Design of Polycationic Micelles by Self-Assembly of Polyethyleneimine Functionalized Oligo[(ε-caprolactone)-*co*-glycolide] ABA Block Copolymers

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Abstract

Cationic polymeric micelles are of interest as delivery materials for nucleotides allowing condensation and transport of anionic macromolecules and enabling the reduction of cytotoxicity of polyethyleneimine (PEI), the current standard of vectors for non-viral nucleic acid delivery. In addition, micelles based on a degradable core would be capable to degrade hydrolytically and release their payload, which should preferably occur after uptake in early endosomes providing a pH of 5.5. We explored whether degradable and amphiphilic ABA block copolymers from hyperbranched PEI A blocks and B blocks based on hydrophobic oligoesters (CG) can be created, which can degrade in a pH range relevant for the early endosomes. CG was synthesized by ring-opening polymerization of ε-caprolactone and diglycolide. Polycationic micelles with particle sizes between 19 ± 1 and 43 ± 2 nm were obtained by self-assembly of the ABA block copolymers with different chain lengths of B blocks and/or co-assembly with a diblock copolymer from poly(ethylene glycol) (PEG) functionalized CG oligoester in phosphate-buffered saline solution. Mixed micelles containing PEG-CG showed a decreased zeta potential, suggesting a shielding by dangling PEG chains at the micelle surfaces. Sizes

of cationic micelles were stable at pH 7.4 over the studied time period of two weeks at 37 °C. The hydrolytic degradation was controlled by the composition of the CG core and was accelerated when the pH was decreased to 5.5 as detected by increasing micelle sizes. In this way, the polycationic micelles may act as an on-demand delivery system of condensed macromolecules.

Keywords: Self-Assembly, Micelles, Degradable Copolymers, Hydrolytically Degradable Copolymers

Introduction

A promising strategy for future disease treatment is the transfection of nucleic acids providing a pharmaceutical significant function into cells.[1, 2] Cationic polymers with a high positive charge, e.g. provided by PEI, were shown to act as gene delivery vectors with a high transfection efficiency.[3, 4] However, one of the factors limiting the application of PEI is its inherent cytotoxicity, which could cause cell membrane damage by necrotic and apoptotic mechanisms.[5, 6] Therefore, cationic polymeric micelles are explored as potential drug and gene delivery carrier systems as they are supposed to exhibit low cytotoxicity.[7-9] These micellar systems can be created by self-assembly of amphiphilic block copolymers and provide a typical core-shell morphology. The condensing capacity, surface charge, and cell compatibility/toxicity of the micelles can be adjusted by variation of the copolymer composition as well as by the introduction of chemically distinct block copolymers, which act as co-assembly agents.[10, 11] Compared to PEI, cationic micelles with integrated ester groups as hydrolytically cleavable bonds would enable the degradation of the micelle components within the endosomes and their excretion after transfection. Numerous cationic micellar systems were prepared as degradable polymeric gene delivery vectors, e.g. with a hydrophobic poly(lactide-*co*-3(*S*)-methyl-morpholine-2,5-dione), poly(ε-caprolactone), or poly(γ-benzyl-glutamate) core, but their hydrolytic degradation, which could contribute to the release mechanism of polyanionic molecules, was rarely investigated.[12-

[19] The cleavage of cationic micellar systems based ABA triblock copolymers with poly(lactide-*co*-glycolide) as hydrophobic core and low molecular weight PEI was analyzed in a neutral pH range at room temperature. These polycationic micelles exhibited a fast degradation-induced reorganization within two days, which was related to changes in the particle size.[20, 21] However, as the micellar system should be stable under storage conditions and for transportation of macromolecules at physiological temperature and neutral pH to their target cells, the degradation should ideally be enabled after uptake of the micelle in early endosomes with a pH of 5.5.

We hypothesized that polycationic micelles including ester bonds and hydrophobic segments in the particle core could provide structures that are relatively stable in terms of hydrolysis in a neutral pH range, while cleavage of ester bonds may be induced on-demand via an acid-catalyzed degradation at low environmental pH as present in early endosomes (Figure 1a). *ε*-Caprolactone (CL) units were selected as building blocks in order to realize hydrophobic polyester segments that would reduce the water uptake within micelles. The CL should be copolymerized with glycolide (GL) including weak ester bonds to control the degradation rate of the micellar structure. Hence, a series of polycationic micelles was created in PBS (pH = 7.4) via self-assembly of amphiphilic ABA block copolymers with a degradable and hydrophobic oligo[(*ε*-caprolactone)-*co*-glycolide] (CG) B blocks of different oligomer chain lengths, which were modified with hyperbranched PEI as A blocks (Figure 1b). Considering the concept of molecular packing parameters, which enables the prediction of molecular self-assembly and the resulting shape of created aggregates, e.g. spherical, rodlike or bilayered structures, the chain length of the hydrophobic segment in amphiphilic block copolymers can control the resulting shape by influencing the volume of the PEI shell.[22] In this context, the molecular weight of CG oligoester should not exceed the chain length of PEI (M_n = 10,000 g·mol^{-1}, M_w = 25,000 g·mol^{-1}) to facilitate the formation of spherically shaped micelles. Furthermore, mixed micelles based on ABA block copolymers and PEG-CG as a co-assembly agent (Figure 1c) were designed, where PEG should enable the suppression of surface charge by a shielding effect. The co-assembly agent was

equipped with a high CL to GL ratio, whereby the adjustment of degradation time might be obtained. Considering potentially effects of micelle composition on physical properties, the influences of CG chain length and formation of co-assembled mixed micelles on size, surface charge, and hydrolytic cleavage at pH = 7.4 and 5.5 will be investigated. We speculated that these systems might provide shape stability at neutral pH to potentially prolong circulation time in later *in vivo* applications and an accelerated and controllable cleavage of ester groups when the pH range is adapted to the range of early endosomes.

