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Optically encoded nanoprobes using single walled carbon nanotube as the building scaffold for magnetic field guided cell imaging

Hong Wang [a], Zhuyuan Wang [b,*], Minglang Ye [b], Shenfei Zong [b], Mingyue Li [b], Peng Chen [b], Xueqin Ma [b], Yiping Cui [b,*]

[a] Department of Laboratory Medicine, the First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, China
[b] Advanced Photonics Center, Southeast University, Nanjing 210096, China

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ABSTRACT

We construct a novel fluorescent, surface enhanced Raman scattering (SERS) encoded and magnetic nanoprobe for live cell imaging. To fabricate this nanoprobe, single walled carbon nanotube (SWNT) is used as the building scaffold while gold nanoparticles (Au NPs), superparamagnetic iron oxide nanoparticles (SPIONs) and quantum dots (QDs) are employed as the building blocks. Here, Au NPs serve as the SERS substrate and QDs act as the fluorescent agent. Au NPs and SPIONs are first adsorbed on the SWNT via electrostatic interactions. Then a silica layer is coated on the SWNT. Finally, QDs are attached on the silica shell. With such a structure, various optical signals can be readily encoded to the nanoprobe simply by using different Raman molecules and QDs with different emission wavelengths. Experimental results show that the as-prepared nanoprobe exhibits well fluorescence and SERS performance. Furthermore, *in vitro* experiments demonstrate that the nanoprobe can fulfill magnetic field guided fluorescence and SERS dual mode imaging of live cells. As a fascinating optical encoding material and a multifunctional nanoplatform, the presented nanoprobe holds genuine potential in future biosensing applications.

1. Introduction

The integration of various functions into a single platform is a thriving research area as it allows simultaneous accomplishment of different tasks [1,2]. As one of the particular nanosized platforms, composite nanoprobes have received massive attention concerned with their fabrication and utilization [3]. Such nanoprobes can be validly applied *in vitro* or *in vivo* due to their small size. Popular materials involved in the construction of nanoprobes include metal nanoparticles (NPs) [4], silica NPs [5], polymers [6], magnetic NPs (MNPs) [7], carbon nanotubes (CNTs) [8], liposomes [9], oligonucleotides [10] and so on. Among these, CNTs are attractive candidates due to their special chemical and physical characteristics, such as large surface area, high mechanical strength, excellent chemical and thermal stability and rich electrical and optical properties [11]. To date, CNTs have been implicated in biomedical studies, including drug delivery [12], cell endoscope [13], immunoassay [14], and photothermal therapy [15,16]. Aside from CNTs, another interesting and promising material is MNPs. Owing to their intrinsic magnetic property, MNPs can be facilely manipulated by an external magnetic field and realize magnetic field guided tumor specific drug delivery or cancer cell imaging [17–19]. Superparamagnetic iron oxide nanoparticle (SPION) with diameter of around 12 nm is one of the commonly used MNPs. With such a tiny size, they can be incorporated into other nanomaterials to form multifunctional nanocomposites [20,21].

A newly emergent kind of multifunctional nanoplatform is the optically encoded nanoprobe, which has been proven to have great applicability in bioimaging and biosensing [22,23]. Fluorescence is well employed in designing optical nanoprobes due to its fast readout and easy operation. Another involved technique is surface enhanced Raman scattering (SERS) as it has good multiplexing ability and can provide rich spectroscopic information of the analyte [24,25]. Despite their impressive progress, nanoprobes encoded with solely fluorescence or SERS signal still suffer from some annoying shortcomings. For example, the wide bandwidth of fluorescence often causes severe spectral overlap, which limits the multiplexing ability of fluorescence technology. While SERS usually requires a relatively long acquisition time due to its weak signal intensity, which hinders its application in high-speed dynamic analysis. As a result, combining both SERS and fluorescence into a single nanoprobe can be an effective solution. Several such SERS and fluorescence dual encoded nanoprobes have been reported [2,26,27]. Recently, we have demonstrated a SERS-fluorescence joint

* Corresponding authors.
E-mail addresses: wangzy@seu.edu.cn (Z. Wang), cyp@seu.edu.cn (Y. Cui).

