Advances in the application of poly(ethylenimine) conjugated bio-reducible dendrimers for gene delivery systems

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Abstract

The bio-reducible dendrimers containing poly(ethylenimine) and disulfides are interested in gene delivery systems as carrier for gene therapy. The synthesis and characterization of poly(ethylenimine) conjugated polymers has been reviewed for the development of gene delivery systems. The linear PEIs and branched PEIs of bio-reducible dendrimers have briefly introduced in this paper. The preparation and application of poly(ethylenimine)s conjugated bio-reducible dendrimers are also discussed for the discovery of gene delivery systems. The bio-reducible poly(ethylenimine)s dendrimers have a great potential as gene carriers in drug delivery systems. It has reported that the bio-reducible PEIs branched dendrimers have a great potential as a gene delivery system consisting of PEI (1.8kDa) with disulfide bonds.

Key-words: Gene therapy, gene delivery system, bio-reducible dendrimer, poly(ethylenimine)(PEI), PEI-conjugated polymer
1. Introduction

Bio-reducible polymers such as poly(ethylenimine)(PEI) derivatives have been recently investigated as efficient carriers of gene delivery systems for gene therapy.\(^1\), \(^2\), \(^3\) PEI is an efficient vector of gene carriers with outstanding gene condensation capacity.\(^2\) However, it is caused higher cytotoxicity. The toxicity and transfection efficiency of PEI have known highly dependent upon their structures and molecular weights. As the PEI of lower molecular weight (LMW) has lower cytotoxicity, but its efficiency of transfection shows lower value. Thus, the PEI (LMW) is not able to use as a non-viral vector for gene delivery systems.\(^4\), \(^5\) The PEI of higher molecular weight (HMW) shows a higher transfection efficiency, but it also induces higher cytotoxicity problem. There are recently appeared several approaches to overcome increasing the effect of cytotoxicity for PEI (HMW). Maintaining the higher efficiency of transfection for PEI (HMW), the PEI (HMW) can combine with bio-reducible polymers to produce the proper non-viral vectors. In order to decrease the cytotoxicity of PEI (HMW), the biodegradable bonds such as ester and disulfide bonds are incorporated to the cationic polymers.\(^6\), \(^7\), \(^8\)

In the present paper, the recent advances in the preparation and application of poly(ethylenimine) conjugated bio-reducible dendrimers for gene delivery have been briefly introduced. The various kinds of polycationic dendrimers for drug delivery systems have synthesized to apply to gene delivery in our laboratory.\(^9\), \(^10\), \(^11\) The PEI conjugated poly(crystamine)-bis(acrylamide)-di aminohexane) has been prepared to decrease weight ratio and increase the transfection efficiency. The polymer is composed of multiple disulfides that is able to cleave in the cytoplasm. The design of PEI-conjugated bio-reducible dendrimer for efficient gene delivery has made to confirm the successful vectors and polyplexes formation with pDNA.\(^12\) Bio-reducible dendrimers for gene delivery have introduced first in the preparation of bio-reducible poly(ethylenimine)s and their application to gene delivery systems.

2. Bio-reducible dendrimers for gene delivery

In the research field of gene delivery systems, an attention has attracted to the polymeric gene carriers for gene therapy. Because they consist many advantages over viral vectors such as non-immunogenicity and no integration of exogenous genes into host chromosome, and convenience of handling and manufacturing.\(^13\), \(^14\) Lot of multiple functionalities for biodegradable polymers can give a specific and
bio-functional activity, including targeting, biological stimuli, and environmentally sensitive degradability. The bio-reducible polymers are able to contain characteristic disulfide linkages, which can degrade specifically in response to redox reaction through thiol-disulfide reaction.\textsuperscript{15} There are also consisted of the stimuli-sensitive polymers, which can give a specific activity for the biological stimuli including environmental sensitive change of their polymer structures.\textsuperscript{16} Temperature, pH, and redox potential are included as stimuli effects. Most intracellular compartments are generally reducing, while extracellular space is generally oxidizing.