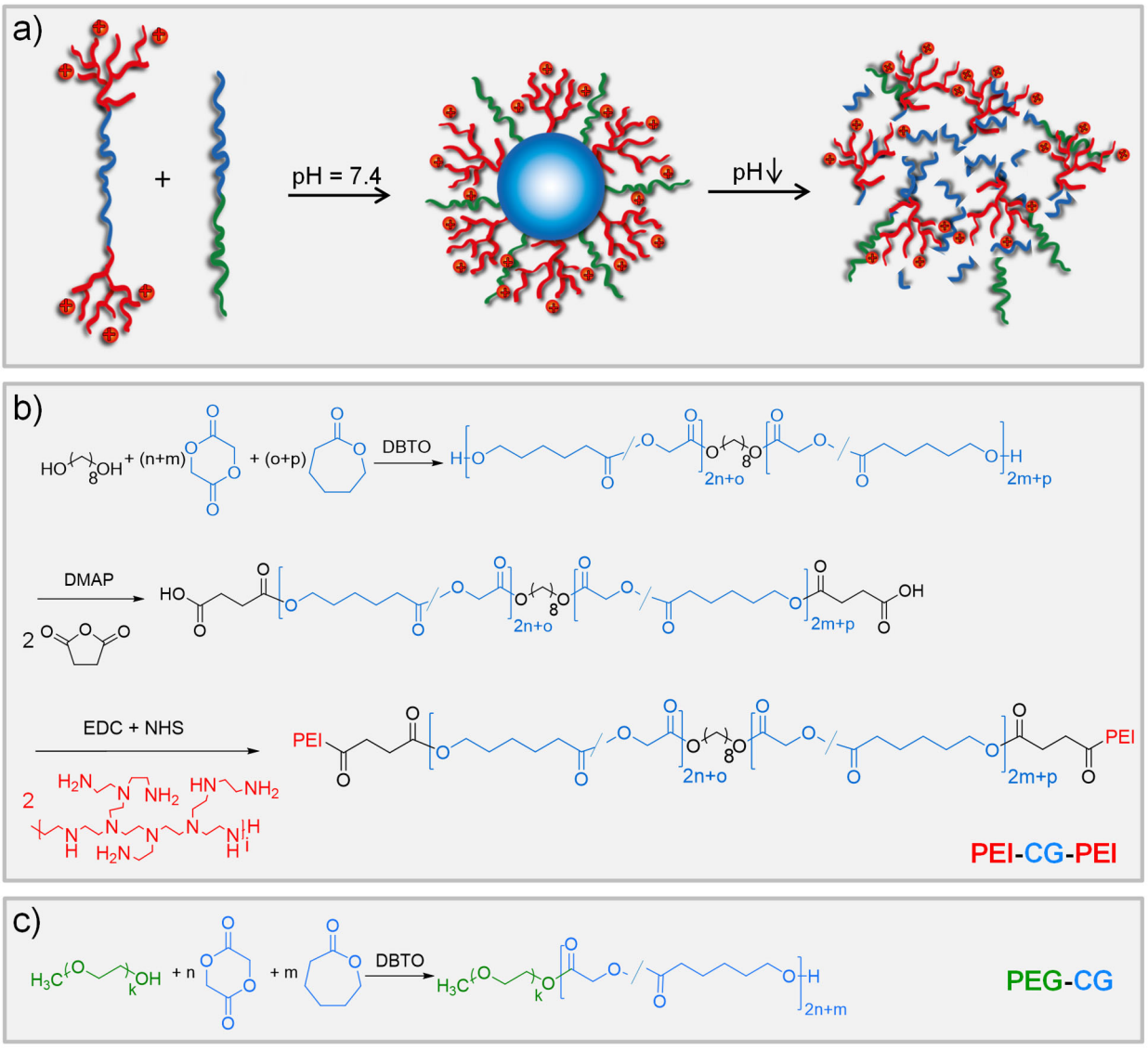


Figure 1: Formation of degradable micelles and from amphiphilic block copolymers. a) Schematic presentation of self-assembly-induced micellization with an on-demand degradation. b) Synthesis of PEI-CG-PEI triblock and c) PEG-CG diblock copolymers.

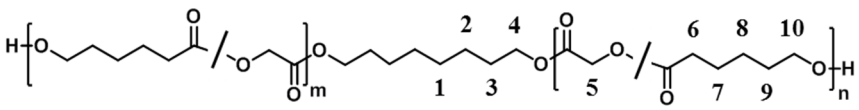
Materials and Methods

Materials

If not otherwise mentioned all chemicals were obtained from Aldrich (Steinheim, Germany). The ε-caprolactone (CL), octanediol, dibutyltin oxide (DBTO), 4-(dimethylamino)pyridine (DMAP), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochlorid (EDC), *N*-hydroxysuccinimide (NHS), succinic anhydride, trimethylamine, polyethyleneimine (PEI) (hyperbranched, M_n = 10,000 g·mol^{-1}, M_w = 25,000 g·mol^{-1}), hydrochloric acid, sodium bicarbonate, sodium chloride, magnesium sulfate, phosphate buffer saline (PBS) (low endotoxin, Biochrom GmbH, Berlin, Germany), and regenerated cellulose tubes with a cut off = 14,000 g·mol^{-1} (Roth, Karlsruhe, Germany) were used as received. Diglycolide (dGL) was recrystallized from acetic anhydride, oligo(ethylene glycol) monomethyl ether (PEG-OH, M_n = 5,000 g·mol^{-1}) was dried in vacuum, and anhydrous 1,4-dioxane was stored over 4 Å molecular sieves before use. All other solvents (Merck, Darmstadt, Germany) were of technical grade and were used as received unless noted otherwise.

