NOVEL CATIONIC POLYMERS DESIGNED FOR NON-VIRAL GENE DELIVERY

Zhiyuan Zhong[1], Chao Lin[1], Yan Song[1], Martin C. Lok[2], Xulin Jiang[2], Lane V. Christensen[3], Johan F.J. Engbersen[1], Sung Wan Kim[3], Wim E. Hennink[2], and Jan Feijen[*,1]

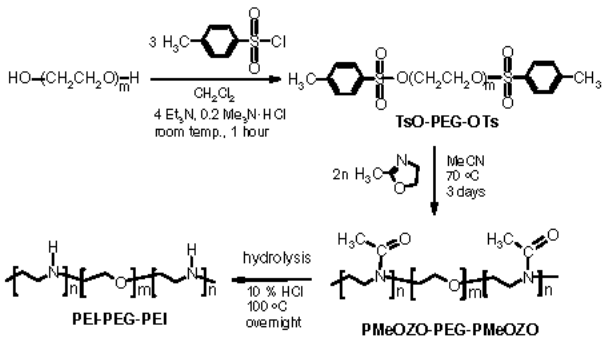
[1]Department of Polymer Chemistry and Biomaterials, Institute for Biomedical Technology (BMTI), Faculty of Science and Technology, University of Twente, P.O. Box 217, 7500 AE Enschede, The Netherlands
[2]Department of Pharmaceutics, Utrecht Institute for Pharmaceutical Science (UIPS), Utrecht University, P.O. Box 80082, 3508 TB Utrecht, The Netherlands
[3]Department of Pharmaceutics and Pharmaceutical Chemistry, University of Utah, Salt Lake City, Utah 84112-5820, USA.
j.feijen@utwente.nl

Introduction

Gene therapy holds great promise for treating various forms of diseases with a genetic origin including cystic fibrosis, different forms of cancer, and cardiovascular disorders. The clinical use of gene therapy treatments is however restricted, mainly because of the absence of safe and efficient gene delivery technologies. In the past decade, non-viral approaches, particularly cationic lipid or polymer-based systems, have received rapidly growing interest, because they offer several advantages over the viral counterparts. As compared to viral vectors, non-viral systems have a low or absent immunogenicity, can relatively easily be scaled-up, and have great flexibility with regard to vector modification and DNA incorporation. Nevertheless, currently investigated gene delivery polymers like polyethylenimine (PEI), poly(L-lysine) (PLL), polyamidoamine dendrimers (PAMAM), and poly(2-(dimethylamino)ethyl methacrylate) (pDMAEMA) have not yet advanced to clinical evaluation, mainly due to their low *in vivo* transfection activity and acute cytotoxicities. In our group, with an aim of developing efficient and nontoxic polymeric gene delivery systems, several novel types of polymeric gene carriers have been designed, synthesized, and evaluated. Herein, I will mainly present our work on low molecular weight linear PEI-PEG-PEI triblock copolymers, degradable hyperbranched poly(ester amine)s, and reduction-sensitive poly(amido amine)s.

Results and discussion

PEI-PEG-PEI triblock copolymers.[1,2] The cationic polymerization of 2-methyl-2-oxazoline (MeOZO) using PEG-bis(tosylate) as a macroinitiator followed by acid hydrolysis afforded linear PEI-PEG-PEI triblock copolymers with controlled compositions (Scheme 1). Two copolymers, PEI-PEG-PEI 2100-3400-2100 and 4000-3400-4000, were synthesized.



Scheme 1. Synthesis of PEI-PEG-PEI triblock copolymers.

