). ROP of the lactides afforded a 98% conversion of monomers to norbornene functionalized polymer **2**.

The norbornene units on polymer **2** were then prepared as initiation sites by addition of polymer **2** dropwise to a pyridine-modified variant of Grubbs' second generation catalyst, $(IMesH_2)(C_5H_5N)_2(Cl)_2RuCHPh$.[35] This catalyst was chosen due to its exceptional functional group tolerance and favorable rates of initiation and propagation to afford well-defined polymers with low dispersity. Polymer **2** was pre-loaded with 1.1 equivalents of Grubbs' catalyst with respect to the norbornene units for 10 min. The polymer was precipitated with methanol and excess/unreacted catalyst washed away to avoid competing polymerization events not originating from the PLA polymer-bound initiation sites. Following polymer resuspension in methylene chloride, phenyl-modified monomer **3** (5 equivalents with respect to norbornene units on the PLA backbone) was added to the catalyst-loaded polymer **2** and allowed to stir for 1 h before quenching with ethyl vinyl ether, generating polymer **3** (Fig. 1).

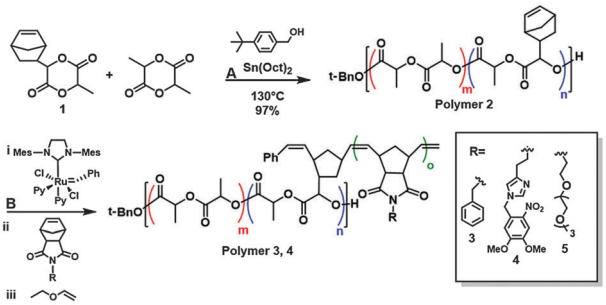

Fig. 1 Synthesis of functionalized PLA-based polymers utilizing a graft-from ROMP strategy. (A) ROP synthesis: 15 mol% of monomer **1** was copolymerized with lactide in the presence of $Sn(Oct)_2$ as the catalyst and 4-*tert*-butylbenzyl alcohol as the initiator (B) ROMP polymer synthesis: (i) polymer **2** was mixed with Ru initiator. (ii) Polymer **2** with catalyst loaded was then mixed with norbornene monomers as shown. (iii) Termination of polymerization using ethyl vinyl ether to generate final polymers **3** and **4**.

This technique allowed for the preparation of polymer **3** without generating any free ROMP polymer, as verified by the presence of a monomodal distribution in the SEC-MALS chromatogram (Fig. S2B, ESI†). SEC-MALS also verified the success of the grafting technique by showing an increase in molecular weight of the PLA polymer **3** from M_n = 32 000 ($Đ$ 1.4) to M_n = 51 000 ($Đ$ 1.8) after the addition of the phenyl monomer (see Fig. S2a and b, ESI†). As a negative control, unfunctionalized PLA polymers were exposed to the same solution conditions and reagents including the Ru-initiator that were used for the preparation of polymer **3**. This treatment yielded unfunctionalized PLA and no free homopolymer of monomer **3**, based on ^{1}H-NMR and SEC-MALS, demonstrating the reliability of the precipitation step to eliminate any free, unreacted initiator from solution (see ESI† and Fig. S3 for more experimental detail). In order to demonstrate that no undesired cross linking of the PLA polymers were taking place, another control reaction was performed. Polymer **2** was resynthesized and this polymer was added to the Grubbs' catalyst. The solution was let to stir for 1 h and then a large excess of ethyl vinyl ether (30 equivalents with respect to norbornene backbone units) was added to the reaction to produce a cross metathesis product and to terminate the opened olefin. Polymer **2** was analyzed by SEC-MALS before the reaction (M_n = 44 000 $Đ$ = 1.1) and after the reaction (M_n = 46 000 and $Đ$ = 1.1). The M_n did not increase a substantial amount, confirming no crosslinked product. (See Fig. S4 ESI,† for more information).To demonstrate graft-from polymers could be prepared as higher molecular weight assemblies, the polymers were allowed to assemble by dissolution in THF followed by slow addition to water and concentration *in vacuo* to form polymeric nanoparticles. Transmission electron microscopy (TEM) was then used to verify the formation of these nanoscale assemblies. Samples for TEM were prepared on carbon TEM grids by drop deposition followed by staining with 1% uranyl acetate solutions. Comparison of these TEM images

Table 1 Physical parameters of the PLA polymers

Polymer	M_n [a] (kg mol^{-1})	M_w [b] (kg mol^{-1})	$Đ$ [c]	DP [d]
PLA	13	15	1.2	—
Polymer **2**	32	45	1.4	—
Polymer **3**	51	93	1.8	5
Polymer **4**	45	—	—	5
Polymer **4b**	59	—	—	10[e]

All data was obtained by SEC-MALS, except for those describing the imidazole containing polymers, which did not elute on the SEC-MALS column and were instead characterized by ^{1}H-NMR. [a] Number-average molecular weight from light scattering or ^{1}H-NMR. [b] Weight average molecular weight from light scattering. [c] The dispersity of each polymer. [d] Experimentally determined degree of polymerization of ROMP block *via* ^{1}H-NMR. [e] Polymer **4b** was polymerized to a degree of 10: DP of 5 for monomer **4** and DP of 5 for the TEG monomer to be able to be utilized for nanoparticle synthesis.

