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Polymer gene delivery vectors encapsulated in thermally sensitive bioreducible shell

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ABSTRACT

Stable, nanosized polyelectrolyte complexes between rationally designed thermally sensitive block copolymers and plasmid DNA (polyplexes) were formed and their in vitro transfection efficiency was tested. The polyplexes were further stabilized through encapsulation into a biodegradable polymer shell. Although reduced as compared to that of the corresponding polyplexes, the encapsulated systems still show acceptable transfection efficiency. That opens the possibility to tune the balance between the safe transport and efficient delivery of DNA into the cells.

Polymer based non-viral gene delivery systems have gained significant attention during the last two decades due to their great potential for the development of safe and efficient vectors.[1,2] Most often cationic polymers are used to form polyelectrolyte complex (polyplex) with the negatively charged DNA molecule in order to condense and protect the genetic material during its transport through the blood stream to the target cells. The main drawback of the first and most widely used cationic homopolymers–polyethyleneimine (PEI) and poly(L-lysine) is their relatively high cytotoxicity.[3,4] The recent advances in macromolecular engineering allow the design and synthesis of more complex polymer architectures.[5] Thus, numerous copolymers of various architectures with cationic segments and potential application in gene delivery systems were synthesized.[6–8] Besides the low toxicity these copolymers may impart other properties to the gene delivery vehicles such as 'smart' response to environmental changes and targeting functions.[9,10] However, in all cases a surplus of positively charged copolymers must be used to form stable polyplexes. The overall positive surface charge leads to the formation of colloidally stable particles and enables their transfection through a non-receptor binding to the highly anionic cell surface proteoglycans.[11] On the other hand, the positively charged polyplexes might be rapidly cleared through non-specific interactions with negatively charged blood stream components.

The rational design of polymer vectors must provide an optimal balance between two conflicting attributes, namely, protection and release of nucleic acids. Consequently, the next level in constructing efficient gene delivery systems based on polyplexes involves various methods for their further stabilization. Those are silica-coating, incorporation on the surface of negatively charged nanoparticles or reversible cross-linking techniques.[12–16] The exploitation of a redox gradient within the intra- and extracellular region might address both the conflicting issues of DNA condensation and release. Bioreducible polymers with disulfide linkages can be sufficiently stable during the circulation and in the extracellular region. However, in a reductive cellular environment they undergo rapid cleavage through glutathion (GSH)-mediated thiol-disulfide exchange reactions.[17,18] The quick responsive chemical degradation kinetics of bioreducible polymers is advantageous over commonly used hydrolytically degradable polymers such as aliphatic polyesters and polycarbonates which exhibit a significantly slower gradual degradation kinetics.

Very recently we introduced a new method for polyplex stabilization through an aqueous seeded radical polymerization of acrylic monomer, performed on the polyplex surface in the presence of different cross-linkers.[19] The method allows fairly simple polyplex encapsulation under mild conditions and the incorporation of various functions into the gene delivery systems, like

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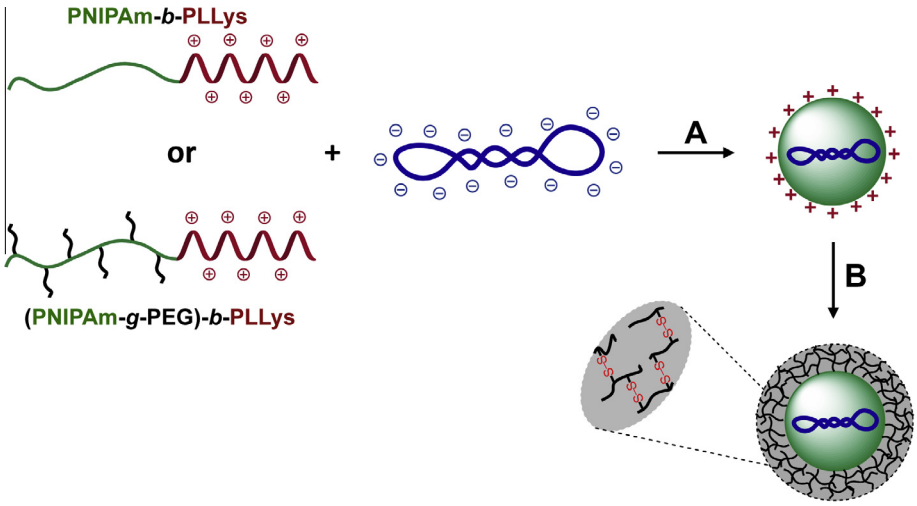
targeting and stabilizing moieties. A rationally designed diblock copolymer with thermally sensitive and polypeptide cationic segments was synthesized and used to condense salmon sperm DNA.[19] Further improvement in the copolymer design involved the incorporation of poly(ethylene glycol) (PEG) grafts into the thermally sensitive block.[20] Both copolymers formed stable nanosized polyplexes with low cytotoxicity and the former was successfully coated with an additional layer of cross-linked polymer. These results encouraged us to continue our investigations with plasmid DNA (pDNA).

