Multifunctional magnetic–plasmonic nanoparticles for fast concentration and sensitive detection of bacteria using SERS

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ABSTRACT

Multifunctional magnetic–plasmonic Fe_3O_4–Au core–shell nanoparticles (Au-MNPs) were prepared for simultaneous fast concentration of bacterial cells by applying an external point magnetic field, and sensitive detection and identification of bacteria using surface-enhanced Raman spectroscopy (SERS). We demonstrated that a spread of a 10 μL drop of a mixture of 10^5 cfu/mL bacteria and 3 μg/mL Au-MNPs on a silicon surface can be effectively condensed into a highly compact dot within 5 min by applying an external point magnetic field, resulting in 60 times more concentrated bacteria in the dot area than on the spread area without concentration. Surrounded by dense uniformly packed Au-MNPs, bacteria can be sensitively and reproducibly detected directly using SERS. The principle component analysis (PCA) showed that three different Gram-negative bacterial strains can be clearly differentiated. We also demonstrated that the condensed multifunctional Au-MNPs dot can be used as a highly sensitive SERS-active substrate and a limit of detection better than 0.1 ppb was obtained in detection of small molecules such as 4-mercaptopyrine. This novel platform significantly simplifies the concentration and detection process, which holds great promise for applications in food safety, environmental monitoring, medical diagnoses, and chemical and biological threat detections.

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1. Introduction

Recently, growing interest has been paid to the development of multifunctional plasmonic magnetic nanoparticles by incorporation of gold nanostructures to superparamagnetic Fe_3O_4 nanoparticles (MNPs) that can combine both plasmonic and magnetic properties in one single nanoparticle (Melancon et al., 2009). Impinged by light, gold nanostructures such as nanodots, nanorods and nanoshells can generate extreme high local electric fields arising from local surface plasmon resonance (LSPR). This is due to collective oscillation of conduction band electrons in response to the electric field of the electromagnetic radiation of the light. Both LSPR wavelength and electric field intensity can be tuned by varying the dimensions of the nanostructures, the spacing between them and the surrounding dielectric media (Willets and Van Duyne, 2007). Therefore, metallic nanostructures have been used for bioimaging (El-Sayed et al., 2005; Jain et al., 2007; Tam et al., 2010), LSPR biosensing (Anker et al., 2008), surface-enhanced Raman spectroscopy (SERS) (Yu et al., 2008), thermotherapy (Bardhan et al., 2009; Ji et al., 2007; Tam et al., 2010), and many other nanophotonic

applications (Atwater and Polman, 2010; Schuller et al., 2010). Plasmonic MNPs have also been developed recently by many research groups with different methods. Several unique particle structures such as Fe_3O_4 core/gold shell (Levin et al., 2009; Ma et al., 2009; Q. Zhang et al., 2010b), dumbbell-like NPs formed by attaching a Au NP to a Fe_3O_4 NP (Yu et al., 2005), and gold nanorods decorated with Fe_3O_4 NPs (Wang et al., 2009; Wang and Irudayaraj, 2010) have been developed. Tunable plasmonic properties were achieved by controlling the thickness of the gold nanoshell or the size of gold nanoparticles (Levin et al., 2009; Yu et al., 2005; Q. Zhang et al., 2010b). One of the fascinating aspects of plasmonic MNPs is that the plasmonic property can be further tuned by varying the inter-particle distance via an external magnetic field (Q. Zhang et al., 2010b). Because the sensitivity of SERS strongly depends on the distance of gold or silver nanoparticles, and single molecule detection can be achieved in the very narrow gap between nanoparticles (Kneipp et al., 1997; Nie and Emery, 1997), it is expected that closely packed plasmonic MNPs could serve as highly sensitive SERS-active substrates for sensing and detection applications. While plasmonic MNPs have been explored for protein concentration/detection using SERS (Zhou et al., 2011), pathogen separation and imaging (Wang and Irudayaraj, 2010), photothermic therapy (Ma et al., 2009; Wang et al., 2009), drug delivery (Melancon et al., 2009; Xu et al., 2009), and immunoassay (Tang et al., 2006; Zhuo et al., 2009), little work has been reported to apply plasmonic MNPs

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for simultaneous concentration and detection of microorganisms using SERS.

Using plasmonic NPs (e.g., Au or Ag NPs) for the detection and differentiation of intact microorganisms such as bacteria or spores by SERS has been extensively studied recently (Guicheteau et al., 2008; Ivleva et al., 2008; Jarvis and Goodacre, 2008). However, the non-uniform attachment of plasmonic NPs on bacteria makes it difficult to conduct quantitative analysis and reproducible detection. Early studies made SERS samples by simply mixing a bacteria solution with colloid plasmonic NPs. The mixture was either dried on a glass slide and then measured by Raman spectrometer (Holt and Cotton, 1989), or directly detected in a cuvette (Sengupta et al., 2006). Although these methods are straightforward and easy to conduct, it was found that NPs non-uniformly attached to bacteria, and NPs and bacteria are non-uniformly distributed on the substrate surface, resulting in low reproducibility and accuracy. Several new approaches were developed for solving this problem. One method was based on "convective assembly" of NPs and bacterial cells by slowly moving a glass slide with an angle to another glass slide having a drop of bacteria and NPs mixture. The relatively uniform distribution of bacteria and NPs on the substrate was obtained and signal reproducibility was notably improved (Kahraman et al., 2008). To further improve the distribution of NPs on the surface, clusters of NPs were assembled in prefabricated nanohole arrays via electron beam lithography (EBL) for SERS detection of bacteria (Yang et al., 2010). Both of the methods could improve detection reliability and reproducibility to some extent. However, these methods always require a relative high concentration of bacteria solution to achieve the detectable SERS signals.