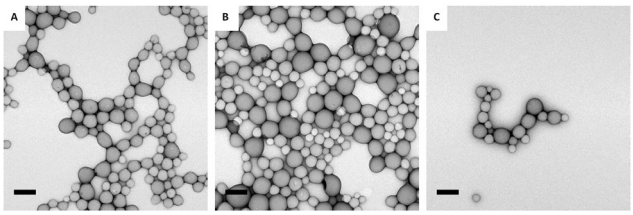

Fig. 2 TEM images of nanoparticles comprised of (A) unfunctionalized PLA, (B) polymer **2**, and (C) polymer **3** with phenyl functionality. Stained with 1% uranyl acetate. Scale bars = 200 nm. These nanospheres were prepared by the solvent evaporation method by dissolving each polymer in THF and adding DI water dropwise. The polymers formed nanospheres after concentrating the solutions *in vacuo*.

(Fig. 2) and dynamic light scattering (DLS) data (Fig. S5, ESI†) of unfunctionalized PLA, polymer **2** and polymer **3**, indicated that each material had very similar morphology, consisting of spherical nanoparticles with diameters of 100–200 nm.

To demonstrate one potential application of this graft-from polymerization technique, graft polymers featuring a stimuli-responsive functional moiety were synthesized. In this example, a grafted PLA polymer was synthesized by incorporating a norbornene monomer functionalized with a 5-dimethoxy-2-nitrobenzyl caged imidazole moiety. This moiety was chosen due to its potential to change its physical properties in response to two separate stimuli; UV light (*via* cleavage of the 2-nitrobenzyl cage) and pH (by way of protonation of the imidazole unit). Moreover, imidazole, in particular, was of interest since its protonation (pK_a of the immidazolium ion is $\sim$7) is within a biologically relevant range (pH 5.0–7.4) and would demonstrate the possibility of triggering a morphology change in this material upon exposure to mild changes in pH environments.

The caged imidazole-containing norbornenyl monomer **4** readily polymerized, yet the resulting polymer **4** formed only amorphous aggregates rather than well dispersed spherical particles. As such, this monomer was copolymerized with tetra(ethylene glycol) norbornene monomer **5** to increase the water solubility of the resulting polymer (**4b**, Fig. 3). The polymers were characterized by ^{1}H-NMR to determine the degree of polymerization (DP) (Fig. S6, ESI†). Note: a detailed characterization of the polymer's molecular weight was not possible by SEC-MALS due to aggregation on the SEC column. Following nanoparticle formation by dialysis from DMSO into buffered water of polymer **4b**, spherical nanoparticles of 100 nm diameter were formed at both pH 7.4 and 5.5 as evidenced by TEM (Fig. 3) and DLS (Fig. S7, ESI†).

Deprotection of the nitrobenzyl caged-imidazole *via* treatment of the nanoparticles with 350 nm UV light for three minutes at either pH 5.5 or 7.4 resulted in a change in shape and diameter of the nanoparticles. At pH 5.5, we observed a change to micron-sized aggregates from discrete spherical structures as evidenced by TEM (Fig. 3D) and DLS (Fig. S7, ESI†). We attribute this change in shape of polymer **4b** to both removal of the bulky nitrobenzyl group and to protonation of the newly liberated imidazole moiety after exposure to UV light in an acidic environment. The disruption of the nanoparticle

