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# Poly(β-amino amine) cross-linked PEIs as highly efficient gene vectors

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ABSTRACT

To increase the release of DNA into the cytoplasm and further improve transgene expression of nucleic acid novel polymeric gene carriers were prepared which would be biodegradable under the reducing conditions in the cytoplasm. Disulfide-containing poly(β-amino amine)s were first synthesized and then used to cross-link low molecular weight polyethyleneimine (1800 Da) through Michael addition to obtain SS-PBAA-PEIs as the final gene carriers. The physicochemical characteristics of SS-PBAA-PEI/DNA complexes were characterized. In vitro transfection mediated by the SS-PBAA-PEIs under serum conditions was carried out. Cell uptake of the gene delivery systems was observed by confocal laser scanning microscopy. The results of the physicochemical characterisation demonstrated that the SS-PBAA-PEIs could efficiently condense DNA. In vitro transfection under serum conditions showed that SS-PBAA-PEIs had comparable or even higher transfection efficiencies than 25 kDa PEI. And SS-PBAA-PEIs showed much lower cytotoxicity compared with 25 kDa PEI. In summary, the SS-PBAA-PEIs possess great potential as non-viral gene vectors and exhibit high transfection efficiency under serum conditions.



## 1. Introduction

Gene therapy is a promising strategy for clinical trials because almost all human diseases, either inherited or acquired, have a genetic component. In the process of gene therapy the delivery of nucleic acids is exceedingly important. A large number of non-viral vectors, which have the advantages of safety compared with viral vectors, have been designed and prepared [1–3] to obtain favorable properties such as high transfection efficiency, non-toxicity, non-immunogenicity, and, preferably, biodegradability.

Polyethyleneimine (PEI) is well known as an effective non-viral gene vector both in vitro and in vivo. It not only has a high cationic charge density to stably condense DNA but also has a high density of near neutral p$K_a$ groups with good buffer capacity and can cause the release of the PEI/NDA complexes into the cytoplasm through the "proton sponge" mechanism [4,5]. However, high molecular weight PEI ($M_w \geqslant 25$ kDa) shows significant cytotoxicity, which evidently limits its clinical application. On the other hand, low molecular weight PEI ($M_w \leqslant 2$ kDa) demonstrates neglectable cytotoxicity but has much lower transfection ability [6]. Therefore, biodegradable PEI derivatives based on cross-linked low molecular weight PEIs have been developed [7,8]. The use of disulfide bonds to cross-link low molecular weight PEI is an attractive strategy [9]. For example, PEI crosslinked by cystamine bisacrylamide exhibited comparable transfection efficiency to and lower toxicity than 25 kDa PEI [10–13]. The disulfide bond-containing PEI derivative could temporarily maintain high stability of the delivery system outside cells, and would be degradable under the reducing condition in the intracellular environment, facilitating the release of therapeutic nucleic acids.

Poly(β-amino ester) (PBAE) and poly(β-amino amine) (PBAA) are two kinds of biodegradable polymers attracting a lot of attention as non-viral gene vectors. They can be easily synthesized by Michael addition between amines and diacrylates or diacrylamides through a one-step reaction without any by-products. Using high throughput synthesis and screening techniques, Langer and coworkers created a series of PBAEs with different structures [14–17]. It was found that the polymer structural characteristics were important for effective gene delivery. And PBAEs containing hydroxyl side-chains and primary amine end groups were favorable for gene delivery [14].

In this study these two kinds of traditional gene vectors, PEI and PBAA, were integrated to obtain a series of remarkable novel gene carriers. Bioreducible linear disulfide-containing PBAA (SS-PBAAs) were first synthesized and then used to cross-link branched low molecular weight PEI to obtain SS-PBAA-PEIs as non-viral gene vectors. Linear SS-PBAAs with different degrees of polymerization and different side-chains were synthesized to study the influence of the component and structural characteristics on gene transfer mediated by these copolymers.

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## 2. Materials and methods

### 2.1. Materials

Cystamine dihydrochloride (98%), branched PEI (bPEI, water-free) with molecular weights of 25 and 1.8 kDa, 2-amino-1-ethanol (EA), and 5-amino-1-pentanol (PA) were purchased from Sigma–Aldrich and used as received. 1,4-Dithiothreitol (DTT) and acryloyl chloride of analytical grade were purchased from Shanghai Chemical Reagent Co. and used as received. QIAfilter™ Plasmid Giga Kits (5) were purchased from Qiagen (Hilden, Germany). GelRed™ was purchased from Biotium (Hayward, CA). Fetal bovine serum (FBS), 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazoliumbromide (MTT), Dulbecco's modified Eagle's medium (DMEM), penicillin–streptomycin, trypsin, and phosphate-buffered saline (PBS) were purchased from Invitrogen. The Micro BCA protein assay kit was purchased from Pierce. Acetate and methanol were of analytical grade and used as received.

