Highly branched poly(β-amino ester)s for skin gene therapy

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ARTICLE INFO

ABSTRACT

Poly(β-amino ester)s (PAEs) have emerged as a promising class of gene delivery vectors with performances that can even be compared to viruses. However, all of the transfection studies (over 2350 PAEs) have been limited to linear poly(β-amino ester)s (LPAEs) despite increasing evidence that polymer structure significantly affects performance. Herein, we describe the development of highly branched poly(β-amino ester)s (HPAEs) via a new "A2 + B3 + C2" Michael addition approach demonstrating 2 to 126-fold higher in vitro transfection efficiencies of different cell types in comparison to their linear LPAE counterparts as well as greatly out-performing the leading transfection reagents SuperFect and the "gold-standard" polyethyleneimine (PEI) - especially on skin epidermal cells. More importantly, the ability to correct a skin genetic defect is demonstrated in vivo utilizing a recessive dystrophic epidermolysis bullosa (RDEB) knockout mouse model. Our results provide evidence that the "A2 + B3 + C2" approach can be controlled and offers sufficient flexibility for the synthesis of HPAEs. The branched structures can significantly improve the transfection efficiency and safety of PAEs highlighting the great promise for the successful application of non-viral gene therapy in skin disease.

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1. Introduction

Gene therapy offers the prospect for the treatment of various inherited and/or acquired diseases [1,2]. However, current efficiency and safety issues [3,4] continue to inspire the development of improved gene delivery vectors [5,6]. In contrast to the viral vectors, non-viral vectors (especially cationic polymers) hold greater promise for clinical application [6–9]. Effective and safe polymeric vectors with simplicity, scalability, low cost and ease of use are of high clinical significance [10–12].

Due to advances in the area of polymer chemistry, various vectors with different polymeric structures have been developed [7,8]. Among them, linear poly (β-amino ester)s (LPAEs) have recently emerged as a new class of highly effective gene delivery vectors [13]. Since they were first developed in 2000, more than 2350 LPAEs have been designed, synthesized and screened by Langer, Anderson and co-workers [14–16]. Several high performance LPAEs have been identified for gene delivery both, in vitro and in vivo [17–19], and their performance is comparable to viruses [19]. However, so far all the transfection studies have been limited to LPAEs. Therefore, the possibility of developing

branched poly(β-amino ester)s (HPAEs) is an important "next step" following the encouraging advancements of LPAEs. Branched polymers have a three-dimensional (3D) spatial structure with multiple terminal groups [4,20,21], thus compositions and structures can be adjusted easily to introduce special functionalities [22–25]. Previous studies have proven that branched structures can significantly improve the interaction of polymers with DNA, prevent DNA degradation by enzymes and increase the cellular uptake of polyplexes [22,25–27]. Branched polyethyleneimine (PEI) [26], branched poly-L-lysine (PLL) [25,27], branched poly [2-(dimethylamino) ethyl methacrylate] (PDMAEMA) [4] and branched poly(2-aminoethyl methacrylamide-st-2-lactobionamidoethyl methacrylamide) [P(AEMA-st-LAEMA)] [20] have proven to be much more efficient gene vectors in comparison to their linear counterparts. Therefore, it is conceivable that HPAEs would have similar structural advantages to further improve the gene transfection performance compared to LPAEs.

Despite the great potential, HPAEs have never been developed for gene delivery because the synthesis of highly branched polymers has always been a big challenge. Previously, only a few HPAE analogues have been synthesized via the "A2 + BB'B''" and "A3 + 2BB'B" type Michael addition under strict reaction conditions utilizing a small set of special monomer systems [28,29] limiting the choice of structures, functionalities and ultimately the transfection capability. Therefore, it is advantageous to develop HPAEs from readily available monomers using a

facile and controllable synthesis approach. Here, we report the design and synthesis of a novel family of HPAEs *via* a new "A2 + B3 + C2" Michael addition approach. This approach means that the HPAEs can essentially be derived from the same functional LPAE components that have already been proven to be highly effective gene delivery vectors. Meanwhile, an additional benefit can be obtained *via* the introduction of branched structures increasing the transfection efficiency to higher levels than the original LPAE. The transfection activity of HPAEs was systematically assessed *in vitro* and compared with LPAE as well as the leading commercially available transfection reagents SuperFect and polyethyleneimine (PEI). The cell types used were primary human adipose derived mesenchymal stem cells (hADSC), human cervical cancer cells (HeLa) and recessive dystrophic epidermolysis bullosa keratinocytes (RDEBK). The best performing HPAE was selected to deliver therapeutic *collagen VII* (C7) DNA (pcDNA3.1COL7A1) for the restoration of expression of the protein in C7 null mice with the RDEB

phenotype to examine the potential application of HPAEs as an efficient delivery vector in skin gene therapy (Fig. 1).