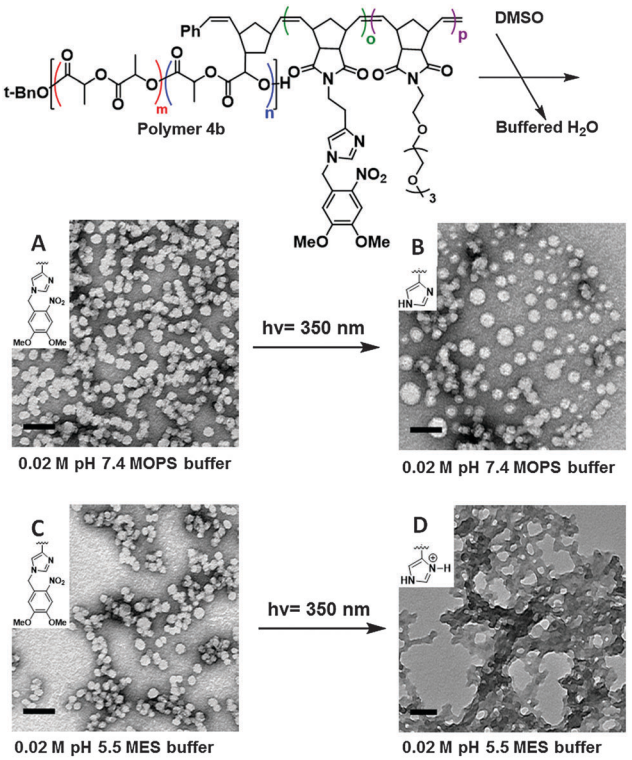

Fig. 3 Responsive imidazole derivatized PLA particles. Polymers were dialyzed from DMSO to buffered water at a concentration of 1 mg mL^{-1}. Spherical nanoparticles of 100 nm diameter were formed at both pH 7.4 (A) and 5.5 (C). After exposing to 350 nm UV light, particles in pH 7.4 buffer remained discrete nanostructures (B) and particles at pH 5.5 transformed from nanospheres to micron scale aggregates (D). This suggests that protonation of the liberated imidazole moiety is necessary for the shape change, due to putative electrostatic repulsion of the positively charged imidazolium ions at low pH. Stained with 1% uranyl acetate. Scale bar = 200 nm.

morphology of **4b** after exposure to UV light is likely due to electrostatic repulsion of the positively charged imidazolium groups and also to the removal of the potentially self-associating 2-nitrobenzyl moieties. Imidazole-containing nanoparticles generated by removal of the 2-nitrobenzyl group from polymer **4b** in pH 7.4 buffered water (Fig. 3A) could be made to form the same aggregated structures as shown in Fig. 3D by decreasing the pH of the solution *via* slow addition of HCl to pH 5.5 (Fig. 3B), indicating the necessity of both stimuli, a reduction in pH and the application of UV light, to facilitate the morphology changed observed for polymer **4b** (Fig. S8, ESI†). Likewise, note that alkyl imidazoles, such as 2-nitrobenzyl protected polymer **4b**, could also become protonated in acidic solutions, however, this material does not aggregate until exposed to UV-light, indicating that a decrease in pH is not alone sufficient for the morphology change (Fig. 3C).

In summary, we have synthesized novel PLA-based materials *via* graft-from ROMP to install a high density of functionality from relativity low density branch points along the PLA backbone. As a proof-of-principle, we synthesized polynorbornyl grafts derivatized with a phenyl group along the PLA backbone and generated well-defined polymers and polymeric nanoparticles.

We then synthesized 2-nitrobenzyl-protected imidazole graft variants to illustrate that chemical handles with increasingly useful functionality can be incorporated into PLA *via* this method by capitalizing on the high functional group tolerance of the Grubbs' second generation modified catalyst. Photoprotected imidazole PLA polymers were formulated into nanoparticles and demonstrated a morphology change only in the presence of both UV exposure and reduced pH as dual stimuli. This work represents a significant step toward highly functionalizable PLA materials that are potentially useful for a wide range of biomedical or materials applications.

This work was partially supported by the NSF (CHE-0847530) and the AFOSR (FA9550-12-1-0435). We thank Dr Matt Thompson for his technical assistance with polymer characterization. The authors also thank Dr Angela Blum for her critical reading of the manuscript and helpful suggestions. We also acknowledge the UCSD Cryo-Electron Microscopy Facility and Norm Olsen for TEM training and assistance.

