Dual Responsive, Stabilized Nanoparticles for Efficient in vivo Plasmid Delivery

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ABSTRACT SUMMARY

Developing safe and efficient non-viral gene vectors for successful in vivo gene therapy remains a significant challenge. In this study, we report dual responsive, stabilized nanoparticles and demonstrate their use for efficient gene transfer to rodent brains. The delivery vehicle is reversibly hydrophobic in response to reducing or acidic conditions, thus providing triggered morphology changes and facilitated cargo release in the intracellular milieu. The material is also pH-sensitive to enable endosomal release. A combination of ring-opening polymerization (ROP) and atom transfer radical polymerization (ATRP) was used to synthesize this ternary copolymer based on an oligoamine tetraethylenepentamine (TEPA)-decorated amphiphilic poly(ε-caprolactone)-SS-poly[(glycidyl methacrylate)-s-(oligo(ethylene glycol) monomethyl ether methacrylate)] (PCL-SS-P(GMA-s-OEGMA)) block-statistical copolymer with a disulfide bond in the block junction. Due to its unique features combining reversible hydrophobilization and statistical hydrophilization, this formulation mediated superior in vitro serum-conditioned gene transfection as well as in vivo gene delivery to the mouse brain by intraventricular injection. This work not only presents a promising platform for efficient delivery of nucleic acids, but also provides new insight into future design and development of advanced vectors for efficient delivery of nucleic acids.

INTRODUCTION

Gene therapy is a highly promising strategy to treat various inherited and acquired human diseases.1 Successful gene therapy largely relies on the development of safe and efficient delivery vectors. Cationic polymer-based gene vectors has attracted broad attention in the past two decades due to their advantages over viral vectors, such as non-immunogenicity, physicochemical flexibility, and facile manufacturing.2 Recently, hydrophobic modification of polycations has been explored as an effective approach towards novel gene vectors with enhanced transfection activity.3 In addition, construction of intelligent vehicles that can adapt its structure to perform a desired function (e.g. detachment of shielding layer to facilitate gene release) in response to the changes in the biological microenvironments, such as acidic conditions or reducing conditions represents an elegant approach to address the dilemma of high stability of polyplex and efficient release of DNA.4

Based on these perspectives, herein we designed and synthesized a reversibly hydrophobilized polycation using a reduction-sensitive disulfide bridge. An oligoamine TEPA-decorated amphiphilic PCL-SS-P(GMA) block copolymer with a disulfide linkage in the block junction was synthesized by a combination of ROP and ATRP using a reducible double-head initiator with both terminal OH and Br groups (OH-SS-iBuBr).5 To further enhance salt stability and serum-tolerance of polyplexes, uncharged hydrophilic OEGMA protecting segments were incorporated into PCL-SS-P(GMA-TEPA) di-block copolymers. OEGMA was incorporated either as a central or terminal block or randomly throughout the cationic block. The resulting ternary amphiphilic cationic copolymers with different architectures were evaluated for their transfection efficacy. The optimal “block-statistical” formulation was further applied to in vivo gene transfer by intraventricular injection of polyplexes to the mouse brain.

EXPERIMENTAL METHODS

PCL-SS-iBuBr homopolymer was prepared by ROP of CL using HO-SS-iBuBr as the initiator and Sn(Oct)2 as the catalyst. PCL-based di-block, block-statistical, and tri-block copolymers were synthesized by ATRP using PCL-SS-iBuBr as the macroinitiator and CuCl/bpy as the catalyst. Decoration of GMA units in all the PCL-based copolymers was carried out according to our recent publication.4 Polyplex formation, cell culture, in vitro and in vivo transfection study were carried out following our previous publications.5

RESULTS AND DISCUSSION

The morphology of various polyplexes formed by PCL-based copolymers was visualized by TEM (Figure 1) at an N/P of 10. All the materials can condense pDNA into regularly spherical nanoparticles with diameter < 50 nm.

Scheme 1 interprets the structure of block-statistical copolymer, formation of polyplex and the proposed intracellular trafficking route for gene delivery. The PCL-40-SS-P[(GMA-TEPA)45-s-OEGMA30] block-statistical copolymer is expected to transfect cells by condensing DNA efficiently to form core-shell-corona (CSC) ternary polyplexes with the hydrophobic PCL as the inner core, P(GMA-TEPA)/DNA electrostatic complex as the middle shell, and the hydrophilic OEGMA units as the outer corona. Once internalized, the polyplexes become localized within the endocytic vesicles. The protonatable amines in TEPA were included to facilitate endosomal escape through the proton sponge effect, and glutathiones in the intracellular environment are expected to degrade the disulfide bridges, leading to
Figure 1. TEM images of polyplexes formed by (a) PCL_{40}-b-P(GMA-TEPA)$_{57}$, (b) PCL_{40}-SS-P(GMA-TEPA)$_{52}$, (c) PCL_{62}-SS-P(GMA-TEPA)$_{55}$, (d) PCL_{40}-SS-P{(GMA-TEPA)$_{41}$-s-OEGMA$_9$}, and (e) PCL$_{40}$-SS-P{(GMA-TEPA)$_{58}$-s-OEGMA$_{10}$} at a fixed N/P ratio of 10 (scale bar: 500 nm).

detachment of the hydrophobic PCL core. The resulting loose P{(GMA-TEPA)$_{58}$-s-OEGMA$_{10}$}/pDNA complex facilitates unpackage of pDNA. Eventually, pDNA is released into the cytosol and transferred to the nucleus for gene expression.

Figure 2. Transfection efficacy in mouse brains two days after intraventricular injection. (n = 6, *p < 0.01, **p < 0.01).

CONCLUSION
Our study revealed that PCL$_{40}$-SS-P{(GMA-TEPA)$_{58}$-s-OEGMA$_{10}$} block-statistical copolymer resolved the stability dilemma of cationic polymer-based gene delivery systems by combining reversible hydrophobicity and statistical stabilization monomers for enhanced polyplex stability in extracellular environments and efficient plasmid release inside cells. The block-statistical copolymers provide high serum-compatible transfection efficacy and efficient in vivo delivery to the mouse brain.

REFERENCES
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The effect of polymer architecture on the transfection efficacy was assessed. The block-statistical copolymer mediates significantly higher transfection efficacy than both tri-block copolymers and bPEI (25kDa) at all the tested N/P ratios in serum condition (data not shown). Given the promising in vitro transfection results, we further carried out in vivo transfection study using glucose, reduction-insensitive PCL$_{40}$-b-P(GMA-TEPA)$_{57}$, reduction-sensitive PCL$_{40}$-SS-P(GMA-TEPA)$_{52}$, and PCL$_{40}$-SS-P{(GMA-TEPA)$_{58}$-s-OEGMA$_{10}$} block-statistical copolymers. The polyplexes formulated at the optimal N/P ratio of 15 were injected into the right lateral ventricle of mice to deliver the luciferase plasmid (Figure 2). The in vivo delivery efficiencies correlate well with the in vitro serum-conditioned delivery efficiency, i.e., the polyplexes based on block-statistical copolymer combining reversible hydrophobilization and statistical hydrophilization displayed ~3.0 and 15.6-fold higher luciferase activity compared to the reduction-sensitive polplexes and reduction-insensitive polyplexes, respectively.