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Gene delivery using dendrimer-entrapped gold nanoparticles as nonviral vectors

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ABSTRACT

Development of highly efficient nonviral gene delivery vectors still remains a great challenge. In this study, we report a new gene delivery vector based on dendrimer-entrapped gold nanoparticles (Au DENPs) with significantly higher gene transfection efficiency than that of dendrimers without AuNPs entrapped. Amine-terminated generation 5 poly(amidoamine) (PAMAM) dendrimers (G5.NH_2) were utilized as templates to synthesize AuNPs with different Au atom/dendrimer molar ratios (25:1, 50:1, 75:1, and 100:1, respectively). The formed Au DENPs were used to complex two different pDNAs encoding luciferase (Luc) and enhanced green fluorescent protein (EGFP), respectively for gene transfection studies. The Au DENPs/pDNA polyplexes with different N/P ratios and compositions of Au DENPs were characterized by gel retardation assay, light scattering, zeta potential measurements, and atomic force microscopic imaging. We show that the Au DENPs can effectively compact the pDNA, allowing for highly efficient gene transfection into the selected cell lines as demonstrated by both Luc assay and fluorescence microscopic imaging of the EGFP expression. The transfection efficiency of Au DENPs with Au atom/dendrimer molar ratio of 25:1 was at least 100 times higher than that of G5.NH_2 dendrimers without AuNPs entrapped at the N/P ratio of 2.5:1. The higher gene transfection efficiency of Au DENPs is primarily due to the fact that the entrapment of AuNPs helps preserve the 3-dimensional spherical morphology of dendrimers, allowing for more efficient interaction between dendrimers and DNA. With the less cytotoxicity than that of G5.NH_2 dendrimers demonstrated by thiazoyl blue tetrazolium bromide assay and higher gene transfection efficiency, it is expected that Au DENPs may be used as a new gene delivery vector for highly efficient transfection of different genes for various biomedical applications.

1. Introduction

Gene therapy is a technique using foreign nucleic acid (such as plasmid, Minivector DNA [1] or siRNA) as medicine to repair defective genes which are responsible for genetic disorders such as Parkinson's disease [2,3], cystic fibrosis [4], severe combined immunodeficiency [5], as well as cancer [6,7]. The success of gene therapy is largely dependent on the development of an ideal delivery system that can selectively and efficiently deliver genetic materials to target cells without causing any associated pathogenic effects [8,9]. Therefore, the main issue of gene therapy is not the cellular expression of an exogenous gene itself, but the development of safe and efficient gene delivery systems [10].

It is well known that the gene delivery systems include both viral and nonviral vector systems, and viral vectors usually exhibit high transfection efficiency [11]. However, the safety problems raised by the toxicity, oncogenicity, and immunogenicity of the viral vectors greatly hamper their routine use in both basic research laboratories and clinical settings [12–15]. Hence, nonviral delivery systems have continuously received considerable attention because they can be structurally varied, are relatively safe, and have an ability to carry large and diverse genetic materials into cells [10]. In general, the nonviral vectors need to overcome three major trafficking barriers when delivering the gene cargos: (1) the passage of DNA across the plasma membrane, (2) protection and release of the DNA molecules, and (3) the passage of DNA across the nuclear membrane (Fig. 1). Of all the three barriers, the biggest problem for the nonviral gene delivery system is the passage of the DNA across the nuclear membrane into the nucleus for expression, which has

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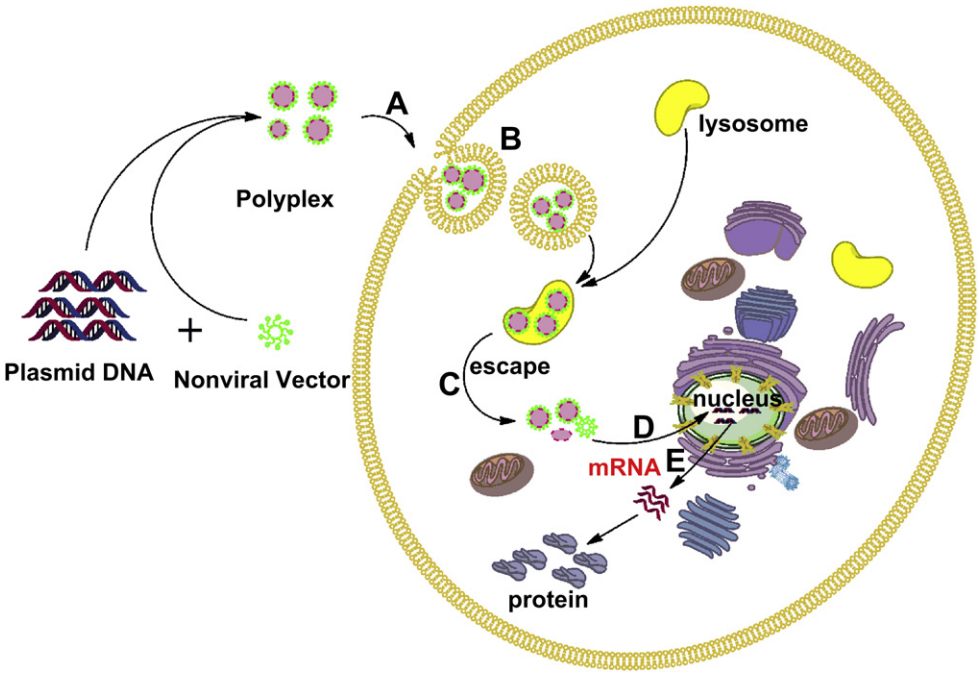

