Published in final edited form as:

Proc SPIE Int Soc Opt Eng. 2015 April 20; 9488: . doi:10.1117/12.2178077.

Bioenabled SERS Substrates for Food Safety and Drinking Water Monitoring

Jing Yang¹, Gregory L. Rorrer², and Alan X. Wang^{1,*}

¹School of Electrical Engineering and Computer Science, Oregon State University, Corvallis, OR, 97331, USA

²School of Chemical, Biological & Environmental Engineering, Oregon State University, Corvallis, OR, 97331, USA

Abstract

We present low-cost bioenabled surface-enhanced Raman scattering (SERS) substrates that can be massively produced in sustainable and eco-friendly methods with significant commercial potentials for the detection of food contamination and drinking water pollution. The sensors are based on diatom frustules with integrated plasmonic nanoparticles. The ultra-high sensitivity of the SERS substrates comes from the coupling between the diatom frustules and Ag nanoparticles to achieve dramatically increased local optical field to enhance the light-matter interactions for SERS sensing. We successfully applied the bioenabled SERS substrates to detect melamine in milk and aromatic compounds in water with sensitivity down to 1 μ g/L.

Keywords

SERS; Food Sensor; Bioenabled Nanomaterials; Photonic Crystals; Surface Plasmons

1. INTRODUCTION

Food and drinking water safety is now an omnipresent problem and is becoming a critical concern of the United States as well as in many developing countries such as China and India. Centers for Disease Control (CDC) of the U.S. estimates that each year roughly 1 in 6 Americans (or 48 million people) get sick, 128,000 are hospitalized, and 3,000 die of foodborne diseases. In China, numerous scandals such as gutter oil, baby formula, and McDonald's meat have raised food safety as the top social issue in recent years. The concern of drinking water is majorly caused by emerging contaminants as pointed out by the Environmental Protection Agency (EPA). For example, synthetic organic compounds are used heavily in our daily life in vast quantities for the production and preservation of food, in industrial manufacturing, as well as for healthcare of human and animals. Most of these organic compounds ultimately enter urban wastewater system. Although dilution and degradation during wastewater and sludge treatment removes significant amounts of the

*Corresponding authors: wang@eecs.oregonstate.edu.

organic contaminants, many of them can still go into sewages sludge and cause groundwater and soil pollution [1].

Among many sensing techniques for food safety and drinking water monitoring, Raman spectroscopy stands out and is currently widely used as a simple and reliable tool for quality control and analysis because: 1) Raman peaks are unique and represent the signatures of the vibrational bonds of many molecules, and therefore Raman spectroscopy does not require costly labeling processes to identify the molecules; 2) water has almost no Raman scattering signals, so it is very convenient to detect very low concentration analytes in solution or from natural biological samples; and 3) due to the narrow bands of Raman peaks, it is very convenient to achieve multiplex sensing by identifying the fingerprint spectra of multiple molecules [2]. As a comparison, infrared (IR) absorption spectroscopy [3], which is another widely used vibrational spectroscopy technique, has very limited potential for the detection of water pollution and food contaminants due to the wideband and strong IR absorption of water. However, spontaneous Raman scattering is typically extremely weak, which is only about 10^{-8} to Rayleigh scattering. Since its discovery in 1977 [4–6], surface-enhanced Raman scattering (SERS) spectroscopy has greatly overcome the limits of Spontaneous Raman scattering and enhanced the Raman scattering signals by several orders of magnitude. Such dramatic improvement comes majorly from the plasmonic resonances of metallic nanoparticles (NPs), usually gold (Au) or silver (Ag) NPs with extremely large local field enhancement factors (EFs) at the NP surfaces to boost the Raman signals.

Although unprecedented sensitivity, reproducibility, and specificity have been achieved, many rationally designed optical sensors using cost-prohibitive top-down nanofabrication techniques [7] may not be widely used as disposable sensors for food safety and drinking water monitoring. On the other hand, nature provides an inspirational source of nanophotonic structures. Many photosynthetic marine micro-organisms efficiently capture sun light for photosynthesis by imbedding inorganic periodic photonic structure, which is very similar to artificial photonic crystals (PCs), into their cell walls. Unlike rationally designed PCs using cost-prohibitive top-down lithographic and reactive ion etching (RIE) techniques, diatoms create their own skeletal shells of hydrated amorphous silica, called frustules that possess photonic crystal-like features by bottom-up approach at ambient temperatures and pressures. Diatoms take up water soluble silicic acid from the environment which is then precipitated into amorphous silica within an intracellular nano-bioreactor to form the frustules. In this work, we demonstrate a simple bioenabled nanophotonic sensor for the rapid detection of melamine and aromatic compounds using diatom frustules decorated with Ag NPs as active SERS substrates. The photonic crystal structures enable diatom frustules to enhance localized surface plasmon resonances of self-assembled silver nanoparticles (Ag NPs) at the surface of diatom frustules [8].