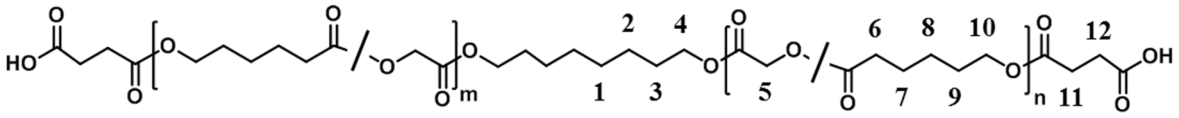
Copolymer Synthesis

Synthesis of CG-diOH copolymers: 394 mmol (45.0 g) CL and 43 mmol (4.9 g) dGL were molten at 130 °C in a Schlenk flask under an argon atmosphere, octanediol (2.5 mmol (0.4 g) for CG10-diOH and 8.3 mmol (1.2 g) for CG6-diOH) and DBTO (0.05 mmol (0.01 g) for CG10-diOH and 0.17 mmol (0.04 g) for CG6-diOH) were added, and the reaction mixture was stirred at 130 °C for 24 hours.[23] Afterwards the obtained solid was dissolved in chloroform, precipitated from cold hexane, filtered off, washed with cold hexane, and dried under vacuum until constant weight was achieved.



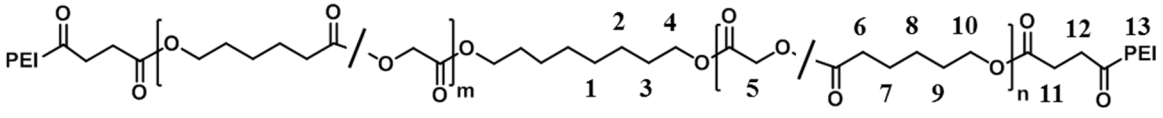
^{1}H-NMR (500 MHz, $CDCl_3$): δ [ppm] = 4.57-4.85 (m, CH_2-5); 3.65-4.26 (m, CH_2-4 and -10); 2.28-2.47 (m, CH_2-6); 1.61-1.75 (m, CH_2-3, -7, and -9); 1.25-1.48 (m, CH_2-1, -2, and -8).

Synthesis of CG-diCOOH copolymers: CG-diCOOH copolymers were synthesized according to the procedure provided in reference.[21] In a Schlenk flask, 4.5 mmol (26.6 g of CG6-diOH and 49.9 g of CG10-diOH) CG-diOH, 97 mmol (11.8 g) DMAP, 92 mmol (9.2 g) succinic anhydride, 4.5 mL (2.9 g) triethylamine were dissolved in 400 mL 1,4-dioxane and the reaction was performed for 24 hours at room temperature under stirring and argon atmosphere. The product of reaction was precipitated from cold ethanol, filtered off, washed with cold ethanol, and was dissolved again in dichloromethane. The organic solution was washed twice with saturated sodium bicarbonate solution, three times with hydrochloric acid (10%), three times with saturated sodium chloride solution, dried over magnesium sulfate, and filtered off. The product was precipitated from cold hexane and was dried under vacuum until constant weight was achieved.



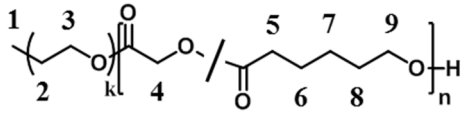
^{1}H-NMR (500 MHz, $CDCl_3$): δ [ppm] = 4.57-4.85 (m, CH_2-5); 3.65-4.26 (m, CH_2-4 and -10); 2.63-2.79 (m, CH_2-11 and -12); 2.28-2.47 (m, CH_2-6); 1.61-1.75 (m, CH_2-3, -7, and -9); 1.25-1.48 (m, CH_2-1, -2, and -8).

Synthesis of PEI-CG-PEI: 1 mmol CG-diCOOH (6.1 g of CG6-diCOOH or 11.3 g of CG10-diCOOH) was dissolved under an argon atmosphere in a Schlenk flask in 200 mL DMSO. 19 mmol (3.6 g) EDC and 21 mmol (2.4 g) NHS were added and the reaction mixture was stirred for 1 hour at room temperature according to the procedure described in reference.[20] This mixture was added dropwise to a PEI solution (10 mmol (100 g) in 200 mL DMSO) in a Schlenk flask under intensive stirring. The reaction mixture was further stirred for 24 hours. The block copolymer PEI-CG-PEI was purified by dialysis and was obtained by freeze drying until constant weight was achieved.



^{1}H-NMR (500 MHz, $CDCl_3$): δ [ppm] = 4.57-4.85 (m, CH_2-5); 3.65-4.26 (m, CH_2-4 and -10); 2.63-2.79 (m, CH_2-11 and -12); 2.51-2.79 (m, CH_2-13); 2.28-2.47 (m, CH_2-6); 1.61-1.75 (m, CH_2-3, -7, and -9); 1.25-1.48 (m, CH_2-1, -2, and -8).

