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Maximizing gene delivery efficiencies of cationic helical polypeptides via balanced membrane penetration and cellular targeting

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ABSTRACT

The application of non-viral gene delivery vectors is often accompanied with the poor correlation between transfection efficiency and the safety profiles of vectors. Vectors with high transfection efficiencies often suffer from high toxicities, making it unlikely to improve their efficiencies by increasing the DNA dosage. In the current study, we developed a ternary complex system which consisted of a highly membrane-active cationic helical polypeptide (PVBLG-8), a low-toxic, membrane-inactive cationic helical polypeptide (PVBLG-7) capable of mediating mannose receptor targeting, and DNA. The PVBLG-7 moiety notably enhanced the cellular uptake and transfection efficiency of PVBLG-8 in a variety of mannose receptor-expressing cell types (HeLa, COS-7, and Raw 264.7), while it did not compromise the membrane permeability of PVBLG-8 or bring additional cytotoxicities. Because of the simplicity and adjustability of the self-assembly approach, optimal formulations of the ternary complexes with a proper balance between membrane activity and targeting capability were easily identified in each specific cell type. The optimal ternary complexes displayed desired cell tolerability and markedly outperformed the PVBLG-8/DNA binary complexes as well as commercial reagent Lipofectamine™ 2000 in terms of transfection efficiency. This study therefore provides an effective and facile strategy to overcome the efficiency-toxicity poor correlation of non-viral vectors, which contributes insights into the design strategy of effective and safe non-viral gene delivery vectors.

1. Introduction

Gene therapy has shown great potentials in treating various genetic diseases, such as cystic fibrosis, diabetes, arthritis, immunological deficiency, and cancer [1–6]. Compared to viral vectors, non-viral gene delivery vectors allow safe delivery of genetic materials with less inherent immunogenicity and oncogenicity. Polycations, capable of condensing the anionic nucleic acids to facilitate their intracellular uptake, are one of the most widely explored non-viral vectors. Although the cationic charge of polycations features strong membrane binding of delivery vehicles and facilitates cellular uptake and transfection of the gene cargos, it meanwhile causes severe associated cytotoxicities [7–9]. Excessive positive charges can ultimately undermine the transfection efficiency [10]. Therefore, it is of particular importance to balance the charge related membrane activity and cytotoxicity in the design of non-viral vectors such that the gene delivery efficiency could be maximized. One promising approach towards this goal is the combinatorial/parallel synthesis that creates a large library of materials and allows identification of the best-performing candidate via screening [11,12]. This technology, although promising, requires tedious task and often suffers from high cost. Alternatively, covalent modification of existing polycations with various charge-reducing moieties—including saccharides [13], hydrocarbons [14], and poly(ethylene glycol) (PEG) [15–17], stands as an effective tool to reduce their toxicities. While the modified polycations benefit from improved safety profiles, they typically suffer from diminished gene delivery capabilities [10]. All these challenges thus necessitate a facile and effective strategy for the development of non-viral vectors which can properly balance the membrane activity and toxicity towards maximized gene delivery efficiency.

Cell penetrating peptides (CPPs), exemplified by HIV-TAT, Arg9, penetratin, and melittin, are sequence-specific short oligopeptides that mediate effective membrane penetration and translocation via either energy-dependent endocytosis or energy-independent transduction [18]. Due to their excellent membrane activities, CPPs are able to facilitate the cellular delivery of a variety of

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exogenous materials, including metals, macromolecules (e.g., proteins and nucleic acids), and nanoparticles [19,20]. However, when used as gene transfer agents, CPPs are often too short (fewer than 25 amino acid residues) and lack sufficient cationic charge density, which raises great challenges for CPPs to condense and deliver genes by themselves. As such, they often act as membrane-active ligands incorporated or conjugated to existing delivery vehicles to enhance their delivery efficiencies [19,21,22]. To address the dearth of CPP-mediated non-viral gene delivery, we recently developed a cationic polypeptide, poly(γ-(4-(((2-(piperidin-1-yl) ethyl) amino) methyl) benzyl-$_L$-glutamate) (PVBLG-8), via a controlled ring-opening polymerization method (Fig. 1A) [23–25] and used PVBLG-8 or its analogs in gene and siRNA delivery [26–28]. PVBLG-8 with stabilized helical structure exhibited desired membrane activity and thus triggered effective cellular uptake as well as gene transfection, which rendered it a better gene delivery vector than traditional oligo-CPPs [23]. However, the appreciable cytotoxicity of PVBLG-8 at higher concentrations makes it unlikely to strength the gene delivery capabilities by increasing the amount used [26], which thus necessitates alternative approaches to maximize its gene transfer efficiencies without causing additional cytotoxicities.

