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Rare-earth-incorporated polymeric vector for enhanced gene delivery

Qiwen Wang [a,1], Weihong Jin [b,1], Guosong Wu [b], Ying Zhao [b], Xue Jin [a,b], Xiurong Hu [a], Jun Zhou [a], Guping Tang [a,b,**], Paul K. Chu [b,*]

[a] Institute of Chemical Biology and Pharmaceutical Chemistry, Zhejiang University, Hangzhou 310028, PR China
[b] Department of Physics & Materials Science, City University of Hong Kong, Tat Chee Avenue, Kowloon, Hong Kong, PR China

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ABSTRACT

Cationic polymer PEI-CyD is doped with Nd by plasma technology to produce the gene vector: Nd@PEI-CyD. Luciferase expression and EGFP transfection experiments performed *in vitro* reveal that Nd@PEI-CyD has significantly higher transfection efficiency than lipofectamine 2000 and PEI-CyD and the mechanism is studied and proposed. The rare-earth element, Nd, stimulates the energy metabolism of cells, enhances cell uptake of complexes/pDNA, and regulates the cellular pathways. These special features suggest a new strategy involving metal-incorporated non-viral gene vectors.

1. Introduction

In recent years, two kinds of gene delivery systems for gene therapies: viral and non-viral [1] have been widely reported [2,3]. Viral gene vectors have high efficiency, but serious safety risks such as immunogenicity, carcinogenicity and inflammation limit their clinical implementation [4]. In comparison, non-viral gene vectors with several advantages over viral ones such as low immunogenicity and toxicity, large DNA loading capacity, and tissue-specific targeting are typically less effective [2]. Therefore, it is critical to develop more efficient non-viral vectors for clinical applications [2,5].

Among the various types of non-viral vectors, polyethylenimine (PEI), especially high-molecular weight (HMW) PEI (~ 25 kDa), is a widely used cationic polymer known as the gold standard [6]. Although HMW PEI (~ 25 kDa) has high gene transfection efficiency, the high toxicity remains a drawback for application *in vivo* [7]. Our previous studies have shown that β-cyclodextrin-polyethylenimine(PEI-CyD), in which β-CyD is crosslinked by low molecular weight PEI (600Da), has lower cytotoxicity than PEI 25 kDa and similar transfection efficiency as PEI 25 kDa [8]. Our preliminary studies suggest that the gene transfection efficiency of PEI-CyD can be improved by surface modification of the polymer [9,10].

Metallic elements play vital roles in biological systems and activities, for example, bones and teeth [11], functional components of proteins [12], activators of enzymes [13], essential parts in the electron transport of the respiratory chain [14], and maintenance of normal functions in cell membranes [15]. In particular, rare earth elements (REEs) can change the mitochondrial metabolic activity [16,17], promote DNA synthesis [18], regulate the activity of calmodulin [19,20], and participate in a variety of physiological and biochemical processes [21]. Although REEs have been used to promote the growth and development of plants and animals [22,23], their use in gene vectors have seldom been explored.

In this work, the benefits of metallic elements in biological processes such as DNA transcription, mRNA translation [24–26] and surface modification of polymeric materials by ion beams and plasmas [27–29] are combined. The cationic polymer PEI-CyD is doped with a rare earth element, neodymium (Nd), to produce Nd@PEI-CyD complexes. To determine the effects of the plasma treatments, the chemical, physical, and biological characteristics of the Nd@PEI-CyD are investigated. The transfection efficiency of Nd@PEI-CyD is compared to that of PEI-CyD and the commercial transfection agent Lipofectamine 2000. To illustrate the underlying mechanism (Fig. 1), the ATP assay is performed to evaluate the

* Corresponding author. Tel.: +86 852 34427724; fax: +86 852 34420542.
** Corresponding author. Institute of Chemical Biology and Pharmaceutical Chemistry, Zhejiang University, Hangzhou 310028, PR China. Tel./fax: +86 571 88273284.
E-mail addresses: tangguping@zju.edu.cn (G. Tang), paul.chu@cityu.edu.hk (P.K. Chu).
[1] Both authors contributed equally to this work.