Synthesis of PEG-CG diblock copolymer: In a Schlenk flask, 91 mmol (10.4 g) CL, and 28 mmol (3.2 g) dGL were molten at 130 °C under an argon atmosphere, 1.6 mmol (8 g) PEG-OH (M_n = 5,000 g·mol^{-1}) and 0.4 mmol (0.1 g) DBTO were transferred to the flask, and the mixture was reacted under stirring at 130 °C for 24 hours. Afterwards, the mixture was dissolved in chloroform, the product of reaction was precipitated from cold hexane, filtered off, washed with cold hexane, and dried under vacuum until constant weight was achieved.



^{1}H-NMR (500 MHz, $CDCl_3$): δ [ppm] = 4.57-4.85 (m, CH_2-4); 4.03-4.26 (m, CH_2-9); 3.61-3.69 (m, CH_2-2 and -3); 4.38 (s, CH_3-1); 2.28-2.47 (m, CH_2-5); 1.61-1.75 (m, CH_2-6 and -8); 1.35-1.48 (m, CH_2-7).

Characterization of Copolymers

Number average of molecular weight (M_n) of the copolymers were determined on a multidetector gel permeation chromatography system (GPC) consisting of a precolumn, two 300 mm × 0.8 mm M columns (PSS, Mainz, Germany), an isocratic pump 2080, an automatic injector AS 2050 (both Jasco, Tokyo, Japan), a RI detector Shodex RI-101 (Showa Denko, München, Germany), and a dual detector T60A (Viscotek Corporation, Houston, USA) using chloroform (0.2 wt% toluene as internal standard, 35 °C, 1.0 mL·min^{-1}) as eluent. For PEG-based copolymers, M_n was determined with a multidetector GPC consisting of a GRAM VS1 precolumn (40 mm × 4.6 mm), a GRAM 30Å 5091312, and a GRAM 1000Å 71111 column (both 250 mm × 4.6 mm) (all PSS, Mainz, Germany), an isocratic pump 980,

an automatic injector 851-AS, a LG 980-02 ternary gradient unit, a multiwave length detector MD-910, a RI detector RI-930 (all Jasco, Gross-Umstadt, Germany), a differential viscometer η-1001 (WGE Dr. Bures, Dallgow-Doeberitz, Germany), a Wyatt miniDawn Tristar light scattering detector (Wyatt Technology Corporation, Santa Barbara, USA) and a degasser ERC-3315α (Ercatech, Berne, Switzerland). DMF was used as eluent (containing 0.4 wt% toluene as internal standard) at 35 °C with a flow rate of 0.25 mL·min^{-1}. Polystyrene samples were used for universal calibration for DMF and chloroform GPC in order to determine the hydrodynamic volume as function of elution volume.

The number of hydroxy end groups was determined by potentiometric titration with a Tritrino 716 (DMS, Metrohm, Filderstadt, Germany).

^{1}H-NMR spectra were recorded at 25 °C in $CDCl_3$ with a Bruker Avance 500 spectrometer (500 MHz, Bruker, Karlsruhe, Germany) using tetramethylsilane as a reference with a relaxation time of 2 s for copolymers without PEI end groups and with a relaxation time of 15 s for PEI modified copolymers.

Thermal properties were determined on a Netzsch DSC 204 (Netzsch Ltd., Selb, Germany) in sealed Al-pans under N_2-atmosphere between -50 and 100 °C with heating and cooling rates of 10 K·min^{-1}.

The degradability of copolymers was investigated at 37 °C in PBS (pH = 7.4) and at 37 °C at pH = 5.5 (pH value of the PBS solution was adjusted to 5.5 with HCl). Degradation products were characterized by GPC measurements.

Preparation and Characterization of Polymeric Micelles

Particles were prepared by dissolving 30 mg of the copolymers (or copolymer mixture) in 6 mL DMSO. Particle compositions were as followed: PEI-CG10-PEI (100 wt%), PEI-CG6-PEI (100 wt%), PEI-CG10-PEI:PEG-CG (50 wt% : 50 wt%), and PEI-CG6-PEI:PEG-CG (50 wt% : 50 wt%). The solution was added dropwise under intensive stirring to 30 mL PBS (pH = 7.4) and the resulting particle suspension was sonicated for 30 min. The obtained suspension was purified by dialysis under intensive stirring for 2 d using regenerated cellulose bags (Roth, Karlsruhe, Germany, cut off =

14,000 g·mol^{-1}) with PBS as extraction media in a 500 mL beaker, whereby the PBS solution was exchanged four times per day.

The particle size and polydispersity index (PDI) were determined by Dynamic Light Scattering (DLS) using a ZetasizerNano (Malvern Instruments, Herrenberg, Germany). The zeta potential was analyzed with the same instrumentation. The measurements were performed at 25 °C using non-diluted samples in disposable cuvettes. The morphology of particles was characterized by Talos F200X transmission electron microscope (TEM) (FEI, Eindhoven, Netherlands). One drop of particle suspension was placed on a copper grid covered with a closed carbon film and dried in air at room temperature.

Degradation experiments were performed with copolymer-based particles at 37 °C (in PBS, pH = 7.4) and at 37 °C, in which the pH value of the PBS solution was adjusted to 5.5 by adding hydrochloric acid solution. At certain time intervals, DLS measurements were performed.

