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Reconfiguring the architectures of cationic helical polypeptides to control non-viral gene delivery

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ABSTRACT

Poly(γ-4-((2-(piperidin-1-yl)ethyl)aminomethyl)benzyl-L-glutamate) (PPABLG), a cationic helical polypeptide, has been recently developed by us as an effective non-viral gene delivery vector. In attempts to elucidate the effect of molecular architecture on the gene delivery efficiencies and thereby identify a potential addition to PPABLG with improved transfection efficiency and reduced cytotoxicity, we synthesized PEG-PPABLG copolymers with diblock, triblock, graft, and star-shaped architectures via a controlled ring-opening polymerization. The PPABLG segment in all copolymers adopted helical structure; all copolymers displayed structure-related cell penetration properties and gene transfection efficiencies. In HeLa and HepG-2 cells, diblock and triblock copolymers exhibited reduced membrane activities and cytotoxicities but uncompromised gene transfection efficiencies compared to the non-PEGylated homo-PPABLG. The graft copolymer revealed lower DNA binding affinity and membrane activity presumably due to the intramolecular entanglement between the grafted PEG segments and charged side chains that led to reduced transfection efficiency. The star copolymer, adopting a spherical architecture with high density of PPABLG, afforded the highest membrane activity and relatively low cytotoxicity, which contributed to its potent gene transfection efficiency that outperformed the non-PEGylated PPABLG and Lipofectamine™ 2000 by 3–5 and 3–134 folds, respectively. These findings provide insights into the molecular design of cationic polymers, especially helical polypeptides towards gene delivery.

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1. Introduction

Gene therapy has been widely regarded as a promising modality that can potentially treat various inquired or congenital diseases in a highly specific manner [1–3]. The success of gene therapy largely depends on the delivery efficiency of the therapeutic gene. Although viral vectors enable highly efficient gene delivery, they cause associated immune responses and oncogenic transformations with several reported fatal cases in clinical trials [4]. Non-viral gene delivery mediated by cationic liposomal and polymeric vectors has emerged as an attractive alternative because of their low toxicity and low immunogenicity [5–7].

Cell penetrating peptides (CPPs), such as oligoarginine, HIV-TAT, and penetratin, are sequence-specific oligopeptides that have excellent membrane activities and can effectively mediate membrane transduction to facilitate cellular internalization of exogenous materials, such as DNA, RNA, and proteins [8,9]. Hence, they have been extensively applied in non-viral gene delivery [6,7,10]. However, CPPs are often too short (less than 25 amino acid residues) and lack adequate cationic charges to efficiently condense and deliver genes. As such, they often function as membrane-active ligands incorporated or conjugated to delivery vehicles to improve the delivery efficiency [6,7].

We have recently developed a high molecular weight (MW), cationic, α-helical polypeptide, termed poly(γ-(4-((2-(piperidin-1-yl)ethyl)aminomethyl)benzyl-L-glutamate) (PPABLG) [11,12], as an effective non-viral gene delivery vector. We demonstrated that the stabilized helical structure of PPABLG markedly contributed to its membrane penetrating capacity via the pore formation mechanism; its relatively high MW and cationic charge density facilitated the efficient condensation of DNA, making it a more efficient vector than traditional CPPs for gene delivery to mammalian cells [12,13]. However, excessive membrane activity of PPABLG may cause irreversible damage to cell membranes. We thus seek to find a strategy to further improve the gene transfection efficiency of PPABLG and reduce its cytotoxicity.

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Polymer physico-chemical properties, such as molecular weight, charge density, three-dimensional structure, chain flexibility, and hydrophilicity/hydrophobicity balance, have significant impact on the efficiency and safety of non-viral gene vectors [14–22]. Therefore, design features to control over these properties may render promising strategy for the identification of an ideal non-viral gene delivery vector with maximized transfection efficiency and minimized toxicity. Incorporation of poly(ethylene glycol) (PEG) moieties in the macromolecular design has been demonstrated an effective strategy to not only reduce the cytotoxicity of the gene delivery vectors but also improve the stability of the polymer/DNA complexes [14,16]. Additionally, PEGylation provides an opportunity to modulate the architecture of the PEG-polymer conjugates, which in turn alters their complexation capacities with DNA, interaction with cellular/endosomal membranes, and ultimately the transfection efficiencies [14,16].