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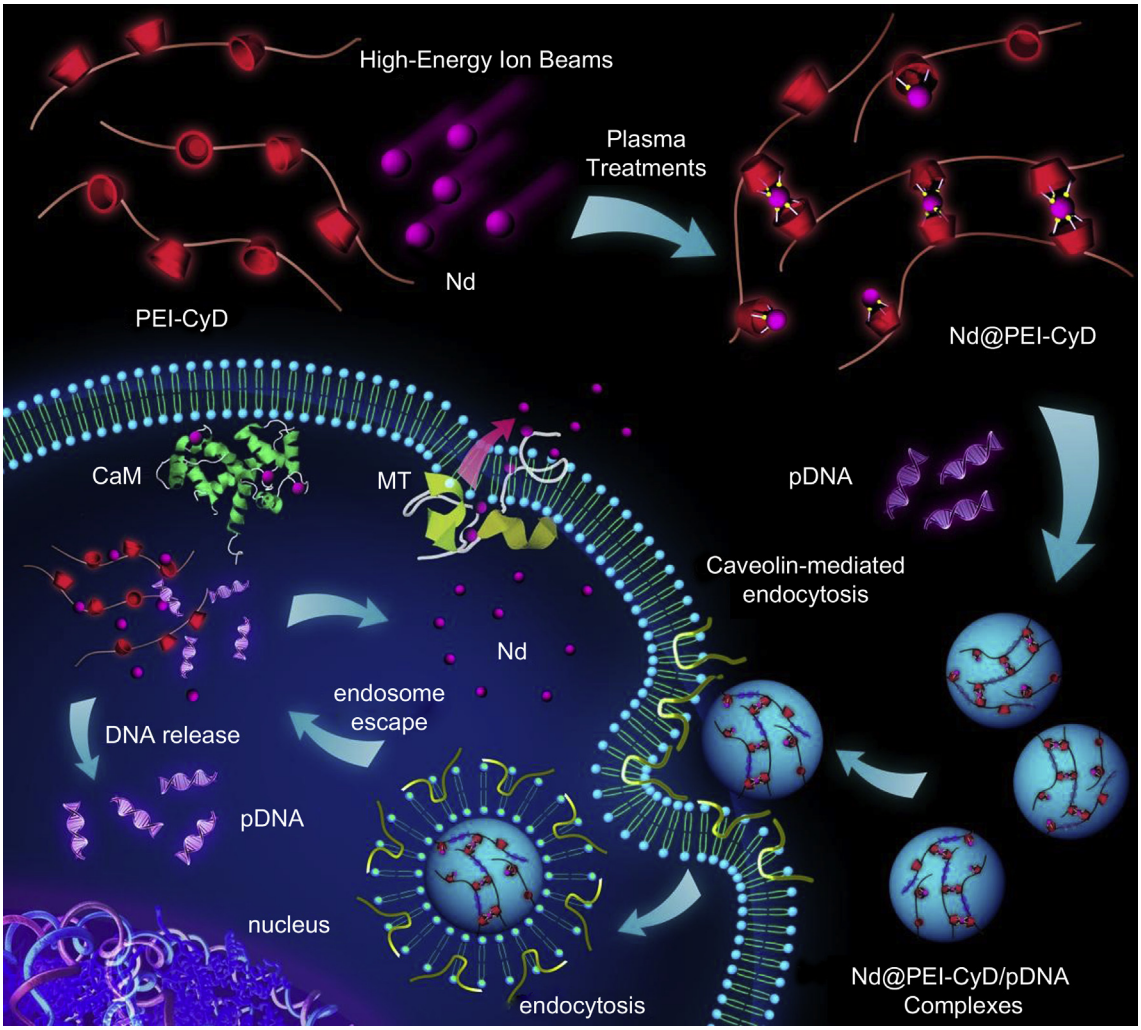

Fig. 1. Illustration of the synthesis of Nd@PEI-CyD and process of gene delivery mediated by Nd@PEI-CyD.

energy metabolism of cells and the reverse transcription-polymerase chain reaction (RT-PCR) and western blot analysis are conducted to assess several mRNA and proteins.

2. Materials and methods

2.1. Materials

Branched polyethylenimine (PEI; average 25 000 Da), linear polyethylenimine (average MW 600), β-cyclodextrin (β-CyD, MW 1135), 1,10-carbonyldiimidazole (CDI, MW 162.15), dimethyl sulfoxide (DMSO, ≥99.5%), N,N-dimethylformanide (DMF, ≥99%), [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (MTT, MW 218.1), phosphate buffered saline (PBS), and triethylamine (Et3N, 99%) were obtained from Sigma (St. Louis, MO, USA). DMF and DMSO were dried by refluxing over CaH2 and distilled before use. Calcium hydride (CaH2) was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

The plasmid DNA (pDNA) encoding firefly luciferase (pGL3-Luc) or green fluorescent protein (pEGFP) were purchased from Promega Corporation (Madison, WI, USA) and Guangzhou Jetway Biotech CO., Ltd. (Guangzhou, Guangdong, China). The BCATM protein assay kit and luciferase activity assay kit were purchased from Pierce Biotechnology, Inc. (Rockford, IL) and Promega Corporation (Madison, WI, USA), respectively. The ATP assay kit was purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, Jiangsu, China).

2.2. Production of the Nd@PEI-CyD

The PEI-CyD was synthesized as described previously [8]. The PEI-CyD films were prepared by dropping the solution containing 10 mg of PEI-CyD dissolved in 200 μL of deionized water onto a 1 cm × 1 cm silicon chip and dried in air overnight.

Table 1
Primers used in RT-PCR of selected gene transcripts.

Gene	Primer sequences	Annealing temperature (°C)
calmodulin	Sense primer: 5′-TGAGATAGGGTTCCTGGTTTG-3′ Antisense primer: 5′-GAGGGTGTAGGGTTTCTGGTT-3′	56
caveolin	Sense primer: 5′-TAGGATGCTCCCTTGTCGC-3′ Antisense primer: 5′-TGCTTCTCGCTCAGCTCGT-3′	56
metallothionein	Sense primer: 5′-CTCAACTTCTTGCTTGGGATC-3′ Antisense primer: 5′-AATGGGTCAGGGTTGTATGG-3′	56
GAPDH	Sense primer: 5′-AAGGTCGGAGTCAACGGATTT-3′ Antisense primer: 5′-AGATGATGACCCTTTTGGCTC-3′	56