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Polyethyleneimine and Poly(ethylene glycol) Functionalized Oligoester Based Polycationic Particles

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ABSTRACT

Polycationic particles based on a degradable oligoester core are interesting candidate materials for the transfection of polyanionic macromolecules like DNA, which would enable the degradation after delivery of condensed molecules. Good transfection efficiencies can be obtained when the size of the polyplex (containing both polycationic nanoparticles and polyanionic macromolecules) does not exceed 120 nm. Therefore, here we explored how size, but also dispersity, and surface charge of these carrier systems can be adjusted by variation of the block copolymer composition or the presence and ratio of a co-assembly agent. Polycationic particles were obtained based on an amphiphilic triblock copolymer from oligo[(ϵ -caprolactone)-*co*-glycolide] (CG) functionalized with polyethyleneimine (PEI) and diblock copolymer based on poly(ethylene glycol) (PEG) modified with CG. A second series of particles was created, in which the oligoester blocks contained only ϵ -caprolactone units, therefore the effect of the presence of glycolide units was also studied. In both series, the ratio between di- and triblock copolymers was systematically varied. Nano-sized particles ranging from 34.5 ± 0.2 nm to 97.9 ± 0.3 nm with controllable positive surface charges between 2.9 ± 0.2 mV and 18.1 ± 0.5 mV were obtained by self-assembly in PBS solution under intensive stirring. The incorporation of PEG-C diblock copolymers resulted in an increase of particle size, however no specific relation between composition, size, and polydispersity was observed. In case of PEG-CG diblock copolymers a rather systematic increase of the particles' size with increasing content of diblock copolymer was shown. Furthermore, with a decrease of content of diblock copolymer in the particle structure zeta potential strongly increased. Additionally, the content of glycolide units in triblock copolymer increased the zeta potential of PEI-CG-PEI-based particles in comparison to PEI-C-PEI-based ones. Therefore, obtained particles could be used as potential target-oriented polycationic macromolecules for carrier systems.

INTRODUCTION

The transfection of nucleic acids into cells constitutes a promising strategy for future disease treatment [1, 2]. Cationic polymers, e.g. polyethyleneimine (PEI), were found to act as efficient gene delivery vectors [3, 4]. However, the reported cytotoxicity of PEI causing cell membrane damage by necrotic and apoptotic mechanisms, strongly limits its potential application [5, 6]. Therefore, cationic polymeric particles, supposed to exhibit low cytotoxic potential, are explored as prospective drug and gene delivery carrier systems [7, 8]. These systems can be created by self-assembly of amphiphilic block copolymers and provide typical core-shell morphology. When the block copolymer compositions are varied or co-assembly agents are incorporated, the characteristics of polycationic particles can be adjusted, e.g. size, surface charge, and as result also condensing capacity and toxicity [9, 10]. Furthermore, the degradation rate of these micellar structures can be controlled by the incorporation of weak ester bonds, e.g., from glycolide (GL) [11-13]. However, a systematic investigation of how the incorporation of GL units or the content of a co-assembly agent will affect the self-assembly process was not performed so far.

We hypothesized that the content of hydrophilic GL units in polycationic particles will strongly influence particles' properties like size and surface charge. Furthermore, the addition of co-assembly agent in form of diblock copolymer based on PEG should enable the suppression of surface charge by a shielding effect, and further adjustment of properties of the resulting particles. Therefore, in the presented study, two series of polycationic particles were prepared via self-assembly of amphiphilic ABA triblock copolymers, in which block B constitutes oligo[(ϵ -caprolactone)-*co*-glycolide] (CG) or oligo(ϵ -caprolactone) (C) (as control block without GL). These degradable and hydrophobic blocks were modified with hyperbranched PEI providing two end blocks A (Figure 1). As a result two types of triblock copolymers were obtained: PEI-CG-PEI and PEI-C-PEI. Furthermore, mixed particles based on ABA triblock copolymers and diblock copolymers of PEG-modified oligo(ϵ -caprolactone) (PEG-C) or oligo[(ϵ -caprolactone)-*co*-glycolide] (PEG-CG) as a co-assembly agent were obtained, and systematic studies of the influence of glycolide (GL) content as well as wt% of triblock copolymers ABA in the mixture of particles on size and surface charge were performed. PEI-C-PEI(X) means mixture of triblock copolymer PEI-C-PEI and diblock copolymer PEG-C containing X wt% of the triblock copolymer. Mixtures of the triblock copolymer PEI-CG-PEI and the diblock copolymer PEG-CG containing X wt% of the triblock are named PEI-CG-PEI(X).

