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Poly(ethylene glycol)-*block*-cationic polylactide nanocomplexes of differing charge density for gene delivery

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ABSTRACT

Representing a new type of biodegradable cationic block copolymer, well-defined poly(ethylene glycol)-*block*-cationic polylactides (PEG-*b*-CPLAs) with tertiary amine-based cationic groups were synthesized by thiol-ene functionalization of an allyl-functionalized diblock precursor. Subsequently the application of PEG-*b*-CPLAs as biodegradable vectors for the delivery of plasmid DNAs (pDNAs) was investigated. Via the formation of PEG-*b*-CPLA:pDNA nanocomplexes by spontaneous electrostatic interaction, pDNAs encoding luciferase or enhanced green fluorescent protein were successfully delivered to four physiologically distinct cell lines (including macrophage, fibroblast, epithelial, and stem cell). Formulated nanocomplexes demonstrated high levels of transfection with low levels of cytotoxicity and hemolysis when compared to a positive control. Biophysical characterization of charge densities of nanocomplexes at various polymer:pDNA weight ratios revealed a positive correlation between surface charge and gene delivery. Nanocomplexes with high surface charge densities were utilized in an in vitro serum gene delivery inhibition assay, and effective gene delivery was observed despite high levels of serum. Overall, these results help to elucidate the influence of charge, size, and PEGylation of nanocomplexes upon the delivery of nucleic acids in physiologically relevant conditions.

1. Introduction

With high delivery efficacy, viral vectors have been extensively used in gene therapy research over the previous decades [1]. Specifically, viral-mediated carriers have been employed in more than 70% of gene therapy clinical trials [2]. However, there are appreciable concerns about viral vectors regarding their biosafety, cytotoxicity, immunogenicity, tumorigenicity, gene capacity, and targeting efficiency [3,4]. These limitations have led to increased interest in the development of safe and efficacious non-viral vectors.

Accordingly, two major categories of non-viral vectors have been developed: cationic lipids (CLs) and cationic polymers (CPs). Since the initial studies by Felgner and co-workers [5], CLs represent the most studied systems for non-viral gene delivery [6]. In contrast, CPs have gained increasing attention due to their inherent flexibility in design and formulation, which allows precise structure and surface modification for specific biomedical applications [7–10]. Both CLs and CPs can spontaneously form nanocomplexes with negatively charged nucleic acids (RNA and DNA) through electrostatic interactions. Within the nanocomplexes, CLs and CPs serve as a protective barrier to prevent nucleic acids from degradation via ubiquitous nucleases. However, CL and CP-based nanocomplexes differ significantly in endosomal/phagosomal escape mechanisms. CL-based systems operate on the basis of lipid mixing, which may involve membrane fusion and formation of transient and local perturbations, thus allowing the release of nucleic acid into the cytosol. In contrast, CP-based systems most likely function through a proton sponge mechanism, in which amine groups of CPs (pKa 5 ~ 7) become protonated during endosome (or phagolysome) acidification due to the inflow of H^+ by the activity of H-ATPase and counter ion Cl^- influx to restore charge neutrality. It is believed that water intake occurs in endosomes to compensate for the increased

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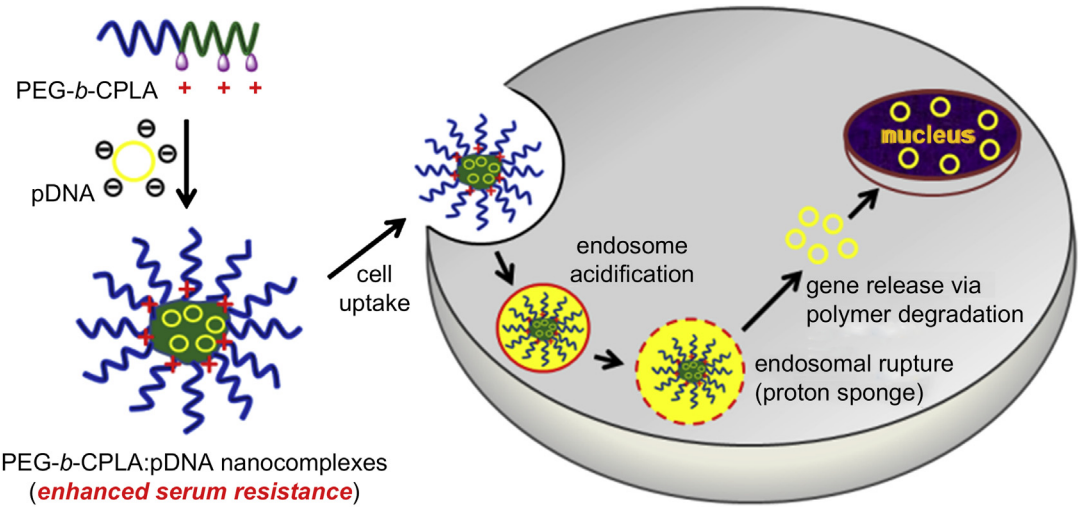

Fig. 1. Conceptual diagram of pDNA gene delivery using PEG-*b*-CPLA.

ion inflow, causing osmotic swelling followed by endosomal rupture. However, reports have questioned the validity of the proton sponge effect [11,12], and recent findings by Rehman and co-workers indicate that cellular administration of CLs and CPs did not result in complete endosomal rupture or the release of nanocomplexes into the cytosol, suggesting the presence of alternative delivery mechanisms [13].

