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Concentration effect on large scale synthesis of high quality small gold nanorods and their potential role in cancer theranostics

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ABSTRACT

Cancer theranostics agents, such as gold nanorods, represent great potential in cancer therapy. However, the big size and the low yield of the gold nanorods reported previously have limited their clinical translation. Therefore, it is significant to develop a new method to prepare the small gold nanorods (width < 8 nm) at larger scale. In this report, a modified seedless method was proposed based on the effect of precursor concentration assisted synthesis of high quality small gold nanorods at large scale. The obtained small gold nanorods exhibit high quality and dimension of (18 ± 5 nm) × (5 ± 1 nm). After modified with biological compatibility reagents, the small gold nanorods behave excellent photoacoustic imaging and photo-thermal therapy ability. These results manifest that the obtained small gold nanorods not only realize the improvements of previously limitations, also are thus supposed to pave the way to cancer theranostics in clinic application.

1. Introduction

Cancer theranostics, which is the combination of imaging diagnosis and therapy, becomes an emerging therapy that has great potential in cancer therapy [1–4]. Generally, theranostics agents are indispensable for the aim to improve the diagnosis and therapeutic effect [5,6]. As a result, it is vital for the exploration and research of new theranostic agents [7–10]. Currently, more and more inorganic materials [11–13] and hybrid materials [14–16], which were used for delivery [17–19], therapy [20], biomimetic process [21,22] and so on, are developed for theranostics agents [23,24]. Among those developed theranostics agents [25–29], gold nanorods have been widely explored by the scientists [27,30–32]. Gold nanorods exhibit excellent properties including superior heat generation under the irradiation of laser, good biocompatibility and easy functionalization [30–34], which are available for both photoacoustic imaging and photo-thermal therapy. But as a matter of fact, most of the gold nanorods that act as photothermal agents are too large, specifically the width is > 8 nm and the length is about 40 nm [35]. On account of the larger size, these gold nanorods are not readily excreted and biodegradable, giving rise to the concerns about their long-term toxicity, which has ultimately limited their clinical translation [36,37]. Thus, the ideal way for safe translation of gold nanorods to clinical agents is to develop a large scale approach to synthesize high quality small gold nanorods (width < 8 nm) with tunable absorption [38].

Several different methods, *i.e.* seed and seedless method, have been developed to synthesize the gold nanorods [39]. Compared with other methods, the seedless method has several advantages [40,41]: (1) it is simple and beneficial for the gram scale synthesis of nanorods; (2) reproducibility is better because of no unstable seeds; (3) the width of the nanorods can be controlled < 8 nm, which is quite crucial for bio-application. When taking the seedless method, several parameters, *i.e.* pH value have been found to play an important role in the mono-dispersity of the small nanorods [39]. However, most of case are synthesized at a low gold concentration (< 0.5 mM) and the quality of the as-synthesized small gold nanorods is not that good. Recently, Richard et al. [42] have discovered that the highly concentrated reagent solutions will promote a robust, scalable, and size-focusing nanoparticle synthesis. In addition, high concentration is able to improve the volumetric production capacity, which greatly favors for economical fabrication at scale. Thus, it inspires us to investigate whether the favourable small gold nanorods with high quality can be prepared under highly concentrated reagent solutions at larger scale.

Herein, the gold nanorods were prepared by a seedless method at a higher gold concentration (2.5 mM). This method can be scaled up easily and the obtained gold nanorods present ultra-small size and high quality. After two-step ligand exchange, the PEGylated gold nanorods with good biological compatibility were well obtained. Furtherly, we investigated the bio-application of the as-prepared PEGylated gold nanorods, including the photoacoustic imaging, the photothermal

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conversion property and the photothermal ablation of the cancer cells *in vitro*.

2. Experiment

2.1. Materials

Chloroauric acid, Silver nitrate, Hexadecyltrimethylammonium bromide (CTAB, 99% purity), Sodium oleate (≥82% fatty acids (as oleic acid) basis, powder), Sodium borohydride (for the determination of hydride formers by AAS, ≥99%), L-Ascorbic acid (reagent grade), Poly(ethylene glycol) 2-mercaptoethyl ether acetic acid (SH-PEG-COOH, average Mn 2100) were purchased from Sigma-Aldrich and used as received without further purification.

2.2. Large scale synthesis of small gold nanorods

Small nanorods were synthesized by a modified seedless method, as described eleswhere [39]. In a typical procedure, 300 mL of CTAB (0.2 M) and 10 mL of Sodium oleate aqueous solution (0.1 M) were mixed well in a 1000 mL round flask by ultrasonic treatment for 30 min. Next, a homogeneous and transparent solution was obtained by stepwise mixing 300 mL of $HAuCl_4$ aqueous solution (5 mM), 16 mL of $AgNO_3$ solution (10 mM), 0.96 mL of HCl (37%) and 9 mL of L-Ascorbic acid (85.8 mM). After each step of mixing, the mixed solution was dispersed by ultrasonic treatment for 1 min. At last, the 3.6 mL of $NaBH_4$ aqueous solution (10 mM, ice cold) was injected by one time to the above mixture solution with gentle shaking. The resultant solution changed to deep brown within a few minutes and was kept in water bath at 30 °C for 4 h. The as-synthesized gold nanorods were purified by centrifugation at 11000 rpm and redispersed into Milli-Q water for next use.

