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Detecting explosive molecules from nanoliter solution: A new paradigm of SERS sensing on hydrophilic photonic crystal biosilica

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ABSTRACT

We demonstrate a photonic crystal biosilica surface-enhanced Raman scattering (SERS) substrate based on a diatom frustule with in-situ synthesized silver nanoparticles (Ag NPs) to detect explosive molecules from nanoliter (nL) solution. By integrating high density Ag NPs inside the nanopores of diatom biosilica, which is not achievable by traditional self-assembly techniques, we obtained ultra-high SERS sensitivity due to dual enhancement mechanisms. First, the hybrid plasmonic-photonic crystal biosilica with three dimensional morphologies was obtained by electroless-deposited Ag seeds at nanometer sized diatom frustule surface, which provides high density hot spots as well as strongly coupled optical resonances with the photonic crystal structure of diatom frustules. Second, we discovered that the evaporation-driven microscopic flow combined with the strong hydrophilic surface of diatom frustules is capable of concentrating the analyte molecules, which offers a simple yet effective mechanism to accelerate the mass transport into the SERS substrate. Using the inkjet printing technology, we are able to deliver multiple 100 pico-liter (pL) volume droplets with pinpoint accuracy into a single diatom frustule with dimension around 30 $\mu m \times 7\ \mu m \times 5\ \mu m$, which allows for label-free detection of explosive molecules such as trinitrotoluene (TNT) down to 10^{-10} M in concentration and 2.7×10^{-15} g in mass from 120 nL solution. Our research illustrates a new paradigm of SERS sensing to detect trace level of chemical compounds from minimum volume of analyte using nature created photonic crystal biosilica materials.

1. Introduction

The detection of a small number of molecules in miniature amount of solution is of pivotal significance in many practical applications including biomedicine, homeland security, forensics, and environmental protection (Liu et al., 2010), among which ultra-sensitive detection of trace amount of explosive chemicals has the top priority. 2, 4, 6-Trinitrotoluene (TNT) is a typical explosive material and is widely used in military as well as for terrorist activities (recent terrorist attacks in Europe and Pakistan). In addition, TNT is also widely used in underwater explosion and many other industrial applications, which could lead to the contamination of soil and ground water (Yang et al., 2010). A variety of technologies such as mass spectroscopy, photoluminescence (PL), chromatography and Raman spectroscopy (GrahamáCooks, 2005; Harvey et al., 1990; He et al., 2015; Zhen et al., 2016) are currently employed to detect TNT in the environment, among which surface-enhanced Raman scattering (SERS) is one of the most popular detection techniques (Dasary et al., 2009; Hamad et al., 2014; Jamil et al., 2015a, 2015b; Zapata et al., 2016). Although ultra-high detection sensitivity has been reported, surface functionalization of the SERS substrates sometimes are required and the Raman signals actually come from the probe molecules rather than TNT itself (Hakonen et al., 2015; He et al., 2015; Yang et al., 2010) because the binding affinity of TNT toward metallic nanoparticle surfaces is very low. Ultra-sensitive label-free detection of TNT with finger print Raman spectra still remains a grand challenge.

SERS spectroscopy is a powerful analytical technique due to its high selectivity and its ability to gather fingerprint information of analytes at trace concentration, even down to single molecules (Kneipp et al., 1997; Nie and Emory, 1997) for some chemical species. In SERS effect, the Raman enhancement are primarily attributed to the electromagnetic field induced by localized surface plasmon resonance (LSPR) as well as chemical interactions between the analyte and metallic substrate (Kong et al., 2013; Willets and Van Duyne, 2007). The LSPR is closely associated with the dielectric environment and the morphology of the nanostructure. The sharp edges, clusters, and narrow gaps of metallic

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nanostructures can significantly enhance localized electromagnetic fields, which generates 'hot spots' with large SERS enhancement factors (EFs) (Driskell et al., 2006). The fabrication of well-defined plasmonic nanostructures to obtain maximum 'hot spots' is one of the most critical aspects in SERS substrate design. Numerous reports have been focused on the design of plasmonic SERS substrates utilizing metallic or bimetallic materials (Chen et al., 2010; Gómez-Graña et al., 2013; Pei et al., 2013; Wu et al., 2012). Porous materials have long been the focus as well because of their unique physical and chemical properties, the rapid mass transport inside the micro-channels, and exceptionally high surface areas that enable them as efficient adsorbents in the liquid phase (Deng et al., 2008). When depositing metallic NPs in the pores of porous materials to form three dimensional (3-D) SERS substrates, it would bring exclusive advantages including large surface area to maximize the number of hot spots and adsorption sites for analyte compared with the planar metallic nanostructures (Ko et al., 2008b). Tsukruk's group has conducted advanced work on fabricating 3-D porous SERS substrates through loading metallic NPs in the micro-channels of porous alumina membranes (PAMs) (Chang et al., 2010; Ko et al., 2008a, 2008b). Silicon, glass and polyoxometalate materials with porous structures were also employed to prepare 3-D SERS substrates by depositing metallic NPs (Lee et al., 2007; Lu et al., 2009; Xu et al., 2013; Zhang et al., 2015). These substrates possess excellent SERS sensitivity for various analytes.