Results and Discussion

Synthesis and Characterization of Degradable Copolymers

The oligoesters CG were synthesized by ring-opening polymerization of CL and dGL catalyzed by DBTO (Figure 1b and c). In case of CG-diOH, octanediol was used as initiator, whereas monohydroxy PEG-OH (M_n = 5,000 g·mol^{-1}) acted as initiator for the PEG-CG synthesis. A GL content of 20 mol% was selected for CG diol synthesis to facilitate a fast hydrolytic degradation, while the content of CL was supposed to be high enough to enable the reduction of the water uptake induced by the high hydrophobic character. A GL content of 45 mol% was used for the synthesis of PEG-CG to enable the adjustment of degradation time. The molecular weight of the synthesized copolymers was varied by altering the molar ratio between initiator and monomers. M_n of CG as determined by GPC, NMR, and titration of hydroxy end groups (Table 1) was adjusted to 5,900 g·mol^{-1} for CG6-diOH, 11,100 g·mol^{-1} for CG10-diOH as well as 10,200 g·mol^{-1} for PEG-CG (average values of the different measurements).

The composition of copolymers was investigated by NMR spectroscopy from which a GL content of 22 ± 2 mol% for CG6-diOH, 18 ± 2% mol% for CG10-diOH, and 44 ± 4% mol% for PEG-CG was determined.[23] The sequence structure of copolymers was determined by ^{1}H-NMR spectroscopy, analyzing the ratios of the specific triads and diads in the copolyester chain segments. The signal at a chemical shift of 4.75 ppm was attributed to the CH_2-group of GL in a neighborhood of GL-GL-GL. When a GL unit is directly linked to CL, the signal is shifted to a higher field (4.64 ppm for GL-GL-CL and 4.53 ppm for CL-GL-CL). Based on this chemical environment, CG10k-diOH exhibited ratios of triads $18_{GL\text{-}GL\text{-}GL}$:$65_{GL\text{-}GL\text{-}CL}$:$17_{CL\text{-}GL\text{-}CL}$. CG6-diOH, which was synthesized with a higher initiator to monomer ratio to enable the synthesis of a lower oligomer chain length, possessed a comparable GL content, but a different distribution of triades: $1_{GL\text{-}GL\text{-}GL}$:$20_{GL\text{-}GL\text{-}CL}$:$79_{CL\text{-}GL\text{-}CL}$. In order to allow similar reaction rates, the initiator to catalyst ratio was kept constant, whereby a higher concentration of DBTO was used for the synthesis of CG6-diOH. In this way, the transesterification process could be more pronounced resulting in a higher concentration of CL-GL-CL triads. In case of PEG-CG, a triad ratio of $4_{GL\text{-}GL\text{-}GL}$:$36_{GL\text{-}GL\text{-}CL}$:$60_{CL\text{-}GL\text{-}CL}$ was determined for the monofunctionalized oligoester. The diad concentrations were analyzed by comparing the integrals of signals from the CH_2-group of the CL unit (CH_2-COO) at 2.24 ppm (CL-CL) and at 2.37 ppm (GL-CL). CG-diOH copolymers exhibited a diad ratio of $82_{CL\text{-}CL}$:$18_{GL\text{-}CL}$ and PEG-CG of $53_{CL\text{-}CL}$:$47_{GL\text{-}CL}$. Here, the PEG-initiated oligoester exhibited a higher molar ratio of glycolide units resulting in a higher concentration of GL-CL diads.

The thermal properties of synthesized CG copolymers were investigated by DSC measurements, from which the endothermic peaks in the second heating run were identified as melting temperatures (T_ms). The oligoester CG6-diOH and CG10-diOH exhibited comparable melting transitions in the range between 40 and 42 °C with a heat of fusion (ΔH_m) between 62 and 65 J·g^{-1}. While oligo(ε-caprolactone) with a M_n of 6,000 and 10,000 g·mol^{-1} was reported to exhibit T_ms ranging from 53 to 55 °C,[24] the incorporation of GL units and the random sequence structure reduced the temperature of this thermal transition. In comparison with these oligoesters, T_m = 51 °C was detected for the diblock copolymer

PEG-CG, which exhibited a higher GL content. Here, it was expected that the high GL content would result in amorphous CG oligoester, whereby the detected endothermic peak correlated to the melting transition of the PEG block.

Table 1. Determination of molecular weight, GL content, and thermal properties of copolymers.

Sample-ID	M_n [g·mol^{-1}]			GL content*	T_m	ΔH_m
	GPC	NMR	Titration	[mol%]	[°C]	[J·g^{-1}]
CG6-diOH	5,400 ± 600	5,900 ± 200	6,300 ± 300	22 ± 2	40 ± 2	62 ± 1
CG10-diOH	9,700 ± 800	10,700 ± 300	13,000 ± 700	18 ± 2	42 ± 2	65 ± 1
PEG-CG	8,300 ± 500	10,800 ± 300	11,400 ± 900	44 ± 4	51 ± 2	52 ± 1

*calculated by NMR spectroscopy

For the formation of ABA block copolymers, the dihydroxy copolyester blocks were first functionalized with succinic anhydride (Figure 1b) with a conversion ratio above 95% for CG6-diCOOH and CG10-diCOOH as determined by NMR spectroscopy. Afterwards, the amphiphilic block copolymers were synthesized by amidation of CG-diCOOH with hyperbranched PEI. The molecular weights of the obtained block copolymers as determined by NMR spectroscopy were 59,900 ± 3,000 g·mol^{-1} for PEI-CG10-PEI and 56,500 ± 2,800 g·mol^{-1} for PEI-CG6-PEI, indicating the formation of ABA triblock copolymers with a hydrophobic CG core and two hydrophilic PEI-based segments attached at both sides. However, according to the polydispersity of the used hyperbranched PEI (PDI = 2.5), a broad distribution of the chain length of ABA block copolymers was expected. The determination of M_n by means of size exclusion using GPC was not successful, which was attributed to a separation of the amphiphilic PEI-based copolymers by polarity.

