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A visual physiological temperature sensor developed with gelatin-stabilized luminescent silver nanoclusters

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ABSTRACT

A visual physiological temperature sensor was successfully developed with newly hydrothermally prepared fluorescent silver nanoclusters (AgNCs) at room temperature using gelatin as the protective and reducing agent. The as-prepared gelatin-stabilized AgNCs was water-soluble, uniform and exhibited a narrow distribution with an average size of 1.16 nm, showing a maximum emission band at 552 nm (2.45 eV) when excited at 445 nm (2.79 eV). The large Stokes shift of 110 nm of the gelatin-stabilized AgNCs makes it actually applicable with very low background and light scattering interferences. It was found that the as-prepared gelatin-stabilized AgNCs is temperature-sensitive over the range from 5 °C to 45 °C, and thus a visual physiological temperature sensor could be developed with the gelatin–AgNCs as under the irradiation of visible light.

1. Introduction

Temperature is one of the most important physical factors for the temperature-dependent systems, such as environment and organism. In life system, the temperature is closely related to the physiological state, and any change of temperature could prompt our bodies to suffer from some diseases. Up to now, numerous temperature sensors have been developed and widely applied in environmental temperature measurement, biomedical sensing, and thermal monitoring of microprocessors [1]. Of these sensors, optical temperature sensing based on the luminescence properties (e.g., excited state lifetime or emission intensity) is a versatile technique to monitor the local temperature [2]. Quantum dots (QDs) [3], inorganic phosphors [2], and organic dyes [4] have been employed for the temperature sensing and indicating. However, the further applications of these luminescent materials in environment or organism are limited because the fluorescence emissions at these cases need stable illuminated and non-toxic fluorescence probes.

Fluorescent noble metal nanoclusters (NCs, i.e., AuNCs, AgNCs, and PtNCs), consisting of several to tens of metal atoms and possessing size comparable to the Fermi wavelength of electrons, have been the subject of intense research for their widely potential applications in bioimaging [5,6], biosensing [7–9], and catalysts [10,11]. Among these clusters, low-nuclearity AgNCs are particularly attractive candidates for their low toxicity and potential to combine small size and potentially ultrabright emission in a variety of scaffolds [12]. Up to now, a lot of efforts have been made to direct the synthesis of fluorescent and water-stable AgNCs with the help of various stabilizers, including polyelectrolytes [13–15], thiolates, [16,17] and biomolecules [18–21]. Especially, proteins or peptides have attracted increasing attention in the synthesis of fluorescent AgNCs due to their excellent biocompatibility and abundant functional groups [20,21].

Herein, we firstly report a novel, rapid one-step synthesis of fluorescent AgNCs starting from Ag^+ using gelatin as protective and reducing agent, the as-prepared gelatin-stabilized AgNCs have a Stokes shift of 110 nm, which is very large and makes the gelatin-stabilized AgNCs makes it actually applicable with very low background and light scattering interferences. In principle, Ag^+ is reduced to Ag^0 by the phenolic group of tyrosine under alkaline conditions and the formed AgNCs are captured by the –SH group of cysteine [21]. Gelatin significantly restricts the size of AgNCs during the nucleation and growth processes [22], and the application of gelatin for AgNCs preparation prompts an new easy one-step synthesis route without any additional reagents compared with previous reports [23], wherein green-emitting AgNCs have successfully prepared with dihydrolipoic acid (DHLA) as an etching ligand to remove the Ag atom on the surface of gelatin-protected Ag nanoparticles (AgNPs). Noteworthily, the as-prepared gelatin–

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AgNCs could respond to physiological temperatures with reversibly and sensitively.

2. Experimental

2.1. Materials

Gelatin from bovine skin (Type B) was purchased from Dingguo Changsheng Biotechnology Co., Ltd. (Beijing, China) and $AgNO_3$ was obtained from Shanghai Shenbo Chemical Co., Ltd. (Shanghai, China). Other reagents were of analytical reagent grade. Mili-Q purified water (18.2 MΩ cm) was used to prepare solutions throughout the experiment.

2.2. Apparatus

The fluorescence spectroscopy and absorption spectroscopy were performed with an F-2500 fluorescence spectrophotometer (Hitachi, Tokyo, Japan) and a UV-3600 spectrophotometer (Hitachi, Tokyo, Japan), respectively. A high resolution transmission electron microscope (Tecnai G2 F20 S-TWIN, FEI Company, USA) with an accelerating voltage of 200 kV was used to record the high-resolution TEM (HRTEM) images. Atomic force microscopy (AFM) images were recorded on a Dimension Icon Scan Asyst atomic force microscope (Bruker Co.) Elemental and functional group analysis were carried out using an ESCALAB 250 X-ray photoelectron spectrometer (XPS) and a FTIR-8400S Fourier transform infrared spectrometer (FTIR, Toyota, Japan), respectively. LabRAM HR800 Laser confocal Raman spectrometer was used to record the Raman spectrum of nature gelatin and gelatin–AgNCs. An FL-TCSPC fluorescence spectrophotometer (Horiba Jobin Yvon Inc., France) measured the fluorescence life time of gelatin–AgNCs. Dynamic laser light scattering (ZEN3600, Malvern) was used to characterize the zeta potential on the surface of AgNCs.

