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Asymmetric polyplex-nanocapsules loaded with photosentisizer for light-assisted gene transfer

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ABSTRACT

Inefficient intracellular gene delivery is still a limitation for the clinical translation of gene therapy. Recently, photochemical internalization (PCI) has emerged with the opportunity to overcome *endo*-lysosomal sequestration in gene delivery, which utilizes photosensitizer (PS) plus light generating reactive oxygen species (ROS) at sub-lethal level to facilitate intracellularly targeted drug delivery. In this work, asymmetric polyplex-nanocapsules were prepared based on the triblock copolymers of PEG-PCL-PEI by using the simple solvent-injection method. Subsequently, the hydrophobic PS was encapsulated in the hydrophobic layer of polyplex-nanocapsules through hydrophobic interaction. The results from agarose gel electrophoresis and fluorescence scanning spectroscopy show that DNA could be condensed effectively and the PS was encapsulated, resulting in the stable polyplex-nanocapsules. The obtained polyplex-nanocapsules were characterized by transmission electron microscopy (TEM) and dynamic light scattering (DLS) measurements with the average size ranging from 200 to 280 nm and the negatively charged surface. Importantly, these polyplex-nanocapsules can be uptaken by Hela cells, resulting in improved gene transfection efficiency in comparison with the case without laser treatment due to the assistance of PCI effect. This work demonstrates a promising strategy to build the light-assisted gene delivery system containing PS and transporting genes simultaneously in one platform.

1. Introduction

Gene therapy has emerged as a potential therapeutical method for various types of human diseases such as genetic diseases, cardiovascular diseases and cancer. However, gene-based biotherapeutics encounters several challenges such as rapid elimination from the circulation, poor bioavailability, low cell permeability, and so on. Safe and efficient gene delivery system is important and necessary in order to achieve good outcome in gene therapy [1–3]. Although viral vectors have shown high transfection efficiency, some shortcomings such as immunogenic and susceptibility to enzyme degradation, have limited their clinical applications. Nonviral gene delivery vectors have attracted much attention, due to their improved safety profile and ease of preparation and manipulation [4,5]. In the past decades, polymeric nanoparticles, micelles and liposomes, which usually contain cationic segment, have been investigated as carriers for gene delivery in many researches [6,7]. Although cationic lipids or polymers are beneficial to the package of negative genetic material, the exposed positive charge is problematic for in vivo application.

According to the theory of polymer self-assembly, the amphiphilic block polymer with specific structure can self-assemble into vesicular nanocapsule (polymersome) in aqueous environment [8]. Like liposomes, polymersomes contain an aqueous interior that is separated from the outer fluids by hydrophobic membranes. The aqueous interior can be used to contain hydrophilic therapeutic drugs such as DNA, RNA and proteins, and hydrophobic intercalation can contain hydrophobic drugs. It is noted that the polymersomes have better stability than liposomes [9,10]. Recently, biodegradable polymersomes have been developed as efficient drug delivery systems to encapsulate small-molecular drug and/or protein [6]. However, little is reported on exploring polymersome-like nanocapsules as gene carriers. Dennis E. Discher et al. reported non-ionic polymersomes for delivery of siRNA and antisense oligonuclestide (AON) by using the amphiphilic block copolymers [11]. These non-ionic polymersomes exhibited efficient siRNA delivery into cancer cells in vitro as well as AON delivery into muscle in vivo, with similar pathways to polymersomes in delivering chemotherapeutic agents. Moreover, Liu et al. reported the preparation of chimaeric polymersomes by protein-induced self-assembly of asymmetric triblock polymer PEG-PCL-PDEA, co-loading with protein and anti-cancer drug to achieve highly efficient intracellular drug delivery [12].