Motivated by these understandings, we designed and synthesized various PEG-PPABLG copolymers with different molecular architectures, attempting to elucidate the effect of polymer topology on the gene delivery efficiencies and thus identify desired PEGylated PPABLG derivative with high gene transfection efficiency and low cytotoxicity. Amine-terminated PEG served as the macro-initiator for the ring opening polymerization (ROP) of γ-(4-vinylbenzyl)-L-glutamate *N*-carboxylanhydride (VB-L-glu-NCA, Supplementary Scheme S1) [23], thus achieving the PEG-PPABLG block polymers. By using mPEG-NH_2 (5 kDa), H_2N-PEG-NH_2 (10 kDa), and 8-arm PEG-NH_2 (40 kDa) as initiators, diblock, triblock, and star copolymers were obtained (Fig. 1A). Furthermore, mPEG-NH_2 (550 Da) was grafted onto PPABLG, yielding the graft copolymer (Fig. 1A). The degree of polymerization (DP) of PPABLG was maintained constant at ~100, along with a comparable weight fraction of PEG segments of ~11%, thus allowing the synthesis of

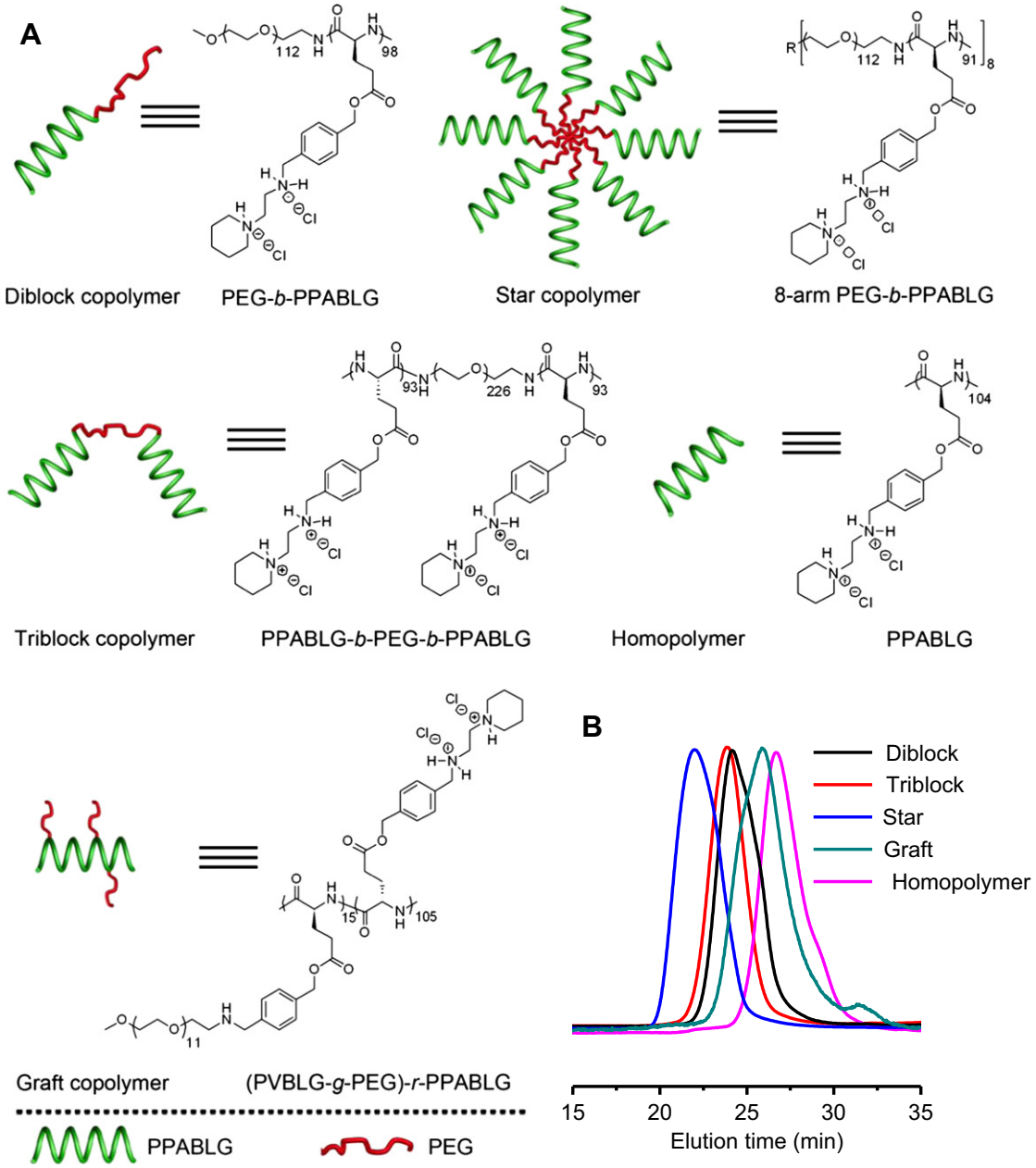

Fig. 1. (A) Schematic representation of PEG-PPABLG conjugates with different architectures. (B) GPC traces of the precursors (PVBLG or PEG-PVBLG conjugates) of various polypeptides.

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