Journal of Materials Chemistry B

Accepted Manuscript

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/materialsB

Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

ARTICLE TYPE

Linear Polyethyleneimine as (multi)Functional Initiator for Organocatalytic L-lactide Polymerization

Olivier Coulembier[a,*], Sébastien Moins[a], Samarendra Maji[b], Zhiyue Zhang[c], Bruno G. De. Geest[c], Philippe Dubois[a], Richard Hoogenboom[b,*]

Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX
DOI: 10.1039/b000000x

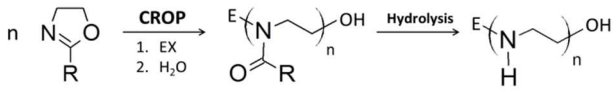
The preparation of polyethylenimine (PEI) – polylactide (PLA) copolymer structures is promising as these materials may find use in gene and/or drug delivery applications. In the current work we have explored the utilization of linear polyethylenimine (L-PEI) as multifunctional initiator for the organocatalytic ring-opening polymerization of lactide. Evaluation of the effect of the amount of catalyst revealed that with high catalyst loadings mixtures of unmodified L-PEI and PEI-PLA were obtained while low catalyst loadings leads to efficient preparation of PEI-PLA graft copolymers. This difference is described to the enhanced polymerization time with lower catalyst loading enabling efficient initiation from up to every second ethylenimine unit. The resulting PEI-PLA were subsequently formulated into nanoparticles of ~400 nm by nanoprecipitation, which could be efficiently labeled with rhodamine octadecylester as model hydrophobic drug. These nanoparticles were efficiently taken up by DC2.4 cells as demonstrated by flow cytometry and fluorescence miscoscopy demonstrating their potential for gene and/or drug delivery applications.

Introduction

Polylactide (PLA) is a popular material for use in biomedical applications based on its biodegradability as well as its good mechanical properties[1-3] The relatively high glass transition temperature of PLA of around 60 °C also facilitates its application in hydrophobic nanoparticle formulations, which have been utilized for controlled release gene delivery[4,5] as well as controlled and sustained delivery of hydrophobic drugs.[4,6-8] To enhance the applicability of PLA for use in drug delivery systems, a wide variety of modified and functionalized PLA (co)polymers have been and are being explored.[9,10] In particular, the formulation of HB-PEI and oligonucleotides in PLA has been demonstrated to lead to controlled release of the oligonucleotides.[11-13] Furthermore, hydrophobic cationically modified PLA has been utilized as nanoparticulate gene delivery formuation,[14] as well as some examples of star-shaped PLA based on HB-PEI as initiator.[15,16] It has been demonstrated that both primary and secondary amines of HB-PEI can act as initiator, but the resulting structures are rather ill-defined due to the broad molar mass distribution of the starting HB-PEI. To the best of our knowledge no reports have yet appeared on the utilization of L-PEI as initiator for the polymerization of lactide to obtain defined PEI-PLA copolymer structures.

Polyethylenimine (PEI) is frequently utilized as non-viral gene delivery vector.[17,18] Despite the popularity of high molecular weight hyperbranched HB-PEI as gene transfection agents related to the so-called "proton-sponge" hypothesis,[19,20] high molecular weight HB-PEI (25 kDa) suffers from an inherent cytotoxicity.[18,21] Although low molecular weight HB-PEI congeners exhibit significant reduced cytotoxicity, they also present a much lower transfection efficiency.[22,23] A systematic investigation of various PEI derivatives interestingly concludes that not only the amine content and functionality are of high importance for polyplex formation, but also the hydro-/lipophilicity balance and the polymer constitution.[24-27] Furthermore, linear PEI (L-PEI) is less toxic than HB-PEI while still showing high transfection efficiency.

Interestingly, the preparation of defined L-PEI is to date best achievable by living cationic ring-opening polymerization (CROP) of 2-oxazoline derivatives. The versatility of this polymerization techniques enables the preparation of highly defined poly(2-oxazoline)s with controlled degree of polymerization (DP) as well as chain-end functionalities that can be introduced during initiation and/or termination (Scheme 1).[28-32] Subsequent hydrolysis of the amidic side chains yields the L-PEI derivatives.[33-37]

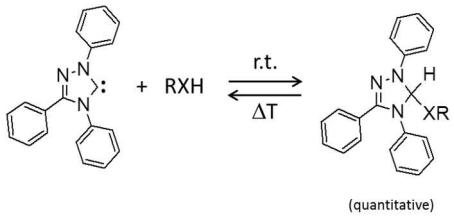


Scheme 1. Linear PEI obtained via CROP of 2-substituted oxazoline (R = H, Me, Et, …; EX = electrophilic initiator).

State-of-the-art approaches to produce well-defined L-PEI-based copolymer structures are limited to multi-step post-polymerization modification and coupling strategies.[38-43] Therefore, there is a clear need for the development of more straightforward methodologies to prepare advanced L-PEI based (block) copolymer structures. As far as we know, no report has been published on the possibility to use the PEI end-group and/or to use both the end-group and the secondary amino functions

present along the L-PEI backbone to directly initiate another polymerization process.

Reasoning that the backbone secondary amines and chain-end hydroxyl group (resulting from water termination of the poly(2-oxazoline) synthesis) of L-PEI have significant different pK_as,[44] and because *N*-heterocyclic carbenes (NHCs) are known to be sensitive to the hydrogen acidity during a proton transfer reaction,[45] we reckoned that it might be feasible to obtain a selective interaction between NHCs with either the NH or the OH functions of L-PEI. Besides their selectivity, NHCs such as 1,3,4-triphenyl-4,5-dihydro-1*H*-1,2,4-triazol-5-ylidene (**C**) are known to react quantitatively and reversibly with both alcohols and secondary amines to form the corresponding alcohol or amino-adducts (Scheme 2).[46] We previously demonstrated the ability of alcohol and amino-adducts of **C** to act as initiators for the controlled polymerization of cyclic esters, either in solution[47-49] or in bulk.[50]



Scheme 2. Reversible generation of alcohol- (with X = O) and amino-adducts (with X = N) of C.