As the synthesized CG copolymers included GL ester groups, the degradation behavior of the hydrophobic B block was investigated by determining the oligomer chain length. Therefore, the influence of different storage conditions on the degradability of CG copolymers was analyzed: 37 °C at pH = 7.4 and 37 °C at pH = 5.5 (representing the environment within endosomes within the early stage after cellular uptake). As presented in Figure 2, the decrease of pH accelerated the degradation process. Here, M_n decreased from 9,700 ± 800 g·mol^{-1} to 7,300 ± 500 g·mol^{-1} for CG10-diOH and from

5,400 ± 600 g·mol^{-1} to 4,500 ± 600 g·mol^{-1} for CG6-diOH within 14 days. In contrast, no significant change of the molecular weight was detected at pH = 7.4. In case of PEG-CG with the highest content of GL, the degradation process was more pronounced compared to CG-diOH copolymers. Here, M_n decreased from 8,300 ± 500 g·mol^{-1} to 2,300 ± 400 g·mol^{-1} under accelerated conditions and also at pH = 7.4 the degradability of GL was monitored.

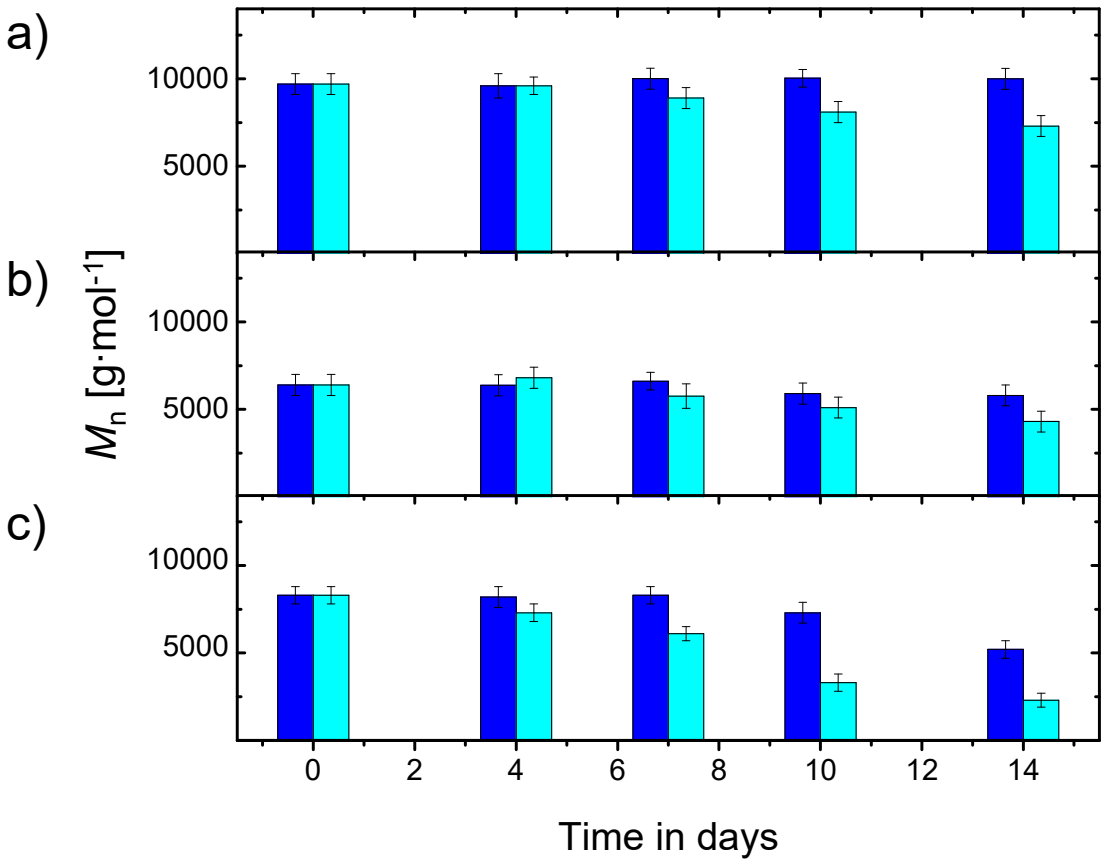


Figure 2. Investigation of degradation behavior of CG copolymers by determining the oligomer chain length as function of time with a pH of 7.4 at 37 °C (■) and with a pH of 5.5 at 37 °C (■). a) CG10-diOH, b) CG6-diOH, and c) PEG-CG.