In the process of gene delivery, the uptake of foreign materials

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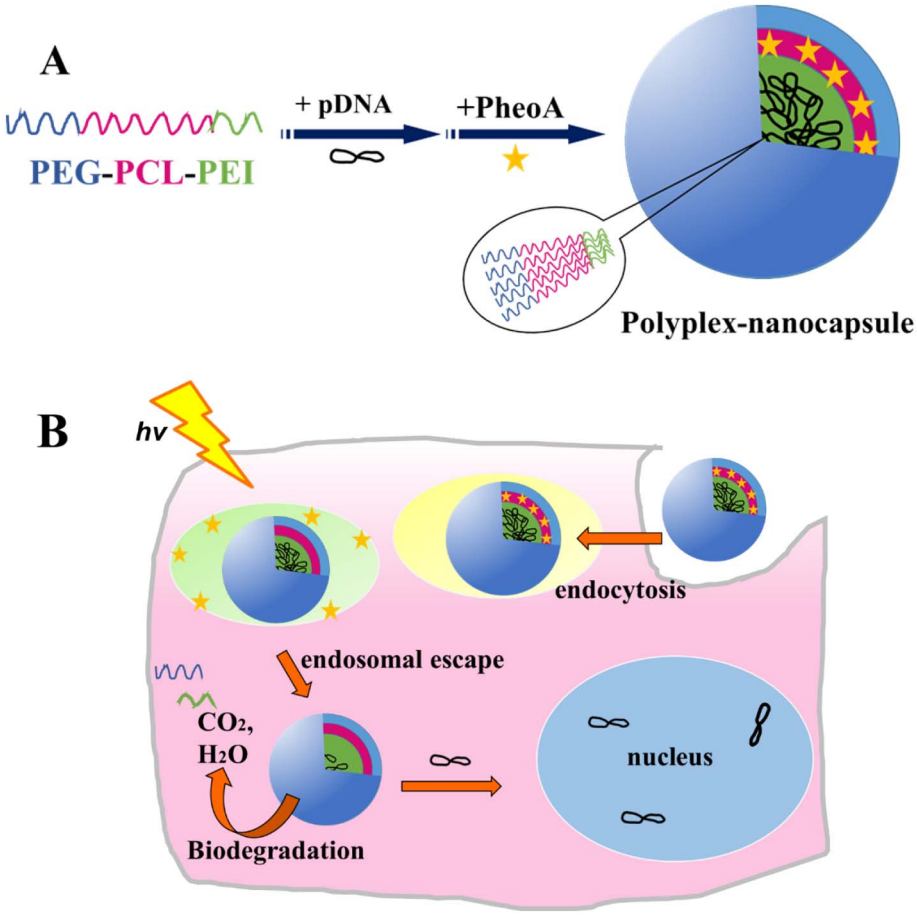

Scheme 1. Schematic presentation of asymmetric polyplex-nanocapsules with photosensitivity. (A) Nano-sized polyplex-nanocapsules were formed by PEG-PCL-PEI in aqueous pDNA solution via the simple solvent-injection method, followed by the loading of PS (PheoA); (B) Internalization of polyplex-nanocapsules, followed by the endosomal escape through PCI effect.

usually relies on the cell's innate endocytic uptake mechanism and the following inefficient endosomal escape is one of the main bottlenecks for efficient gene delivery. Photochemical internalization (PCI) has been utilized as a technique to improve intracellular delivery of therapeutic agents through destructing the *endo*-lysosomal membranes by reactive oxygen species (ROS) generated from activated photosensitizer (PS) [13–15]. However, the greatest challenge in systemic PCI-mediated gene transduction is the simultaneous delivery of the gene (plasmid DNA; pDNA) and the PS to the target cell [16–18]. Polymeric nanocaplsues provide a platform to incorporate both the pDNA and the PS.

Therefore, polyplex-nanocapsules with the similar structure as polymersomes were proposed in this study based on the ternary tri-block copolymers of poly(ethylene glycol)-b-polycaprolactone-b-poly (ethylene imine) (PEG-PCL-PEI) for highly efficient encapsulation and delivery of plasmid DNA (pDNA) into cells with the cooperation of photosensizers (PheoA) loading. As presented in Scheme 1, the complexation between cationic linear PEI segment and pDNA induced the formation of asymmetric nanocapsules, where the PEI block will located inside the nanocapsules together with pDNA, the hydrophilic PEG block will be preferentially oriented at the nanocapsules outlayer and the hydrophobic PCL block serves as hydrophobic intermediate domain to encapsulate the photosensizers by hydrophobic interaction. Here, photosensizers are introduced to exploit polyplex-nanocapsules as smart nanocontainers with tunable capability: light-assisted release of cargo genes into intracellular compartment by photochemical internalization (PCI) effect.

2. Materials and Methods

2.1. Materials

The ternary tri-block copolymer of PEG-PCL-PEI, designated as mPEG5k-PCL18k-lPEI2500, was synthesized as the method reported by T. Kissel's group [19]. Pheophorbide-a (PheoA) was purchased from Frontier Scientific, Inc. (UT, USA). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), Ethidium bromide (EB), bisBenzimide H 33342 trihydrochloride (Hochest 33,342), trypan blue, acetone and dimethyl sulfoxide (DMSO) were obtained from Sigma Co., Ltd. (USA). YOYO-1 and Lyso Traker Red were obtained from Invitrogen Molecular Probes (USA). The Dulbecco's Modified Eagle Medium (DMEM), penicillin–streptomycin, fetal bovine serum (FBS), 0.25%(w/v) trypsin-0.03% (w/v) EDTA solution and Phosphate buffer solution (PBS) were purchased from Gibco BRL (USA). Water was purified by distillation, deionization, and reverse osmosis (Milli-Q plus). All other reagents were analytical grades and used without further purification. Endotoxin-free GFP-encoding plasmid DNA (pCMV-GFP) was provided by Plasmid Factory (Bielefeld, Germany).

2.2. Cell Culture

The human cervical carcinoma cell line (Hela cell) was kindly donated by Professor Richard Dequan Ye (School of Pharmacy, Shanghai Jiao Tong University). The cells were grown in DMEM supplemented with 50 μg ml^{-1} penicillin/streptomycin and 10% (v/v) FBS in a 5% CO2/air incubator at 37 °C. Cells grown to confluence were subcultured every other day after trypsinized with 0.25% trypsin-EDTA and diluted in fresh growth medium.