In this paper, we report the preparation of various L-PEI/ PLA copolymers with proposed resulting topologies ranging from block copolymers to comb-like and grafted structures. The main aspect of this work is to investigate the effect of the amount of NHC catalyst **C** relative to the amount of L-PEI on the resulting copolymer structure (Figure 1). Moreover, the resulting PLA-PEI graft copolymers are formulated into nanoparticles and their cellular uptake is demonstrated.

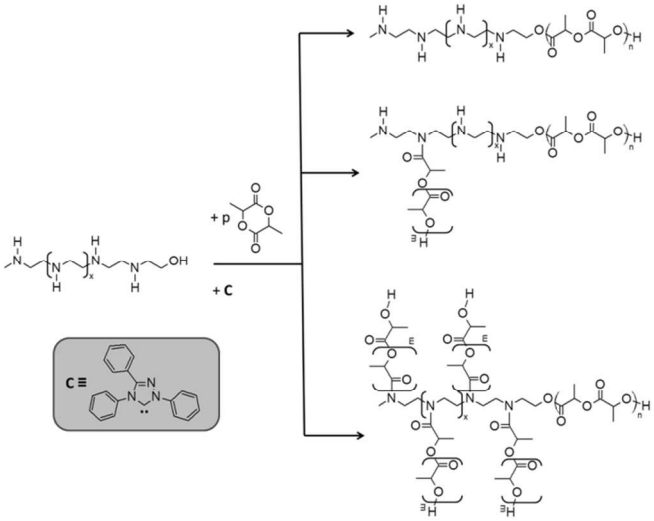


Figure 1. Proposed possible PLA-PEI polymer structures that may be obtained by adjusting the relative amount of polymerization catalyst.

Experimental Section

Materials.

L-Lactide (LA; Boehringer-Ingelheim, 99+%) was recrystallized three times in dried toluene (20% wt/vol) at 70°C and then dried under vacuum overnight. 5-Methoxy-1,3,4-triphenyl-4,5-dihydro-1*H*-1,2,4-triazol-5-ylidene (Acros,98%) was purified and degassed at r.t. by three nitrogen/vacuum treatments prior to any carbene generation.

Polymerization Protocol

In a previously flame-dried schlenk flask are introduced 16.5 mg of 5-methoxy-1,3,4-triphenyl-4,5-dihydro-1*H*-1,2,4-triazol-5-ylidene (1 eq.). After thermal stabilization at 90°C and a vacuum treatment of two hours (in-situ generation of **C**), the medium is cooled down, introduced in a dry box and complemented by the addition of 50 mg of L-PEI20 (1eq.). Out of the box, the entire medium is dried under vacuum at 60°C for an additional hour. In the dry box, the dried schlenk was filled with 0.72 gr of recrystallized L-LA (100 eq.) and sealed under vacuum. The L-LA polymerization is then performed at 110°C for 5 hours. Conv.$_{grav.}$ = 99%, M_{nSEC}DMA = 14600 g.mol-1; Đ = 1.65.

Characterization methods

Proton nuclear magnetic resonance spectra (^{1}H-NMR) of the polymers were recorded on a Bruker Avance 500 MHz spectrometer using CDCl3 as solvent. Data were acquired and processed by TOPSPIN 3.2 (Bruker, Germany). For all the samples 256 scans were used. All experiments were recorded at 298 K.

Size-exclusion chromatography (SEC) using hexafluoroisopropanol (HFIP) as eluent were performed on a Agilent 1260-series HPLC system equipped with a 1260 online degasser, a 1260 ISO-pump, a 1260 automatic liquid sampler, a thermostatted column compartment, a 1260 diode array detector (DAD) and a 1260 refractive index detector (RID). Analyses were performed on a PLHFIPgel column (250 x 4.6 mm) at 40°C. HFIP containing 3g/L of sodium trifluoroacetate was used as eluent at a flow rate of 0.3 ml/min. The spectra were analysed using the Agilent Chemstation software with the GPC add on. Number average molar mass ($M_{n,SEC,HFIP}$) and dispersity (Đ) values were calculated against PMMA standards.

Size-exclusion chromatography (SEC) using DMA (N,N-dimethylacetamide) as eluent were performed on a Agilent 1260-series HPLC system equipped with a 1260 online degasser, a 1260 ISO-pump, a 1260 automatic liquid sampler, a thermostatted column compartment, a 1260 diode array detector (DAD) and a 1260 refractive index detector (RID). Analyses were performed on a PSS Gram30 column in series with a PSS Gram1000 column at 50 °C. DMA containing 50 mM of LiCl was used as eluent at a flow rate of 0.593 ml/min. The spectra were analysed using the Agilent Chemstation software with the GPC add on. Number average molar mass ($M_{n,SEC,DMA}$) and dispersity (Đ) values were calculated against PMMA standards.

Nanoparticle formation

PEI-PLA (entries 3 and 5) were dissolved in THF at a concentration of 50 mg/mL. To this solution 1 µL of a 10 mg/mL rhodamine octadecylester solution in ethanol was added as a fluorescent marker. 100 µL of the resulting solution was added drop wise to 5 mL of a stirring solution of 1% PVA (85% hydrolyzed, 85-124 kDa) in PBS. This leads to a final polymer concentration of 1 mg/mL. After 1 min stirring the solution was placed in a heating bath set at 40 °C to evaporate the THF. After 4h, the particles were stored at 4°C prior to further use. Particle size was measured by dynamic light scattering using a Malvern Nano S in backscatter mode.