In most practical SERS sensing applications, the target molecules are dispersed in solutions and are free to diffuse into the liquid volume. During the process of mass transport into the SERS substrate, the surface property of SERS substrate may lead to the spread of the aqueous sample over a bigger area than that of the plasmonic sensing surface (Shao et al., 2015), which makes it difficult to concentrate the analyte molecules for high detection sensitivity. Therefore, much recent attention has been paid toward the surface wettability of SERS substrates (De Angelis et al., 2011; Hakonen et al., 2016; Shao et al., 2015). Fabrizio and co-workers reported SERS substrates with super-hydrophobic artificial surfaces(De Angelis et al., 2011), which could concentrate target samples at the SERS sensing area and enable the detection of Rhodamine 6G (R6G) at atto-molar concentration. The SERS active metallic colloids with hydrophilic surface were also employed to confine analyte molecules by spotting them onto a hydrophobic substrate. But these approaches require specific fabrication processes such as optical lithography and ion etching or functional polymers to modify the surface of the SERS substrates.

Diatoms are unicellular, photosynthetic biomineralization marine organisms that possess a biosilica shell called a frustule. PL-based diatom biosensors have been successfully developed, including the detection of TNT down to 3.5×10^{-8} M (Zhen et al., 2016). Compared with traditional porous substrates, the two dimensional (2-D) periodic pores on the 3-D diatom frustule with hierarchical nanoscale photonic crystal features can enhance the local optical fields at the surface of and inside diatom frustules (Jeffryes et al., 2008b; Yang et al., 2011). Our previous studies have shown that such unique photonic crystal features are capable of enhancing LSPRs of self-assembled metal NPs on the surface of diatom frustules, which will enable additional enhancement of SERS signals of the molecules adsorbed on the metallic NPs (Ren et al., 2014; Ren et al., 2013; Yang et al., 2014). In this work, we demonstrated a simple and fast method to integrate Ag NPs by an in-situ growth method from electroless deposited seeds within the photonic nanoporous diatom for the fabrication of 3-D hybrid plasmonic–photonic crystal biosilica SERS substrates. The advantages of in-situ growth diatom-Ag NPs compared with self-assembled diatom-Ag NPs come from two aspects: 1) the higher density Ag NPs at the surface that will introduce more hot-spots; and 2) many more Ag NPs in the pores of diatom frustules as our numerical simulation results have proved that in-pore Ag NPs can provide even higher SERS enhancement factors (Ren et al., 2013). In addition to the well-known photonic crystal effect, diatom also offers unique surface properties, which have been rarely investigated before. Compared with conventional flat glass slides, the abundant hydroxyl groups on the surface make diatom biosilica very hydrophilic. The highly hydrophilic surface and highly ordered nanopores of diatom frustules could drive the liquid flow from glass towards the diatom frustule due to capillary forces. Continuous mass transport can be sustained as the liquid evaporates (Buffone and Sefiane, 2004; Sefiane and Ward, 2007). As a result, the highly hydrophilic and porous diatom frustule provides a driving force to concentrate the target molecules, which can help to improve the detection limit by orders of magnitudes.

In addition to the optimization of SERS substrates, another practical challenge of biosensors is how to efficiently deliver miniature amount of analyte solution to the sensor surface. Microfluidic devices could perform assays at nanoliter-volume scale. However, the complicated microfluidic chips, expensive control systems, and assay-specific surface modifications are indispensable. Inkjet printing is a fast, simple and cost effective technology, which provides the possibility of pinpoint accuracy and miniature amount of analyte consumption (Baluya et al., 2007; Wang et al., 2009). When combined with diatom frustules that have comparable size as the inkjet droplets, inkjet printing could effectively accumulate the analyte molecules into a single or a few diatom frustules, and therefore improve the detection limit. We developed a novel strategy to precisely dispense miniature amount of analyte into a single diatom by inkjet printing as shown in Fig. 1. Each analyte droplet is controlled around 100 pico-liter (pL) and multiple droplets can be precisely delivered to the same diatom frustule. The multiple cycles of droplet dispense-evaporation provide a simple yet effective mechanism to accelerate the mass transport into the hybrid plasmonic-biosilica SERS substrate. The hydrophilic porous nanochannels and the photonic-plasmonic coupling effect of diatom biosilica are combined in a synergistic way to allow ultra-sensitive TNT detection in miniature volume of solutions.

2. Experimental section

2.1. Materials and reagents

Silver nitrate ($AgNO_3$) was obtained from Alfa Aesar. Ethylenediaminetetraacetic acid (EDTA), Tin (II) chloride ($SnCl_2$), ascorbic acid and poly-diallyldimethylammonium chloride (PDDA) were purchased from Sigma-Aldrich. Trisodium citrate ($Na_3C_6H_5O_7$), hydrochloric acid (HCl), sodium hydroxide (NaOH), ethanol and acetone were purchased from Macron. Rhodamine6G (R6G) was obtained from TCI. All the reagents were used as received without further purification. Water used in all experiments was deionized and further purified by a Millipore Synergy UV Unit to a resistivity of $\sim$18.2 MΩ cm.

2.2. Fabrication of diatom biosilica substrates

Diatom cells were (*Pinnularia sp.*) cultivated according traditional microbiological cultivation with modification (Jeffryes et al., 2008a). Diatoms were cultured in flasks for 7 days, 400 mL of diatom suspension were centrifuged and dispersing with 40 mL of sterile filtered artificial seawater. The suspended cells were transferred into new centrifuge tube using a 20 μm mesh filter to collect isolated cells, the cell density was diluted to 2.5×10^5 cells/mL for seeding. Each coverslip was placed into