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Integration of antimicrobial peptides with gold nanoparticles as unique non-viral vectors for gene delivery to mesenchymal stem cells with antibacterial activity

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ABSTRACT

Gold nanoparticles (AuNPs) have emerged as attractive non-viral gene vectors. However their application in regenerative medicine is still limited partially due to a lack of an intrinsic capacity to transfect difficult-to-transfect cells such as primary cells or stem cells. In current study, we report the synthesis of antimicrobial peptide conjugated cationic AuNPs (AuNPs@PEP) as highly efficient carriers for gene delivery to stem cells with antibacterial ability. The AuNPs@PEP integrate the advantages of cationic AuNPs and antibacterial peptides: the presence of cationic AuNPs can effectively condense DNA and the antimicrobial peptides are essential for the cellular & nucleus entry enhancement to achieve high transfection efficiency and antibacterial ability. As a result, antimicrobial peptides conjugated AuNPs significantly promoted the gene transfection efficiency in rat mesenchymal stem cells than pristine AuNPs, with a similar extent to those expressed by TAT (a well-known cell-penetrating peptide) modified AuNPs. More interestingly, the combinational system has better antibacterial ability than free antimicrobial peptides *in vitro* and *in vivo*, possibly due to the high density of peptides on the surface of AuNPs. Finally we present the concept-proving results that AuPs@PEP can be used as a carrier for *in vivo* gene activation in tissue regeneration, suggesting its potential as a multifunctional system with both gene delivery and antibacterial abilities in clinic.

1. Introduction

Gold nanoparticles (AuNPs) have emerged as attractive nonviral gene vectors in last decades, due to their ease of synthesis, tunable size and shape, flexible surface modification, and tunable optical and electronic properties [1–5]. For example, Mirkin et al. developed AuNPs-oligonucleotide nanoconjugates as effective intracellular gene regulation agents [6]. Several groups have demonstrated that polyethylenimine (PEI)-conjugated AuNPs are able to deliver plasmid DNAs (pDNAs) to various types of cells with comparable or even better efficiency to PEI molecules [7–10]. Wang et al. and Gunaratne et al. demonstrated that AuNPs are capable of delivering microRNAs and small interfering RNAs into cells and efficiently down-regulated target genes and modulated cell functions [11,12].

Although AuNPs based gene delivery vectors are effective for *in vitro* gene transfection, the efficiency is greatly reduced *in vivo*. It is well recognized that once the surface of NPs is covered by biomolecules (proteins, sugars, lipids and so on), upon the contact with biological systems, it results in the formation of a protein "corona" that is strongly associated with the NPs' surface. This process defines how living organisms "see" the NPs in a biological

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milieu [13–20]. Therefore, NPs covered by proteins with negative charge may aggregate and reduce their internalization in cells. In addition, it was reported that non-viral gene complexes enter the nucleus preferentially upon the disassembly of the nuclear envelope during mitotic cell division [21]. Therefore, AuNPs would be extremely inefficient when delivering DNA to the cells with slow mitosis cycle and low proliferation rate, such as normal tissue cells and stem cells [22–27].

Conjugation of ligands that are able to bind specific receptors on cell membrane have been demonstrated to enhance the targeting ability and the transfection efficiency of vectors [28,29]. Recently, functional peptides have been widely used in complex with genes or in combination with other transfer vehicles such as cationic polymers, due to their cell targeting ability, membrane destabilization activity and nuclear localization function [30–33]. For example, in our previous study, HIV-1 twinarginine translocation (TAT) peptide with a sequence derived from the "transduction domain" of Tat protein, was conjugated onto AuNPs. The obtained AuNPs@TAT integrated the advantages of AuNPs and peptides: the presence of AuNPs can effectively decrease the cytotoxicity of cationic molecules and condense pDNAs, while the cell penetrating peptides are essential for enhanced cellular and nucleus entry to achieve high transfection efficiency [34].

Antimicrobial peptides, known as natural antibiotics, can combat drug-resistant bacteria, mainly because their distinctive amino sequences can insert into and subsequently disintegrate bacterial cell surfaces [34–39]. They can insert into plasma membrane of human cells too, shielding light on their potential to facilitate cellular uptake of nanomaterials. Since the membrane of mammalian cells is different from that of bacteria in terms of chemical composition and surface charge, the interaction of antimicrobial peptides with plasma membrane of mammalian cells is much weaker than that with bacteria. Consequently, the cytotoxicity of antimicrobial peptides to mammalian cells is much lower than that to bacterial cells [40]. Besides, the extremely low concentration of peptides (lower than 0.1 μM *in vitro*) used for decoration of AuNPs, is 1–2 order of magnitude lower than that of the minimal inhibitory concentration of typical antimicrobial peptides, suggesting the safety to use antimicrobial peptides to functionalize AuNPs as gene delivery vectors. More interestingly, the combinational system may have antibacterial function, leading to a platform for both gene delivery and inhibiting infection. Since infection happens frequently in clinic, for examples, after injury or implant [41,42], such vectors possess a great potential for regenerative medicine. All these properties suggested that antimicrobial peptide may act as a promising component for the gene vector construction. However, to our best knowledge, using antimicrobial peptide as a part of gene delivery carrier has not been reported elsewhere.