In vitro cell uptake

The immortalized dendritic cell line DC2.4 was a kind gift from Prof. Dr. Ken Rock, Dana-Farber Cancer Institute and University of Massachusetts. Cells cultured in DMEM supplemented with 10% FBS. For flow cytometry, the cells were plated at a density of 105 cells per well in a 25 well plate and allowed to adhere overnight. Subsequently cells were pulsed for 1h with different concentrations of PEI-PLA nanoparticles (i.e. 5, 25 and 50 µL of a 1 mg/mL nanoparticle formulation). Afterwards the cells were washed with PBS and detached from the well plates by treatment with trypsin-free cell dissociation buffer. Particle uptake was measured by flow cytometry using a BD Accuri flow cytometer and data processing was done in FlowJo. Statistical analysis was done in GraphPad.

For microscopy, cells were plated in culture dishes with a flat optical bottom at a density of 50.104 cells per well and allowed to adhere overnight. Subsequently cells were pulsed for 1h with 20 µL PEI-PLA nanoparticle formulation. Afterwards the cells were washed with PBS, fixated with 4 % PFA and stained with Hoechst (cell nuclei) and AlexaFluor488-conjugated cholera toxin subunit B (Life Technologies) (cell membrane.) Fluorescence microscopy images were recorded on a LeicaDM2500P microscope equipped with a 63x (1.40 NA) oil immersion objective and a 360FX camera. Image processing was done in ImageJ.

Results and discussion

The L-PEI was prepared following our previously reported methodology.[20] In brief, poly(2-methyl-2-oxazoline)s with DP 20 and 40 were prepared by microwave-assisted living cationic ring-opening polymerization at 140 °C using methyl tosylate as initiator and acetonitrile as solvent.[51,52] The polymerization was quenched by the addition of water. Subsequent hydrolysis of these polymers by refluxing in 6N HCl yielded well-defined hydroxy-functionalized L-PEIs (no remaining oxazoline units could be detected by ^{1}H NMR spectroscopy). In the following, these two L-PEIs will be named L-PEI20 and L-PEI40 for which values of 20 and 40 correspond to their DP. Due to their partial insolubility in usual organic solvents, SEC analysis was performed with hexafluoroisopropanol (HFIP) as eluent revealing their well-defined nature (Table 1).

Table 1. Molecular characterizations of L-PEI/PLA copolymers as obtained from bulk ROP of LA from L-PEI in bulk at 110°C and catalyzed by C.

entry	PEI type	DP_{th} in LA	$[PEI]_0/[C]_0$	Pol. time (h)	Number of PLA chains [a]	M_n SEC_{HFIP} [b] (g/mol)	$Đ_{HFIP}$ [b]	M_n SEC_{DMA} [c] (g/mol)	$Đ_{DMA}$ [c]
1	PEI20	-	-	-	-	3300	1.17	-	-
2	20	100	1/10	0.5	2.9	5800	2.37	n.m.*	n.m.*
3	20	100	1/5	1	4.4	6100	3.45	17600	1.90
4	20	100	1/1	5	7.4	5900	2.73	14600	1.65
5	20	100	1/0.3	12	10.0	6900	2.39	16950	1.55
6	20	100	1/0.1	24	7.7	7300	2.29	20600	1.33
7	20	200	1/0.1	48	7.9	12700	2.28	17200	2.15
8	20	400	1/0.1	72	11.1	16500	2.40	24900	2.06
9	PEI40	-	-	-	-	5600	1.29	-	-
10	40	100	1/0.3	24	10.2	7100	2.96	15000	1.90
11	40	200	1/0.3	48	21.2	8500	2.88	12300	2.73
12	40	400	1/0.3	72	12.9	15100	2.95	23000	2.38

[a] Number of initiated PLA chains from ^{1}H-NMR spectroscopy using the following formula $DP_{th}/(a/a')$). [b] Experimental number-average molecular weight and dispersity determined by SEC in HFIP at 40 °C and referenced to PMMA standard. [c] Experimental number-average molecular weight and dispersity determined by SEC in DMA at 50 °C and referenced to PMMA standard. Note that L-PEI is not soluble in DMA and these data therefore correspond to the pure L-PEI-PLA copolymers. *not measured

As the L-PEIs are also insoluble in the common solvents used for ring-opening polymerizations (ROP), L-PEI20 and L-PEI40 have been used to initiate the ROP of L-lactide (LA) in bulk at 110°C in presence of C as catalyst. As already demonstrated by some of us,[50] performing the LA ROP at such temperature allows maintaining a high level of control by eradication of backbiting and reshuffling (transesterification) reactions. The first set of experiments were performed with L-PEI20, a ratio of LA to L-PEI20 of 100 (= 5 LA units per L-PEI repeat units) and varying amounts of C with regard to the L-PEI. The polymerization times were adapted to collect samples with a LA conversion higher than 90% (Table 1; entries 2-5). ^{1}H NMR spectroscopic analyses, after purification by precipitation, revealed remarkably different spectra for the L-PEI-PLA copolymers obtained with different ratios of C (Figure 2). All spectra show the characteristic PLA signals at 5.2 ppm (a; CH from the backbone) and 4.3 ppm (a'; CH from the final monomer unit) confirming successful polymerization of LA in presence of L-PEI. The characteristic L-PEI signals at 2.8-3.2 ppm are still clearly visible for the L-PEI-PLA samples prepared with low $[PEI]_0/[C]_0$ ratios of 1/5 and 1/10. In addition there is a broad signal in between 3.2 and 4.0 ppm, which is very similar to the chemical shift of poly(2-oxazoline) backbone protons demonstrating that partial initiation from the secondary amine groups occurred. When lowering the amount of C to 1, 0.3 and 0.1, the initiation from the secondary amines becomes more efficient and almost no original L-PEI signal is retained. A more quantitative analysis results from the integral ratios of a and a', which in combination with the assumption of full monomer conversion, that is 100 LA units, provides the average number of PLA chains initiated by the L-PEI20. It is evident that decreasing the amount of C from 10 to 0.3 leads to a gradual increase in the initiation efficiency from 2.8 chains per L-PEI20 to 10 chains per L-PEI20, whereby these

numbers include initiation from the terminal alcohol group of the L-PEI20. The value of 10 corresponds to a L-PEI-PLA graft copolymer in which one out of every two secondary amine groups has initiated a LA polymerization.

