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Targeted surface-functionalized gold nanoclusters for mitochondrial imaging

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ABSTRACT

Due to mitochondria involved in both apoptotic and necrotic cell death, labeling and imaging mitochondria has attracted considerable interest. However, conventional organic dyes used for mitochondrial imaging are limited because of their poor photostability. Considering that gold nanoclusters (AuNCs) possess some advantages over considerable interest, such as excellent photostability and strong fluorescence emission, we herein prepared a mitochondria-targeted fluorescent probe, AuNCs@CS-TPP, based on a covalent link between triphenylphosphonium (TPP) cations and chitosan-coated AuNCs (AuNCs@CS). The as-prepared AuNCs@CS-TPP exhibited a bluish fluorescence emission at 440 nm with a quantum yield of 8.5%. Meanwhile, the fluorescence intensity of AuNCs@CS-TPP labeled HeLa cells did not show apparent decrease after 8 min irradiation. Cytotoxicity assay showed that AuNCs@CS-TPP did not display any appreciable cytotoxicity on cells even at a concentration of 60 μg mL^{-1}. In addition, the result of fluorescence co-localization imaging in vitro indicated that AuNCs@CS-TPP could selectively accumulate into mitochondria of HeLa cells and HepG2 cells. These findings demonstrated that AuNCs@CS-TPP possessed superior photostability, low cytotoxicity, high sensitivity and target-specificity to mitochondria, allowing labeling and imaging of the mitochondria in living cells.

1. Introduction

Mitochondria play a central role in cellular energy metabolism (Green and Reed, 1998; Hoppins and Nunnari, 2012). Recent discoveries have showed that mitochondrial dysfunctions severely affected human health and led to many diseases (Vafai and Mootha, 2012; Wallace, 1999). Therefore, the fundamental understanding of mitochondrial behavior in living cells is of great importance for clear explanation of biological processes in mitochondria, and advancing early diagnosis and drug development in biomedicine (Smith et al., 2011).

Fluorescent labeling and imaging mitochondria in living cells is very likely to be a powerful tool for understanding of mitochondrial function and changes. To observe the biochemical events in living cells for long time, the fluorescent probe must be photostable under the continual irradiation (Leung et al., 2013). As is well known, conventional organic dyes have been developed in mitochondrial imaging, but their poor photostability often limits their applications in living cells studies, leaving much to be desired (Lee and Chen, 2011; Derfus et al., 2004). To overcome this limitation, recent advances in the development of fluorescent quantum dots (QDs) have emerged as an alternative method for live-cell imaging with strong photoluminescence and excellent photostability (Biju et al., 2010; Kuo et al., 2011; Hsieh et al., 2011). Nevertheless, their intrinsic toxicity (the release of heavy metals like cadmium, selenium and lead) of II-VI QDs is the main concerns for their applicability in live-cell studies (Chan et al., 2006; Lee et al., 2009; Li et al., 2011).

In contrast, gold nanoclusters (AuNCs) have recently attracted enormous interests, because they possess extraordinary advantages over QDs and conventional fluorescence dyes such as low toxicity, robust resistance to photobleaching and facile synthesis (Chen et al., 2012; Palmal et al., 2013; Wang et al., 2011). However, it has been reported that bare gold nanomaterials could accumulate in the liver and spleen at high level, and these accumulations can cause potential cytotoxicity (Zhang et al., 2012; Du et al., 2012; Jia et al., 2009). To reduce the potential cytotoxicity of AuNCs, many biocompatible materials such as poly(lactic-*co*-glycolic acid (PLGA), chitosan (CS), bovine albumin (BSA), and glutathione (GSH) were used as protection ligands coated onto the surface of the AuNCs (Geng et al., 2012; Chen et al., 2012; Xie et al., 2009; Zhou et al., 2011). Among these various coatings, CS has attracted much attention due to its low-toxicity, good biocompatibility, biodegradability and non immunogenicity

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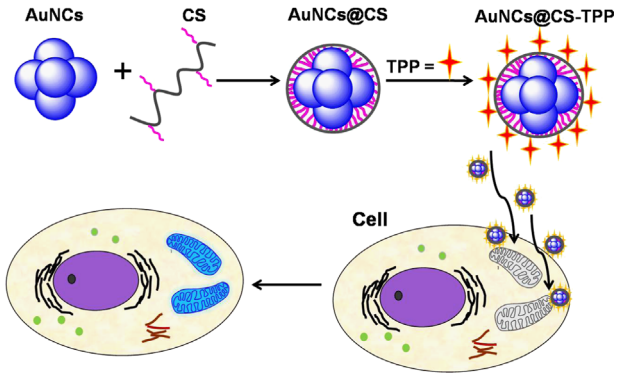

Scheme 1. Schematic of the formation and live-cell imaging of mitochondria-targeted fluorescent probe (AuNCs@CS-TPP). AuNCs@CS was synthesized through hydrophobic interaction between AuNCs and CS, and then was functionalized with the mitochondria-targeted molecule (TPP) to produce the fluorescent probe, AuNCs@CS-TPP. AuNCs@CS-TPP can enter into cells, selectively accumulate in mitochondria, and then be used for mitochondrial imaging in living cells.

(He et al., 2012; Patel et al., 2013). In addition, in our previous study, we prepared amphiphilic CS, which are able to form self-assembled nanoparticles in an aqueous environment (Han et al., 2012). Based on above considerations, to further improve the safety of the AuNCs, CS is an ideal material for its surface coating.

