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Transfection and intracellular trafficking properties of carbon dot-gold nanoparticle molecular assembly conjugated with PEI-pDNA

Jinhwan Kim, Juhee Park, Hyunwoo Kim, Kaushik Singha, Won Jong Kim*

Center for Self-assembly and Complexity, Institute for Basic Science, and Department of Chemistry, Polymer Research Institute, Pohang University of Science and Technology (POSTECH), Pohang 790-784, Republic of Korea

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ABSTRACT

The work employs carbon dot (CD) which has been emerging as a fluorescent nanomaterial with excellent biocompatibility and perceived as a promising alternative to quantum dot (QD), to monitor the association/dissociation of polymeric carrier/plasmid DNA (pDNA) complex during transfection. To shed light on the underlying post-endosomal events and provide the insight to design rational and efficient gene delivery vector, the adopted strategy exploited the quenching of the fluorescence of CD by Au nanoparticles. The surface of CD and Au was modified with highly cationic polymer, polyethylenimine (PEI) and subsequent treatment with non-labeled pDNA gave rise to quenched delivery complex. High salt concentration triggered the dissociation of the complex with accompanied fluorescence recovery arising due to the increase in distance between CD and Au. The studies revealed the potential of the developed CD-PEI/Au-PEI/pDNA ternary nano-assembly as a highly efficient hybrid transfecting agent with high cell viability under the optimum condition. The changes occurred at the intracellular level during transfection especially post-endosomal step were monitored by fluorescence measurement using fluorescence microscope. This nano-assembly system was found to be very effective at monitoring the carrier/pDNA dissociation in a non-labeled manner, thus provides efficient strategy to study the mechanistic aspect of polymer-mediated pDNA delivery.

1. Introduction

The impetus to develop polymeric vectors for gene delivery has been drifted from viral vectors mainly due to the safety and less viable production concerns associated with viral vectors [1–5]. However, polymeric vectors are impaired significantly with low delivery efficiency compared to viral vectors [6–8], and thus to overcome diverse impediments plethora of research activities have been undertaken employing various strategies and hybrid integrated systems, to mention a few, polymer-inorganic particle hybrid system, crosslinking of polymer system, and so on [9–14]. Nevertheless, for practical clinical realization it is essential to find out rational design method for polymeric gene delivery system with high efficiency. To develop biologically viable and efficient delivery constructs, it is of paramount importance to decipher the underlying mechanisms of polymer-mediated delivery which comprises several extra- or intracellular events such as circulation in blood stream, cellular uptake, endosomal escape, and dissociation of cationic polymer/gene complex (polyplex) [15–17]. However, post-endosomal escape the dissociation of polyplex is considered as the rate-limiting step for efficient trafficking of gene to nucleus. A prompt and opportune dissociation of polyplex bears an immense importance in achieving high gene transfection efficiency, however, only a few work has highlighted this aspect [18,19]. Therefore, there is a pressing need to develop tools which can monitor the dissociation of polyplex in real-time without compromising the delivery efficiency and the safety issues.

In practice, organic dyes are employed to label polymeric vectors and plasmid DNA (pDNA) to study polymer/pDNA dissociation and trafficking [20], however, organic dyes generally show photobleaching and instability which render them inappropriate for monitoring of polyplex dissociation under biological setup [21]. As an alternative quantum dot (QD) which possesses excellent optical properties, such as good photostability, less photobleaching, narrow fluorescence spectra, and size-tunable emission has been used for labeling the materials [22–24]. Especially, its use in combination with organic dyes to obtain fluorescence resonance energy transfer (FRET) in constructing vector and pDNA complexes with distance dependent optical properties is worth mentioning and provides the provision to monitor association and dissociation of

* Corresponding author. Tel.: +82 54 279 2104; fax: +82 54 279 3399.
E-mail address: wjkim@postech.ac.kr (W.J. Kim).

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polyplexes [25–27]. However, such systems raise a detrimental biocompatibility issues due to the toxic nature of heavy metal which can be accumulated in the body and thereby elicit potential long term toxicity [28–31]. Furthermore, such systems require complicated modifications of nucleotide and dye-labeled pDNA and are known to impede intracellular transcription to mRNA; hence this approach is hard to apply in image-guided gene delivery system.

Recently, quantum sized carbon-dot (CD), which is composed of carbon and oxygen, and being passivated by polymer has been reported and the fluorescent nanomaterial shows excellent optical properties like high quantum yield and excitation wavelength dependent multi-color emission as well as environmental and biological compatibility [32–34]. Furthermore, its chemical inertness, good colloidal stability, and easy preparation promote CD as an appropriate alternative to QD. Above all, heavy metal-free CD shows low cytotoxicity which is one of the most important requisite for biological applications. Many strategies for synthesizing CD have been developed such as laser ablation, thermal carbonization, electrochemical preparation, and microwave-assisted pyrolysis [32,33,35,36]. In particular, microwave-assisted pyrolysis has been widely used due to its easy, fast, and environmental friendly green synthesis [36–40]. All these favorable optical, chemical properties and the facile synthesis render CD an enticing and promising candidate for bioimaging exploitations.

The aim of this study is to demonstrate the capability of enhanced gene delivery and real-time monitoring of intra-cellular movement and fate of the carriers and concomitant gene transfection utilizing polyethylenimine (PEI)-functionalized CD (CD-PEI) and PEI-functionalized gold nanoparticle (Au-PEI) nanohybrids. Herein, we develop a delivery system to monitor carrier/pDNA dissociation with intracellular gene delivery by modulating molecular assembly of nanohybrids.