### 2.1 Preparation of bio-reducible poly(ethylenimine)s (PEIs)

Since PEI had first used as a gene carrier in 1995, it is one of most efficient polymeric gene carriers that are able to condense pDNA at low molecular ratios due to its high charge density effect.\textsuperscript{17-20} However, the evaluation of PEIs (HMW25k) had shown severe cytotoxicity accumulated polycations with the high charge density of molecular weights. The PEI of low molecular weight (LMW, 800Da) had tested by Lee and his coworkers.\textsuperscript{21} The result had showed significantly to improve the toxicity of gene delivery to Chinese hamster ovary (CHO) cells after reducible crosslinking with homo-bi-functional amines. There are two kinds of morphology in PEIs, which exist in either a linear and branched structures. The linear form of PEIs are synthesized by cationic ring-opening polymerization of 2-substituted 2-oxazoline monomers, followed by acid-catalyzed hydrolysis.\textsuperscript{22, 23} On the linear PEI molecular structures, the protonation of amine group occur about 90\% in the physiological pH condition. In addition, branched PEI forms are synthesized by ring-opening polymerization of aziridine monomers catalyzed in acid.\textsuperscript{24} The branched PEI has a high density of amine group, which is in two third remaining un-protonated in physiological environments.\textsuperscript{25} The un-protonated amine groups are able to absorb protons as pH is lower regions. The unique property gives an extraordinary buffering capacity over the wide range of pH in the solution. That offers PEI-carrying nuclei acid drugs an opportunity to escape from the acidic endolysosomal compartment via a hypothetical ‘proton capture sponge’ effect.\textsuperscript{17} A proton sponge effect plays an important role in the efficiency of PEI-based gene delivery systems. The further alteration in the proton-buffering capacity of PEIs enhance significantly the overall transfection efficiency.\textsuperscript{26} The charge neutrality of the PEI/DNA complexes gives a best transfection results, comparable to \textit{in vitro} transfection results using neural cells. The branched PEI
consists of primary (25%), secondary (50%), and tertiary (25%). It can easily modify to optimize the gene delivery system on its own activity and cytotoxicity, because of PEIs having many primary amines. In order to overcome the limitation of PEI itself in gene delivery systems, PEIs (LMW) were developed to impart with biodegradable core molecules. Bio-reducible PEIs with disulfide groups have also developed to impart with biodegradable properties maintaining the amine groups. The controlled preparation of dendrimers are resulted in the low polydispersity, while the linear synthetic polymers have a high polydispersity. Dendrimers have a well-defined numbers of terminal groups for the conjugation of biodegradable polymers. The conjugation of PEI (1.8kDa) with dendritic core molecules have made successfully to increase the molecular weight distribution. Increasing molecular weights give an increase of transfection efficiency. Synthesizing the core molecules of dendrimer, PEI (1.8kDa) have conjugated with the core molecules containing disulfide bonds.

2.2 Application of bio-reducible poly(ethylenimine)s (PEIs)

A series of linear PEI with various cationic density and molecular weight were prepared from poly(2-ethyl-2-oxazoline) by the controlled acid hydrolysis and examined the transfection efficiency. On the results, linear PEI 22kDa demonstrated higher transfection efficiency in vitro as well as in vivo than that of branched PEI 25kDa. The trans-gene expression has affected by the size of the complex particles and the number of nitrogen in PEI per the number of phosphate in DNA. The linear PEI (22kDa) has popularly employed due to its reduced cytotoxicity and consistent transfection efficiency. Enhanced transgene expression and increased diffusion in the brain could be achieved by formulating DNA (22KDa)/linear PEI in glucose. A complexation of linear PEI/DNA has been produced the physicochemical behaviors different from the complexation of branched PEI/DNA. The local delivery of PEI/DNA complexes to mouse lung has successfully carried out using a nebulization. Administration of PEI/DNA complex to liver showed significantly higher expression of a gene in the liver than naked DNA. Intracerebral delivery of PEI/DNA complexes to brain led to significant expression of a gene in the brains of adult. Intrathecal dose of PEI (25kDa)/DNA complexes to the lumbar subarachnoid single space demonstrated trans-gene expression 40 times higher than naked DNA in the spinal cord.
The prepared PEI derivatives containing disulfide bonds show higher transfection efficiency, as compared to non-degradable PEI (25kDa), Lipofectamine®, and FuGENE®. Moreover, the PEI containing disulfide bonds have relatively lower cytotoxicity due to the degradability of the other polymers. The therapeutic targeting of chitosan-PEG-folate complexed oncolytic adenovirus has made for active and systemic cancer gene therapy. Evaluation of dendrimer type bio-reducible polymer as a siRNA delivery carrier was done for cancer therapy. VEGF therapeutic gene delivery using dendrimer type bio-reducible polymer has applied into human mesenchymal stem cells (nMSCs). The oncolytic adenovirus coated with multi-degradable bio-reducible core-cross-linked poly(ethyleneimine) was used for cancer gene therapy. Human relaxin gene expression delivered by bio-reducible dendrimer has applied to post-infarct cardiac remodeling in rats. The cleavable modifications to reducible poly(amido-ethyleneimine)s are applied to enhance nucleotide delivery. Tumor targeting RGD conjugated bio-reducible polymer has developed for VEGF siRNA expressing plasmid delivery systems. Targeted therapeutic gene delivery to tumor site is critical for successful and safe cancer gene therapy. The research results give a demonstration for a tumor targeting bio-reducible polymer with an anti-angiogenic therapeutic gene for efficient cancer gene therapy.

Conclusion

Poly(ethyleneimine)s conjugated bio-reducible dendrimers for gene delivery have been extensively investigated during past decades. Utilizing their functional groups of high stability exists in their structures and in extracellular physiological condition. The prepared PEIs conjugated bio-reducible dendrimers containing disulfide bonds show higher transfection efficiency, as comparing with non-degradable PEI (25KDa). The PEIs derivatives with disulfides have relatively lower cytotoxicity than that of PEIs themselves due to the degradability. It has found that these polymers and dendrimers have great plasmid condensing capacity. It has been also concluded that the bio-reducible polymers and dendrimers have a great potential as a gene carrier, especially in PEI (1.8kDa) consisting of disulfide bonds.

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References


25. Suh J, Paik HJ, Hwang BK. Ionization of poly(ethyleneimine) and poly(allylamine) at


