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Nanoparticles for multi-modality cancer diagnosis: Simple protocol for self-assembly of gold nanoclusters mediated by gadolinium ions

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ABSTRACT

It is essential to develop a simple synthetic strategy to improve the quality of multifunctional contrast agents for cancer diagnosis. Herein, we report a time-saving method for gadolinium (Gd^{3+}) ions-mediated self-assembly of gold nanoclusters (GNCs) into monodisperse spherical nanoparticles (GNCNs) under mild conditions. The monodisperse, regular and colloidal stable GNCNs were formed via selectively inducing electrostatic interactions between negatively-charged carboxylic groups of gold nanoclusters and trivalent cations of gadolinium in aqueous solution. In this way, the Gd^{3+} ions were chelated into GNCNs without the use of molecular gadolinium chelates. With the co-existence of GNCs and Gd^{3+} ions, the formed GNCNs exhibit significant luminescence intensity enhancement for near-infrared fluorescence (NIRF) imaging, high X-ray attenuation for computed tomography (CT) imaging and reasonable r1 relaxivity for magnetic resonance (MR) imaging. The excellent biocompatibility of the GNCNs was proved both *in vitro* and *in vivo*. Meanwhile, the GNCNs also possess unique NIRF/CT/MR imaging ability in A549 tumor-bearing mice. In a nutshell, the simple and safe GNCNs hold great potential for tumor multi-modality clinical diagnosis.

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1. Introduction

Nowadays, there is an increasing interest in the development of molecular imaging technology for applications in early-stage cancer imaging diagnosis [1,2]. Among various imaging modalities, near-infrared fluorescence (NIRF) [3], computed tomography (CT) [4] and magnetic resonance (MR) [5] imaging are three of the most widely used imaging techniques for disease diagnosis. NIRF imaging possesses high sensitivity. But it cannot guarantee an adequate spatial resolution [6]. CT imaging is a time-saving and low cost imaging technique with excellent density and spatial resolution. Despite of high-resolution images of anatomic structures, CT imaging is trapped in soft-tissue imaging applications due to the inadequacy of sensitivity [4]. Besides, MR imaging is a non-invasive technique with high sensitivity, good tissue penetration depth, and detailed profile ability of soft-tissues. But it is unable to detect lesions of bone structures [7]. Due to the limitations of the individual imaging technique discussed above, the use of dual mode or multimode imaging is indispensable for comprehensive and accurate cancer diagnosis, especially for early-stage cancer diagnosis [8–10]. To present day, the development of nanoparticle-based contrast agents provide a promising way to integrate the advantages of different imaging techniques for accurate cancer diagnosis and for extending the application scope of molecular imaging [11–14]. Nevertheless, most of the contrast agents used in the clinic

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are only suitable for single modality imaging. Meanwhile, complicated architectures and synthetic processes are always required to synthesize multifunctional nanoparticle-based contrast agents for multi-modality cancer diagnosis [11,15,16]. Thus, the development of a simple and time-saving protocol to synthesize a single nanostructure bearing various contrast agents for different imaging modalities would bring a great improvement towards an accurate cancer diagnosis.

Among the numerous nanoparticles described nowadays [12,13,17,18], the noble metal nanoclusters (NCs) are ultrasmall nanoparticles (with a core size smaller than 2 nm) with well-defined sizes, components and charges [19,20]. They possess strong luminescence and enhanced catalytic activity which make them especially attractive in biomedical areas for biosensing [21], bioimaging [22,23], and therapy [24,25]. Generally speaking, polymers, dendrimers, biological molecules or polyelectrolytes are the main templates used to synthesize NCs in solution [24,26–28]. Glutathione (GSH)-capped gold nanoclusters (GNCs) are one kind of noble metal nanoclusters that are synthesized using glutathione as thiolate ligand, and can be denoted as $Au_n(SG)_m$ (where n is the number of gold atoms, and m is the number of glutathione molecules) [29–31]. The GNCs composed of a dozen to about a hundred gold atoms possess many advantages for biomedical applications, such as excellent biocompatibility, good water solubility, faster clearance by normal tissues, longer retention time in the tumor, and easily functionalized surface ligands [26,32,33]. Moreover, the intrinsic characteristics of GNCs, red-near-infrared fluorescence, large two-photon excitation and excellent X-ray attenuation, make them highly suitable for both *in vitro* and *in vivo* NIRF and CT imaging [29,33–35]. Therefore, the GNCs may have a great potential for multi-modality cancer diagnosis. Nowadays, the lanthanide ion Gd^{3+} based contrast agents have been widely studied for cancer diagnosis [16,36,37]. But the development of sensitive, biocompatible, and stable Gd^{3+}-based contrast agents without the use of molecular gadolinium chelates still remains as an interesting approach and great challenge. Based on the qualities of GNCs and lanthanide Gd^{3+} ions, an exciting methodology for biomedical applications could be development of an effective and simple method to assemble GNCs and Gd^{3+} ions into a single multifunctional spherical nanoparticle for cancer multi-modality imaging. According to a previous report [38], the GNCs could be assembled into monodisperse and uniform spheres via selectively inducing interaction of electrons between divalent cations and negatively-charged GNCs. In this scenario, the GNCs can sever as a new family of ion mimics ("nanoions") in aqueous solution where the self-assemble process is analogous to the theory of formation of ionic crystals from real ions. Using the above theory, the hydrophilic GNCs could be assembled into monodisperse spherical shapes via the strong interaction of electrons between negatively-charged GNCs and trivalent counter cations of gadolinium. If properly implemented, this method to combine GNCs and Gd^{3+} ions would be a simple and time-saving approach to assemble them into a single nanoparticle for multi-modality tumor imaging.

