Nanomaterials Optimized to Improve Delivery Efficiency for Gene Therapy

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ABSTRACT

Polycationic nanoparticles are potential as polycationic nonviral vectors (polyplexes) to overcome the defects of traditional viral vectors. The low transfection efficiency in vivo was attributed to poor circulation of polyplexes with high residual positive charges on the surface, which cause them susceptible to agglomerate with negatively charged serum components and to be scavenged by macrophages, whereas, the positive charges are likewise beneficial polyplexes to interact with negative-charged plasma membranes of targeted cells and specifically increase intracellular transfection efficiency of polyplexes. Numerous studies focused on revealing the relationship between the structure of polycationic vectors and the transfection efficiency in vivo. The designed polyplexes in our group to extend nanoparticles circulation is possible to overcome other barriers including endosome escape, cytoplasmic transport and nuclear entry for ultimately successful gene transfection in vivo. Polyplexes circulation was prolonged by modifying with polycations such as PEIs, PDMAEMA, chitosan et al. It was found that PEGylation of polyplexes could improve the stability of polyplexes in vivo but dramatically decrease therapeutic efficiency due to reduced transfection. In addition, the strong hydrophilicity of the PEG segments could dissociate PEGylated polyplexes under the physiological environment and attenuate polycation carrying with target genes. polycationic nonviral gene delivery vectors in order to improve the circulation time and transfection efficiency in vivo for efficient gene therapyin order to improve the circulation time. Polyplexes might be potential to be alternative nonviral gene delivery vectors for efficient clinical therapy.

Keywords: polyplexes, gene delivery, cancer therapy

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1 INTRODUCTION

Gene therapy is defined as the transfer of genetic information into specific cells to modulate protein expression for treatment of inherited or acquired diseases [1,2]. Generally, recombinant plasmid DNA (pDNA) (encoding therapeutic proteins) and oligonucleotides (relatively small fragments of synthetic DNA or RNA) have been used as a “drug” to modulate gene expression associating with a particular disease. Since it is difficult for negative charged pDNA and oligonucleotides to be taken up by targeted cells, the clinic use of naked pDNA and oligonucleotides is severely limited due to rapid degradation by nucleases in serum and quick clearance by immune-system. Therefore, successful gene therapy largely depends on the development of efficient delivery vectors [3].

Two classes of primary gene vehicles including recombinant viruses and non-viral vectors have been developed [4]. Viral vectors were widely applied in gene delivery studies because of its evolutionarily rooted efficiency as gene delivery vehicle [5,6]. However, safety concerns on the random recombination immunogenicity, limited nucleic acid loading capacity and high cost hinder the wide clinical application of recombinant viruses [7-9]. Clinical trials have underscored the safety risks of viral vectors because of concomitant development of cancer [10] and even death [11] with viral gene therapy. Therefore, new attention has been focused on non-viral vectors due to their potential advantages such as low immunogenic response, easy modification, capability to carry large inserts and facile manufacturing even though the transfection efficiency of non-viral vectors is lower than that of viral vectors [12,13]. The flexibility in formulation design provides a great promising potential in improving the transfection efficiency of non-viral vectors to the level comparable to that of viral vectors.

2 CONCLUSION AND DISCUSSION

2.1 Polycations employed as non-viral vectors

Cationic polymers (usually directly designated as polycations) as gene carriers have been widely used. Besides their potential advantages such as lower safety risks and greater flexibility, polycations have advantage for carrying large DNA molecules, facile manufacturing and easy modification compared to viral vectors [12-14]. When complexed with DNA, excessive polycation are required to effectively encapsulate pDNA or oligonucleotides. The

residual positive charges on polyplexes surface are beneficial to polyplexes for adsorptive endocytosis, because it is easy for the positively charged polyplexes to interact with negatively charged glycoproteins, proteoglycans and glycerophosphates on the cell membrane for activated endocytosis. However, residual positive surface charges could also result in toxicity at the cellular and/or the systemic level. In particular, the in vivo applicability of such polyplexes seems to be potentially hazardous, since residual surface charge leads to non-specific interaction with components of circulation system. It often causes a rapid removal of polyplexes from the blood stream as well as unwanted events such as organ embolism due to aggregate formation with erythrocytes [13].