2.3. Ligand exchange with SH-PEG-COOH

First step: The small gold nanorods, as above, were purified twice by centrifugation. Under stirring, 25 mg SH-PEG-COOH was dispersed in 2 mL DI water. Then, 50 mL small gold nanorods (1 mg/mL) were added to above SH-PEG-COOH solution with stirring for 24 h. The displaced CTAB and excessed SH-PEG-COOH were removed by centrifugation.

Second step: Firstly, 25 mg SH-PEG-COOH was dissolved in 2 mL the mixture of water and ethanol (90% *v*/v ethanol). After that, the above solution was introduced into the product of first step under gentle swirling. 24 h later, ethanol, unreacted SH-PEG-COOH and displaced CTAB were deprived by centrifugation and the gold nanorods coated with PEG were redispersed in DI water for the next biochemical experiments.

2.4. Characterization

Sizes, morphologies, and microstructures of the small gold nanorods were determined by a high-resolution transmission electron microscope (HRTEM; JEM-2010F). UV–visible absorption spectra were conducted on a Shimadzu UV-2550 ultraviolet-visible-near-infrared spectrophotometer using quartz cuvettes with an optical path of 1 cm. The concentration of gold nanorods in the solution was obtained by a Leeman Labs Prodigy high-dispersion inductively coupled plasma atomic emission spectroscopy (ICP-AES). The zeta potential of small gold nanorods before and after ligand exchange was measured by laser Doppler anemometry (Malvern Zetasizer). The temperature of the gold nanorods dispersed in water under the laser irradiation was recorded by the FLIR A300 thermal camera.

2.5. Photoacoustic imaging

For the small gold nanorods capable of photoacoustic imaging ability, 3% agarose was used as a tissue-mimicking phantom to mimic tissue scattering. Firstly, the small gold nanorods with different concentration (0, 2.5, 6.25, 12.5, 25 and 37.5 μg/mL) were mixed with agarose. After the agarose solidified, the photoacoustic imaging of the small gold nanorods were tested by a multispectral optoacoustic tomography system (iThera Medical).

2.6. Cytotoxicity assay

The *in vitro* cytotoxicity was evaluated using the methyl thiazolyl tetrazolium (MTT) assay in both normal (HUVEC) and cancer (human primary glioblastoma, U87 MG) cell line (purchased from Shanghai Institutes for Biological Sciences, Shanghai, China). Cells growing in a log phase were seeded into 96-well cell-culture plate at 5×10^4/well in DMEM at 37 °C with the presence of 5% CO_2 for 24 h, and then the cells were incubated with the small gold nanorods by different concentration (0, 5, 10, 25 and 50 μg/mL) for 12 or 24 h. Subsequently, 10 μL of MTT (5 mg/mL) was added to each well of the 96-well assay plate and incubated for another 4 h. After the addition of DMSO (150 L/well), Multiskan MK3 monochromator-based multifunction microplate reader was used to measure the absorbance of each well with background subtraction at 492 nm. The cytotoxicity was calculated through the percentage of material treated cell viability compared with untreated cells.

Hemolysis assays were carried out to evaluate the biocompatibility of small gold nanorods according to the previous work [43]. Firstly, mouse erythrocytes were obtained from health BALB/c nude mouse (Shanghai SLAC Laboratory Animal Co., Ltd., Shanghai, China). Then, 2% (volume ratio) packed red blood cells were mixed with deionized water, PBS, or small gold nanorods PBS dispersion (5, 10, 25 and 50 μg/mL) by volume ratio 0.4 to 1. Here, deionized water and PBS group are negative and positive controls, respectively. After that, the mixtures were incubated at 37 °C for one hour, followed by centrifugation at 3000 rpm for 5 min. At last, the absorbance of the supernatant at 540 nm was recorded by a Beckman Coulter DU 730 UV/vis spectrophotometer. Percentages of hemolysis were calculated using the equation: hemolysis% = (At − Anc)/(Apc − Anc) × 100%, where the At, Apc and Anc are the absorbance of the test, positive control and negative control groups, respectively, at 540 nm.

2.7. Photothermal ablation of cancer cells in vitro

Photothermal ablation of cancer cells of the small gold nanorods *in vitro* were carried out by two different methods (MTT assay and trypan blue staining method).

MTT assay: For the MTT assay, U87 MG cell line growing in a log phase were seeded into 96-well cell-culture plate at 5×10^4/well in DMEM at 37 °C and in the presence of 5% CO_2 for 24 h. Then the cells were randomly divided into four groups: control, gold nanorods, control with laser, and gold nanorods with laser. Next, the DMEM was taken out from the wells, and the cells were washed for three times with PBS. The 0.1 mL PBS and small gold nanorods dispersed in PBS (50 μg/mL) were then added into wells respectively. The cells of the PBS group and a small gold nanorods group were irradiated for 10 min using an 808-nm laser with an output power density of 2 W/cm^2. After incubated for 1 h, the cell viabilities were measured by standard MTT assays. All the tests were independently performed in quadruplicate.

Trypan blue staining method: U87 MG cells were plated in cell-culture dish at a density of 15,000 cells/mL in DMEM at 37 °C with the presence of 5% CO_2 for 24 h, prior to treatment. After 24 h of incubation, the U87 MG cells were divided to four groups: control, gold nanorods, control with laser, and gold nanorods with laser. The 0.1 mL PBS and small gold nanorods dispersed in PBS (50 μg/mL) were then added into wells respectively. The cells of the control with laser and small gold nanorods with laser groups were irradiated for 10 min using an 808-nm laser with an output power density of 2 W/cm^2. Subsequently, the additives in all