To understand this large difference in initiation efficiency by variation of the amount of C we need to consider the higher reactivity of alcohol groups compared to secondary amine groups for ROP of LA. During the fast polymerization with a large amount of C, the faster ROP on the alcohol group of the chain end and those resulting from a first LA addition to a secondary amine will be kinetically favored over further LA addition to the remaining secondary amine groups. Slowing down the polymerization by using much less of C leads to better control over the ROP, whereby LA addition to alcohol groups and secondary amines will simultaneously proceed.

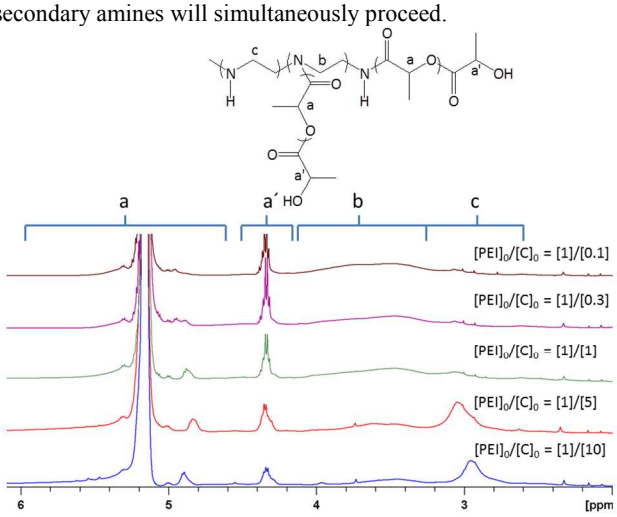


Figure 2. ^{1}H NMR spectra of the L-PEI-PLA copolymers in different quantity of carbene **C** to the L-PEI initial macroinitiator content for the targeted DPs of LA 100.

Further analysis of the resulting copolymers was performed by SEC analysis. The SEC traces in HFIP are shown in Figure 3 demonstrating bimodal peaks with a low molecular weight shoulder around 19-20 minutes corresponding to the starting L-PEI20 (the signals at 25 minutes are solvent peaks). However, this shoulder is most apparent with high **C** loading (10 equivalents) while only a minor shoulder is present with 0.3 equivalent of **C**. The presence of this low molar mass shoulder is largely responsible for the high dispersities (Đ) that were obtained as demonstrated by the large decrease in Đ when measuring in *N,N*-dimethylacetamide (DMAc) in which the L-PEI is not soluble. As such, the SEC analysis in DMA is representative for the resulting PLA-PEI copolymers in absence of L-PEI. To confirm whether the shoulder is due to pure low molar mass L-PEI20 or PLA oligomers, the copolymer **5** was dissolved in chloroform and extracted with water. Subsequent ^{1}H NMR analysis showed a strong enrichment in PLA content confirming that there was a significant amount of free L-PEI present after the ROP of LA with 10 eq of **C**. The fact that some unreacted L-PEI also remains after the polymerization with 0.3 eq of **C** suggests that besides the kinetic control over initiation and polymerization, also the limited solubility of L-PEI20 plays a role in the secondary amine initiation efficiency. These results demonstrate that utilization of L-PEI as initiator for the ROP of LA is feasible for the efficient preparation of graft copolymers as comb polymers are restricted by steric crowding and block copolymer synthesis with large quantities of catalyst **C** leads to a major fraction of L-PEI that does not initiate the LA polymerization.

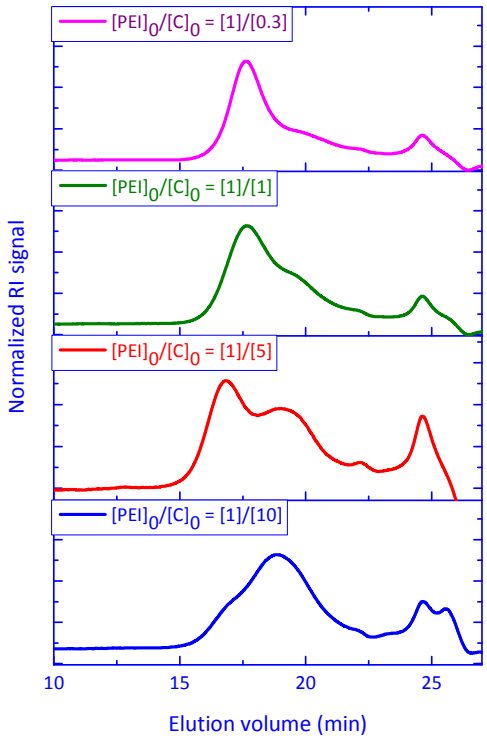


Figure 3. SEC traces of the L-PEI-PLA copolymers in different quantity of carbene **C** to the L-PEI initial macroinitiator content for the targeted DPs of LA 100 (eluent: hexafluoroisopropanol; solvent peaks appear at 25 minutes).

Based on the observation that a low amount of catalyst **C** can be utilized for the preparation of relatively defined L-PEI-PLA graft copolymers, the scope of these optimal conditions was further explored with L-PEI20 and L-PEI40 as initiators with 0.1 and 0.3 equivalents of **C** per L-PEI polymer chain and varying amounts of LA. It should be noted that the amount of catalyst **C** was even further lowered in an attempt to further suppress the presence of unreacted L-PEI after the ROP. The SEC traces (HFIP) in Figures 4 and 5 clearly demonstrate the possibility of both extending the L-PEI main chain length as well as the PLA graft side chain lengths, albeit the longer L-PEI results in a somewhat larger amount of retaining L-PEI in the final precipitated sample. ^{1}H NMR spectroscopy revealed that the L-PEI20 initiated 8-11 PLA chains per L-PEI chain while the L-PEI40 initiated 10-21 PLA chains per L-PEI, confirming that one PLA chain per two secondary amines is the maximum attainable grafting density with this synthetic approach.