Cationic fluorophore and cationic quencher should form a complex with negative charged pDNA through an electrostatic interaction, leading to the fluorescence quenching. Any suppression or lessening of the attractive electrostatic force is expected to facilitate the dissociation of the complex by allowing the cationic components to part with the negatively charged pDNA, and consequently the distance between fluorophore and quencher is increased more than the Foster distance due to charge repulsion, inducing the fluorescence recovery. Excellent optical property and biocompatibility make CD an enticing material and has been employed as a fluorophore after being surface functionalized with PEI, a prevalently used cationic polymer for gene delivery. Au-PEI is used as a quencher to investigate molecular assembly. We envisioned that pDNA induced complex formation between negatively charged pDNA and the CD-PEI and Au-PEI carriers, should trigger the quenching of the fluorescence arising from CD-PEI due to reduced distance between CD-PEI and Au-PEI. It is expected that upon internalization of the complex, pDNA should dissociate from the complex which is essential not only for initiating transfection but also for eliciting recovery of fluorescence from CD-PEI. Therefore, a simple measurement of the fluorescence changes at the intracellular level could enable us to monitor the association and dissociation of carrier/pDNA easily in real-time without any labeling of pDNA and further facilitate to achieve efficient gene delivery (Fig. 1).

As there is no effort that has been directed to utilize such molecular assembly system comprising Au-PEI and CD-PEI to accomplish gene transfection we consider this approach could provide significant insight onto its potential as delivery agent with unique ability to assist in monitoring of pDNA release after internalization.

2. Materials and methods

2.1. Materials

Glycerol (ultrapure, molecular biology grade) was purchased from Affymetrix (Cleveland, USA). Polyethylenimine (PEI, Mw = 25 kDa and 1.2 kDa) was obtained from Polysciences, Inc. (Warrington, UK). Gold chloride hydrate ($HAuCl_4$), sodium citrate tribasic dehydrate (NaCit), α-lipoic acid, potassium bromide (KBr), 1-ethyl-3-[3-(dimethylamino)propyl] carbodiimide hydrochloride (EDC), sodium chloride (NaCl), 4-(2-hydroxy-ethyl)-1-piperazineethanesulfonic acid (HEPES) buffer solution, and suberic acid bis(N-hydroxysuccinimide ester) (DSS) were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). N-hydroxysuccinimide (NHS) was obtained from Fluka (China). Acetonitrile (ACN, HPLC ultra gradient solvent) was received from J.T.Barker (USA). All commercial reagents were used without further purification. Dialysis membrane (MWCO: 25 kDa) was purchased from Spectrum Laboratories (Rancho Dominguez, CA, USA). TOTO-3 iodide was purchased from Invitrogen, Inc. (Eugene, Oregon, USA), and mounting medium for fluorescence was purchased from VECTOR (Burlingame, USA).

2.2. Synthesis of PEI-functionalized carbon dot (CD-PEI)

CD-PEI was synthesized by microwave-assisted hydrolysis of glycerol [40]. Glycerol (22 g) was mixed with PEI (0.5 g, MW = 25 kDa) and phosphate buffer (6 mL, 10 mM, pH = 7.4) and stirred vigorously overnight. Then, it was heated using commercial microwave oven (700 W) for 10 min, allowed to cool down. The sample was diluted with distilled water and dialyzed using cellulose membrane dialysis bag (MWCO = 25 kDa) against pure water for 3 days. The collected yellow product was lyophilized. The final product was dissolved in D.I. water and stored at 4 °C without any further purification.

2.3. Synthesis of citrate-capped gold nanoparticle (Au-cit)

Au nanoparticle was synthesized by reduction of $HAuCl_4$ at high temperature in the presence of citrate ion which is mild reductant and stabilizer [41]. 100 mL of

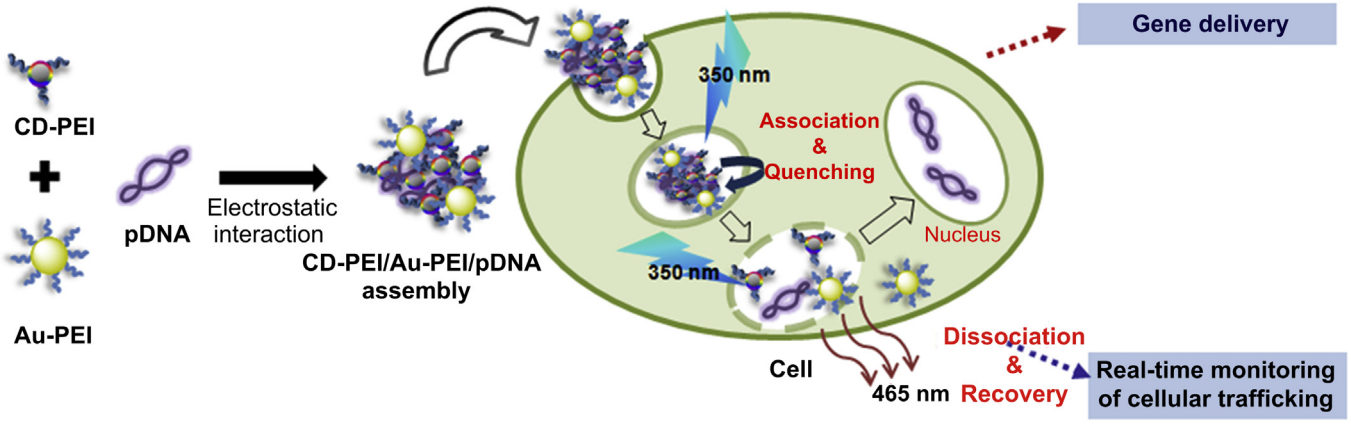

Fig. 1. A schematic illustration for the gene delivery and real-time monitoring of cellular trafficking utilizing CD-PEI/Au-PEI/pDNA molecular assembly of nanohybrids.