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A facile strategy to functionalize gold nanorods with polycation brushes for biomedical applications

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ABSTRACT

The fabrication of highly efficient nonviral gene carriers with low cytotoxicity remains a challenge in gene therapy. This paper reports a facile strategy to combine the advantages of gold nanorods (Au NRs) and polycations through surface functionalization. Different Au NR carriers with a controlled amount of poly(2-(*N*,*N*-dimethyl amino)ethyl methacrylate) (PDAEMA) brushes could be readily synthesized via surface-initiated atom transfer radical polymerization to achieve optimized nanohybrids for gene transfection. The obtained gene carriers demonstrate much higher gene transfection efficiency and lower cytotoxicity compared with polyethylenimine (∼25 kDa, gold standard of nonviral gene vector) in both COS7 and HepG2 cell lines. In addition, the potential of the PDMAEMA-grafted Au NR carriers to be utilized as a computed tomography contrast agent for the imaging of cancer cells has also been investigated. This strategy may realize the gene therapy and real-time imaging within one nanostructure and facilitate biomedical applications.

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1. Introduction

Gene therapy provides a promising way to cure cancer and genetic disorders while the development of efficient and safe gene vectors is still the main challenge. Many efforts have been devoted to the design of nonviral vectors such as cationic polymers and inorganic nanoparticles [1,2]. On the other hand, in situ and real-time imaging of living tissues without causing injuries remains a target in the diagnosis and therapy areas. Gold nanoparticles are attractive candidates for biomedical applications since they are biocompatible, nontoxic and easy to synthesize and functionalize [3–7]. Furthermore, gold nanoparticles could be employed as probes for cancer cell imaging [8,9], which makes them ideal models for multifunctional therapeutic and diagnostic platforms [10]. After suitable surface functionalization, gold nanoparticles could form inorganic/organic nanohybrids to construct highly efficient nonviral gene vectors. Recent studies have demonstrated that gold nanoparticle-based nanohybrids could protect DNA from nuclease degradation and target cells successfully without immunogenicity or cytotoxicity [11–13]. However, the strategy to functionalize gold nanorods (Au NRs) for gene vectors is still limited. Due to the high atomic number, electron density and X-ray absorption coefficient [14], gold nanoparticles could be used as contrast agents for computed tomography (CT) imaging of living tissues with high resolution. Although many nonviral gene vectors with different structures have been designed, ideal safe and efficient gene vectors have not been achieved yet. Cationic polymers synthesized up to now still have the limitation of either low efficiency or high cytotoxicity [15]. Therefore, the development of a more effective and safe gene delivery strategy for clinical applications is pressing.

Atom transfer radical polymerization (ATRP) has played an important role in the preparation of biopolymers with specific functions [16,17]. Various biopolymers with a narrow-molecular-weight distribution have been synthesized and applied in gene therapy. Poly(2-(*N*,*N*-dimethyl amino)ethyl methacrylate) (PDMAEMA) has been proved to protect the plasmid DNA (pDNA) and facilitate cellular transfection efficiently [18]. High-molecular-weight PDMAEMA exhibits excellent gene transfer efficiency but high cytotoxicity. Recently, we have demonstrated that novel comb and star shaped cationic gene vectors with high transfection efficiency and low cytotoxicity can be readily achieved via ATRP by linking PDMAEMA to polysaccharide backbones like hydroxypropyl cellulose [18], chitosan [19], dextran [20,21] and cyclodextrin [22,23]. In particular, we have successfully functionalized layered

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double hydroxide nanoparticles [24] and hydroxyapatite nanoparticles [25] with PDMAEMA for advanced gene delivery. These results have inspired us to explore the functionalization of gold nanoparticles with PDMAEMA brushes to produce advanced vectors for both gene therapy and diagnosis.

Herein, we report a facile preparation of organic–inorganic hybrid gene vectors with the function of CT imaging based on PDMAEMA-grafted Au NRs. Au NRs were selected as the platform since they demonstrated greater potential for imaging and cancer therapy compared with conventional spherical Au nanoparticles, probably due to their longitudinal plasmonic adsorption in the near-infrared (NIR) region and the difference in curvature and cellular uptake of different-shaped nanoparticles [10,26,27]. The Au NR–bovine serum albumin (BSA) conjugate is first constructed to improve the biocompatibility and stabilization of Au NRs [28] and introduce ATRP initiator [29] for the preparation of different PDMAEMA-grafted Au NRs (Au-g-PD) (Scheme 1). Then, the gene transfection and in vitro CT imaging have been investigated in detail through a series of experiments to demonstrate therapeutic and diagnostic applications of the obtained organic–inorganic hybrids.