In the present communication we report on the polyplex formation through electrostatic complexation between the copolymers poly(*N*-isopropylacrylamide)-block-poly(L-lysine) (PNIPAm-*b*-PLLys) or [poly(*N*-isopropylacrylamide)-graft-poly(ethylene glycol)]-block-poly(L-lysine) (PNIPAm-*g*-PEG)-*b*-PLLys) and pDNA (Scheme 1). Further polyplex stabilization via a bioreducible polymer outer shell was performed and the transfection abilities of the systems have been evaluated and discussed.

The polyplex formation was performed in aqueous media through a dropwise addition of pDNA solution (50 μg/ml pEGFP-C2, 4700 bp) to an equal volume of vigorously stirred polymer solution. The optimal molar ratio between the copolymers cationic groups and pDNA phosphate groups (N/P) was 20. Under these conditions positively charged polyplexes with sizes in the 115–165 nm range were formed as determined by dynamic light scattering (DLS) and zeta-potential measurements (Fig. 1). The



Scheme 1. Route to: (A) polyplex formation; and (B) coating with cross-linked bioreducible polymer layer.

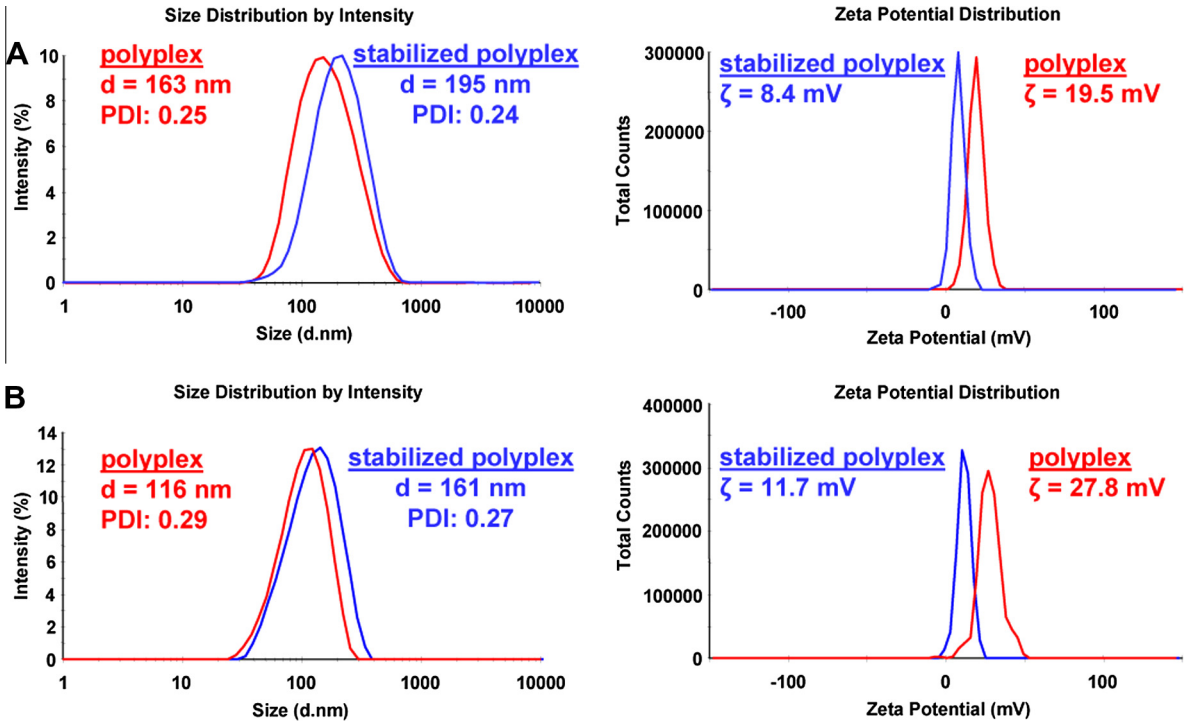


Figure 1. Average diameters and zeta potentials before and after coating of the polyplexes formed at N/P = 20 from pDNA and: (A) PNIPAm-*b*-PLLys; (B) PNIPAm-*g*-PEG)-*b*-PLLys.