Therefore, in current study, we use a facile method that uses antimicrobial peptides (PEP) to functionalize cationic gold nanoparticles. PEP is a peptide sequence from lactoferrin, an iron-binding protein from the innate immune system with anti-fungal and antibacterial properties. Positively charged PEI molecules are used to serve as capping agents to prepare AuNPs. Then the nanoparticles are further functionalized via partial ligand exchange with thiol groups of functionalized peptides (see Fig. 1 as a schematic illustration). The immobilized peptide amount on AuNPs can be adjusted via peptide feeding concentration during the ligand exchange process. To verify the versatility and feasibility of peptide-conjugated AuNPs as gene carriers, their transfection activity in bone marrow derived mesenchymal stem cells (MSCs), which has a wide application in tissue engineering, is investigated. TAT peptide conjugated AuNPs are used as positive controls (AuNPs@TAT). The cellular uptake and intracellular distribution of PEP conjugated AuNPs (AuNPs@PEP) are also studied to elucidate the gene delivery mechanism. Furthermore, the antibacterial effect of AuNPs@PEP was also evaluated *in vitro & in vivo*. Finally, the transfection ability of AuNPs@PEP *in vivo* was demonstrated with a full skin excision with infection model on rats.

2. Experimental section

2.1. Materials

$HAuCl_4$, polyethelyimine (PEI, Mw 25 kD), amiloride-HCl, amantadine-HCl, genistein, cytochalasin D (CytD) and 4′,6-diamidino-2-phenylindole (DAPI) were purchased from Sigma. Methylthiazoletetrazolium (MTT) was purchased from AMRESCO. TAT (H-Cys-Cys-Tyr-Gly-Arg-Lys-Lys-Arg-Arg-Gln-Arg-Arg-Arg-OH, Mw 1559 D) and PEP (H-Cys-Ala-Cys-Trp-Gln-Val-Ser-Arg-Arg-Arg-Arg-Gly-OH, Mw 1478 D) peptides with thiol functional groups were purchased from Sangon Biotechnology Inc., Shanghai, China. Micro-bicinchoninic acid (MicroBCA) protein assay kit was purchased from Beyotime Biotechnology Inc., Nantong, China. Ethidium bromide (EB) was purchased from Fluka. Plasmid DNA encoding luciferase (pDNA-LUC) was provided by the Institute of Infectious Diseases, Zhejiang University, China. Plasmid DNA encoding VEGF165 (pDNA-VEGF) was obtained from Flygene company (Hangzhou, China). The plasmid DNAs were stored at −20 °C until the transfection experiments. Sprague Dawley (SD) rats were supplied by Zhejiang Academy of Medical Sciences, China. VEGF ELISA kit was obtained from Boster Company (Wuhan, China). All other reagents were analytical grade and were used as received. Milli-Q water was used throughout the experiments.

2.2. Isolation and culture of MSCs

The isolation and culture of MSCs were performed as previously described [43]. Sprague–Dawley rats were supplied by Zhejiang Academy of Medical Sciences, China. All experimental procedures were in accordance with the Zhejiang University guidelines for the welfare of experimental animals. Briefly, rat femurs were cut away from the epiphysis, and bone marrow was flushed out using a syringe with Dulbecco's Modified Eagle Medium (DMEM, Gibco, USA) supplemented with 10% fetal bovine serum (FBS, Hyclone, USA), L-glutamine, penicillin (50 U/mL), and streptomycin (50 U/mL). The cell suspension was placed into a 25 cm^2 flask (IWAKI Glass Co. Ltd., Japan) and cultured at 37 °C in 5% CO_2. The medium was changed every 3 days. Subconfluent first passage cells were detached from the flask with phosphate-buffered saline (PBS) containing 0.25 wt% trypsin and 0.02 wt% ethylenediaminetetra acetic acid (EDTA) for 5 min at 37 °C. Subconfluent second passage cells were used for all experiments [44,45].

2.3. Preparation and characterization of antimicrobial peptide (PEP) modified AuNPs

Firstly, PEI capped gold nanoparticles (AuP) were synthesized by reduction of $HAuCl_4$ (150 μg/mL, 0.44 mM) with $NaBH_4$ (10 mg/mL, 260 mM) in the presence of PEI (3 mg/mL). The reaction mixture (50 mL) was vigorously stirred at room temperature for 15 min and then stored for at least 1 h. After centrifugation at 15000× g to remove excess PEI, the AuP (100 μg/mL) were mixed with TAT or PEP or TAT/PEP (1:1 w/w) mixture solutions to reach a variable final peptide concentration within 0–50 μg/mL. The solution (50 mL) was kept under constant stirring for 12 h to prepare AuP@TAT, AuP@PEP, AuP@TAT/PEP, respectively. The excess ligands were removed by dialysis using a membrane with cut-off molecular weight of 100 kD.

The MicroBCA method was used to quantify surface-