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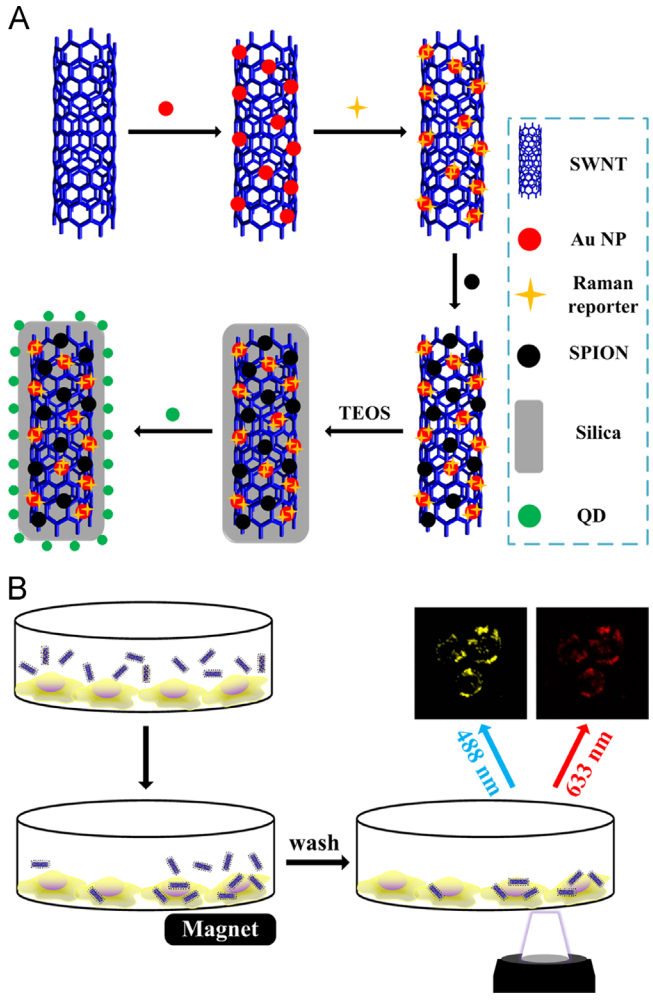

Scheme 1. (A) Fabrication procedures of the multifunctional nanoprobe. (B) Magnetic field guided SERS-fluorescence dual mode imaging of living cells.

spectral encoding (SFJSE) method using nanoprobes composed of metal NPs, silica and quantum dots (QDs). Such SFJSE based nanoprobes can be fruitfully employed in high-throughput bioanalysis [28].

Here, we present a new kind of SERS and fluorescence encoded magnetic nanoprobe for cell imaging. The structure of the nanoprobe is illustrated in Scheme 1A. Basically, single walled carbon nanotube (SWNT) is used as the building scaffold since it can provide numerous anchor spots for smaller NPs. SPIONs and Au NPs are electrostatically adsorbed onto the SWNT. The Au NPs are used as the SERS substrate and Raman molecules are linked to these Au NPs to generate SERS signals. Then a silica shell is coated to bury the Au NPs and SPIONs inside. Finally, CdSe/ZnS core–shell QDs are adsorbed to the silica shell to produce fluorescence signals, thus the multifunctional nanoprobe is obtained. *In vitro* experiments using human breast cancer cells (SKBR3 and MCF7) as the model cells confirm that the presented nanoprobe can realize magnetic field guided SERS and fluorescence dual mode imaging of live cells.

2. Material and methods

2.1. Materials

Single walled carbon nanotubes (SWNTs) were purchased from Nanoport Co. Ltd. (Shenzhen, China). CdSe/ZnS core–shell quantum dots with emission peaked at 530 nm and 580 nm were purchased from Wuhan Jiayuan Quantum Dot Technological Development Co., Ltd. Hydrogen tetrachloroaurate(III) trihydrate ($HAuCl_4 \cdot 3H_2O$), tetraethoxysilane (TEOS), polyethyleneimine (PEI, Branched, M.W. 10000) and 4-aminothiophenol (4ATP) were purchased from Alfa Aesar. 3-mercaptopropionic acid (MPA) and 5, 5'-dithiobis (2-nitrobenzoic acid) (DTNB) were purchased from Sigma-Aldrich. Ammonia water, H_2SO_4, HCl and HNO_3 were purchased from Shanghai Zhongshi Chemical Co., Ltd. Absolute ethanol was purchased from Nanjing Chemical Reagent Co., Ltd. NaCl was purchased from Guangdong Xilong Chemical Co., Ltd. All the reagents were used as received. Deionized water (Millipore Milli-Q grade) with a resistivity of 18.2 MΩ/cm was used in all the experiments.

