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Non-viral gene transfection in vitro using endosomal pH-sensitive reversibly hydrophobilized polyethylenimine

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ABSTRACT

Reversibly hydrophobilized 10 kDa polyethylenimine (PEI) based on rapidly acid-degradable acetal-containing hydrophobe was designed for nontoxic and highly efficient non-viral gene transfer. Water soluble PEI derivatives with average 5, 9 and 14 units of pH-sensitive 2,4,6-trimethoxybenzylidene-tris(hydroxymethyl)ethane (TMB-THME) hydrophobe per molecule, denoted as PEI-*g*-(TMB-THME)$_n$, were readily obtained by treating 10 kDa PEI with varying amounts of TMB-THME-nitrophenyl chloroformate. Gel retardation assays showed that all PEI-*g*-(TMB-THME)$_n$ derivatives could effectively condense DNA at an N/P ratio of 5/1. Notably, polyplexes of PEI-*g*-(TMB-THME)$_n$ derivatives had smaller sizes (about 100∼170 nm) and higher surface charges (+25 ∼ +43 mV) than the parent 10 kDa PEI at the same N/P ratios ranging from 10/1 to 40/1. MTT assays revealed that these PEI-*g*-(TMB-THME)$_n$ derivatives were practically non-toxic at polymer concentrations used in transfection experiments. The acetal degradation of PEI-*g*-(TMB-THME)$_9$ was shown to be highly pH dependent in which half lives of 1.3, 2.8 and 11 h were determined for pH 4.0, 5.0 and 6.0, respectively, while negligible hydrolysis (<12%) was observed after 24 h at pH 7.4. Gel electrophoresis, dynamic light scattering (DLS) and zeta potential analyses indicated that polyplexes formed at an N/P ratio of 10/1 were dissociated following 5 h incubation at pH 5.0, highlighting the importance of hydrophobic TMB-THME moieties in DNA condensation and supporting that acetal hydrolysis in endosomes would facilitate DNA release. Notably, *in vitro* transfection experiments performed at N/P ratios of 10/1 and 20/1 in HeLa, 293T, HepG2 and KB cells using plasmid pGL3 expressing luciferase as the reporter gene showed that reversibly hydrophobilized PEIs had superior transfection activity to 25 kDa PEI control. For example, polyplexes of PEI-*g*-(TMB-THME)$_{14}$ showed about 235-fold and 175-fold higher transfection efficiency as compared to 10 kDa PEI in HeLa cells in serum-free and 10% serum media, respectively, which were approximately 7-fold and 16-fold higher than 25 kDa PEI formulation at its optimal N/P ratio under otherwise the same conditions. Confocal laser scanning microscope (CLSM) studies confirmed that PEI-*g*-(TMB-THME)$_{14}$ efficiently delivered Cy5-labeled DNA to the nuclei of HeLa cells. These endosomal pH-sensitive reversibly hydrophobilized PEIs have great potentials for safe and efficient non-viral gene transfection.

1. Introduction

In the past decade, non-viral vectors in particular cationic polymers have gained rapidly growing interests for gene transfection in that they present several advantages over viral systems including facile synthesis and tuning of vector structures and properties, large DNA loading capacity, possible repeated administration, and reproducible large-scale pharmaceutical grade production [1–4]. Moreover, polymeric vectors can be modified to achieve prolonged circulation and deliver therapeutic genes to the targeted cells *in vivo* [5–10]. It should be noted, however, that despite their obvious merits, few polymeric systems have advanced to the clinical trials, primarily due to a low transfection activity as compared to the viral counterparts [11,12].

Polyethylenimine (PEI) is one of the most efficient non-viral gene carriers that are able to deliver DNA to a variety of cells due to its unique combination of high charge density and proton sponge