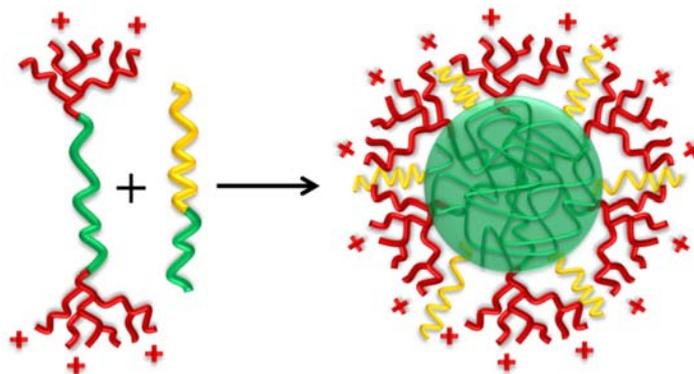


Figure 1. Schematic illustration of particle formation via self-assembly using an amphiphilic triblock copolymer with polycationic and hyperbranched end groups and a neutral amphiphilic diblock copolymer. The polyethyleneimine (PEI) segments are shown in red color, the oligo(ϵ -caprolactone) or oligo[(ϵ -caprolactone)-*co*-glycolide] (C/CG) segments in green color, and the PEG blocks in yellow color.

EXPERIMENTAL DETAILS

If not otherwise mentioned, chemicals were obtained from Aldrich (Steinheim, Germany). The phosphate buffered saline (PBS) (low endotoxin, Biochrom GmbH, Berlin, Germany) was used as received, poly(ϵ -caprolactone) (PCL) (linear, $M_n = 10,000 \text{ g}\cdot\text{mol}^{-1}$) (Solvay Caprolactones Warrington, UK), polyethyleneimine (PEI) (hyperbranched, $M_w = 25,000 \text{ g}\cdot\text{mol}^{-1}$), and oligo(ethylene glycol)monomethyl ether (PEG-OH, $M_n = 5,000 \text{ g}\cdot\text{mol}^{-1}$) was dried under vacuum, glycolide (GL) was recrystallized from acetic anhydride, and anhydrous 1,4-dioxane was stored over 4 Å molecular sieves before use. All solvents (Merck, Darmstadt, Germany) were of commercial grade and were used as received unless noted otherwise. Regenerated cellulose tubes with a cut off = 14,000 $\text{g}\cdot\text{mol}^{-1}$ (Roth, Karlsruhe, Germany) were used as received.

Copolymer Synthesis

The synthesis of diblock copolymers PEG-C and PEG-CG, as well as triblock copolymers PEI-CG-PEI and PEI-C-PEI were performed according to the procedure described in references [11, 12]. The molecular weight and dispersity index (D) of single diblock copolymers were determined by Gel Permeation Chromatography (GPC) in DMF as eluent. In case of PEG-C $M_n = 7,700 \pm 800 \text{ g}\cdot\text{mol}^{-1}$ (D = 1.4) was determined, and $M_n = 7,800 \pm 800 \text{ g}\cdot\text{mol}^{-1}$ (D = 1.3) in case of PEG-CG. Error of the measurement method was estimated as 10% based on measurements of PS standards. The molecular weight of single triblock copolymers was calculated based on NMR spectroscopy (chloroform used as a solvent) and was $M_n = 57,300 \pm 2,800 \text{ g}\cdot\text{mol}^{-1}$ and $M_n = 56,500 \pm 2800 \text{ g}\cdot\text{mol}^{-1}$ in case of PEI-C-PEI and PEI-CG-PEI, respectively. Error of the method was estimated based on error of integral peak area (around 5%).