2.2 Strategies to improve cell uptake efficiency and circulation time of polyplexes *in vivo*

Polycation/plasmid DNA complexes are usually prepared in the presence of an excess amount of polycation to effectively condense DNA for preventing the access of nucleases to plasmid DNA and improving resistance of plasmid DNA against enzymatic degradation. Therefore, the finally formed complex has a net positive charge [13,14]. Although the positively charged complexes can interact with negatively charged cell membranes and facilitate cellular uptake of the polymer/plasmid DNA complexes via endocytosis, the net positive charge of complexes makes them prone to bind with negative charged serum proteins in circulation system, which results in rapid clearance by macrophages and reduction of delivery efficiency. The nonspecific binding may also be the cause for cytotoxicity [15]. However, the ability of polyplexes to circulate in vivo for a prolonged period of time is often a prerequisite for successful gene therapy.

To increase biocompatibility and transfection efficiency of polyplexes, it is necessary to mask the net positive charge to enable them circulating in vivo. Poly(ethylene glycol) (PEG) was widely used to conjugate the cationic polymers to prevent the inter-particle aggregation of the complexes and to increase complex stability in the presence of serum proteins [9, 15]. PEG-conjugated copolymers have advantages for gene delivery: 1) The PEG-conjugated copolymers show low cytotoxicity to cells in vitro and in vivo, 2) PEG increases water-solubility of the polymer/DNA complex, 3) PEG reduces the interaction of the polymer/DNA complex with serum proteins and increases circulation time of the complex, 4) PEG can be used as a spacer between a targeting ligand and a cationic polymer, 5) PEG can prevent the capture and clearance by macrophages [14].

2.3 PEGylated cationic polymer-based gene delivery systems

So far, numerous of PEGylated PLL, PEI and PDMAEMA polymers have been synthesized in order to increase the polyplexes circulation time and reduce their cytotoxicity in vivo. Studies indicate that the properties of the PEGylated PLL/PEI conjugates are strongly dependent on the degree of PEGylation and the molecular weight of PEG [16].

Besides increased cytotoxicity, high molecular weight PLL/DNA complexes have shown a tendency to aggregate and precipitate depending on the ionic strength of the solution. The block copolymers of PLL with PEG (PLL-b-PEG) were prepared to prohibit the formation of insoluble precipitates [17]. The polyplexes of PLL-b-PEG and DNA or oligodeoxynucleotides show reduced sizes regardless of the NaCl concentration in the buffer and are resistant to deoxyribonuclease I (DNase I) digestion. Comb-shaped PEG grafted PLL (PEG-g-PLL) was first synthesized with different PEG-grafted ratios (PEG: 550; PLL: 25,000). PEG-g-PLL formed a complex with plasmid DNA at or above a 1:1 weight ratio. DNase I protection assay indicated that 10 mol% PEG-g-PLL could completely protect plasmid DNA for more than 60 min. In addition, PEG-g-PLL had dramatically low toxicity to HepG2 cells. Recently, the PEGylated dendritic PLL was found to enhance blood residence and reduce hepatic accumulation compared to dendritic PLL .

Various synthetic strategies have adopted to conjugate PEI with PEG. The primary amino groups of PEI molecules can react with N-hydroxysuccinimide activated PEG to form PEGylated PEI. Alternatively, in a two-step procedure, PEG can be activated with either epoxide or isocyanate groups followed by reaction with the amino groups of PEI. Two series of branched PEI-grafted-PEG (BPEI-g-PEG) was synthesized by Petersen et al [18]. PEI (25 kDa) was grafted to PEG (5 kDa) with different degrees of substitution in first BPEI-g-PEG. The second branched PEI-grafted-PEG contains PEG with molecular weight (MW) from 550 Da to 20kDa. 5 kDa PEG significantly reduced the diameter of spherical complexes from 142 ± 59 to 61 ± 28 nm. With increasing of the grafted PEG, complexation with DNA was impeded and complexes lost their spherical shape. Copolymers with PEG 20 kDa yielded small and compact complexes with DNA (51 ± 23 nm) whereas copolymers with PEG 550 Da resulted in large and diffuse structures (130 ± 60 nm). The zeta potential of complexes was reduced with increasing PEG grafted with molecular weight more than 5 kDa [18]. The synthesized PEG-block-linear PEI (PEG-LPEI) with linear 22 kDa polymer can form complex with DNA and allow the preparation of higher concentrated polyplexes compared to unmodified PEI. High transgene expression was accessed by PEI–PEG/DNA complexes administrated by nasal instillation in mice. Recently, a new biodegradable chitosan-g-PEI-g-PEG-OH with a hydroxyl group at the PEG chain end was prepared for constructing amphiphilic copolymers by grafting reaction of chitosan, polyethylenimine (PEI) and heterobifunctional PEG. The amphiphilic and end-functionalized copolymer may have a

potential utility in conjugating with targeting moieties for targetable gene or drug delivery.