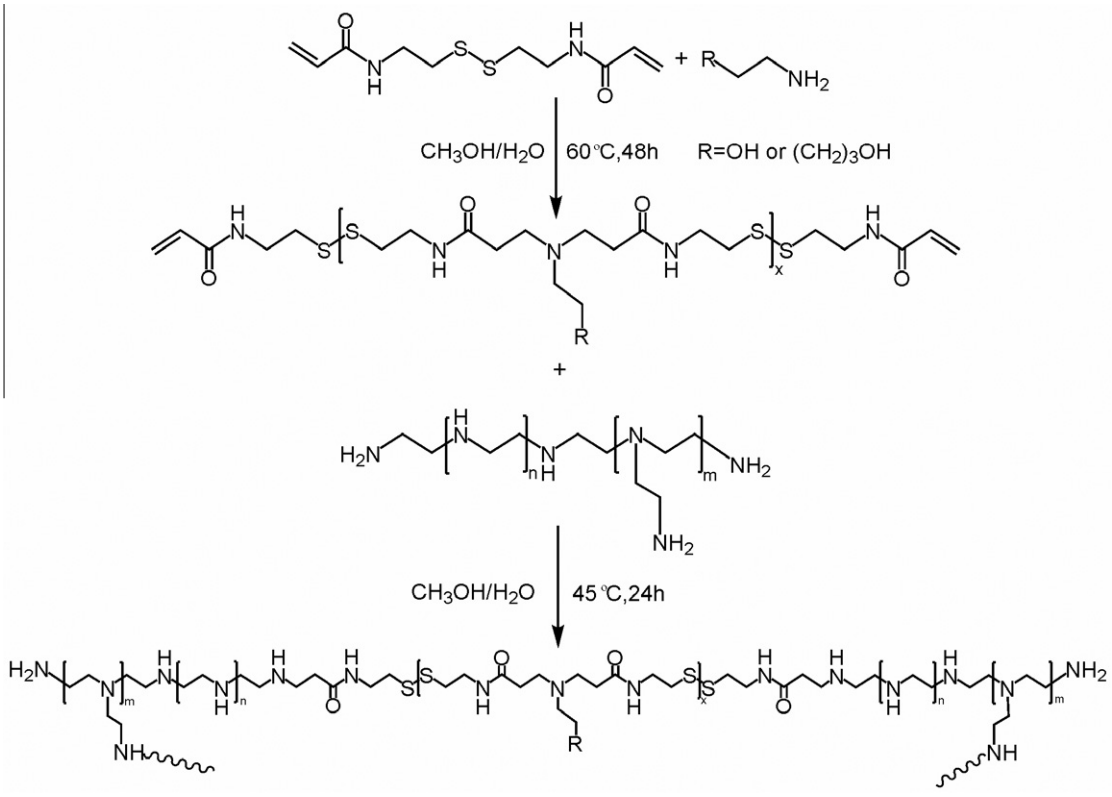
### 2.2. Synthesis of disulfide-containing poly(β-amino amine) cross-linked PEIs (SS-PBAA-PEIs)

Cystamine bisacrylamide (CBA) was synthesized according to the literature [18]. Briefly, 22.5 g cystamine dihydrochloride (0.1 mol) and 8.0 g NaOH (0.2 mol) were dissolved in water (100 ml) in a three-necked flask. After the solution was cooled to 0 °C acryloyl chloride dichloromethane solution (0.2 mol, 20 ml) and aqueous NaOH solution (0.2 mol, 10 ml) were added simultaneously. The reaction mixture was stirred continuously at room temperature for 16 h. The raw product was obtained by filtration and washed three times with water. After purification by crystallization using ethyl acetate CBA was obtained by removing the solvent under vacuum.

The synthesis of SS-PBAA-PEIs is shown in Scheme 1. SS-PBAAs with terminal diacrylamide groups were synthesized by adding 2-amine-1-ethanol (EA) and 5-amino-1-pentanol (PA) to CBA [19]. By varying the stoichiometric ratio ($r$) of the monomers (i.e. $r_{EA/CBA}$ or $r_{PA/CBA}$) four SS-PBAAs oligomers were obtained. Taking SS-PBAA$_1$-PEI as a typical example, PBAA$_1$ oligomer was prepared by stirring a mixture of EA (0.109 g, 1.8 mmol) and CBA (0.520 g, 2 mmol, stoichiometric ratio $r_{EA/CBA}$ = 0.9) in 5 ml of 25% aqueous methanol in the dark at 60 °C under a nitrogen atmosphere. The reaction was allowed to proceed for 48 h to yield a homogeneous solution. Subsequently, 0.276 g PEI was dissolved in 2 ml of 25% aqueous methanol and then added to SS-PBAA$_1$ solution to obtain SS-PBAA$_1$-PEI. The molar ratio of primary amine in PEI to reactive group in SS-PBAA$_1$ was 4:1. The reaction was allowed to proceed for another 24 h at 45 °C under a nitrogen atmosphere. The transparent solution was diluted with water to 30 ml and acidified with 6 M HCl to pH 4, then dialyzed against distilled water (molecular weight cut-off 8000) for 2 days to remove the unreacted reactants. After that the solution was lyophilized for 2 days to obtain SS-PBAA$_1$-PEI. SS-PBAA$_2$-PEI ($r_{EA/CBA}$ = 0.5), SS-PBAA$_3$-PEI ($r_{PA/CBA}$ = 0.9) and SS-PBAA$_4$-PEI ($r_{PA/CBA}$ = 0.5) were synthesized in a similar way. The four SS-PBAA-PEI polymers were all prepared with the molar ratio of primary amine in PEI to reactive group in SS-PBAA of 4:1.

### 2.3. Polymer characterization

$^1$H nuclear magnetic resonance spectroscopy (NMR) was carried out in a Varian Unity 300 MHz spectrometer. DMSO-$d_6$ and $D_2O$ were used as the solvents for CBA and SS-PBAA-PEIs, respectively. The molecular weights and polydispersities ($M_w/M_n$) of PBAAs and SS-PBAA-PEIs were determined by gel permeation chromatography (GPC) using a Waters 2690D HPLC equipped with Ultrahydrogel 120 and 250 columns. HAc–NaAc buffer solution (0.03 M, pH 2.8) was used as eluent at a flow rate of 1.0 ml min$^{-1}$.




**Scheme 1.** Synthesis of disulfide-containing poly(β-amido amines) cross-linked PEI.

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