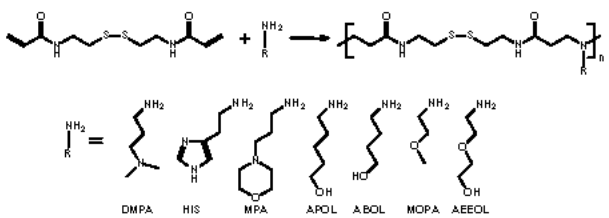
Both copolymers were shown to interact with and condense plasmid DNA effectively to give polymer/DNA complexes (polyplexes) of small sizes (< 100 nm) and moderate zeta-potentials (~ +10 mV) at polymer/plasmid weight ratios ≥ 1.5/1. These polyplexes were able to efficiently transfect COS-7 cells and primary bovine endothelial cells (BAECs) *in vitro*. The transfection activity of polyplexes of PEI-PEG-PEI 4000-3400-4000 in BAEC cells using luciferase as a reporter gene was threefold higher than for polyplexes of linear PEI 25k. The presence of serum in the transfection medium had no inhibitive effect on the transfection activity of the PEI-PEG-PEI polyplexes. These PEI-PEG-PEI triblock copolymers displayed also an improved safety profile in comparison with high molecular weight PEIs.

Degradable hyperbranched poly(ester amine)s.[3] Nine poly(ester amine)s were obtained in high yields through a Michael-type conjugate reaction between three diacrylate monomers and three trifunctional amine monomers (monomers see Table 1). Analysis of degradation products using liquid chromatography-mass spectroscopy (LC-MS) demonstrated that all poly(ester amine)s had a hyperbranched structure with a degree of branching of approximately 0.30. These poly(ester amine)s were readily water-soluble and degradable under physiological conditions (pH 7.4, 37 °C), in which more than 10 % ester bonds were hydrolyzed within 4 hours. Moreover, these hyperbranched poly(ester amine)s showed high buffering capacities between pH 5.1 and 7.4. Three out of nine synthesized polymers were shown to effectively condense plasmid DNA into small-sized and positively charged complexes. The highest transfection level was observed for p(HDDA-AEP) polyplex which had a transfection efficiency higher than or comparable to that of polyplexes of PEI and pDMAEMA. Furthermore, these poly(ester amine)s revealed no or low cytotoxicity.

Table 1. Monomers selected for the synthesis of hyperbranched poly(ester amine)s.

Diacrylate	Trifunctional Amine
EGDA	AMP
BDDA	AEP
HDDA	MEDA

Reduction-sensitive poly(amido amine)s (SS-PAAs) (unpublished results). The SS-PAA polymers were prepared by Michael addition of selected primary amines to cystamine bisacrylamide (CBA) (Scheme 2). The polymerizations were performed in methanol/water (4/1, v/v) at 45 °C under nitrogen atmosphere. ^{1}H-NMR spectra (D_2O, 300 MHz) indicated that all polymers have the expected compositions. GPC measurements showed that these polymers have M_w values in the range from 3000 to 8000 g/mol with polydispersity indexes (PDI) ranging from 1.26 to 1.66.



Scheme 2. Synthesis of reducible poly(amido amine)s (SS-PAAs).

Dynamic light scattering (DLS) and zeta potential measurements showed that five out of seven synthesized polymers (except pMPA and pAEEOL) are capable to effectively condense plasmid DNA into small (< 200 nm) and positively charged (>+20 mV) polyplexes. Importantly, these polyplexes were stable at physiological pH, but are

rapidly dissociated in the presence of 2.5 mM dithiothreitol (a reducing agent), mimicking the reductive intracellular environment. The transfection efficiency and cell viability experiments showed that polyplexes of several SS-PAAs have much higher transfection efficiency than that of the reference polymer pDMAEMA. In particular, a remarkably high transfection was observed for poly(CBA-APOL) at a polymer/DNA weight ratio of 66/1 in the absence of serum, with more than 8-fold higher efficiency than that of the reference polymer. Nearly all SS-PAA polyplexes are practically nontoxic (~100% cell viability) at polymer/DNA ratios showing optimal transfection efficiency.

References

[1] Zhong, Z.; Lok, M. C.; Dijkstra, P. J.; Hennink, W. E.; Feijen, J. *Journal of Controlled Release* **2005**, *101*, 406-408.
[2] Zhong, Z. Y.; Feijen, J.; Lok, M. C.; Hennink, W. E.; Christensen, L. V.; Yockman, J. W.; Kim, Y. H.; Kim, S. W. *Biomacromolecules* **2005**, *6*, 3440-3448.
[3] Zhong, Z. Y.; Song, Y.; Engbersen, J. F. J.; Lok, M. C.; Hennink, W. E.; Feijen, J. *Journal of Controlled Release* **2005**, *109*, 317-329.