2.2. Fabrication of the multifunctional nanoprobe

Several kinds of NPs, which were employed as the building blocks of the nanoprobe, were prepared beforehand according to previously published literature, including Au NPs [29], SPIONs [30] and water soluble MPA capped CdSe/ZnS QDs [31].

To fabricate the nanoprobe, first, SWNTs were sonicated in a mixture of H_2SO_4 and HNO_3 (3:1) for 24 h and subsequently exposed to 1 M HCl [32,33]. Excess acids were removed by dialysis and the resultant SWNTs were vacuum dried and redispersed in deionized water. Next, 200 μL of SWNTs (0.5 mg/mL) was added to 5 mL of PEI solution (10 mg/mL in 0.5 M NaCl) and sonicated for 1 h. Excess PEI was removed by centrifugation (12000 rpm, 20 min) for at least 3 times. Then the PEI wrapped SWNTs (denoted as SWNT@PEI) were redispersed in 200 μL of deionized water and centrifuged at 3000 rpm for 20 min. The supernatant was collected and the precipitate containing severely aggregated SWNTs was abandoned. To adsorb Au NPs, 200 μL of SWNT@PEI was added to 20 mL of Au NPs and sonicated for 1 h. The mixture was subsequently centrifuged thrice at 3000 rpm for 20 min to remove redundant Au NPs. The precipitate (denoted as SWNT@Au) was dispersed in 1 mL of deionized water. In order to make these nanoprobes SERS active, 6 μL of the Raman reporter (4ATP or DTNB, 10 mM in ethanol) solution was added respectively to SWNT@Au and aged for 1 h. After that, SPIONs were adsorbed to the SWNT@Au following a procedure similar to the adsorption of Au NPs. 1 mL of the SERS tagged SWNT@Au was mixed with 5 mL of PEI (10 mg/mL in 0.5 M NaCl) and sonicated for 1 h. Centrifugation was conducted again to remove excess PEI and the precipitate (denoted as SWNT@Au@PEI) was dispersed in 5 mL of deionized water containing SPIONs (0.1 mg/mL). The mixture was sonicated for 1 h to allow the adsorption of SPIONs and excess SPIONs were removed also by centrifugation. The precipitate (denoted as SWNT@Au–SPION) was dispersed in 40 mL of absolute ethanol. Then a silica layer was coated on the SWNT@Au–SPION via the Stöber method [34]. 2 mL of deionized water, 10 μL of TEOS and 2 mL of ammonia water (25%) were added to the as-prepared 40 mL of SWNT@Au–SPION ethanol solution. The mixture was sonicated for 2 h and 10 μL of TEOS was added thereafter, followed by continuous sonication for 6 h and aging overnight to complete the growth of silica shell. Finally, the silica coated SWNT@Au–SPION (denoted as SWNT@Au–SPION@SiO_2) was collected by centrifugation and magnetic separation. The sediments were dispersed in 5 mL of deionized water. Before attaching CdSe/ZnS QDs, SWNT@Au–SPION@SiO_2 was first modified with PEI as follows. 1 mL of SWNT@Au–SPION@SiO_2 was added to 2 mL of PEI solution (10 mg/mL in 0.5 M NaCl) and sonicated for 1 h. Excess PEI was removed by centrifugation and the sediment (denoted as SWNT@Au–SPION@SiO_2@PEI) was dispersed in 1 mL of deionized water. Next, 1 mL of MPA capped QDs solution (2 μM) was added to 1 mL of the SWNT@Au–SPION@SiO_2@PEI. The mixture solution was shaken for 2 h to allow the attachment of