2.3. The synthesis of luminescent gelatin–AgNCs

Gelatin–AgNCs were efficiently synthesized by one-pot. Briefly, 20 mg/mL of gelatin was mixed with 30 mM of freshly prepared $AgNO_3$ and the aqueous solution was under vigorous magnetic stirring in the dark at 25 °C for 5 min. After that, 1 M of NaOH was added to adjust the pH of solution approximately to 12 and the mixture was kept stirring at 25 °C for 4 h, which was further purified through centrifugation at 10,000 rpm for 20 min and stored at 4 °C for further use. The solid AgNCs was prepared by freezing at −80 °C and dried under vacuum for cytotoxicity investigation and characterization by Fourier transform infrared spectroscopy.

2.4. Cytotoxicity investigation of gelatin–AgNCs

Human epidermoid cancer cells (Hep-2) were used to investigate the cell viability of the as-prepared AgNCs through CCK-8 method. Briefly, 100 μL of Hep-2 cells, approximately 2×10^6 cells per mL in Roswell Park Memorial Institute 1640 medium (RPMI 1640), were plated into a 96-well plate and cultured at 37 °C, 5% CO_2 for 24 h. Then, 100 μL of RPMI 1640 containing 10 μL of AgNCs at different concentrations (0.01 mg/mL, 0.05 mg/mL and 0.1 mg/mL) replaced the culture medium for culturing Hep-2 cells. After 24 h incubation, the culture medium was removed from every cell well, which was washed with PBS buffer twice and contained 90 μL RPMI 1640. Finally, the cells were further incubated for 1 h and the optical density (OD) of the mixture was measured at 450 nm with a Microplate Reader Model. The following equation was used to estimate the cell viability (V_{cell}):

$$V_{Cell}(\%) = \frac{OD_{treated} - OD_{blank}}{OD_{control} - OD_{blank}} \quad (1)$$

wherein, the $OD_{treated}$ and $OD_{control}$ were obtained in the presence and absence of AgNCs, respectively, and OD_{blank} could be obtained only in the presence of PBS buffer.

2.5. Temperature sensing using gelatin–AgNCs

The temperature sensing based on gelatin–AgNCs was as follows. Typically, 400 μL of as-purified AgNCs was put into a 1.5 mL of Eppendorf (Ep) tube. After incubated in a series of temperature, increasing from 5 °C to 45 °C, for 10 min, the fluorescence spectra emitted at 552 nm and absorption spectra at 425 nm of AgNCs were recorded on an F-2500 fluorescence spectrophotometer and UV-3600 spectrophotometer, respectively.

3. Results and discussion

3.1. Synthesis and characterizations of as-prepared gelatin–AgNCs

As depicted in Scheme 1, gelatin, $AgNO_3$, and NaOH were added into one pot and continuous stirred at 25 °C for 4 h. Ag^+ could be reduced to Ag^0 by the phenolic group of tyrosine under alkaline conditions and the AgNCs were captured by the –SH group, released under alkaline conditions, of Cysteine.[21]No fluorescent AgNCs could be obtained without gelatin, or NaOH (Fig. S1, ESI†). The as-prepared AgNCs were yellowish-brown under daylight and yellow under 365 nm UV lamp, respectively (Inset of Fig. 1A). As we could see, the AgNCs showed an absorption band over 260 nm to 700 nm with an obvious peak at 425 nm (Fig. 1A). It was interesting that the emission showed significantly red shift when excited with the light beam of wavelength lower than 440 nm, and the red shift greatly reduced when the excitation wavelength was higher than 440 nm (Fig. 1B), indicating that the distribution of as-prepared AgNCs was very uniform [24]. When excited with 445 nm, the gelatin–AgNCs showed a strong fluorescence peak at 552 nm with a nearly 110 nm Stokes shift, which implied that they could be used as excellent fluorescent nanoprobes for chemo/biosensing and imaging [25]. The average fluorescence life time of AgNCs was calculated as 0.38 ns (Fig. S2,Table S1, ESI†), indicating the luminescence mechanism was most likely the radioactive recombination nature of excitations [26,27].

The uniform distribution of the gelatin–AgNCs could be further identified by measuring the morphology of AgNCs. Transmission electron microscopy (TEM) and atomic force microscopy (AFM) as shown in Fig. 2A and B implied that the AgNCs were mono-dispersed and nanospherical with a narrow size and height distribution. The average size of gelatin–AgNCs was 1.16 nm with a lattice spacing of 0.35 nm (Upper left inset of Fig. 2A). The height distribution (Fig. S3, ESI†) of gelatin–AgNCs was uniform and approximately 0.5–1.6 nm, which was close to the diameter of AgNCs. What's more, the zeta potential, measured with dynamic laser light scattering (DLS), was approximately −25.0 mV (Fig. S4, ESI†), revealing that the gelatin–AgNCs were negatively charged on their surface because of the capping agent gelatin.

The X-ray photoelectron spectrometer (XPS) survey spectra of gelatin–AgNCs implied the presence of the expected elements, including Na, O, N, Ag, C, and S, which were derived from NaOH and gelatin (Fig. S5A, ESI†). As shown in Fig. 2C, the binding energy of 367.72 eV and 373.77 eV were assigned to the Ag 3d5/2 and Ag 3d3/2 of AgNCs, respectively, while the S 2p peak (Fig. S5B, ESI†) at 162.28 eV and 160.88 eV indicated the interaction of AgNCs and gelatin through the covalent binding of Ag–S. The weakened of the gelatin S–S stretching frequency located at 527 cm^{-1} in the Raman