Preparation and Characterization of Polymeric Particles

Particles were prepared from a single triblock (PEI-C-PEI, PEI-CG-PEI) or diblock copolymer (PEG-C, PEG-CG) or from mixtures of tri- and diblock copolymers containing 25 wt%, 50 wt% or 75 wt% of the triblock copolymer. Particles were obtained by dissolving 30 mg of the copolymers (or copolymer mixture) in 6 mL DMSO. Exact molar ratios of components and concentration of particles in the solutions are listed in Table 1.

The copolymer solution was added dropwise to 30 mL PBS (pH = 7.4) under intensive stirring. Then the resulting particle suspension was sonicated in Branson B-Series Ultrasonic Cleaner 5510MTH for 30 min and purified by dialysis under intensive stirring for 2 d. For this purpose regenerated cellulose bags (Roth, Karlsruhe, Germany, cut off = 14,000 $\text{g}\cdot\text{mol}^{-1}$) with PBS as extraction medium in a 500 mL beaker were used, whereby the PBS solution was exchanged four times per day.

The particle size, polydispersity index (PDI), and the zeta potential were determined by Dynamic Light Scattering (DLS) in PBS buffer using a ZetasizerNano (Malvern Instruments, Herrenberg, Germany). Determination of zeta potential was performed at 25 °C using non-diluted samples in disposable cuvettes.

Table 1. Composition and concentration of particles in DMSO solutions.

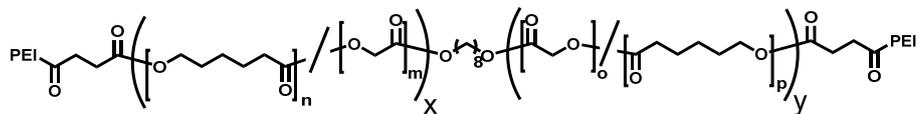
Sample	Composition	Triblock copolymer content (wt%)	Triblock copolymer content (mol%)	Concentration of particles in DMSO solutions (10^{-4} mmol·mL ⁻¹)
PEI-CG-PEI(100)	PEI-CG-PEI	100 ± 0	100 ± 0	1.7 ± 0.2
PEI-CG-PEI(75)	PEI-CG-PEI + PEG-CG	75 ± 1	44 ± 6	2.8 ± 0.3
PEI-CG-PEI(50)	PEI-CG-PEI + PEG-CG	50 ± 1	21 ± 3	4.1 ± 0.4
PEI-CG-PEI(25)	PEI-CG-PEI + PEG-CG	25 ± 1	8 ± 2	5.2 ± 0.5
PEG-CG	PEG-CG	0 ± 0	0 ± 0	6.4 ± 0.6
PEI-C-PEI(100)	PEI-C-PEI	100 ± 0	100 ± 0	1.7 ± 0.2
PEI-C-PEI(75)	PEI-C-PEI + PEG-C	75 ± 1	44 ± 1	2.9 ± 0.3
PEI-C-PEI(50)	PEI-C-PEI + PEG-C	50 ± 1	20 ± 3	4.1 ± 0.4
PEI-C-PEI(25)	PEI-C-PEI + PEG-C	25 ± 1	8 ± 2	5.3 ± 0.5
PEG-CG	PEG-C	0 ± 0	0 ± 0	6.5 ± 0.6