Herein, we report the assembly of gold nanoclusters into monodisperse regular and stable spherical nanoparticles (GNCNs) by a selective electrostatic interaction for enhanced tumor multi-modality imaging (Scheme 1). This simple and time-saving electrostatic interaction occurs between negatively-charged carboxylic groups on the gold nanoclusters and trivalent cations of gadolinium (Gd^{3+}) in aqueous solution under mild conditions. The assembly behavior only occurs when the ratio of Gd^{3+} ions and carboxylate groups exceeds certain threshold values. Such behavior is different from the random aggregation of colloidal nanoparticles by counter ions. With the co-existence of GNCs and Gd^{3+} ions, the formed GNCNs exhibit an enhanced fluorescence, high X-ray attenuation and reasonable r1 relaxivity. All of these properties ensure that GNCNs could be used as NIRF/CT/MR multi-modality imaging contrast agents for accurate diagnosis of different types of tumor. The physicochemical properties of the synthesized GNCNs were extensively characterized via various techniques. In addition, both *in vitro* and *in vivo* experiments are carried out to carefully evaluate the biocompatibility, cellular uptake efficacy and tumor NIRF/CT/MR multi-modality imaging performance. We believe that the multifunctional spherical GNCNs are novel, simple and safe nanoparticles, which hold great potential for cancer NIRF/CT/MR multi-modality clinical diagnose.

2. Materials and methods

2.1. Materials

Tetrabutylammonium borohydride (TBAB; 95%) and Gadolinium chloride hexahydrate ($GdCl_3 \cdot 6H_2O$) were obtained from Aladdin Reagent Co. Ltd. (Shanghai China). Sephadex G-150 was purchased from Pharmacia (Uppsala, Sweden). 3-[4,5-Dimethylthiazol-2yl]-2,5-diphenyltetrazolium bromide (MTT), Gold chloride trihydrate ($HAuCl_4 \cdot 3H_2O$, 99%) and Hoechst 33342 were received from Sigma Chemical Corporation (St Louis, USA). The reagents related to cell culture were obtained from Hyclone. Ultrapure water with resistivity of 18.2 MΩ was used in the preparation of all aqueous solutions.

2.2. Synthesis of red-emitting GNCs

The procedure to synthesize red-emitting GNCs is similar to our previous study [24]. Briefly, the freshly prepared $HAuCl_4$ (5 mL, 0.02 M) and GSH (2 mL, 0.15 M) were diluted with 87 mL cold deionized water under vigorous stirring for 3 min. Then the mixture was added with icy cold TBAB solution (6 mL, 0.186 M) under a quickly vigorous stirring for 5 min. After the mixture was maintained for 2 h without vigorous stirring in ice bath, 1M HCl was added to adjust the pH of the reaction to about 3.0. Then the insoluble Au(I)−thiolate complex were separated by centrifugation. After another 12 h incubation of the mixture in ice bath, the supernatant was added with NaCl (to about 10×10^{-3} M) and cold methyl alcohol to collect the precipitates. The precipitates were dialyzed for three days against ultrapure water to obtain the GNCs.

2.3. Gd (III) ions-induced assembly of red-emitting GNCs

Before the reaction, the above prepared GNCs (1.25 mg/mL) and the $GdCl_3 \cdot 6H_2O$ in specific concentration were dissolved in the aqueous solution, respectively. Then the pH of the each solution was adjusted to about 6.5. The $GdCl_3$ solution was freshly prepared and sonicated for 3 min by an ultrasonicator at 100 W before used. Taking $R^{3+}_{[Gd]/[GSH]} = 0.08$ as an example, 0.25 mL of 0.73 mM $GdCl_3$ aqueous solution was added dropwise into GNCs aqueous solution (1.25 mg/mL, 1 mL) under stirring and maintained at 30 °C for 3 h. The gel filtration method (Sephadex G-150 column equilibrated with the ultrapure water) was used to purify the assembled GNCs (GNCNs). Assembly of GNCs by Gd^{3+} ions in other concentration was also synthesized in the similar steps.

2.4. Characterization

The size and the morphology of GNCNs were evaluated by transmission electron microscopy (TEM), high resolution TEM (HR-TEM) and field emission scanning electron microscopy (SEM). The composition of GNCNs was measured by energy-dispersive X-ray spectroscopy (EDX) and scanning transmission electron microscopy

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