An acid-labile block copolymer was recently synthesized using a PEG as macroinitiator with an acid-cleavable end group by atom transfer radical polymerization of DMAEMA. PEG and PDMAEMA segments were connected through a cyclic ortho ester linkage (PEG-a-PDMAEMA) at pH 7.4, PEG-a-PDMAEMA polyplexes exhibited smaller particle size, lower surface charge, reduced interaction with erythrocytes, and less cytotoxicity compared to PDMAEMA-derived polyplexes [14]. The strong hydrophilicity of the PEG segments could dissociate PEGylated polyplexes under the physiological environment and attenuate polycation carrying with target genes. To circumvent this issue, Won et al. incorporated the hydrophobic middle block of poly(n-butyl acrylate) (PnBA) into the PEG-PDMAEMA and achieved a triblock PEG-b-PnBA-b-PDMAEMA polymer. The properties of the triblock copolymer/DNA complexes are better than that of PDMAEMA homopolymer and PEG-PDMAEMA diblock copolymer, which had comparable molecular weights for individual blocks.

In addition, polyanion was also utilized to assembly with polyplexes or polycation to screen the positive charge [19], which may be the next hotspot in polymeric gene vectors. The unique advantage of this strategy is that polyanion conjugated with PEG can assembly with different polyplexes to dissimulate the positive charge. Therefore, intricate PEGylation synthetic steps of different polycation can be avoided. Moreover, the PEG density on the polyplexes surface can be easily controlled by tuning the amount of added polyanion. Polymethacrylic acid-b-polyethylene oxide (PMAA2100-b-POE5000) was used to complex poly-L-lysine. Micelles showed a hydrodynamic diameter of 30 nm with a peculiar core organized with hydrogen bonds as well as hydrophobic domains. The micelles proved high stability in physiological conditions (pH and ionic strength) and were also able to disassemble under acidic conditions mimicking acidic endolysosome. Ni et al. designed a novel polyanion named MePEG2000-block-poly(methacrylic acid) carring partial thiol groups (MePEG2000-b-PMAASH). MePEG2000-b-PMAASH was used to coat the poly[(dimethylamino)ethyl methacrylate] end-capped with cholesterol moiety (Chol-PDMAEMA30)/DNA complexes via electrostatic interaction between PDMAEMA and PMAASH. Hydrogen peroxide solution was then added to oxidize the mercapto groups to form bridging disulfide linkages between the MePEG2000-b-PMAASH chains and to stabilize the complexes. This system can provide a better stability during systemic circulation, and disintegrate and release DNA once the disulfide linkages are destroyed in a cell reducing environment [20].

3 PERSPECTIVES

Undoubtedly, PEI is still the most widely used and effective non-viral gene vector. The modification of its structure is required further study to not only reduce its cytotoxicity but also improve or at least not impair its transfection efficiency. PAEs are a promising series of polycations whose transfection efficiency can rival PEIs, and their biodegradation performance is an overwhelming advantage compared to PEIs. Design and synthesis of new PAEs is an important direction in non-viral vectors for gene therapy. PDMAEMA seems one unique non-viral vector independent of “proton sponge” mechanism. Although PDMAEMA does not show exceptionally high transfection efficiency compared to other cationic polymers, PDMAEMA serves as an excellent model for structure-transfection studies. Moreover, it is known that PDMAEMA is a thermosensitive macromolecule. Some interesting phenomena will be found with the progress of research for non-viral vectors based on PDMAEMA.

PEGylation of polycations is an effective approach to improve the polyplexes circulation performance in vivo and reduce their cytotoxicity. The introduction of targeting ligands in the terminal of PEG can compensate the decrease of cell uptake efficiency of PEGylated polyplexes. After internalization, however, the shielding PEG chains are required to be removed at least partially or completely, re-exposing the surface charge of the polyplexes at endosomal pH condition, which would promote membrane disruption and endosomal escape of the polyplexes and thus enhance gene transfection efficiency. It owns a great significance to develop intelligent delivery systems based on physiologically triggered, reversible shielding technology.

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