2. Materials and methods

2.1. Materials

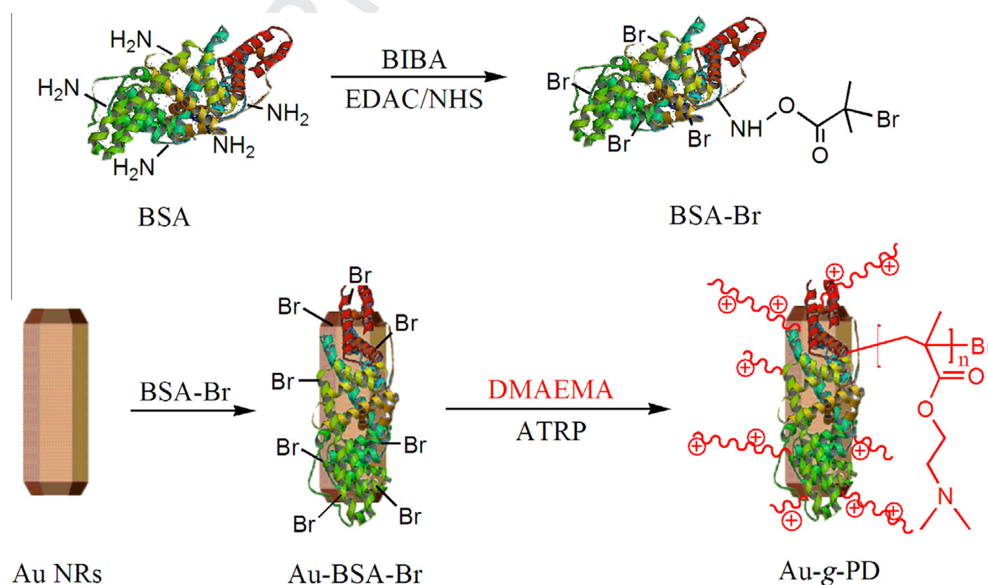
Chloroauric acid ($HAuCl_4$) and ascorbic acid (AA) were obtained from Sinopharm Group Co. Ltd. Silver nitrate ($AgNO_3$, 99.8%), *N*,*N*-dimethylformamide (DMF), sodium borohydride ($NaBH_4$, 98%), copper(I) bromide (CuBr, 98%), cetyltrimethylammonium bromide (CTAB, 99%), branched polyethylenimine (PEI, Mw ~25,000 Da), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDAC, 98%), *N*-hydroxysuccinimide (NHS, 98%), α-bromoisobutyric acid (BIBA, 98%), triethylamine (TEA), *N*,*N*,*N*′,*N*′,*N*″-pentamethyl diethylenetriamine (PMDTETA) and 2-(dimethylamino)ethyl methacrylate (DMAEMA, 98%) were obtained from Sigma–Aldrich Chemical Co., St Louis, MO. DMEAMA was used after removal of the inhibitors in a ready-to-use disposable inhibitor-removal column (Sigma–Aldrich). 3-(4,5-Dimethylthiazol-2yl)-2,5-diphenyl tetrazolium bromide (MTT), penicillin and streptomycin were purchased from Sigma Chemical Co., St Louis, MO. COS7 and HepG2 cell lines were purchased from the American Type Culture Collection (ATCC, Rockville, MD). The plasmid (encoding Renilla luciferase, pRL-CMV) was from Promega Co., Cergy Pontoise, France, and was cloned originally from the marine organism *Renilla reniformis*. The pDNA was amplified in *Escherichia coli* and purified according to the protocol of Qiagen GmbH, Hilden, Germany. The purity and concentration of the purified DNA were determined by absorption at 260 and 280 nm and by agarose gel electrophoresis. The purified pDNA was resuspended in tris–EDTA (TE) buffer and kept in aliquots of 0.5 mg ml^{-1} in concentration. All other chemicals were used as received and were of analytical grade.

2.2. Synthesis of Au NRs

Au NRs were synthesized using the typical seed-mediated growth method with minor modification [30]. Au seeds were prepared by chemical reduction of $HAuCl_4$ with $NaBH_4$. 2.5 ml of 0.2 M CTAB was mixed with 120 µl of 15 mM $HAuCL_4$ and 1 ml of water. Then, 0.5 ml of 10 mM ice-cold $NaBH_4$ solution was added to the mixture and the solution was stirred vigorously for 2 min. The Au seeds were placed in a water bath at 27 °C for 1 h before use. For growth solution, 25 ml of 0.2 M CTAB, 1.4 ml of 4 mM $AgNO_3$, 2.5 ml of 15 mM $HAuCl_4$ and 20 ml of H_2O were mixed to give a yellowish solution. Then, 620 µl of 0.0788 M AA was added and the solution turned colorless. Finally, 500 µl of Au seeds was injected and the growth solution was kept constant at 27 °C for 24 h. Au NRs were collected by centrifugation and had a longitudinal surface plasma resonance band at 790 nm in water.

2.3. Immobilization of ATRP initiator on the surface of Au NRs

Bromoisobutyryl-functionalized BSA (BSA-Br) was first synthesized in the following steps. BIBA (0.1 g) was activated in the presence of EDAC (0.29 g) and NHS (0.15 g) in 2 ml of DMF containing 500 µl of TEA at 30 °C for 4 h. Then, 0.2 g of BSA in 5 ml of PBS buffer was added and the resultant mixture was stirred at 37 °C for 48 h. BSA-Br was collected using lyophilization after being dialyzed against water using a dialysis membrane (molecular weight cutoff, MWCO 3500). The as-prepared Au NRs were purified by centrifugation at 12,000 rpm for 10 min, washed with water and



Scheme 1. Schematic illustration of the preparation processes of Au-g-PDs via ATRP.

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