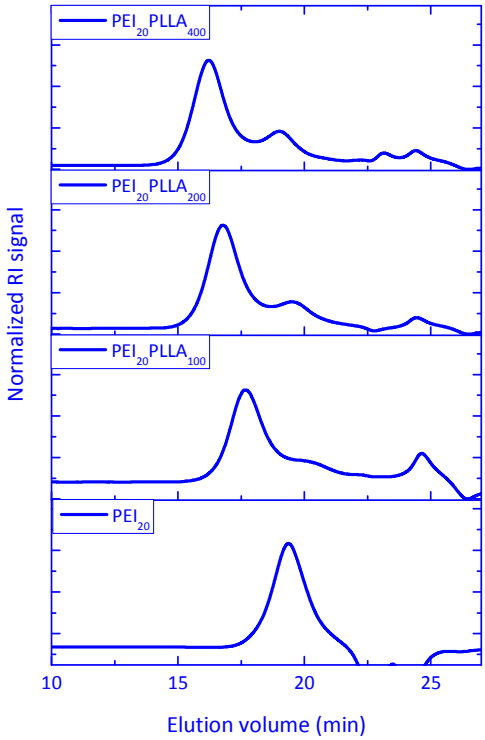


Figure 4. SEC traces of the L-PEI-PLA copolymers for the targeted DPs of LA 100, 200 and 400 respectively (L-PEI20 was used to initiate the ROP of L-lactide in bulk at 110°C in presence of **C**).

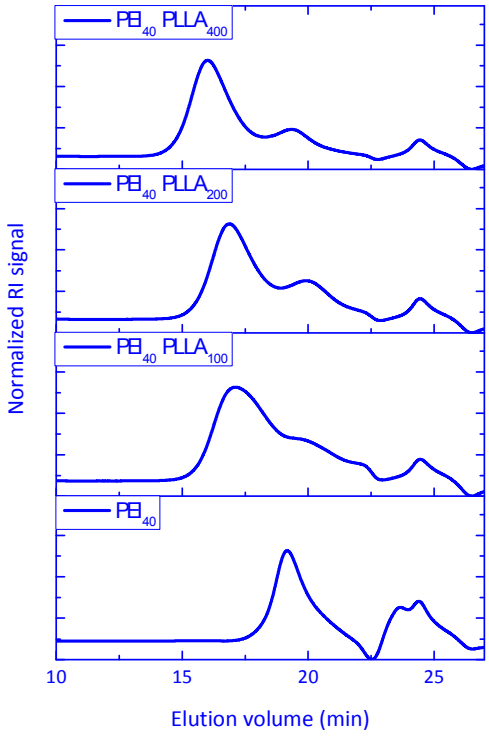


Figure 5. SEC traces of the L-PEI-PLA copolymers for the targeted DPs of LA 100, 200 and 400 respectively (L-PEI40 was used to initiate the ROP of L-lactide in bulk at 110°C in presence of **C**).

The newly developed synthetic methodology provides access to hydrophobic L-PEI-PLA graft copolymers that still have a significant number of secondary amine groups in the structure making them potentially interesting for gene delivery applications or the encapsulation and release of (negatively charged) hydrophobic drug molecules. Therefore, an initial assessment of the formation nanoparticles as well as their cellular uptake was undertaken with two L-PEI/PLA graft copolymers based on L-PEI20 having on average 4 and 10 PLA side chains (entries 3 and 5 in Table 1; referred to as L-PEI-PLA3 and L-PEI-PLA 5). Nanoparticles were prepared by nanoprecipitation, whereby a polymer solution in tetrahydrofuran containing rhodamine octadecylester as fluorescent marker and as model for hydrophobic drugs was first prepared leading to a turbid solution due to insolubility of the remaining L-PEI in the polymer sample, which could easily be removed by filtration. This filtered, and thus purified, L-PEI-PLA solution in THF was added dropwise to a solution of poly(vinyl alcohol) (PVA) in PBS, followed by evaporation of the tetrahydrofuran by stirring at 40°C. Note that the role of PVA is to stabilize the precipitated L-PEI-PLA nanoparticles as is commonly used for the preparation of PLGA (i.e. poly(lactic-co-glycolic acid)) nanoparticles. This procedure led to the formation of defined nanoparticles (without filtration) with a hydrodynamic size of 435 nm ± 10 nm for L-PEI-PLA3 and 395 nm ± 6 nm for L-PEI-PLA5 as determined by dynamic light scattering (DLS; Figure 6). The narrow size distributions indicate that spherical particles are formed as the mathematical correlation of the DLS correlation curve into a size distribution is only valid for spherical particles. The particles remained stable over prolonged periods of time under refrigerated conditions. Fluorescence microscopy confirmed the incorporation of the rhodamine fluorescent marker as bright red nanoparticles were clearly visible (Figure 6). It should be noted that due to the limited resolution of optical microscopy the particles appear larger than observed by DLS. Also the amount of hydrophobic dye was taken sufficiently low to avoid precipitation of non-encapsulated dye.