Along with the development of polymer chemistry, the applicability of CPs in gene delivery has been enhanced by incorporating biodegradability within their macromolecular structures. A variety of biodegradable CPs such as poly(β-amino ester)s [14–17], poly[α-(4-aminobutyl)-L-glycolic acid] [18], poly(4-hydroxy-L-proline ester) [19], poly(D-glucaramidoamine) [20], cationic poly(α-hydroxy acid) [21], and cationic cyclodextrin [22] have been successfully synthesized and used in gene delivery studies. In addition to protecting therapeutic genes from nuclease degradation, synthetic design of CPs can be directed to optimize their biodegradability and improve their biocompatibility for repeated administration of gene-based therapies [23,24]. In particular, the intracellular cleavage of the biodegradable polymer backbone aids nanocomplexes in unpacking gene payloads, and effective cytosolic release of nucleic acids from nanocomplexes was reported to be positively correlated with enhanced gene transfection [25,26].

Although biodegradable CPs may be used as safe gene delivery scaffolds, most of the resulting nanocomplexes are unstable under physiological conditions due to undesired interactions with serum proteins and salts, resulting in breakdown or aggregation and subsequent clearance [27]. To address this issue, certain studies have suggested the use of anionic polymers [28,29]; however, most polymer delivery systems require excess cationic charge to remain stable. On the other hand, as observed in polyethyleneimine (PEI)-based systems, excess cationic charge of nanocomplexes can destabilize plasma membranes of red blood cells and cause aggravated cell damage, resulting in necrosis, apoptosis, and autophagy [30]. To ameliorate such concerns, PEGylation has been used to sterically shield CPs, thereby hindering and minimizing undesired interactions between the cationic nanocomplex and serum albumin [31–33]. Accordingly, colloidal stability and transfection efficiency of PEGylated nanocomplexes under physiological conditions can improve as compared with their unPEGylated analogs. Recently, several studies using PEGylated CPs for enhanced gene transfection efficiency in serum have been reported. For example, Won and co-workers synthesized poly(ethylene glycol)-poly(*n*-butyl acrylate)-poly(2-(dimethylamino)ethyl methacrylate) (PEG-PnBA-PDMAEMAs) and formulated them into micelles for siRNA delivery [34]. The micelle/siRNA nanocomplexes, i.e. micelleplexes, demonstrated better gene silencing efficiency than PDMAEMA/siRNA nanocomplexes. Moreover, improved accumulation of micelleplexes in tumor tissues was observed and credited to PEG shielding and size effects. However, the backbone of PEG-PnBA-PDMAEMAs is unable to degrade under physiological aqueous conditions, which may significantly hamper potential clinical applications. Kwon and co-workers have prepared PEG-conjugated poly(ketalized serine) (PEG-poly(kSer)) for DNA complexation [35]. Possessing ketal-linkages, such nanocomplexes can effectively release DNA payloads into the cytoplasm through acid-hydrolysis. PEG-poly(kSer)/DNA nanocomplexes exhibited three times higher transfection efficiency than poly-L-lysine (PLL) in NIH3T3 cells but were not as effective as typical commercial polymeric vectors (such as 25 kDa branched PEI), presumably due to their insufficient buffering capacity. Similarly, poly(ethylene glycol)-*block*-poly(L-lysine) (PEG-*b*-PLL) has been synthesized by Jin and co-workers and utilized to form nanocomplexes with siRNA for gene silencing [36]. However, gene knockdown efficiency via such nanocomplexes was lower than commercially available Lipofectamin™ 2000, presumably because of low dissociation and release efficiency of siRNA from the nanocomplexes. Although PEGylation can be important for the rational design of CP vectors, it remains a challenge to successfully synthesize PEGylated degradable CPs that possess high complexation ability, desirable safety profile, and high transfection efficiency.

Recently, we synthesized biodegradable cationic polylactides (CPLAs) through ring-opening polymerization (ROP) and thiol-ene functionalization, and further utilized them for delivery of siRNA to prostate cancer cells and pDNA to macrophage and fibroblast cells [37,38]. As compared with commercial gene delivery vectors (Fugene 6), CPLAs provided enhanced transfection efficiencies due in part to high complexation ability and degradability at physiological conditions. However, despite possessing promising transfection and low-toxicity properties, preliminary studies demonstrated that CPLA-based nanocomplexes suffer from problems of low stability and decreasing transfection efficiency with increased serum concentrations. Thus, to further enhance the potential clinical applicability of CPLAs for gene delivery, PEGylation of CPLAs is required to limit nonspecific interactions at physiological conditions.

In this study, we report the synthesis of well-defined poly(-ethylene glycol)-*block*-cationic polylactides (PEG-*b*-CPLAs) with