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Multi-talented applications for cell imaging, tumor cells recognition, patterning, staining and temperature sensing by using egg white-encapsulated gold nanoclusters

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ABSTRACT

We herein provide a facile and green strategy for the fabrication of red fluorescence gold nanoclusters (AuNCs@ew) with low toxicity, good stability and excellent fluorescence (λ_{em} = 650 nm, λ_{ex} = 320 nm) by mixing aqueous tetrachloroauric acid with diluted egg white under alkaline environment using microwave-assisted method. Confocal fluorescence imaging showed that the AuNCs@ew can enter living cells and distribute in the cytoplasm. In addition, the fluorescence of AuNCs@ew is sensitive to the response of Cu^{2+} and Hg^{2+} with a detection limit of 14.48 nM and 0.81 nM, respectively. Interestingly, we found that the fluorescent signal of the quenched AuNCs@ew-Hg(II) system could be recovered in the presence of the glutathione (GSH) in Tris-HCl medium (pH 6.5). This assay displayed a rapid response and highly selectivity toward GSH with a detection limit of 91.9 nM. More excitingly, the fluorescent signal of the AuNCs@ew-Hg(II) system (OFF) could be activated in living tumor cells (ON) such as HepG2 cells rather than in living normal cells (HT22 cells, OFF) without any exogenous GSH. The result showed that the system can be successfully applied for tumor cells recognition, which implied its great potential application in early prevention of cancer. Furthermore, the AuNCs@ew can also be used for patterning, staining and temperature sensing. We propose that the highly fluorescent AuNCs@ew can be eco-friendly and potentially large-scale development for versatile applications and so merits further study.

1. Introduction

Fluorescent nanomaterials, such as silica dots, semiconductor quantum dots, carbon dots and gold nanoclusters (AuNCs), have exhibited size-dependent tunable electronic, optical and mechanical properties, so they have drawn widespread attention in many fields including electronics, photonics, energy, catalysis, and medicine [1]. Among fluorescent nanomaterials, AuNCs becomes an object of keen scientific interest in recent years, owing to its advantages of long lifetime, large Stokes shift, biocompatibility and fluorescence from the visible to near-infrared (NIR) region [2–4]. Therefore, the excellent properties contribute the AuNCs for various applications, including solar energy harvesting, sensing, catalysis, bioimaging, drug delivery and photothermal cancer therapy [5]. Recently, water-soluble fluorescence AuNCs has been synthesized by various approaches. Approaches based on chemical reduction have been applied to prepare AuNCs in the presence of peptides [6] or proteins [7], where the proteins can act as both reducing and capping agents. Since the AuNCs was synthesized using bovine serum albumin by Xie et al. [8], inspired by this discovery, a number of researches have been carried out exploring new proteins (e.g. lysozyme [9], insulin [10] and transferrin [11]) for the synthesis of fluorescent gold nanoclusters. Meanwhile, their biosensing applications have already received great attention.

One of the most noteworthy applications of various probes is toward glutathione (GSH) selectivity and sensitivity, which is involved in many biological processes. For example, GSH plays the essential roles in the body's important antioxidant and free radical scavengers [12]. The content changes of GSH are likely a direct result of aging, Parkinson's disease, and the incidence of cancer [13,14]. A number of methods for the detection of GSH have been currently available described, such as capillary electrophoresis, mass spectrometry, high-performance liquid chromatography,

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and fluorescence spectroscopy [12,15,16]. Compared to the mentioned methods, fluorescence-based strategies get a lot of attention owing to its high sensitivity, simplicity, and short response time. Recently, Yang and co-workers designed a dye BODIPY-based fluorescence sensor to detect GSH in living cells [17]. But most of the dye derivatives are water-insoluble, the dyes often emit fluorescence less than 600 nm, and toxicity can not be ignored. In addition, quantum dots have also been used to measure the GSH [18], yet this probe has low sensitivity due to the high probe background signals. Tian et al. built up a BSA-capped AuNCs-Hg(II) system, which applied in GSH sensing in living cells [12]. Similarly, Zhang et al. concluded that the fluorescence intensity of L-histidine modified AuNCs showed difference in tumor cells with different GSH level [19]. Unfortunately, the preparation of above both AuNCs took a long time and the latter produced high fluorescence background signals.

Considering to the proteins being expensive, large-scale development of the application of AuNCs is limited. Recently there have been several studies on the inexpensive chicken egg white as the materials for red fluorescence AuNCs synthesis. For example, Li et al. demonstrated the application of AuNCs@ew for H_2O_2 sensing [20]. K. Selvaprakash et al. have further characterized the AuNCs@ew well and it was applied to the detection of ATP and PPi [21]. To our knowledge, the deep research on the AuNCs@ew is rare, involving in the optimal preparation conditions and its multi-talented applications. For instance, since most of the gold nanoclusters can be used for the detection of Hg(II), whether it can be made a test strip which is similar to a pH test strip? Since AuNCs-Hg(II) system can be used to detect GSH in living tumor cells, and as well know that GSH level is different between tumor cells and normal cells, whether the AuNCs-Hg(II) system can be used to distinguish between tumor cells and normal cells? In addition, since AuNCs is the water-solubility, luminescence and low toxicity, how can we use it in other ways?

In this work, we attempted to prepare a red fluorescence egg white protein-encapsulated gold nanoclusters (AuNCs@ew, λ_{em} = 650 nm) probe by a green, eco-friendly, simple and rapid process. Due to the advantageous properties of AuNCs@ew, such as low toxicity, good stability and excellent fluorescence, as-synthesized AuNCs@ew has been successfully applied for cell imaging, the applications in a Hg(II) detection test strip, patterning, staining and temperature sensing. More importantly, the AuNCs@ew-Hg(II) system (OFF) was founded to endow good sensitivity and selectivity between the tumor cells (ON) and normal cells (OFF), which showed the potential application in tumor cells recognition and may contribute to the early prevention of cancer.