With an attempt to balance the transfection efficiency and cytotoxicity, we first developed a PVBLG-8-based random copolypeptide (PVBLG-8-*r*-7) (Fig. 2A) which contains glucosamine side chains that allow mannose receptor targeting as well as reduce the material cytotoxicity via saccharide-mediated charge shielding. Although the cytotoxicity was slightly decreased, this approach shares the similar disadvantage of PEGylation and the resulting

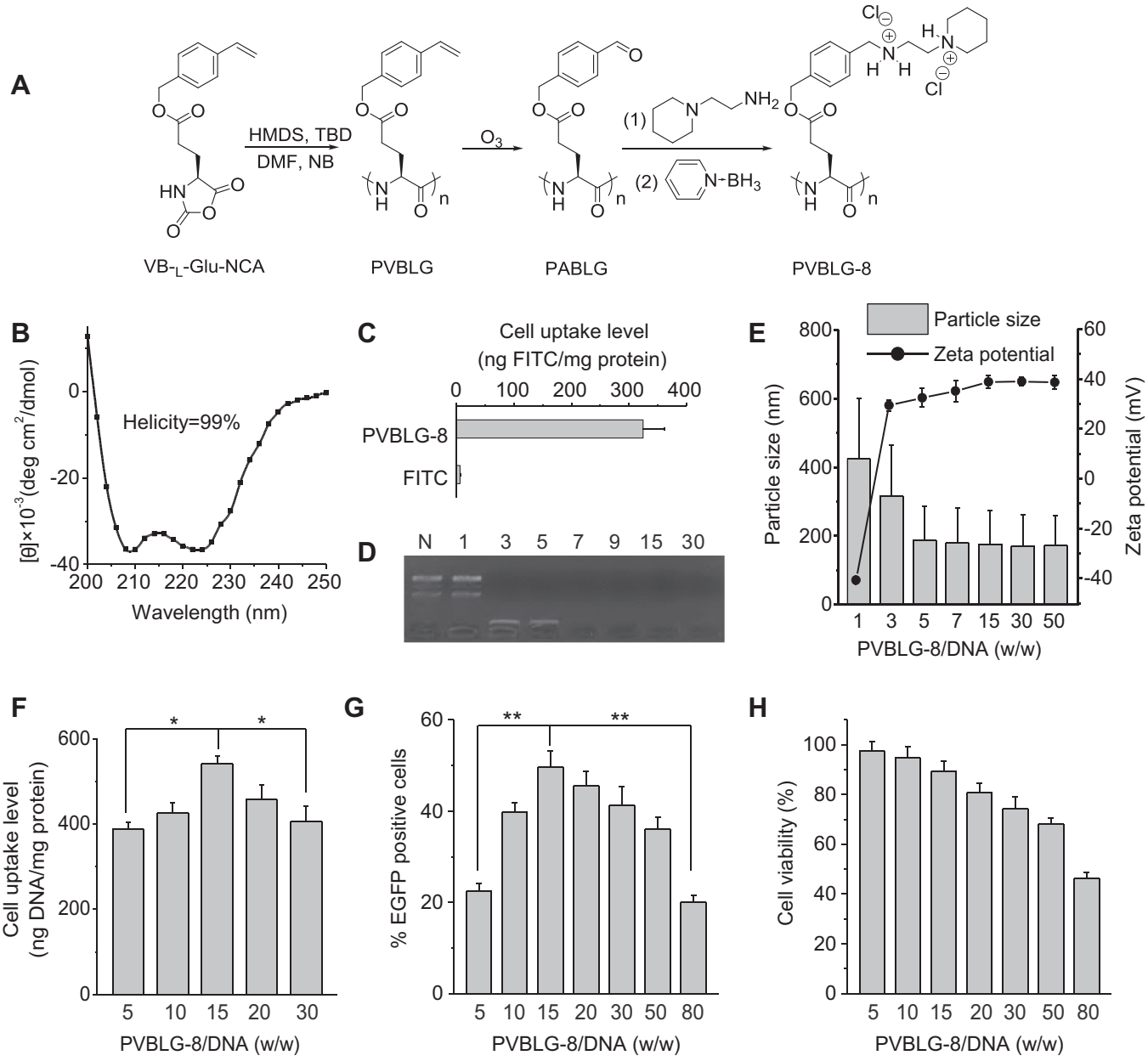

Fig. 1. PVBLG-8 allows effective transfection while induces appreciable cytotoxicity. (A) Reaction scheme of PVBLG-8. (B) CD spectrum of PVBLG-8 in DI water (0.1 mg/mL) at pH 7. (C) FITC-Tris uptake level in HeLa cells following co-incubation with PVBLG-8 for 2 h at 37 °C (D) DNA condensation by PVBLG-8 at different PVBLG-8/DNA weight ratios as evaluated by the gel retardation assay. N represents naked DNA. (E) Particle size and zeta potential of PVBLG-8/DNA complexes. (F) Uptake level of PVBLG-8/YOYO-1-DNA complexes in HeLa cells following incubation at 37 °C for 4 h ($n = 3$). (G) *In vitro* transfection efficiency of PVBLG-8/pEGFP complexes in HeLa cells at different weight ratios ($n = 3$). (H) *In vitro* cytotoxicity of PVBLG-8/pEGFP complexes in HeLa cells at different weight ratios as determined by the MTT assay ($n = 3$).