To achieve the specificity to mitochondria, several strategies have been used, particularly conjugation to peptides and triphenylphosphonium (TPP) (Smith et al., 2011). Although mitochondria-targeted peptides have been widely utilized to date, the peptides are susceptible to degradation upon high pH and prolonged normal storage (Derfus et al., 2004; Szeto, 2006; Liu et al., 2006). The other strategy to the selective delivery of fluorescence AuNCs into mitochondria is using TPP or its derivatives. TPP is a kind of delocalized lipophilic cations, including three phenyl groups, which result in highly lipophilic and delocalization of positive charges on phosphonium into three aromatic rings (Murphy, 1997). This facilitates TPP cations pass easily through phospholipid bilayers and accumulate into highly negatively charged mitochondria of living cells (Blaikie et al., 2006; Zhou et al., 2013). In addition, a number of mitochondria targeted probes, antioxidants or anticancer agents have been developed by conjugation to TPP cations (Dickinson and Chang, 2012; Yamada and Harashima, 2008). The results demonstrate that TPP cations could successfully enhance cellular uptake, and be targeted to mitochondria in living cells.

In this work, we synthesized small-sized chitosan-coated fluorescent AuNCs (AuNCs@CS) in water, and then functionalized AuNCs@CS with the TPP cations for the purpose of targeting mitochondria in living cells (Scheme 1). The resultant fluorescent probe (AuNCs@CS-TPP) was characterized by UV-visible spectroscopy, fluorescence spectroscopy, fluorescence lifetime spectroscopy, scanning electron microscopy (SEM), dynamic light scattering (DLS), Fourier transform infrared spectroscopy (FT-IR), X-ray photoelectron spectroscopy (XPS) and energy dispersive X-ray spectroscopy (EDS). To examine the suitability of the mitochondria-targeted fluorescent probe for living cells imaging, we conducted a photostability experiment of AuNCs @CS-TPP, and incubated HeLa cells and HepG2 cells with the fluorescent probe to evaluate its optical properties, cytotoxicity and mitochondria-selective optical imaging.

2. Material and methods

2.1. Reagents and chemicals

CS (MW = 80 kDa, degree of deacetylation = 85%), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC), *N*-hydroxysuccinimide (NHS) hydrogen tetrachloroaureate (III) trihydrate ($HAuCl_4 \cdot 3H_2O$), 1-undecanethiol and *N*,*N*-dimethylformamide (DMF) were purchased from Sigma-Aldrich (St Louis, MO). (4-Carboxybutyl)triphenylphosphonium bromide was obtained from J&K Chemical Ltd. (Beijing, China). Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum, penicillin, streptomycin, MitoTracker® Red CMXRos (Mito Tracker) and phosphate buffered saline (PBS) were purchased from Invitrogen Co. (Beijing, China). Ultrapure water (Milli-Q plus, Millipore Inc., Bedford, MA) was used throughout. Unless specified, all other chemicals were of analytical reagent grade and were used without further treatment.

2.2. Instrumentation

UV–visible absorption spectra were recorded in a conventional quartz cell (light path 10 mm) by using a Hitach UV-3310 spectrophotometer (Japan). Fluorescence measurements were performed on a Hitachi F-2500 fluorescence spectrophotometer (Tokyo, Japan). FT-IR scans were collected on a Bruker Tensor-27 Fourier-transform infrared spectrometer (Bruker, Germany). The fluorescence lifetimes were measured with a compact fluorescence lifetime spectrometer C11367 (Hamamatsu, Japan). High resolution transmission electron microscopy (HRTEM) was determined on a JME-2100F (JEOL) instrument by placing colloidal solutions on the carbon-coated copper grid and then drying at room temperature. SEM and EDS measurements were made on a S-4800 scanning electron microscope (Hitachi, Japan) at an accelerating voltage of 30 kV. The samples for SEM and EDS characterization were prepared by dropping the colloidal solutions onto silicon slice, and then spraying platinum onto its surface. DLS measurements were performed using a Zetasizer nano ZS (ZEN3600) instrument (Malvern, England). XPS measurements were recorded with a ESCALab220i-XL (VG, England). The absorbance for MTT analysis was recorded on a microplate reader (BIO-TEK Synergy HT, USA) at 490 nm. All measurements were herein performed at room temperature.

2.3. Synthesis of AuNCs

DMF-protected AuNCs were prepared according to the previously reported method (Kawasaki et al., 2010). Aqueous $HAuCl_4$ solution (150 μL, 0.1 M) was added to 15 mL DMF at 140 °C, and the DMF solution was refluxed by simply heating with an oil bath (140 °C for 6 h) with vigorous stirring. After evaporating the excess solvent under a vacuum, the residue was redissolved in methanol or water.

The hydrophilic DMF-protected AuNCs were then ligand exchanged with 1-undecanethiol to make AuNCs hydrophobic. To a stirred methanol solution of AuNCs (3 mL) was added 100 μL of 1-undecanethiol solution in methanol (5×10^{-2} M) dropwise. The mixture was stirred and maintained in the dark for more than 24 h. After removal of the solvent, the residue (1-undecanethiol-protected AuNCs) was redissolved in methanol.

2.4. Synthesis of AuNCs@CS and AuNCs@CS-TPP

Stock solutions of 1-undecanethiol-protected AuNCs (2 mg mL^{-1}) and CS (10 mg mL^{-1}) were prepared by dissolving the solid in chloroform and methanol, respectively. Then, the solutions of the AuNCs (250 μL) and CS (200 μL) were successively added to a 10 mL water (50 °C), and mixed thoroughly with vigorous stirring. After 5 min, the chloroform and methanol were removed under vacuum. Finally, 3 μL glutaraldehyde (25%) was added and stirring for 2 h at 40 °C. The product, AuNCs@CS, was then freeze-dried.

TPP was conjugated to amino groups on the surface of AuNCs@CS to form AuNCs@CS-TPP conjugates. The AuNCs@CS-TPP was synthesized by following several steps: First, (4-carboxybutyl)