In practical applications of pathogen detection and identification, potential samples from fields or patients are always in low concentration. Culturing and growing these bacteria require prolonged time and strictly controlled environments, therefore, methods that can concentrate bacteria samples to improve detection accuracy are highly desired (J.Y. Zhang et al., 2010a). Several concentration methods have been developed by using, for example, magnetic beads (Wang et al., 2011), electrodynamic (Zhou et al., 2006), and nano/microfluidic systems (Park et al., 2009; Wang et al., 2007; J.Y. Zhang et al., 2010a). However, to fulfill the criteria by World Health Organization for concentration of bacterial samples (i.e., simple-to-use, rapid, low-cost, sensitive, accurate, specific, and robust (Mabey et al., 2004; J.Y. Zhang et al., 2010a)), new methods have to be developed.

In this work, we developed the multifunctional magnetic–plasmonic Fe₃O₄–Au core–shell NPs and demonstrated that they can be used for simultaneous fast concentration of bacterial cells by applying an external point magnetic field, and sensitive detection and identification of bacteria using SERS. This novel platform significantly simplifies the concentration and detection process, which holds great promise for applications in food safety, environmental monitoring, medical diagnoses, and chemical and biological threat detections.

2. Materials and methods

2.1. Materials

$FeCl_2 \cdot 4H_2O$ (>99%), $FeCl_3 \cdot 6H_2O$ (>99%), $HAuCl_4$ (>99.9%), citric acid (>99.5%), polyethyleneimine (PEI, M.W. ∼ 60,000), NH_2OH solution (50% in water), 4-mercaptopyridine (4-MP, 95%) were purchased from Sigma–Aldrich. NaOH (>99%) was purchased from J.T. Baker. Three Gram-negative bacterial strains, *Acinetobacter calcoaceticus (A. calcoaceticus), Escherichia coli K12 (E. coli K12)* and *Pseudomonas aeruginosa (P. aeruginosa)*, were purchased from American Type Culture Collection (ATCC).

2.2. Preparation of Fe₃O₄–Au NPs

The details to prepared PEI coated superparamagnetic Fe₃O₄ NPs are described in the supporting information. To make gold coated MNPs (Au-MNPs), 0.1 mg PEI-MNPs were dispersed in 5 mL deionized water (DI water), to which 50 μL of 1% $HAuCl_4$ solution and 10 μL of 1% NaOH solution were added. The growth of Au on the PEI-MNPs was started by adding 25 μL 0.2 M NH_2OH solution and the reaction was conducted under sonication at room temperature for 30 min (Goon et al., 2009). The products were collected by a permanent magnet, and re-dispersed in 5 mL DI water. In order to grow a fully covered gold nanoshell, the reaction was repeated 4 more times. Before the Raman tests, the Au-MNPs were dried and cleaned in an oxygen plasma cleaner for 10 min. The cleaned Au-MNPs were re-dispersed in DI water to form a 30 μg/mL colloid solution.

2.3. SERS tests of 4-mercaptopyridine

All the samples in this study were prepared on silicon chips, which were thoroughly rinsed with DI water and acetone, dried under a stream of nitrogen gas, and cleaned in a UV/ozone cleaner for 20 min before use. A 10 μL drop of 3 μg/mL Au-MNPs solution was made on a silicon chip. In order to condense the Au-MNPs to a dot on the chip, one corner of a permanent magnet was put underneath the chip. After about 5 min, a dot of Au-MNPs was formed, and the liquid was completely evaporated after about another 5 min. Four chips prepared by this method were immersed in four different aqueous solutions with the 4-MP concentration of 100 nM (∼10 ppb), 10 nM (∼1 ppb), 1 nM (0.1 ppb) and 0 nM, respectively, and incubated for 3 h (McLellan et al., 2006; Yu et al., 2008). The chips were rinsed with DI water and dried under a stream of nitrogen gas. SERS spectra of 4-MP were collected using a Renishaw InVia Raman spectrometer attached to a Leica DMLM upright microscope with a 50×/N.A. 0.8 objective. The excitation laser was 785 nm and the laser power at the sample was 0.5 mW. Each spectrum was collected with 10 s exposure time and 3 accumulations.

2.4. SERS tests of bacteria

E. coli K12 (ATCC 10798) *and P. aeruginosa* (ATCC 10145) are wild types grown in Lysogeny broth for 10 h at 37 °C and *A. calcoaceticus* (ATCC 23055) grown at 30 °C. After sub-culturing from single colonies three times, the biomass was collected carefully and washed with DI water three times. The final biomass concentration was diluted to 2×10^5 cfu/mL with DI water.

To make the SERS test sample, 9 μL bacteria solution (2×10^5 cfu/mL) was mixed with 1 μL Au-MNPs (30 μg/mL) solution, and then the mixture was dropped onto a clean silicon chip. The Au-MNPs along with the bacteria were condensed into a small dot by placing one corner of a permanent magnet underneath the chip. SERS spectra were collected using the same Renishaw Raman system and the 50×/N.A. 0.8 objective. The excitation laser was 785 nm and the laser power was 0.25 mW at the sample. Each spectrum was collected with 10 s exposure time and single accumulation. To increase the statistics, 15 spectra were collected for each sample at different places within the dot area.

3. Results and discussion

3.1. Synthesis and characterization of Fe₃O₄–Au NPs

The Fe₃O₄ NPs, synthesized using the traditional co-precipitation method (Zhang et al., 2009), are composed with a number of superparamagnetic Fe₃O₄ nano-crystals (∼15 nm) and possess a final hydrodynamic size of ∼70 nm determined by