RESULTS AND DISCUSSION

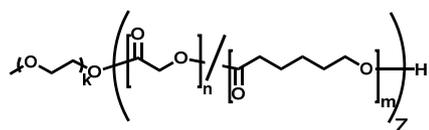
Synthesis of Copolymers

The oligoesters CG were synthesized in a ring-opening polymerization of CL and GL catalyzed by DBTO according to the method described in reference [12]. M_n of CG in PEI-CG-PEI was adjusted to 9,700 g·mol⁻¹ and to 2800 g·mol⁻¹ for PEG-CG. The copolymers without GL units exhibited a molecular weight of the C segment about 10,000 g·mol⁻¹ for PEI-C-PEI and 2700 g·mol⁻¹ for PEG-C. In addition, the composition of copolymers was investigated by NMR spectroscopy from which a GL content of 18 ± 2 mol% in CG of PEI-CG-PEI and 44 ± 4 mol% in the CG block of PEG-CG was determined [11, 12]. The amphiphilic triblock copolymers PEI-CG-PEI(X) and PEI-C-PEI(X) (Figure 2) were obtained after conversion of the hydroxyl end groups with succinic anhydride and subsequent amidation with hyperbranched PEI.

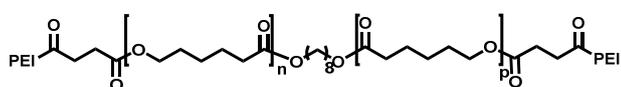
PEI-CG-PEI:



PEG-CG:



PEI-C-PEI:



PEG-C:

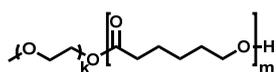


Figure 2. Chemical structures of synthesized tri- and diblock copolymers.

Particle Formation

Particles, which were designed by self-assembly of the synthesized tri- and diblock copolymers were characterized by their Z-average values (intensity weighted mean hydrodynamic size) obtained from DLS (Figure 3a, Table 2). Z-average values ranging from 40.6 ± 0.2 nm and 91.8 ± 0.2 nm were determined for the different compositions of particles based on PEI-C-PEI triblock and PEG-C diblock copolymers. Polymeric particles with the triblock PEI-CG-PEI and diblock PEG-CG copolymers were substantially smaller than those from PEI-C-PEI, Z-average values were lower and ranged from 34.5 ± 0.2 nm to 97.9 ± 0.3 nm. Here, a strong influence of the presence of glycolide units on the PDI (polydispersity index) was observed. In case of particles based on PEI-C-PEI/PEG-C, values of PDI ranged between 0.19 and 0.50. However no evident trend was observed. On the other hand, in case of PEI-CG-PEI/PEG-CG the resulting PDI was lower, more uniform, and clearly decreased with an increase of the PEG-CG diblock copolymer content. Smaller sizes of the particles as well as more homogeneous PDI could be assigned to the different macromolecular organizations in case of glycolide units as part of the hydrophobic block. As the ring-opening polymerization of CL in the presence of GL would result into a reduced degree of crystallinity for CL units, PEI-CG-PEI would exhibit a higher amorphous character when compared to PEI-C-PEI. As a consequence of disturbed crystallization in PEI-CG-PEI/PEG-CG and as particles create a dynamic system, the enhanced mobility of polymer chains of the tri- and diblock copolymers would support the self-assembly process.

The surface charge of the polycationic particles was investigated by determining the zeta potential (Figure 3b, Table 2). This important parameter gives information about the ability of condensing of polyanionic macromolecules like DNA, siRNA or other potentially bioactive molecules. The zeta potential ranged between 2.9 ± 0.2 mV and 15.0 ± 0.8 mV in case of PEI-C-PEI-based compositions, and between 6.7 ± 0.8

mV and 18.1 ± 0.5 mV in case of PEI-CG-PEI-based systems. It was observed that the presence of more hydrophilic glycolide units in triblock copolymers increases the zeta potential. It can be related to the higher content of amorphous phase present in glycolide, which enhanced mobility of the copolymer chains. Therefore, the ability to form structures with more pronounced charges on the surface of particles can increase. Furthermore, a strong decrease of the zeta potential was observed when the content of the co-assembly agents PEG-CG or PEG-C raised.

