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Preparation of biocompatible fluorescent core–shell nanoparticles for cellular imaging

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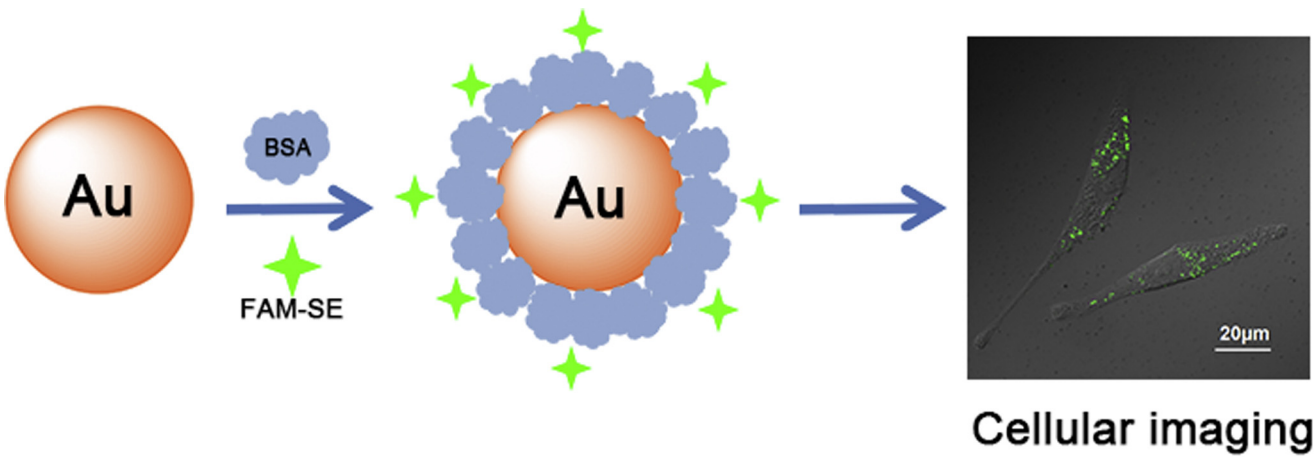
GRAPHICAL ABSTRACT



HIGHLIGHTS

- Core–shell fluorescent nanoparticles with good biocompatibility were synthesized.
- The fluorescence enhancement was attributed to the structure of these nanoparticles.
- The nanoparticles are efficient for cellular imaging.

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ABSTRACT

In this work, we report a facile method to prepare biocompatible fluorescent core–shell structured nanoparticles. The as-prepared nanoparticle was composed of a gold core enclosed in a protein shell. An amine-reactive fluorophore, 5-(and-6)-carboxyfluorescein-succinimidyl ester, was assembled onto the outer shell completing the fluorescent nanoparticle. Fluorescence quenching of the fluorophore was efficiently avoided because of the presence of the shell, which served as a spacer layer between the fluorophore and the gold core. There was an increase in fluorescence intensity, when compared to the fluorophore in its free state that can be attributed to the metal-enhanced fluorescence effect of the gold core. Well-dispersed green fluorescent faculae were observed when the as-prepared nanoparticles

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Assembly
Biocompatibility
Cellular imaging

were studied by the confocal laser scanning microscope, demonstrating the successful assembly of the fluorophores. The nanoparticles were readily uptaken by A549 cancer cells and the results of cell viability assays evidenced their good biocompatibility. Cumulatively, these findings indicate their potential application for cellular imaging.

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1. Introduction

Fluorescence detection is one of the most useful tools in analytical research, especially in the biological fields. It usually requires the bright fluorescence of fluorophores so as to improve the detection sensitivity caused by the high signal-to-noise ratio [1–8]. Compared with conventional organic dyes, fluorescent nanoparticles have superior chemical and optical properties, such as greater brightness and photostability [9–18]. Semiconductor quantum dots [10], conjugated polymer nanoparticles [11–15] and upconversion nanoparticles [16] are some of the most studied fluorescent nanoparticles. Their excellent properties and size make them one of the most useful tools in fluorescence detection, especially in enabling intracellular assays for biosensing and bioimaging purposes.

Gold (Au) nanoparticles are known to be chemically inert and stable and to possess good biocompatibility. Their synthesis routes are simple and usually performed in aqueous solution [19,20]. They are reported to possess unique optical properties that can enhance the emission intensity of a fluorophore located in close proximity to their surface. This phenomenon was usually referred to as metal-enhanced fluorescence (MEF) [21], a distance-dependent effect [22] usually observed in noble metal structures. When a fluorophore directly interacts with a metal nanoparticle surface, the fluorescence is greatly quenched owing to the resonant energy transfer from fluorophore to metal. When a spacer layer is employed to separate the fluorophore and the metal surface, fluorescence quenching can be avoided and efficient fluorescence enhancement is achieved by optimizing the spacer layer thickness [23–29]. The MEF provides a new approach to realizing high sensitivity in fluorescence detection assays.

