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Self-assembly of bacitracin-gold nanoparticles and their toxicity analysis

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ABSTRACT

As the widely use of gold nanoparticles (AuNPs) in drug delivery, the precise control on the size and morphology of the AuNPs is urgently required. In this scenario, traditional synthesis methods cannot meet current requirement because of their inherent defects. We have depicted here a novel method for fabricating monodispersed large size gold nanoparticles, based on the self-assembly of bacitracin. The AuNPs could be facilely, low-cost, and green synthesized with repeatability and controllability in this method. The Bac gold nanoparticles (Bac-AuNPs), composed by bacitracin core and gold shell, exhibited a spherical morphology in TEM and a face-centered cubic crystal structure in X-Ray diffraction and selected area electron diffraction. The mean diameter of the Bac-AuNPs was 89 nm. The nanoparticles were mono-dispersed and the zeta potential of the nanoparticles was 4.1 ± 0.64 mV. Notably, in cell viability assay, the Bac-AuNPs showed less toxicity to HepG2 cells and HEK293 cells compared to small size AuNPs. Collectively, the size, rheological characteristic and the biocompatibility supported the use of the gold nanoparticles as intracellular delivery vehicles for drug delivery, especially for tumor therapy. And this study could provide a maneuverable, controllable and green strategy for the synthesis of AuNPs, which would be applied in disease diagnosis and therapy with biosafety.

1. Introduction

The rapid development of nanotechnology has had an invaluable impact on the biomedical field. Nanoparticles have been utilized in therapeutic delivery (reviewed in [1]). Owing to their nanometer size, they exhibit large surface-to-volume ratios that can be leveraged in therapeutic delivery applications (reviewed in [2,3]). In the past decade, scientists have made great strides in the development of gold nanoparticle (AuNPs) based drug delivery systems, not only due to their nanometer size, but also their ability to bind multiple drug molecules [4], enhancing drug efficacy [5], the ability to control drug release *via* biological stimuli (internal) [6] or light activation (external) [6–9], as well as their noncytotoxic, nonimmunogenic, and biocompatible properties [10]. Except the application in drug delivery, gold nanoparticles, which possessing photothermal property, have also been applied in optogenetics in the therapy for nervous system diseases [11] and the adjuvant therapy for cancer [9].

The AuNPs with diverse morphology have exhibited diverse properties and been applied in various fields [9,12], and the spherical AuNPs are the most commonly used and researched AuNPs types. In different organs, spherical AuNPs with different size are required in transporting drugs. For example, 6 nm is the optimal size for nanoparticles the blood-brain barrier [13]. To cancer therapy, larger size of the nanoparticles are more applicable, because tumor vessels are more permeable to large molecules than many normal vessels [14], and tumors retain large molecules because of poor clearance [15]. For example, the pore cutoff size of tumor vessels is between 380 and 780 nm *versus* ~60 nm for normal tissues in maximum (except hepatic sinusoids) [16,17]. This enhanced permeability and retention (EPR) effect has been demonstrated as a key pharmacokinetic feature of nanomedicines, not only enhancing the efficiency of these agents, but also providing these agents with selective delivery properties that reduce their toxicity in normal tissues [18]. Most long-circulating liposomes and viral vectors proposed for therapeutic use, are between 100 and 300 nm, and could extravasate through the pores of microvessels [19]. Therefore, gold nanoparticles with a larger size, may be larger than 100 nm, could be better when they are designed as drug carrier for tumor therapy.

Kinds of method have been employed to the synthesis of AuNPs (reviewed in [20]), however, the precise control of particle size with a low polydispersity of spherical AuNPs remains difficult for particles larger than 30 nm. Though following a modified Turkevich citrate-reduction procedure, spherical citrate-stabilized AuNPs (xCS-AuNP), could be obtained with a maximum diameter about 100 nm [21], this method has its inherent defects that the colloidal concentration is low and the particle size distribution range is too wide, therefore the

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production of nanoparticles needs extra complicated purification and separation steps. And more, the synthesis procedure is energy exhausted and completed at high temperature. In this case, biotemplated synthesis of materials have gained more attention for the controllable formation of gold nanostructures with specific geometric characteristics [22]. The template-directed assembly method could operate at room temperature without energy consumption, provides a more efficient synthetic pathway through a one-step process and is effective in producing well-defined AuNPs [23]. Meanwhile, compared to the synthesis of solid AuNPs, the biotemplated method significantly reduced the cost, for gold can be deposited on inexpensive biotemplate cores and required in lesser amount than usual [24].

Bacitracin (Bac) is a group of 12 amino acids cyclic peptides produced by organisms of the licheniformis group of *Bacillus subtilis* var. Tracy. The simple structure of bac facilitates the control of the formation of nanomaterials. In acid solution, Bac tends to form a special spatial conformation, which keeps the hydrophobic parts buried internally [25], and exposes the divalent metal ions binding domain on the surface [26,27]. Bacitracin undergoes a self-assemble process during the co-incubation with metal salts solutions, which would further form unique morphologies according to the designs [28].

Here in this context, we designed a novel biotemplated strategy for the synthesis of gold particles by using Bac as the template, hoping to synthesis gold nanostructures with desired size, shape and properties. We compared the pH of reaction solution, the reaction incubation time, the reducing agent and the thermal stability of the product in order to find an optimal reaction condition. Finally, we inspected the morphology and property of the gold nanostructure in detail, and evaluated its biocompatibility in considering its practical application in drug delivery. This study could provide a maneuverable, controllable and green strategy for the synthesis of AuNPs, which would be applied in disease diagnosis and therapy with biosafety.