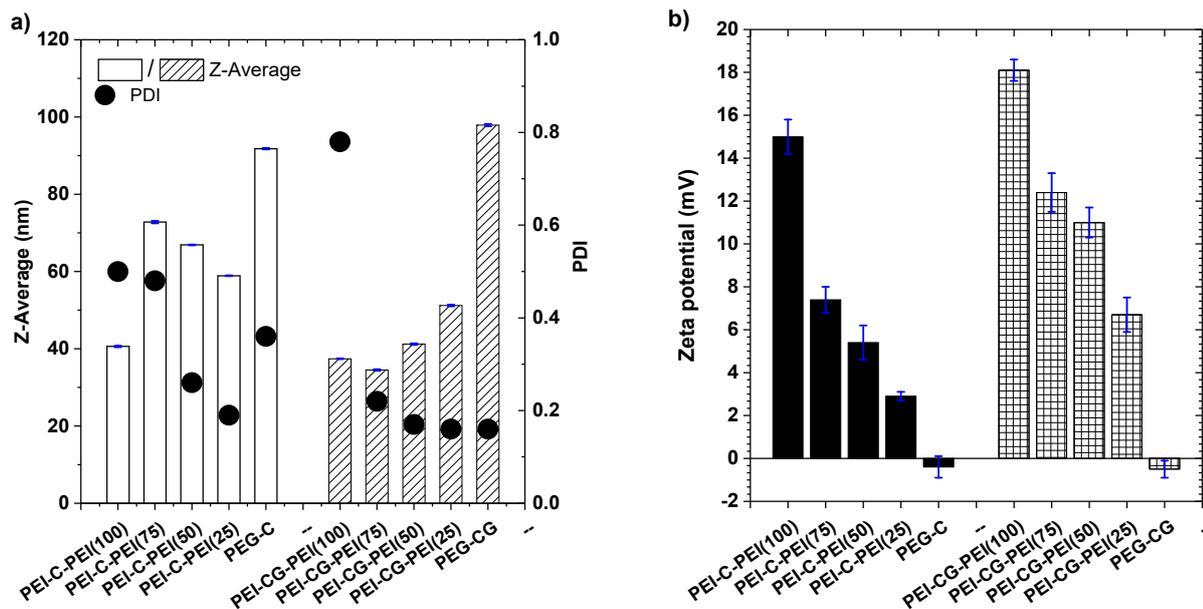


Figure 3. Characterization of polycationic particles. a) Z-average and PDI (black circles) of mixture of triblock PEI-C-PEI with diblock PEG-C copolymers (series white) and mixture of triblock PEI-CG-PEI with diblock PEG-CG copolymers (series with striped pattern). b) Zeta potential of mixture of triblock PEI-C-PEI with diblock PEG-C copolymers (series black) and mixture of triblock PEI-CG-PEI with diblock PEG-CG copolymers (series with plaid pattern). Number in brackets indicates the wt% of triblock copolymer in the mixture.

Table 2. Characteristics (Z average, polydispersity index PDI, and zeta potential) of particles based on various compositions of di- and triblock copolymers.

Sample	Z average ^{a)} (nm)	PDI ^{a)}	Zeta potential ^{a)} (mV)
PEI-CG-PEI(100)	37.4 ± 0.2	0.78 ± 0.02	18.1 ± 0.5
PEI-CG-PEI(75)	34.5 ± 0.3	0.22 ± 0.01	12.4 ± 0.9
PEI-CG-PEI(50)	41.2 ± 0.1	0.17 ± 0.02	11.0 ± 0.7
PEI-CG-PEI(25)	51.2 ± 0.1	0.16 ± 0.01	6.7 ± 0.8
PEG-CG	97.9 ± 0.2	0.16 ± 0.01	-0.5 ± 0.4
PEI-C-PEI(100)	40.6 ± 0.1	0.50 ± 0.01	15.0 ± 0.8
PEI-C-PEI(75)	72.8 ± 0.2	0.48 ± 0.03	7.4 ± 0.6
PEI-C-PEI(50)	66.9 ± 0.2	0.26 ± 0.02	5.4 ± 0.8
PEI-C-PEI(25)	58.9 ± 0.2	0.19 ± 0.01	2.9 ± 0.2
PEG-C	91.8 ± 0.3	0.36 ± 0.02	-0.4 ± 0.5

^{a)} Particle size (Z average), polydispersity index (PDI), and zeta potential were determined by Dynamic Light Scattering (DLS) in PBS buffer. Average values and standard deviations were calculated based on results of three samples of each composition.