Notes and references

1 A.-C. Albertsson and I. K. Varma, *Biomacromolecules*, 2003, **4**, 1466–1486.
2 A. M. Reed and D. K. Gilding, *J. Polym.*, 1981, **22**, 494–498.
3 C. E. Holy, J. A. Fialkov, J. E. Davies and M. S. Shoichet, *J. Biomed. Mater. Res.*, 2003, **65**, 447–453.
4 T.-Y. Kim, D.-W. Kim, J.-Y. Chung, S. G. Shin, S.-C. Kim, D. S. Heo, N. K. Kim and Y.-J. Bang, *Clin. Cancer Res.*, 2004, **10**, 3708–3716.
5 K. S. Lee, H. C. Chung, S. A. Im, Y. H. Park, C. S. Kim, S.-B. Kim, S. Y. Rha, M. Y. Lee and J. Ro, *Breast Cancer Res. Treat.*, 2008, **108**, 241–250.
6 D. Bourissou, S. Moebs-Sanchez and B. Martín-Vaca, *C. R. Chim.*, 2007, **10**, 775–794.
7 Y. Yu, J. Zou and C. Cheng, *Polym. Chem.*, 2014, **5**, 5854–5872.
8 T. Trimaille, M. Móller and R. Gurny, *J. Polym. Sci., Part A: Polym. Chem.*, 2004, **42**, 4379–4391.
9 J. A. Castillo, D. E. Borchmann, A. Y. Cheng, Y. Wang, C. Hu, A. J. García and M. Weck, *Macromolecules*, 2012, **45**, 62–69.
10 R. E. Drumright, P. R. Gruber and D. E. Henton, *Adv. Mater.*, 2000, **12**, 1841–1846.
11 M. Yin and G. L. Baker, *Macromolecules*, 1999, **32**, 7711–7718.
12 X. Jiang, E. B. Vogel, M. R. Smith and G. L. Baker, *Macromolecules*, 2008, **41**, 1937–1944.
13 W. W. Gerhardt, D. E. Noga, K. I. Hardcastle, A. J. García, D. M. Collard and M. Weck, *Biomacromolecules*, 2006, **7**, 1735–1742.
14 M. Leemhuis, J. H. van Steenis, M. J. van Uxem, C. F. van Nostrum and W. E. Hennink, *Eur. J. Org. Chem.*, 2003, 3344–3349.
15 M. Rubinshtein, C. R. James, J. L. Young, Y. J. Ma, Y. Kobayashi, N. C. Gianneschi and J. Yang, *Org. Lett.*, 2010, **12**, 3560–3563.
16 H. K. Hall and A. K. Schneider, *J. Am. Chem. Soc.*, 1958, **80**, 6409–6412.
17 I. A. Barker, D. J. Hall, C. F. Hansell, F. E. Du Prez, R. K. O'Reilly and A. P. Dove, *Macromol. Rapid Commun.*, 2011, **32**, 1362–1366.
18 Y. Yu, J. Zou, L. Yu, W. Ji, Y. Li, W.-C. Law and C. Cheng, *Macromolecules*, 2011, **44**, 4793–4800.
19 J. Zou, C. C. Hew, E. Themistou, Y. Li, C.-K. Chen, P. Alexandridis and C. Cheng, *Adv. Mater.*, 2011, **23**, 4274–4277.
20 R. J. Williams, I. A. Barker, R. K. O'Reilly and A. P. Dove, *ACS Macro Lett.*, 2012, **1**, 1285–1290.
21 C. Feng, Y. Li, D. Yang, J. Hu, X. Zhang and X. Huang, *Chem. Soc. Rev.*, 2011, **40**, 1282–1295.
22 M. Zhang and A. H. E. Müller, *J. Polym. Sci., Part A: Polym. Chem.*, 2005, **43**, 3461–3481.
23 H. Lee, J. Pietrasik, S. S. Sheiko and K. Matyjaszewski, *Prog. Polym. Sci.*, 2010, **35**, 24–44.
24 S. S. Sheiko, B. S. Sumerlin and K. Matyjaszewski, *Prog. Polym. Sci.*, 2008, **33**, 759–785.
25 Y. Xia, B. D. Olsen, J. A. Kornfield and R. H. Grubbs, *J. Am. Chem. Soc.*, 2009, **131**, 18525–18532.
26 I. Czelusniak, E. Khosravi, A. M. Kenwright and C. W. G. Ansell, *Macromolecules*, 2007, **40**, 1444–1452.
27 F. Leroux, V. Montembault, S. Pascual, W. Guerin, S. M. Guillaume and L. Fontaine, *Polym. Chem.*, 2014, **5**, 3476.
28 S. Jha, S. Dutta and N. B. Bowden, *Macromolecules*, 2004, **37**, 4365–4374.
29 D. Mecerreyes, B. Atthoff, K. A. Boduch, M. Trollsås and J. L. Hedrick, *Macromolecules*, 1999, **32**, 5175–5182.
30 F. Tasaka, Y. Ohya and T. Ouchi, *Macromolecules*, 2001, **34**, 5494–5500.
31 K. Fukushima, R. C. Pratt, F. Nederberg, J. P. K. Tan, Y. Y. Yang, R. M. Waymouth and J. L. Hedrick, *Biomacromolecules*, 2008, **9**, 3051–3056.
32 W. Dai, J. Zhu, A. Shangguan and M. Lang, *Eur. Polym. J.*, 2009, **45**, 1659–1667.
33 R. J. Williams, R. K. O'Reilly and A. P. Dove, *Polym. Chem.*, 2012, **3**, 2156.
34 M. S. Sanford, J. A. Love and R. H. Grubbs, *J. Am. Chem. Soc.*, 2001, **123**, 6543–6554.
35 J. A. Love, J. P. Morgan, T. M. Trnka and R. H. Grubbs, *Angew. Chem., Int. Ed. Engl.*, 2002, **41**, 4035–4037.