Fig. 1. Schematic illustration showing the three major trafficking barriers of gene delivery: (1) the passage of DNA across the plasma membrane, (2) protection and release of the DNA molecules, and (3) the passage of DNA across the nuclear membrane. The process can be divided into (A) DNA complex formation and cell binding, (B) cellular uptake and endocytosis, (C) escaping of the complex from endosome with limited stability, (D) cytosolic transit and nuclear entry, and (E) gene expression.

been identified as a rate-limiting step for gene transfection [16]. Consequently, development of highly efficient and less toxic gene carriers is the most challenging work in the field of nonviral gene therapy [17]. Cationic polymers are the commonly used nonviral gene vectors that have been extensively investigated due to their synthetic controllability and multivalent-functionalized surface amino groups, as well as their ability to compact nucleic acid [10,18–20]. For instance, cationic poly-L-lysine, polyethyleneimine, diethylaminoethyl-dextran, and chitosan have been proven to be able to transfect genes to different cell lines [18,21,22].

Dendritic polymers are well known by their well-defined three-dimensional molecular architecture with low polydispersity index and controlled surface functionalities based on stepwise synthesis using divergent or convergent method [23–26]. Among them, poly(amidoamine) (PAMAM) dendrimers are emerging as promising nonviral gene delivery vehicles [25,27]. The use of PAMAM dendrimers as a vector for gene delivery was first separately reported by the groups of Szoka [28] and Baker [29]. PAMAM-DNA complex between PAMAM dendrimers and plasmid DNA can be readily formed, and the DNA can be successfully transfected into different cell lines such as Hela, HepG2, K562, and Jukart cells. However, the application of PAMAM dendrimers as gene delivery vectors is quite limited because of their inherent cytotoxicity and low transfection efficiency. Much effort has been devoted to enhance their gene delivery efficiency and specificity while simultaneously decreasing the cytotoxicity of the dendrimers. It has been reported that partial PEGylation [30–33], acetylation [34], alkylation [35,36], and peptide-conjugation [37,38] of PAMAM dendrimers can greatly enhance their gene delivery efficiency and specificity and reduce their cytotoxicity.

Gold nanoparticles (AuNPs) have been identified as a suitable platform for drug/gene delivery due to their unique physicochemical properties, such as size- and shape-dependent optical properties, high surface area to volume ratio, and rich surface chemistry allowing for facile modification with different functionalities [39–45]. It has been reported that lysine dendron-functionalized AuNPs are 28-fold superior to polylysine in reporter gene expression [46]. The super gene transfection performance of the lysine dendron-functionalized AuNPs is likely due to the biomimetic design of the particles that has a size more or less similar to the nucleosome core proteins (~6 nm) having a large proportion of basic residues (lysine and arginine) that form electrostatic bonding with the phosphate backbone of DNA [47]. Therefore, through appropriate surface functionalization of AuNPs, a highly efficient and less toxic nonviral gene delivery vector may be developed for various biomedical applications.

For effective gene delivery, the nonviral vector should be able to effectively compact and condense DNA. Given the ability and advantage of dendrimers as a gene delivery vector and the remarkable role played by AuNPs in the gene delivery applications, it may be advantageous to combine both dendrimers and AuNPs within one particle system to enhance the gene delivery efficiency. Dendrimers are considered to be soft NPs with flexible molecular structures. A higher generation dendrimer (generation 4 or larger) often loses its original 3-dimensional (3D) spherical morphology upon interaction with solid surfaces or interfaces [48,49], thereby losing a significant portion of binding sites and possibly making its ability to compact DNA weakened. Our previous work has shown that AuNPs can be entrapped within the dendrimer templates [50–53]. It is expected that the entrapment of the rigid AuNPs within the interiors of dendrimers helps reserve the 3D spherical shape of the dendrimers, consequently featuring much more binding sites with DNA than dendrimers without Au cores [54]. Our hypothesis is that Au DENPs could be able to significantly improve the capability of dendrimers to compact pDNA, which is essential for improved gene delivery.

To prove our hypothesis, in this study, we synthesized Au DENPs using amine-terminated generation 5 PAMAM dendrimers ($G5.NH_2$) as templates with different Au atom/dendrimer molar ratios. The number of the primary amine groups of the $G5.NH_2$ dendrimers and Au DENPs was first determined, and then agrose-gel electrophoresis retardation assay was used to examine the