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pH-Triggered DNA delivery based on multilayer film of DNA polyplexes and charge-reversible poly(ethylenimine)

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ABSTRACT

Development of materials with stimuli-responsive properties is of interest for biotechnical applications including gene delivery and regenerative medicine. Here, we report a multilayer film through layer-by-layer self-assembly of DNA polyplexes and charge-reversible poly(ethylenimine) (cPEI). Through functionalizing PEI with cyclohexanedicarboxylic acid, cPEI showed negatively charged and therefore was used for electrostatic self-assembly with positively charged DNA polyplexes. Side chains of cPEI can be hydrolyzed in acidic environment while it is stable in neutral condition. Such pH-triggered hydrolysis led to charge reverse of cPEI from negative to positive, which consequently led to a disassembly of multilayer film. Both UV–vis and ellipsometry spectrum measurements suggested that the multilayer film grew with a thickness of 150 nm for twelve bilayers. Under low pH condition, the multilayer film collapsed and DNA polyplexes were released. The multilayer film containing cPEI could be served as a local gene delivery system in specific low pH conditions such as extracellular acidity of solid tumor and lysosomal.

1. Introduction

Development of efficient gene delivery system is an active field in gene therapy. Viral vectors such as adenoviral and retroviral/lentiviral have been developed as highly efficient methods for gene delivery to a variety of tissues [1]. However, some inherent problems such as immune response significantly restrict its clinical use. Non-viral vectors that thereafter emerge as an alternative provide a versatile and simple approach for gene delivery [2]. Traditionally, DNA polyplexes are formed by condensation between DNA and cationic polymers (e.g., poly(ethylenimine), PEI) or lipids (e.g., Lipofectamine). DNA polyplexes with positively charged surface and highly stable structure proved to be effective for transfection of target cells [3]. In *in vitro* studies, DNA polyplexes are typically directly added into culture media, resulting in internalization of polyplexes and transfection of cells. However, in the case of *in vivo* applications, several barriers inhibit delivery of polyplexes to target cells such as reticuloendothelial cells, blood–brain barrier and immune system. Thus, the development of system for effective delivery of DNA polyplexes to target cells/tissues *in vivo* is a challenge. Substrate-mediated delivery of DNA polyplexes [4], also termed solid phase delivery or reverse transfection, have the potential to overcome the barriers *in vivo*. For instance, Shea et al. have reported series of studies, in which DNA polyplexes were retained at the solid substrates through specific or nonspecific interactions [4–6]. By means of it, DNA polyplexes could be immobilized on biomedical devices and be directly delivered to local environment of target cells.

Recently, we reported multilayer films containing DNA polyplexes and anionic polymers (e.g., poly(L-glutamic acid), PGA) through layer-by-layer (LbL) self-assembly [7–9]. LbL self-assembly is a powerful and simple tool to construct biomacromolecule-loaded films [10,11]. Typically, this technique allows control in nanoscale over the deposition of polyanions and polycations from aqueous solutions through electrostatic interactions. More important, it is in principle applicable to a variety of surfaces including biomedical devices, tissue engineering scaffold. We demonstrated that DNA polyplexes were stably incorporated into multilayer film with PGA or hyaluronan.

Designs of films that provide temporal and spatial control over DNA polyplexes disassembly under specific stimulus, such as pH, could significantly increase the efficiency of gene transfection. Previous studies demonstrated that charge-reversible polymers could be served as a trigger to functional disassembly and release of DNA or drugs [12–14]. Zhang and Lynn reported a polymer with amine-functionalized side chains, which can be degraded through a hydrolysis mechanism, resulting in charge reversal of polymer from positive to negative [13].

In this report, a charge-reversible PEI (cPEI) was used to form LbL multilayer film with DNA polyplexes. We synthesized cPEI by the reaction of 1,2-cyclohexanedicarboxylic anhydride with PEI. Because of amides with neighboring carboxylic acid groups [15], the cPEI can be pH-dependently hydrolyzed, leading to a negative-to-positive charge-reversal. Zeta potential and UV–vis measurements demonstrated that the DNA polyplexes/cPEI multilayer film was fabricated.

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In an acidic solution, the DNA polyplexes/cPEI multilayer film disassembled and the polyplexes were released locally. Such pH-triggered delivery of DNA polyplexes could be potentially applied in the low pH environments such as extracellular acidity of solid tumor (pH<7) and lysosomal (pH 4–5) [16].

2. Experimental section

2.1. Materials

Poly(ethylenimine) (PEI, branched, Mw 25000) and 1,2-cyclohexanedicarboxylic anhydride were purchased from Sigma. DNA (fish sperm sodium salt) was purchased from Bio Basic Inc. 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES, free acid) was purchased from Sangon Biotech (China). All other materials were used as received without further purification. All solutions were prepared by using Milli-Q purified water (>18.0 MΩ cm).

2.2. Synthesis and characterization of cPEI

As shown in Fig 1. cPEI was synthesized by following previous report with minor modification [16]. Briefly, both 0.7 g PEI and 1.6 g 1,2-cyclohexanedicarboxylic anhydride were dissolved in 15 mL dimethyl sulfoxide (DMSO). The reaction was kept for 72 h with continuous stirring and the protection of N_2 at room temperature. After reaction, the product was purified by the precipitation using diethyl ether. The purified product was then dried in a vacuum at 40 °C for 8 h. For characterization, the product was dissolved in DMSO-6D and HEPES buffer (20 mM HEPES with 20 mM NaCl, pH 7.4) for ^{1}H nuclear magnetic resonance (NMR) measurement (DMX500, Bruker) and the measurement of zeta potential (Delsa Nano, Beckman coulter), respectively. For characterization of pH-triggered hydrolysis, cPEI was dissolved in a HAc-NaAc solution at pH 4.0. The solution was kept at room temperature and measured for zeta potential at appropriate time intervals.