CONCLUSION

In this work structure – properties relationships of polyester-based particles created by self-assembly of ABA triblock copolymers and co-assembly with diblock copolymers were explored. The hydrophobic block B constituted oligo(ϵ -caprolactone) or a copolymer of glycolide and ϵ -caprolactone, whereas hyperbranched hydrophilic PEI was utilized as block A. By variation of the molecular parameters, e.g. type of B block as well as the content of PEG-CG or PEG-C as co-assembly agent, nano-sized particles ranging from 34.5 ± 0.2 nm to 97.9 ± 0.3 nm with controllable positive surface charges between 2.9 ± 0.2 mV and 18.1 ± 0.5 mV were obtained. Particles basing solely on triblock copolymers exhibited the highest zeta potential values, which decreased when the content of diblock copolymer increased. Additionally, the content of glycolide units in triblock copolymer increased the zeta potential of PEI-CG-PEI-based particles in comparison to PEI-C-PEI-based ones. In case of incorporation of PEG-C diblock copolymers, an increase of particle size was observed in contrast when PEI-C-PEI was incorporated. However no specific relation between composition and size and polydispersity were found. The systematic incorporation of PEG-CG diblock copolymer resulted in a more pronounced and systematic increase of particle size. Therefore, it can be assumed that the self-assembly process can be supported by the increase of the amorphous phase content (in this case glycolide units) according to reduced crystallinity and enhanced mobility of polymer chains.

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REFERENCES

1. S. L. Ginn, I. E. Alexander, M. L. Edelstein, M. R. Abedi and J. Wixon, *J. Gene Med.* **15** (2), 65-77 (2013).
2. J. C. Burnett, J. J. Rossi and K. Tiemann, *Biotechnol. J.* **6** (9), 1130-1146 (2011).
3. W. T. Godbey, K. K. Wu and A. G. Mikos, *J. Controlled Release* **60** (2-3), 149-160 (1999).
4. O. Boussif, F. Lezoualc'h, M. A. Zanta, M. D. Mergny, D. Scherman, B. Demeneix and J. P. Behr, *Proc. Natl. Acad. Sci. U. S. A.* **92** (16), 7297-7301 (1995).
5. S. M. Moghimi, P. Symonds, J. C. Murray, A. C. Hunter, G. Debska and A. Szewczyk, *Mol. Ther.* **11** (6), 990-995 (2005).
6. V. Kafil and Y. Omid, *Bioimpacts* **1** (1), 23-30 (2011).
7. G. Navarro, J. Pan and V. P. Torchilin, *Mol. Pharmaceutics* **12** (2), 301-313 (2015).
8. Z. K. Zhang, R. J. Ma and L. Q. Shi, *Acc. Chem. Res.* **47** (4), 1426-1437 (2014).
9. Y. Zhong, W. Yang, H. Sun, R. Cheng, F. Meng, C. Deng and Z. Zhong, *Biomacromolecules* **14** (10), 3723-3730 (2013).
10. F. Gu, L. Zhang, B. A. Teply, N. Mann, A. Wang, A. F. Radovic-Moreno, R. Langer and O. C. Farokhzad, *Proc. Natl. Acad. Sci. U. S. A.* **105** (7), 2586-2591 (2008).
11. A. Lendlein, M. Colussi, P. Neuenschwander and U. W. Suter, *Macromol. Chem. Phys.* **202** (13), 2702-2711 (2001).
12. M. Balk, M. Behl, J. Yang, Q. Li, C. Wischke, Y. Feng and A. Lendlein, *Polym. Adv. Technol.* **28** (10), 1278-1284 (2017).
13. W. Wang, M. Balk, Z. Deng, C. Wischke, M. Gossen, M. Behl, N. Ma and A. Lendlein, *J. Controlled Release* **242** (Supplement C), 71-79 (2016).