Micelle Formation and Degradation Behavior of Particles

The particle sizes were analyzed by DLS (Figure 3a), from which Z-average values (intensity weighted mean hydrodynamic size) were obtained. Z-average values ranging from 19 ± 1 nm to 43 ± 2 nm were determined for the different compositions of particles with PDI (polydispersity index) values between 0.18 and 0.52. Polymeric micelles of the ABA triblock copolymer PEI-CG6-PEI were substantially smaller than those from PEI-CG10-PEI and confirmed that already relatively small structural changes

of the core affect the micelle size. This effect is diminished once that PEG-CG is added to create mixed micelles resulting in a different micellar organization. In addition, the dimensions of particles determined by DLS were exemplarily confirmed for PEI-CG10-PEI by TEM analysis (Figure 3b), indicating individual micelle sizes in the range of 12 to 78 nm, which is in good accordance with data from DLS. The surface charge of the polycationic micelles, which is an important parameter to enable condensing of polyanionic macromolecules like DNA, siRNA or other potentially bioactive molecules, was investigated by determining the zeta potential (Figure 3c). The zeta potential, as influenced by the composition of polymeric micelles, ranged between 11.4 ± 0.3 mV and 17.8 ± 0.9 mV. Overall, the zeta potential increased with decreasing B block length of CG, and decreased when PEG-CG was utilized as co-assembly agent. In this way, in the future, an adjustment of condensing efficiency might be obtained as the content of polyanionic molecules like genes, which can be linked to polycationic micellar structures by electrostatic interactions is highly dependent on the surface charge of the particles.

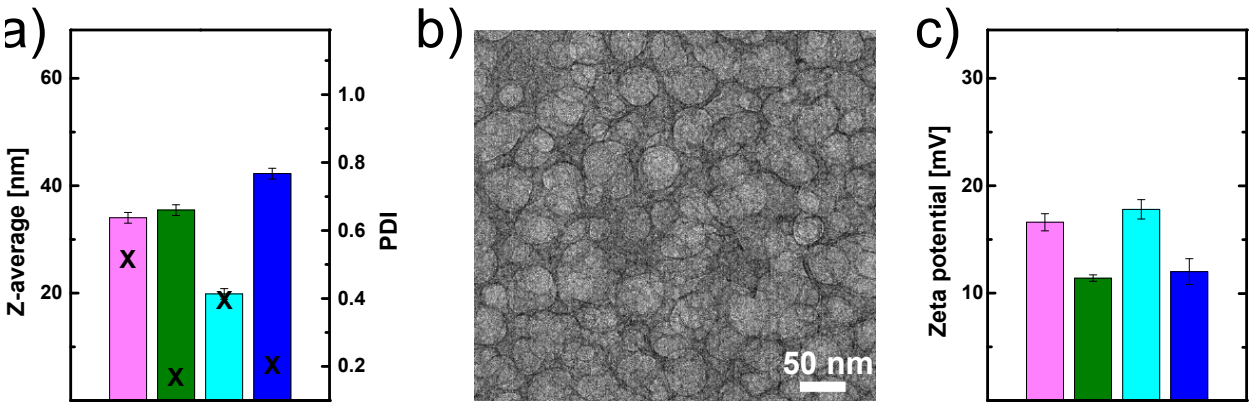


Figure 3. Micelle characterization. a) Z-average and PDI (**X**) of PEI-CG10-PEI (■), PEI-CG10-PEI:PEG-CG (■), PEI-CG6-PEI (■), and PEI-CG6-PEI:PEG-CG (■). b) TEM image of particles based on PEI-CG10-PEI. c) Zeta potential of PEI-CG10-PEI (■), PEI-CG10-PEI:PEG-CG (■), PEI-CG6-PEI (■), and PEI-CG6-PEI:PEG-CG (■).

Furthermore, it was expected that the *in vitro* degradation of the oligoester-based core in polycationic micelles can be accelerated in an acidic environment as present in early endosomes (pH ~ 5.5). The sizes of particles were relatively stable within 14 days at 37 °C at pH = 7.4 (Figure 4) as determined

by DLS under different storage conditions, thus indicating that no particle aggregates were formed and the ester groups of copolymers were hydrolytically stable within this time period (Figure 2). However, at reduced pH, the Z-average particle size increased within 14 days from 20 ± 1 nm to 480 ± 30 nm for PEI-CG6-PEI (Figure 4a). This shift could be attributed to the degradation of hydrophobic segments, which could cause a reorganization/rearrangement of the micellar structure. The hydrolysis of a triblock copolymer within the hydrophobic block would result in two diblock copolymers with an altered balance of hydrophilicity/hydrophobicity. One also has to consider that this hydrolysis will not necessarily result in a uniform length of the hydrophobic segments, which may have a strong disturbing effect on the self-organization of the micelles, which could contribute to micelle size alteration.