2. Materials and methods

2.1. Synthesis of AuNPs on Bac templates

0.7 mg of Bac was dissolved in 1 mL of acid solution (pH = 2.0) or weak alkaline solution (pH = 8.0) to obtain the 0.5 mM of template solutions. Then, 200 μL of aqueous $HAuCl_4$ solutions with a concentration of 2.5 mM were mixed with 200 μL of ready-prepared bacitracin solutions respectively, and the mixtures were kept at room temperature in an air bath shaking table (120 rpm) for 24 h. After the co-incubation, the freshly prepared NaBH4 (5 mM, 10 mM) was added dropwise, and then the color of solutions changed gradually from yellow to gray black which indicated that the Bac-AuNPs were fabricated successfully.

2.2. Characterization of Bac-AuNPs

The size of the samples was observed on a TEM (Hitachi-7650) operated at 80 kV. TEM samples were prepared by placing drops of gold nanoparticles (AuNPs) suspension onto carbon-coated copper grids. After 10 min, the excess liquid was removed with filter paper and the TEM grids were dried at room temperature. The chemical composition of the samples was characterized by EDS, which was carried out on an X-ray spectrometer (Oxford Link-ISIS) instrument operated at an accelerating voltage of 200 kV. EDS analysis is a typical microanalysis system with small irradiation range in which the electron beam can irradiate on targets, then the chemical composition of samples be analyzed by collecting the X-ray. XRD and SAED spectra were employed to examine the structure of the materials. They were also carried on JEM-2010 instrument operated at an accelerating voltage of 200 kV. Malvern Zetasizer ZS (Malvern Instruments, UK) was used to measure the sizes and surface zeta potentials of the prepared nanoparticles. Zeta potentials of the Bac-AuNPs were determined by electrophoretic mobility measurements. Absorption spectra were recorded using a WFZ- 26A UV–Vis spectrophotometer between 200 and 800 nm. Our sample was characterized by adding deionized water till 3 mL aliquots, and deionized water was used as the reference.

2.3. Biocompatibility of Bac-AuNPs

The cytotoxic activity of the Bac-AuNPs was evaluated using MTT assay. In brief, HepG2 cells/HEK 293 cells (1.9×10^4 cells/mL) were cultured for 24 h on 96-well microplates in 5% CO_2 incubator at 37 °C. The cells were incubated for 24 h with the Bac-AuNPs nanoparticles, Bac, and AuNPs ($HAuCl_4$ after reduction with $NaBH_4$). Bac was adjusted to pH 2.0 with HCl. All drugs with gradient concentrations (2.5×10^{-1}, 2.5×10^{-2}, 2.5×10^{-3}, 2.5×10^{-4}, 2.5×10^{-5}, 2.5×10^{-6} mM) were filtered using a 0.22 μm sterile filter. Then 10 μL MTT (5 mg/mL) was added and cultured for 4 h. Then formazan crystals were dissolved in DMSO for another 4 h, the absorbance at wavelength 560 nm was measured with a MK3 Microplate reader (Thermoelectric Instrument Co., Ltd). All experiments were performed in triplicate. Absorbance of untreated cells was set to be 100% cell viability.

3. Results and discussion

3.1. Effect of pH value on Bac-AuNPs formation

Controllable synthesis is of importance for the scale production of nanomaterial with programmed structures and properties. Theoretically, solution pH, incubation time, reaction temperature and reducing condition determined the synthesis process of AuNPs.

The template Bac is a kind of peptide with pI at 6.4, therefore the pH of the solution directly influences the charge status of bacitracin template as well as precursor, and eventually leads to various assembling forms of Bac and AuNPs. Thus, the morphologies of products are explored in the solutions of acid (pH = 2.0) or weak alkali (pH = 8.0). As shown in TEM images (Fig. 1A), in pH 2 solution, Bac was coated completely with AuNPs and presented a globular morphology with a diameter around 90 nm, the products were mono-dispersed and generally homogeneous. As expected, TEM observation reveals that in the acid environment, gold complex ions ($[AuCl_4]^-$) bound to bacitracin through electrostatic interactions. This is ascribed to the fact that when the pH value of aqueous solution is below the isoelectric point (pI = 6.4) of bacitracin, the template will possess more positive charges which are apt to combine with $[AuCl_4]^-$.

On the contrary, as shown in Fig. 1B, the irregular Bac-AuNPs in the aqueous alkali appeared stacking spherical chains-liked morphology with little metal particles on the surface. Such phenomenon is attributed to the higher pH value which leads to the formation of negative charges on the surface of bacitracin. It should be noticed that although massive metal particles cannot combine with the biotemplate through the electrostatic interactions, there are still a few composites formed (Supplementary Fig. 1). The main reason lies on that specific binding sites, possibly the lysine at the sixth amino acid of Bac acting as the electron donor, are exposed at the outside of Bac, which attracted the gold particles distinctively.

3.2. Effect of reduction agent concentration on Bac-AuNPs formation

A final reduction process is used to produce Bac nanoparticles with a uniform layer of gold, that is, a gold nanoshell. In the reduction process, the “seeded” gold particles which are covalently bonded to the Bac core serve as nucleation sites where an aged mixture of chloroauric acid is reduced in solution in the presence of sodium borohydride. This process forms a highly-crystallized gold shell through Oswald ripening. Therefore, the strength of reducing agents is crucial for the morphologies of nanomaterials. In order to investigate the effects of different