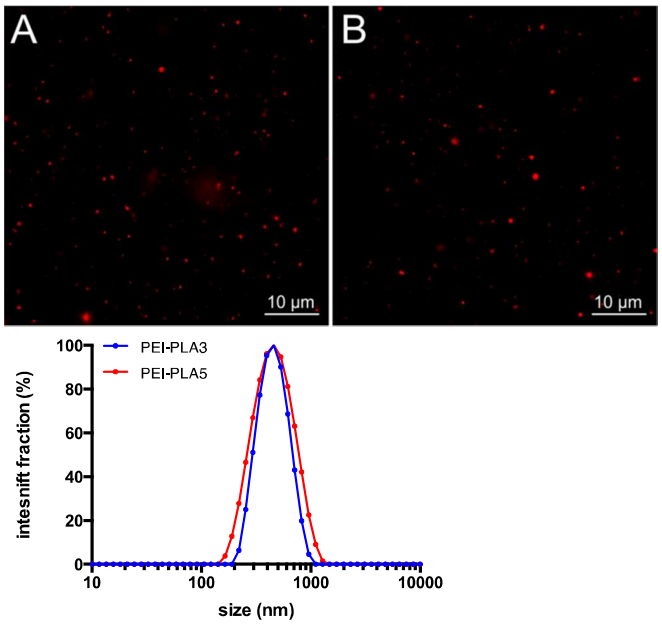


Figure 6. Confocal microscopy images and DLS size distributions of L-PEI-PLA3 (A) and L-PEI-PLA5 (B) nanoparticles prepared by nanoprecipitation.

Next qualitative cell uptake was determined by fluorescence microscopy. Therefore, DC2.4 cells (an immortalized mouse dendritic cell line) were incubated with the nanoparticle suspensions and the fluorescence microscopy images clearly show that a significant number of red-labelled nanoparticles appear inside the cells, demonstrating that the L-PEI-PLA nanoparticles are efficiently taken up by cells (Figure 7).

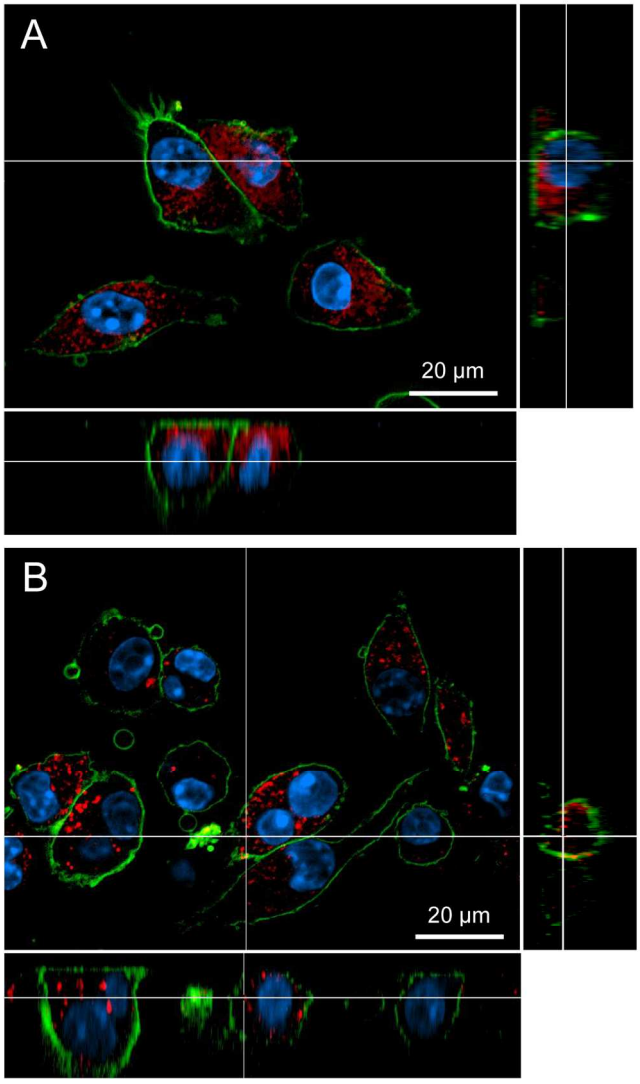


Figure 7. Confocal microscopy images of DC2.4 cells pulsed with (A) L-PEI-PLA3 and (B) L-PEI-PLA5 nanoparticles. Nanoparticles were labeled red fluorescent with rhodamine-octadecylester, cell nuclei were labeled blue fluorescent with Hoechst and the cell membrane was labeled green fluorescent with AlexaFluor488-conjugated cholera toxin subunit B.

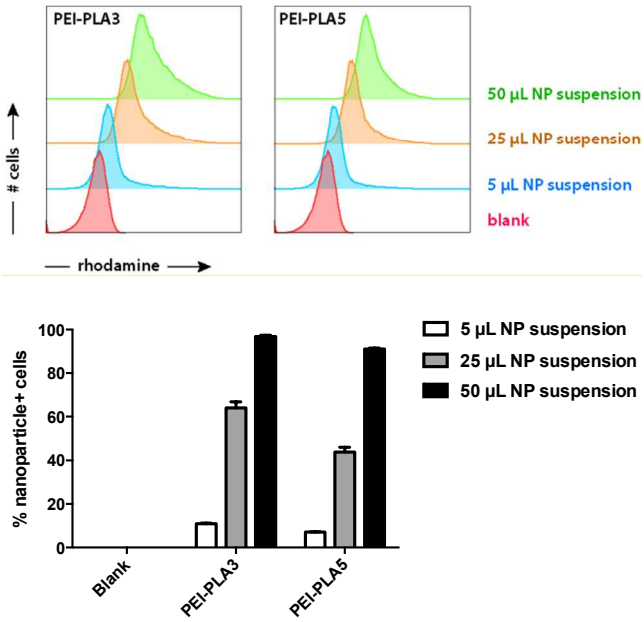


Figure 8. (A) Flow cytometry histograms of DC2.4 cells, untreated (blank) and pulsed with different amounts of L-PEI-PLA nanoparticle suspensions. (B) Quantification of nanoparticle uptake by cells measured by flow cytometry and expressed as the fraction of cells that have internalized nanoparticles. n=3.

Subsequently, the nanoparticle uptake was quantified by flow cytometry as shown in Figure 8. It is clearly seen that the L-PEI-PLA nanoparticle uptake is dose dependent and that with 50 µL of nanoparticle suspension already close to 100% of the cells contain the nanoparticles. Furthermore, there is no significant difference in cellular uptake for nanoparticles prepared from L-PEI-PLA3 and L-PEI-PLA5. Note than when the hydrophobic rhodamine dye was diluted from THF into water, i.e. similarly as for the L-PEI-PLA nanoprecipitation, macroscopic precipitation of the dye occurred. Thus, due to its hydrophobicity it was not possible to perform a free dye control experiment. This further attributes to the attractiveness of the L-PEI-PLA nanoparticles that do allow for intracellular delivery of a hydrophobic payload.

