MethodsX 2 (2015) 306–315

Contents lists available at ScienceDirect

MethodsX

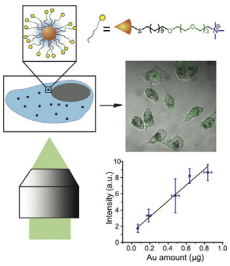
journal homepage: www.elsevier.com/locate/mex

Cellular imaging of endosome entrapped small gold nanoparticles

Chang Soo Kim[1], Xiaoning Li[1], Ying Jiang, Bo Yan, Gulen Y. Tonga, Moumita Ray, David J. Solfiell, Vincent M. Rotello*

Department of Chemistry, University of Massachusetts Amherst, 710 North Pleasant Street, Amherst, MA 01003, USA

GRAPHICAL ABSTRACT



ABSTRACT

Small gold nanoparticles (sAuNPs, <10 nm in a core diameter) have been used for drug delivery and cancer therapy due to their high payload to carrier ratio. Information about the amount and location of sAuNPs in cells and tissues is critical to many applications. However, the current detection method (i.e., transmission electron microscopy) for such sAuNPs is limited due to the extensive sample preparation and the limited field of view. Here we use confocal laser scanning microscopy to provide endosome-entrapped sAuNP distributions and to quantify particle uptake into cells. The quantitative capabilities of the system were confirmed by inductively coupled plasma-mass spectrometry, with an observed linear relation between scattering intensity and the initial cellular uptake of sAuNPs using 4 nm and 6 nm core particles.

The summary of the method is:

- This non-invasive imaging strategy provides a tool for label-free real-time tracking and quantification of sAuNPs using a commercially available confocal laser scanning microscope.
- Scattering intensity depends on particle size.

* Corresponding author.
E-mail address: rotello@chem.umass.edu (V.M. Rotello).
[1] Equal contributions by both authors.

http://dx.doi.org/10.1016/j.mex.2015.06.001

- The linear relation established between scattering intensity and uptaken gold amount enables simultaneous quantitative assessment through simple image analysis.

ARTICLE INFO
Method name: Confocal measurement of cellular uptake of gold nanoparticles
Keywords: Gold nanoparticles, Confocal laser scanning microscopy, Nanoparticle quantification, ICP-MS, Reflectance imaging
Article history: Received 18 March 2015; Received in revised form 26 May 2015; Accepted 5 June 2015; Available online 10 June 2015

Method details

We report a simple, rapid, and non-invasive approach for the imaging of sAuNPs within cells by using a standard confocal laser scanning microscope (CLSM). No additional optical or imaging system is required for this approach. A single-wavelength laser excitation was used to excite sAuNPs within the cell, and the reflective images were recorded to explore the size-dependent visibility of the AuNPs. These studies demonstrate that sAuNPs as small as 4 nm in core size can be readily imaged. Image analysis was carried out to explore the correlation between the sAuNP scattering intensity and sAuNP quantities inside cells.

Step 1: surface-functionalized gold nanoparticle synthesis

Materials

All the reagents/materials required for nanoparticle synthesis were purchased from Fisher Scientific, except for hydrogen tetrachloroaurate(III) hydrate, which was obtained from Strem Chemicals Inc. The organic solvents were from Pharmco-Aaper or Fisher Scientific and used as-received except for dichloromethane, which was distilled in the presence of calcium hydride. HeLa cells (human cervical-cancer cell line) were purchased from ATCC. Dulbecco's Modified Eagle's Medium (DMEM; Sigma, D5523) and fetal bovine serum (FBS; Fisher Scientific, SH3007103) were used in cell culture.

Procedure

Gold nanoparticles (AuNPs) were synthesized and characterized according to previous reports with slight modifications [1]. Briefly, Brust–Schiffrin two-phase synthesis method [2] was used to prepare dodecanethiol-protected AuNPs (AuNPs-DT) with 2 nm core diameter. AuNPs-DT (4 and 6 nm) were grown from 2-nm AuNPs according to Miyake's heat-induced size-evolution strategy [3] with a slight modification.

- 2-nm AuNPs-DT were heated to 154 °C and 165 °C for 4 and 6 nm AuNPs-DT, respectively, with a heating rate of 2 °C/min and held for 30 min at that temperature.
- Murray's place-exchange method [4] was then used to prepare functionalized AuNPs.

The sizes of AuNPs were characterized by TEM and dynamic light scattering (DLS) (Fig. S2). The surface functionalities of AuNPs have been characterized by proton nuclear magnetic resonance (^{1}H NMR) and laser desorption/ionization mass spectroscopy (LDI-MS) (Figs. S3 and S4). Zeta-potential values were measured using a Malvern Zetasizer Nano ZS instrument.

Step 2: cell culture and cellular uptake of AuNPs

Prior to the cellular uptake study of AuNPs, HeLa and MCF 7 cells were seeded into a 24-well plate at a density of 25,000 cells/well with low-glucose DMEM supplemented with 10% FBS and 1% antibiotic.

Procedure

- The cultures were maintained at 37 °C in a humidified atmosphere with 5% CO_2.