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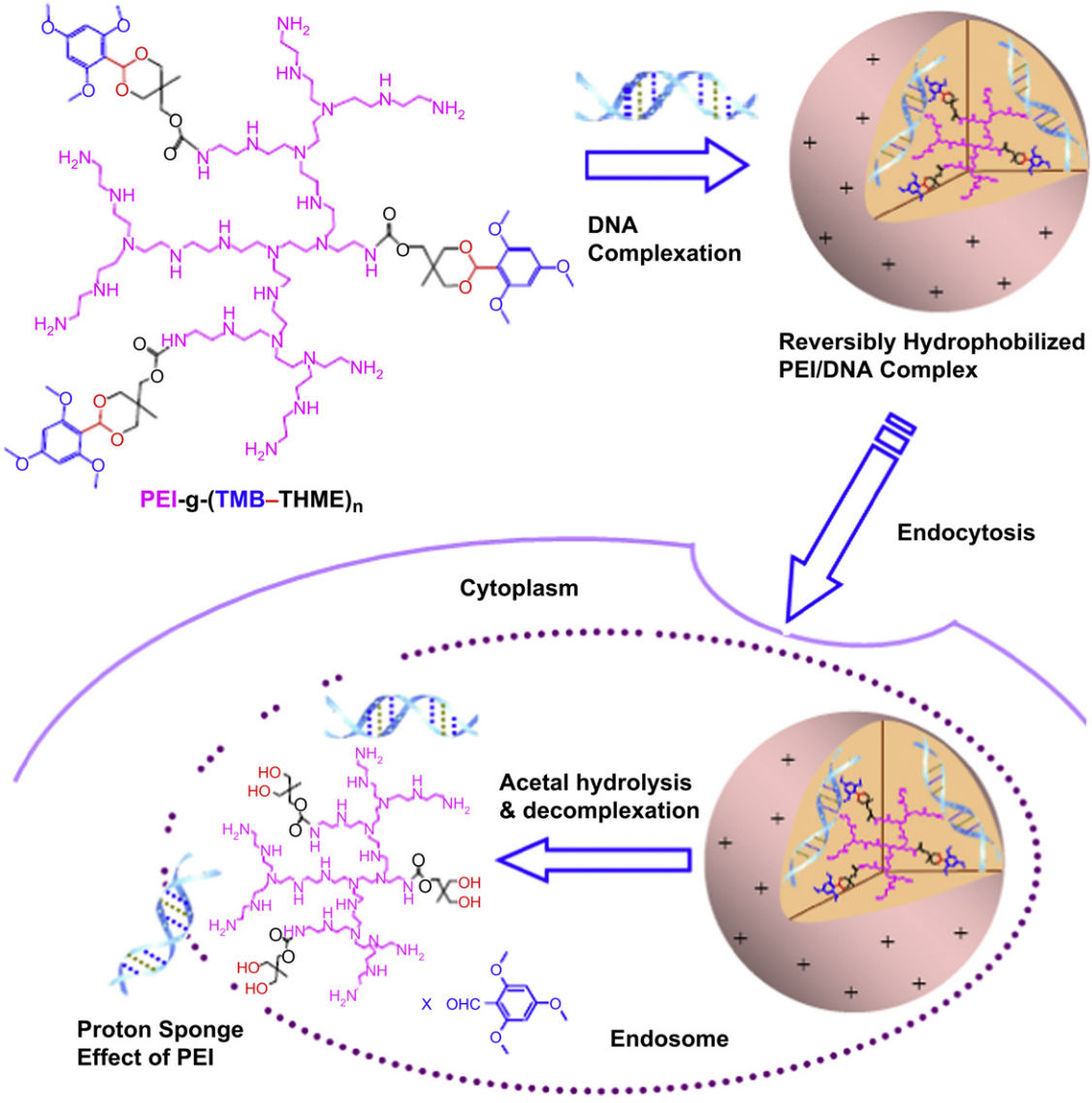
effect [13–15]. The transfection performance of PEI depends on its macromolecular structures and molecular weights, in which 25 kDa branched PEI (denoted as 25 kDa PEI) and 22 kDa linear PEI have turned out to be the best and are currently applied as golden standards for non-viral transfection [16,17]. These PEI reagents are, nevertheless, associated with varying levels of cytotoxicity and furthermore their transfection activity remains moderate as compared to viral vectors. In recent years, based on the fact that low molecular weight PEIs have low cytotoxicity, various types of hydrolytically or reductively degradable PEI polymers and networks have been designed for *in vitro* transfection [18–26]. These degradable PEIs have shown significantly enhanced transfection activity as compared to the parent low molecular weight PEIs, with transfection efficiency approaching or in a few cases exceeding that of 25 kDa PEI control. It should be noted, nevertheless, that the synthesis of degradable PEI polymers and networks are mostly not controlled due to the involvement of coupling reactions between reagents with multiple reactive centers, yielding usually ill-defined vectors in terms of structure as well as molecular weight. Hydrophobic modification represents another effective approach to improve the transfection activity of low molecular weight PEIs [27,28]. For example, Kim reported that cholesterol-modified 1.8 kDa PEI had higher transfection efficiency in CT-26, 293T and A7R5 cells than the parent 1.8 kPEI [29,30]. Klibanov and coworkers reported that dodecylation of 2 kDa PEI resulted in 5-fold higher transfection efficiency relative to that of 25 kDa PEI [31]. Uludag reported that substitution of 2 kDa PEI with aliphatic lipids including caprylic, myristic, palmitic, stearic, oleic and linoleic acids led to a transfection efficiency comparable to 25 kDa PEI [32]. Ramezani and coworkers reported that 10 kDa PEI after conjugation with alkyl-oligoamine had an increased transfection activity in N2A murine neuroblastoma cells to a level similar to that of 25 kDa PEI [33]. The enhanced transfection activity by hydrophobic modification is likely due to its balanced protection and release of DNA as well as enhanced interactions with cellular membranes [34].

In this paper, we report on reversibly hydrophobilized 10 kDa PEIs based on rapidly acid-degradable acetal-containing hydrophobe for nontoxic and marked enhanced non-viral gene transfection (Scheme 1). The commercial 10 kDa PEI has an approximate



Scheme 1. Illustration on reversibly hydrophobilized 10 kDa PEI for efficient intracellular delivery and release of DNA. Hydrophobic modification of 10 kDa PEI enhances its DNA condensation ability and cellular interactions while reversal of hydrophobic modification in endosomes facilitates intracellular release of DNA.