Conclusions

In this work we have demonstrated the successful use of L-PEI as initiator for organocatalytic ROP of lactide, whereby it was found that low concentration of NHC catalyst **C** (0.1 or 0.3 equivalents) should be used to obtain well-defined graft copolymers in which on average one secondary amine group on two of the L-PEI has initiated a PLA chain. Using higher amounts of catalyst leads to the formation of hetereogeneous product mixtures containing L-PEI-PLA copolymers as well as pure unreacted L-PEI, which is ascribed to a too fast propagation/initiation on alcohol groups compared to initiation on secondary amine groups. Furthermore, it was demonstrated that the optimized polymerization protocol allows tuning of the resulting L-PEI-PLA copolymer structure with regard to both main chain and side-chain lengths. Finally, nanoparticles were prepared from the L-PEI-PLA via nanoprecipitation and the resulting nanoparticles were taken up by DC2.4 cells in a dose dependent manner.

All together, these results clearly indicate that the developed synthetic methodology and the resulting L-PEI-PLA graft copolymers are interesting candidates to be further explored for drug and/or gene delivery purposes, whereby the remaining secondary amine groups may be exploited for interactions with DNA, RNA or other (negatively charged) hydrophobic drug molecules.

Notes and references

[a]. Laboratory of Polymeric and Composite Materials, Center of Innovation and Research in Materials and Polymers (CIRMAP), University of Mons, Place du Parc 23, B-7000 Mons, Belgium.
[b]. Supramolecular Chemistry Group, Department of Organic Chemistry, Ghent University, Krijgslaan 281 S4, B-9000 Ghent, Belgium.
[c]. Department of Pharmaceutics, Ghent University, Harelbekestraat 72, 9000 Ghent, Belgium.†
This work has been supported by the European Commission and Région Wallonne FEDER program (’Revêtements fonctionnels’ project - Smartfilm), OPTI²MAT program of excellence, by the Interuniversity Attraction Pole Programme (P7/05) initiated by the Belgian Science Policy Office, the Région Wallonne EMBIOSE project and by FNRS-FRFC. O.C. is Research Associate and J.C. a Research Director of the F.R.S.-FNRS. BDG and RH acknowledge FWO for financial support.
‡ Footnotes should appear here. These might include comments relevant to but not central to the matter under discussion, limited experimental and spectral data, and crystallographic data.

1 Pandey, A. K. *Adv. Mater. Lett.* **2014**, doi: 10.5185/amlett.2013.6489.
2 Lasprilla, A.J.; Martinez, G.A.; Lunelli, B.H.; Jardini, A.L.; Filho, R.M. *J. Biotechnol. Adv.* **2012**, *30*, 321-328.
3 Nampoothiri, K.M.; Nair, N.R.; John, R. P. *Bioresource Technol.* **2010**, *101*, 8493-8501.
4 Munier, S.; Messai, I.; Delair, T.; Verrier, B.; Ataman-Onal, Y. *Coll. Surf. B: Biointerfaces* **2005**, *43*, 163–173.
5 Mahapatro, A; Singh, D.K. *J. Nanobiotechnology* **2011**, *9*, 55.
6 Soppimatha, K.S.; Aminabhavia, T.M.; Kulkarnia, A.R.; Rudzinski, W.E. *J. Controlled Release* **2001**, *70*, 1-20.
7 Musumecia, T.; Ventura, C.A.; Giannone, I.; Ruozi, B.; Montenegro, L.; Pignatello, R.; Puglisi, R. *Int. J. Pharmaceutics* **2006**, *325*, 172-179.
8 Rancan, F.; Papakostas, D.; Hadam, S.; Hackbarth, S.; Delair, T.; Primard, C.; Verrier, B.; Sterry, W.; Blume-Peytavi, U.; Vogt, A. *Pharmaceutical Res.* **2009**, *26*, 2027-2036.
9 Wang, S.; Cui, W.; Bei, J. *Anal. Bioanal. Chem.* **2005**, *381*, 547-556.
10 Rasala, R.M.; Janorkarc, A.V.; Hirt, D.E. *Prog. Polym. Sci.* **2010**, *35*, 338-356.
11 De Rosa, G.; Quaglia, F.; La Rotonda, M.I.; Appel, M.; Alphandary, H.; Fattal, E. *J. Pharm. Sci.* **2002**, *91*, 790-799.
12 Oster, C.G.; Kim, N.; Grode, L.; Barbu-Tudoran, L.; Schaper, A.K..; Kaufmann, S.H.; Kissel, T. *J. Controlled Release* **2005**, *104*, 359-377.
13 Sirsi, S.R.; Schray, R.C.; Wheatley, M.A.; Lutz, G.J. *J. Nanobiotechnol.* **2009**, *7*, 1-12.
14 Jones, C.H.; Chen, C.-K.; Jiang, M.; Fang, L.; Cheng, C.; Pfeifer, B.A. *Mol. Pharm.* **2013**, *10*, 1138-1145.
15 Zhang, C.-X.; Wang, B.; Chen, Y.; Cheng, F.; Jiang, S.-C. *Polymer* **2012**, *18*, 3900-3909.
16 Adeli, M.; Haag, R. J. Polym. Sci., Part A: Polym. Chem. **2006**, 19, 5740-5749.
17 Aoki, K; Furuhata, S.; Hatanaka, K.; Maeda, M.; Remy, J.-S.; Behr, J.-P.; Terada, M.; Yoshida, T. *Gene Ther.* **2001**, 8, 508-514.
18 Boussif, O.; Lezoualc’h, F.; Zanta, M.A.; Mergny, M.D.; Scherman, D.; Demeneix, B.; Behr, J.-P. *Proc. Natl. Acad. Sci. USA* **1995**, 92, 7297-7301.
19 Grund, S.; Bauer, M.; Fischer, D. *Adv. Eng. Mater.* **2011**, 13, B61.