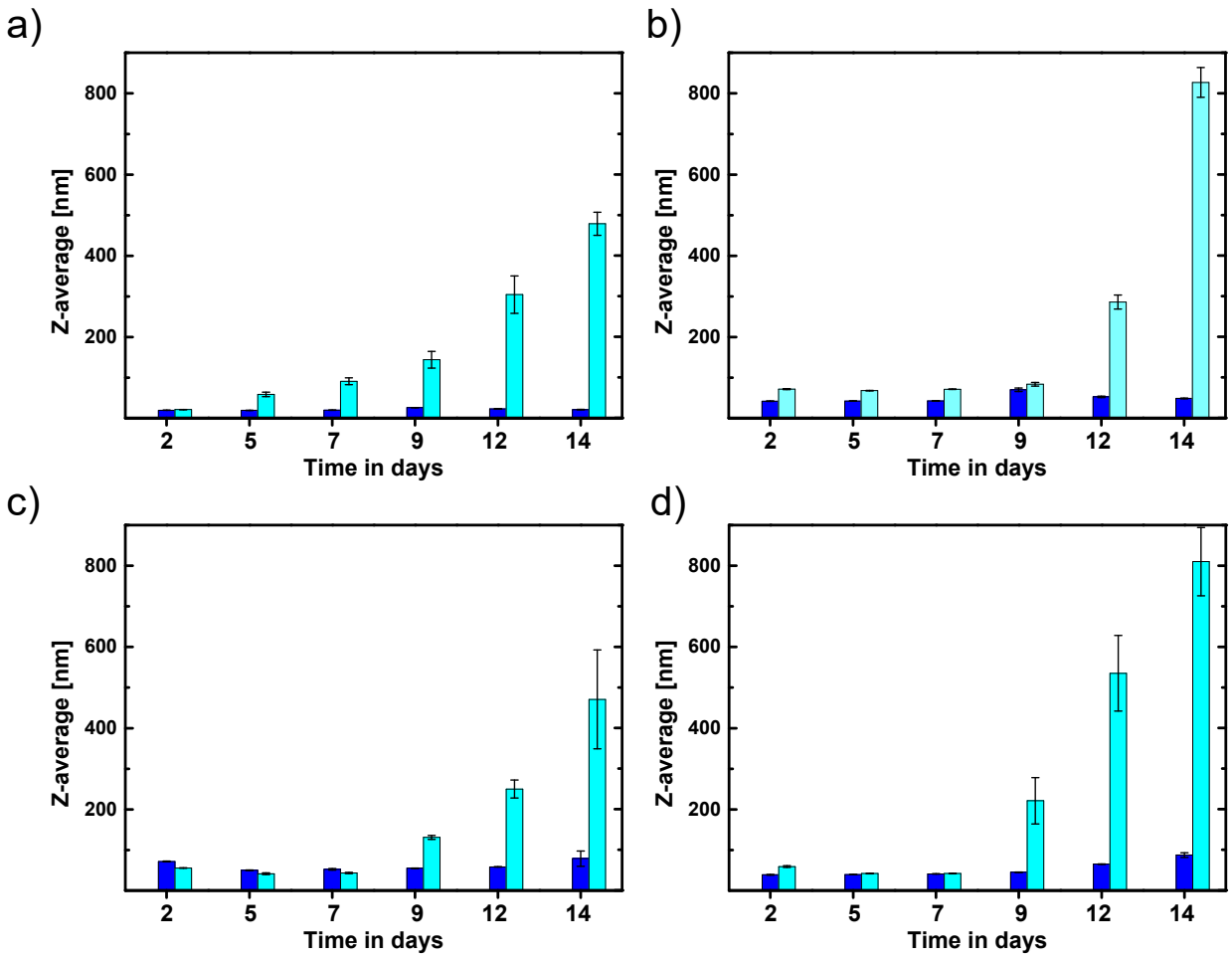


Figure 4. Investigation of degradation behavior of polycationic micelles. Z-average of cationic particles as function of time with a pH of 7.4 at 37 °C (■) and with a pH of 5.5 at 37 °C (■). a) PEI-CG6-PEI, b) PEI-CG6-PEI:PEG-CG, c) PEI-CG10-PEI, and d) PEI-CG10-PEI:PEG-CG.

When PEG-CG was introduced in the micellar system as co-assembly agent, the composition of the degradable CG core was altered, which also affected the degradation behavior. As presented in Figure 4b, the sizes of particles were almost constant at 37 °C in a neutral pH range. When the pH was adjusted to 5.5, an increase in Z-average from 42 ± 1 nm (37 °C, pH = 7.4) to 830 ± 40 nm (37 °C, pH = 5.5) was observed for PEI-CG6-PEI:PEG-CG within 14 days. Therefore, the hydrolytic cleavage *in vitro* was more pronounced for a micellar system having a higher content of GL, which would enable the control of degradation time in the pH range of early endosomes. The variation of the chain length of the hydrophobic segment, where the ratio between GL and CL was constant, exhibited no difference in the rearrangement of the micellar structure as function of time (Figure 4a and c).

Conclusion

In this work we explored, whether polyester-based micelles with a controllable degradation behavior could be created. The micelles were synthesized by self-assembly of ABA triblock copolymers and co-assembly with diblock copolymers. The hydrophobic B block was obtained by ring-opening polymerization of dGL and CL and was afterwards modified with hyperbranched hydrophilic PEI as A block. By variation of the molecular parameters, e.g. chain length of B blocks as well as the introduction of PEG-CG as co-assembly agent, nano-sized micelles ranging from 19 ± 1 nm to 43 ± 2 nm with controllable positive surface charges between 11.4 ± 0.3 mV and 17.8 ± 0.9 mV can be obtained. The polycationic micelles with the ABA block copolymer were relatively stable within two weeks at 37 °C in neutral pH range, which would enable a high shelf-life and a stable attachment of anionic macromolecules in the future. The cleavage of the B block was enhanced by an acid-catalyzed degradation when the pH range was decreased to 5.5. Micelles basing solely on triblock copolymers exhibited an increase in the Z-average values by about one order of magnitude during degradation, whereas the incorporation of PEG-CG diblock copolymer resulted in a more pronounced increase of

micelle size. Here, the degradation behavior was directed by the CL to GL content in the hydrophobic CG core, which was varied when PEG-CG was introduced as co-assembly agent. Thus, the designed polycationic micelles with highly hydrophobic CL segments and hydrolytically cleavable GL units in the micellar core may present an on-demand release system for gene therapy with a controllable degradation time.

Acknowledgements

This work was in part financially supported by the Helmholtz Association (program-oriented funding), and the German Research Foundation (Collaborative Research Center 1112, project A3), as well as by the International Science & Technology Cooperation Program of China (Grant No. 2013DFG52040).

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