2. Materials and methods

2.1. Materials

For polymer synthesis and characterization, commercially available amine monomer 4-amino-1-butanol (S4), acrylate monomers trimethylolpropane triacrylate (TMPTA), bisphenol A ethoxylate diacrylate (BE) and end-capping agent 3-morpholinopropylamine (MPA) were purchased from Sigma-Aldrich and used as received. Lithium bromide (LiBr) for GPC measurements was purchased from Sigma-Aldrich. Solvents dimethyl sulfoxide (DMSO), dimethylformamide (DMF), tetrahydrofuran (THF) and diethyl ether were purchased from Fisher Scientific. Deuterated chloroform (CDCl$_3$) was purchased from
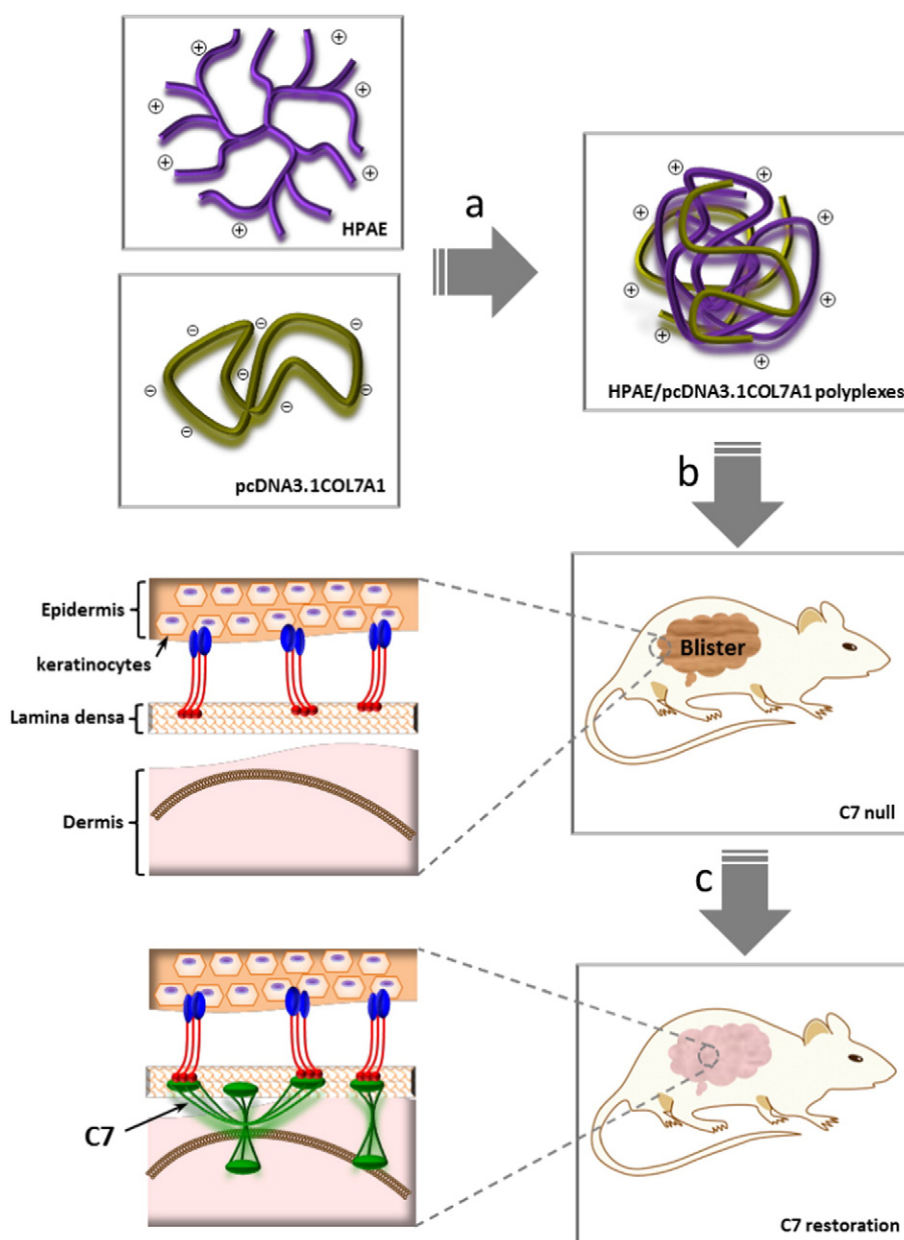

Fig. 1. Schematic illustration of HPAE delivering pcDNA3.1COL7A1 gene to correct the collagen VII gene defect in RDEB mouse. (a) HPAE condenses pcDNA3.1COL7A1 to form positively charged polyplexes by electrostatic interaction; (b) Polyplexes are directly injected into the dermis of blister area in C7 null RDEB mouse; (c) Collagen VII protein is produced.