20 Akinc, A.; Thomas, M.; Klibanov, A.M.; Langer, R. *Gene Med*. **2005**, 7, 657.
21 Bonetta, L. *Nat. Methods* **2005**, 2, 875-883.
22 Kunath, K.; von Harpe, A.; Fischer, D.; Peterson, H.; Bickel, U.; Voigt, K.; Kissel, T. *J. Controlled Release* **2003**, 89, 113-125.
23 Godbey, W.T.; Wu, K.K.; Mikos, A.G. *J. Biomed. Mater. Res*. **1999**, 45, 268-275.
24 Jäger, M.; Schubert, S. Ochrimenko, S.; Fischer, D.; Schubert, U.S. *Chem. Soc. Rev*. **2012**, 41, 4755-4767.
25 Neu, M.; Fischer, D.; Kissel, T. *J. Gene Med*. **2005**, 7, 992.
26 Forrest, M.L.; Meister, G.; Koerber, J.; Pack, D. *Pharm. Res*. **2004**, 21, 365.
27 Thomas, M.; Klibanov, A.M. *Proc. Natl. Acad. Sci. U.S.A*. **2002**, 99, 14640.
28 *Handbook of Ring-Opening Polymerization*, Ph. Dubois, O. Coulembier and J.-M. Raquez, Eds, Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, 2009, p.151.
29 Hoogenboom, R. *Angew. Chem., Int. Ed.* **2009**, *48*, 7978−7994.
30 Kobayashi, S. *Prog. Polym. Sci.* **1990**, *15*, 751-823.
31 Aoi, K.; Okada, M. *Prog. Polym. Sci.* **1996**, *21*, 151-208.
32 Hoogenboom, R. *Macromol. Chem. Phys*. **2007**, *208*, 18-25.
33 Saegusa, T.; Ikeda, H.; Fujii, H. *Macromolecules* **1972**, *5*, 108–108.
34 Saegusa, T.; Kobayashi, S.; Yamada, A. *Macromolecules* **1975**, *8*, 390–396.
35 Tanaka, R.; Ueoka, I.; Takaki, Y.; Kataoka, K.; Saito, S. *Macromolecules* **1983**, *16*, 849–853.
36 Lambermont-Thijs, H. M. L.; Van der Woerdt, F. S.; Baumgaertel, A.; Bonami, L.; Du Prez, F. E.; Schubert, U. S.; Hoogenboom, R. *Macromolecules* **2010**, *43*, 927-933.
37 De la Rosa, V.R.; Bauwens, E.; Monnery, B.; De Geest, B.G.; Hoogenboom, R. *Polym. Chem.* **2014**, DOI: 10.1039/C4PY00355A
38 Lee, Y.; Mo, H.; Koo, H.; Park, J.-Y.; Cho, M.Y.; Jin, G.-W.; Park, J.-S. *Bioconjugate Chem.* **2006**, 18, 13.
39 Pons, B.; Mouhoubi, L.; Adib, A.; Godzina, P.; Behr, J.-P.; Zuber, G. *ChemBioChem* **2006**, 7, 303.
40 Liu, J.; Jiang, X.; Xu, L.; Wang, X.; Hennink, W.E.; Zhuo, R. *Bioconjugate Chem* **2010**, 21, 1827.
41 Becer, C.R.; Hoogenboom, R.; Schubert, U.S. *Angew. Chem., Int. Ed*. **2009**, 48, 4900.
42 Velluto, D.; Thomas, S. N.; Simeoni, E.; Swartz, M. A.; Hubbel, J. A. *Biomaterials* **2011**, *32,* 9839-9847.
43 Bauhuber, S.; Liebl, R.; Tomasetti, L.; Rachel, R.; Goepferich, A.; Breunig, A. *J. Controlled Release* **2012**, *162*, 446-455.
44 Ferrari, S.; Moro, E.; Pettenazzo, A.; Behr, J.-P.; Zacchello, F.; Scarpa, M. *Gene Ther*. **1997**, 4, 1100-1106.
45 Movassaghi, M.; Schmidt, M.A. *Org. Lett*. **2005**, 7, 2453-2456.
46 Enders, D.; Breuer, K.; Raabe, G.; Runsik, J.; Teles, J.-H.; Melder, J.-P.; Ebel, K.; Brode, S. *Angew. Chem. Int. Ed. Engl*. **1995**, 34, 1021-1023.
47 Coulembier, O.; Dove, A.R.; Pratt, R.C.; Sentman, A.C.; Culkin, D.A.; Mespouille, L.; Dubois, Ph.; Waymouth, R.M.; Hedrick, J.L. *Angew. Chem. Int. Ed*. **2005**, 44, 4964.
48 Coulembier, O.; Mespouille, L.; Hedrick, J.L.; Waymouth, R.M.; Dubois, Ph. *Macromolecules* **2006**, 39, 4001.
49 Coulembier, O.; Kiesewetter, M.K.; Mason, A.; Dubois, Ph. Hedrick, J.L.; Waymouth, R.M. *Angew. Chem. Int. Ed.* **2007**, 46, 4719.
50 Coulembier, O.; Delcourt, C.; Dubois, Ph. *The Open Macromolecules Journal* **2007**, 1, 1-5.
51 Wiesbrock, F.; Hoogenboom, R.; Leenen, M. A. M.; Meier, M. A. R.; Schubert, U. S. *Macromolecules* **2005**, 38, 5025–5034.
52 Wiesbrock, F.; Hoogenboom, R.; Abeln, C. H.; Schubert, U. S. *Macromol. Rapid Commun*. **2004**, 25, 1895–1899.

Table of contents graphic