In this work, we synthesized a fluorescent core–shell structured nanoparticle using a facile method. Gold nanoparticles were first synthesized and used as the core, then bovine serum albumin (BSA) was coated onto the nanoparticle surfaces to serve as the biocompatible shell. Subsequently, an amine-reactive fluorophore, 5-(and-6)-carboxyfluorescein-succinimidyl ester (FAM-SE), was assembled onto the BSA shell surfaces, resulting in the formation of Au@BSA/(FAM-SE) fluorescent nanoparticles. Fluorescence quenching of FAM-SE on gold nanoparticle surfaces was efficiently avoided because of the presence of the BSA shell. An increase of the fluorescence of FAM-SE was observed when compared to free FAM-SE solution. Well-dispersed faculae with green fluorescence, as observed in the confocal laser scanning images of the as-prepared nanoparticles, showed the successful attachment of FAM-SE. Cell viability assays demonstrated the low cytotoxicity of these nanoparticles. Their suitable size facilitates the quick internalization into cells, making them a potential nanomaterial for biosensing and bioimaging.

2. Experimental

2.1. Materials

Gold(III) chloride hydrate ($HAuCl_4{\cdot}3H_2O$), hydroxylamine hydrochloride, BSA and sodium hydroxide (NaOH) were purchased from Sigma–Aldrich (USA). FAM-SE was purchased from Life Technologies Co. (USA). Other reagents used in the experiments were purchased from Sigma–Aldrich (USA), and were used without further purification, unless otherwise noted. Distilled water was used throughout the experiment.

For cell assay, the human lung carcinoma (A549) cells were purchased from the Cell Culture Center of Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences (Beijing, China). Dulbecco's modified eagle medium (DMEM) and phosphate buffered saline (PBS) was purchased from Sigma–Aldrich (USA).

2.2. Characterization

Ultraviolet–visible absorption spectra were collected on a Hitachi U-3900H spectrophotometer (Hitachi, Ltd., Japan). Fluorescence spectra were obtained at room temperature, using a Hitachi F-7000 fluorescence spectrometer (Hitachi, Ltd., Japan) equipped with a Xenon lamp excitation source. Transmission electron microscope (TEM) images were recorded with Hitachi H-7650B (Hitachi, Ltd., Japan) at an accelerating voltage of 120 kV. The fluorescence images of the prepared nanoparticles and the cells were recorded using a confocal laser scanning biological microscope (CLSM; Olympus FV1000-IX81, Olympus Corp., Japan). Zeta (ζ) potential of the nanoparticles was measured by diluting the nanoparticles in PBS buffer (pH 7.4) and using a Zetasizer Nano ZS90 instrument (Malvern Instrument Ltd., England) and its dispersion technology software (DTS) at room temperature. Cell viability was detected using a Spectra MAX 340PC plate reader (Molecular Device, LLC, USA).

2.3. Synthesis of BSA coated Au nanoparticles (Au@BSA)

The Au@BSA nanoparticles were synthesized via a modified version of a previously published method [30]. Au nanoparticles were first synthesized by reducing Au^{3+} at the presence of hydroxylamine hydrochloride. Briefly, 200 μL of $HAuCl_4{\cdot}3H_2O$ (20 mg/mL) and 8 mg hydroxylamine hydrochloride were added into 40 mL of water and stirred at 600 rpm, at room temperature, for 5 min. Then, 400 μL of NaOH solution (10 mg/mL) was quickly added to the mixture, and the colour of the mixture changed from colourless to blue immediately. One minute later, the stirring was stopped and the mixture was kept undisturbed under darkened conditions. The obtained colloidal solution reached its stable state after 12 h storage, with a further color change from blue to magenta.

To coat the prepared Au nanoparticles with the BSA shell, 100 μL of BSA solution (50 mg/mL) was added into 1 mL of Au nanoparticle solution under ultrasonic shaking. The mixture was then kept undisturbed as a shell of BSA formed on the Au nanoparticle surfaces 2 h after incubation. The resulting Au@BSA nanoparticles were washed and purified by centrifugation at 8600 × *g* for 6 min, and re-dispersed in 100 μL of distilled water.

2.4. Preparation of Au@BSA/(FAM-SE) fluorescent nanoparticles

Au@BSA nanoparticle solution (100 μL) was diluted with 900 μL of distilled water followed by the addition of 10 μL of FAM-SE

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