2. Materials and methods

2.1. Materials

All the chemicals of the AuNCs synthesis and detection were of analytical grade and used without further purification. Throughout all the experiments doubly distilled deionized water was used and it was prepared using SZ series of automatic water distiller (Shanghai,China). Hydrogen tetrachloroaurate (III) tetrahydrate ($HAuCl_4{\cdot}4H_2O$) was bought from Tianjin, Fresh chicken eggs were obtained from a supermarket near our university. Glutathione reduced (GSH) was purchased from Shanghai. L-cysteine (Cys) was purchased from Solarbio (China), and tris(hydroxymethyl)-aminomethane (Tris) was bought from Tianjin. Sodium hydroxide (NaOH) was obtained from Beijing Chemicals (Beijing, China). Riboflavin 5′-monophosphate sodium salt hydrate was purchased from Aladdin (Shanghai, China). Stock solutions (0.01 mol L^{-1}) of all the cations and biological molecules (Na^+, K^+, Ni^{2+}, Pb^{2+}, Ba^{2+}, Cd^{2+}, Co^{2+}, Mg^{2+}, Zn^{2+}, Mn^{2+}, Ca^{2+}, Al^{3+}, Fe^{3+}, Cu^{2+} and Hg^{2+}; Lysine (Lys), Phenylalanine (PHE), Tyrosine (Tyr), Glycine (Gly), Dithiothreitol (DTT), glucose) were prepared by directly dissolving in doubly-distilled water.

2.2. Apparatus

The list equipments and apparatus used are as follows: the synthesis of AuNCs@ew was reacted in Discover SP (CEM, America), the UV–vis absorption spectra was obtained on a Cary 50 Bio UV–vis Spectrophotometer (America). Fluorescence measurements were carried out using a Cary Eclipse Fluorescence Spectrophotometer (Australia). The slit wavelengths for excitation and emission were both set as 5 nm. Transmission electron microscopy (TEM) image was obtained on a JEM-2100 (JEOL Ltd, Tokyo, Japan) at an accelerating voltage of 200 kV. Fourier transform infrared spectroscopy (FTIR) was recorded on a FTIR-8400S (Shimadzu Corporation, Kyoto, Japan). The size distribution of AuNCs was recorded on Malvern Nano Particle Sizer (ZETA Sizer, Nano-ZS90, England). X-ray photoelectron spectroscopy (XPS) data was obtained by using an AXIS ULTRA DLD electron spectrometer (Shimadzu Japan). X-ray diffraction (XRD) data was recorded on Bruker D8 Advance (Germany). The fluorescence lifetime was carried out by using the X Fluorescence Spectrophotometer (England). The relative fluorescence QY (Φ) of the AuNCs was calculated using the equation: $\phi_x = \phi_{std}F_xA_{std}\eta_x^2/(F_{std}A_x\eta_{std}^2)$. The optical densities were measured using a Cary 50 Bio UV–vis Spectrophotometer and a Cary Eclipse Fluorescence Spectrophotometer. Riboflavin 5′-monophosphate sodium salt hydrate in water was chosen as a standard with a quantum yield Φ_{std} = 26% at 370 nm. The absorbances of all the samples in a 1.0 cm cuvette were kept under 0.05 at the excitation wavelength to minimize re-absorption effects. The cellular uptake and the localization of materials in the intracellular spaces were visualized via laser confocal scanning microscopy. Images were obtained using a Confocal laser scanning microscope (LSM880+Airyscan, Zeiss).

2.3. Microwave-assisted synthesis of fluorescent ew-capped AuNCs

All vessels were washed with aqua regia (HCl/HNO_3 (V:V= 3:1)) and rinsed with doubly distilled deionized water for experiments. In a typical microwave-assisted synthesis experiment, egg white was separated carefully from the whole egg. 4.0 mL egg white was taken out and was diluted to 100 mL by doubly-distilled water, stirred and centrifuged. $HAuCl_4$ (2.5×10^{-3} M, 1.0 mL) was added to a 2 mL resultant clear egg white solution. The mixture was thoroughly mixed by shaking for 5 min, followed by 40 μL of 0.1 M NaOH solution with continuous shaking for 2 min. Then the solution was added in the microwave vessel and was placed in reaction chamber and sealed. The microwave apparatus was set at 150 W, 90 °C, and 250 Psi and then was turned on to react for 30 min with high stirring rate. The mixture was cooled to room temperature in the equipment quickly and it changed from turbid light yellow to clear yellow. Experiments were carried out in different reaction temperature, time and concentration to determine the optimum conditions. Without any megascopic impurities after centrifugation, the solution was stored at 4 °C for further use.

2.4. Specificity investigation

In a typical test, 200 μL of the as-prepared ew-capped AuNCs (AuNCs@ew) and 800 μL of buffer solution of pH 6.5 (0.1 M Tris-HCl) were added to 1.0 mL doubly-distilled water containing various metal ions, such as Na^+, K^+, Ni^{2+}, Pb^{2+}, Ba^{2+}, Cd^{2+}, Co^{2+}, Mg^{2+}, Zn^{2+}, Mn^{2+}, Ca^{2+}, Al^{3+}, Fe^{3+}, Cu^{2+} and Hg^{2+}, respectively. In order to evaluate AuNCs@ew on the sensitivity and selectivity of ions, after two