2.3. Formation and characterization of DNA polyplexes

The formation of DNA polyplexes was conducted as previously described [8]. DNA at 100 μg/mL and PEI at 65 μg/mL were dissolved in HEPES buffer (20 mM HEPES with 20 mM NaCl, pH7.4). DNA polyplexes were formed by adding an equal volume of DNA solution to PEI solution, followed by intense stirring and equilibration at room temperature for 20 min before being characterized or used for experiments. Transmission electron microscopy (TEM, Jem-1230, JEOL) was employed for imaging of DNA polyplexes at an accelerating voltage 80 kV. Briefly, a small drop of DNA polyplexes solution was deposited onto a carbon-coated copper grid. The sample was then fully dried for measurements. The zeta potential of DNA polyplexes was determined by laser Doppler anemometry (Zetasizer 3000HS, Malvern Instruments, Malvern, UK). Briefly, samples containing polyplexes were placed in an electrophoretic cell. The sample time was set automatically by instrument. Average values were calculated with the data from three runs. All measurements were carried out at room temperature.

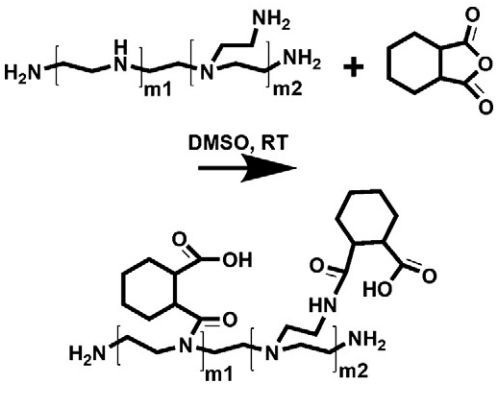

Fig. 1. Synthesis of the cPEI (charge-reversible PEI) through introduction of cyclohexanedicarboxylic anhydride to PEI.

2.4. Construction and characterization of (DNA polyplexes/cPEI) multilayer film

The (DNA polyplexes/cPEI) multilayer film was deposited onto the quartz substrates. Briefly, the layer of cPEI was adsorbed onto the surface of the substrate by immersing in the cPEI solution (pH 7.4) for 10 min. The substrates were then washed in water, followed by drying under a stream of N_2. DNA polyplexes were then attached to the cPEI layer by subsequently immersing the quartz substrate in the polyplexes solution for 10 min followed by a water wash. The process was repeated until a desired number of bilayers had been deposited. Experiments were carried out at room temperature. The buildup of multilayer film was followed by UV–vis spectrometry on a UV–vis spectrophotometer (UV-2505, Shimadzu) through measuring DNA absorbance at 260 nm. The thickness of multilayer film with different bilayer number was measured on an ellipsometry (M-2000, Woollam, USA). Continuous wavelength ranging from 124 to 1700 nm and angle of incidence of both 65° and 70° were chosen for the ellipsometry measurements. Δand Ψ values measured at wavelength of 600–1700 nm were chosen for data analysis. Cauchy model was used for determining the thickness of multilayer films. Parameters of A_n and B_n for Cauchy layer were set as 1.45 and 0.01 respectively as fit parameters. Then the thickness that best fit the multilayer films can be automatically calculated from ellipsometry instrument.

2.5. Characterization of multilayer film hydrolysis and release kinetics

The substrates coated with (DNA polyplexes/cPEI) multilayer film were incubated in the HAc-NaAc solutions (pH = 4.0, 5.0 or 6.0). The incubation solutions were then sampled at predetermined intervals for characterization of released polyplexes by the UV–vis spectrophotometer (UV-2505, Shimadzu). The surface topographical features of the multilayer film, before and after hydrolysis, were imaged by a scanning electron microscope at an accelerating voltage 5 kV (SEM, SiRion 100). For determining structure of DNA polyplexes that released from the multilayer film, the incubation solution was sampled and dropped onto a copper grid for characterization by a transmission electron microscopy (TEM, at an accelerating voltage 80 kV, Jem-1230, JEOL).

3. Results and discussion

3.1. Synthesis of charge-reversible cPEI

In the present study, we prepared multilayer film through LbL assembly of DNA polyplexes and anionic cPEI. The central hypothesis of our approach is the introduction of anionic cPEI with charge-reversible property. Negatively charged cPEI should insure the fabrication of LbL electrostatic self-assembly. In addition, through a pH-triggered hydrolysis of cPEI, it would reverse cPEI from the negatively to positively charge, which could lead to a disassembly of multilayer film. We selected branched PEI as polymer for modification for two reasons: (1) it has a dense amine groups for introduction of other functionalities, (2) PEI has been used as non-viral vector in many studies for DNA delivery.

PEI is a positively charged polyelectrolyte with a zeta potential of +37 mV [3]. We functionalized PEI through introduction of 1,2-cyclohexanedicarboxylic anhydride to PEI, as shown in Fig. 1. The 1,2-cyclohexanedicarboxylic anhydride reacted with amine group of