2. PREPARATION OF BIOENABLED NANOPHOTONIC SERS SUBSTRATES

We present the fabrication and characterization of the bioenabled SERS substrates by integrating Ag NPs into diatom frustules using cost-effective fabrication techniques. Diatoms (*Pinnularia sp.*) were prepared by traditional microbiological cultivation approach which requires two-steps [9]. In the first step, glass substrates will be seeded with live

diatom cells from uniform cell suspension and removed from liquid media. The seed cells will be cultured and then be harvested during active growth phase. Cells will be collected and washed with fresh medium and filtered using a 20 μm polypropylene mesh filter to separate aggregated diatom cells. Biologically prepared monolayers will be treated to remove organic cellular material without disruption of the frustule biosilica structure. In the second step, seeded substrates will be incubated in a humidified chamber under illumination at $100\mu\text{mol m}^{-2} \text{sec}^{-1}$, with a 14:10 light: dark cycle for 48–72 hours. It is expected that the thin liquid film present over the growing cells will result in uniform monolayer growth, which proves the possibility of forming a monolayer of dense diatom frustules. To improve the adhesion of diatom frustules to the glass substrates, the samples were annealed at low temperature. Then the diatom frustules were functionalized with a positive charged thin film of Poly-diallyldimethylammonium chloride (PDDA), and then were immersed into Ethylenediaminetetraacetic acid (EDTA)-reduced Ag NPs for the self-assembling of Ag NPs [10]. Compared with our previous research, the Ag NPs prepared by this method are uniform and have a narrow size distribution of 40–50 nm in diameters as shown in Fig. 1(a). The scanning electron microscopy (SEM) image of Fig. 1(b) shows a representative image of a diatom frustules functionalized with Ag NPs, and the zoomed view of the frustules with its fine structures is shown in Fig. 1 (c).

3. SERS SENSING OF MELAMINE

Melamine is an industrial material which is mainly utilized for resin production, and polymer manufacturing [11]. Due to its high nitrogen rate (67% by mass), melamine was intentionally and illegally added into food ingredients, such as milk and candy, to produce an incorrectly high reading in the measurement of the protein content. The safety limits of melamine for infant formula and milk made by the World Health Organization (WHO) are 1 mg/L and 2.5 mg/L, respectively [12]. Ingestion of high level of melamine can cause renal failure and even death in humans because it can lead to the formation of insoluble crystals which were responsible for renal failures [13]. Instant detection of travel level of melamine requires ultra-sensitive, low cost, and highly specific sensors.

In a proof-of-concept experiment, standard melamine solution (10 μL) from Sigma Aldrich, Inc. with different concentrations was pipetted onto the surface of the bioenabled SERS substrates. After drying, the SERS signals of melamine were collected via a Horiba HR800 spectrometer with an integration time of 20 seconds. The quantitative SERS signals of melamine on flat glass slides and on the bioenabled nanophotonic sensors were shown in Fig. 2 (a) (b), respectively. Each spectrum represents the average measurement results of 10 randomly selected spots on diatom frustules. The most prominent Raman peak at 680 cm^{-1} is the characteristic Raman peak of melamine. The intensity of the SERS signals of melamine shows a strong correlation with the concentration of melamine. Diatom frustules provide stronger SERS signals of melamine when compared with flat glass slides. A quantitative comparison of the 680 cm^{-1} peak is presented in Fig. 2(c). The curves show the Raman intensity of melamine at 680 cm^{-1} vs. the log-scale concentrations of melamine on diatom frustules and flat glass slides. The limit of detection (LOD) of melamine on diatom frustules is $1\mu\text{M}$ (0.126 mg/L), which is much lower than the safety standard by WHO. We shall also notice that the LOD of melamine on the bioenabled nanophotonic sensor is

improved by 10× when compared with the LOD of melamine on Ag NPs functionalized flat glass slides.

4. SERS SENSING OF AROMATIC COMPOUNDS

Organic compounds, especially, aromatic compounds, are major pollutants of water. The potential persistence and toxicological effects to the environment and their biodegradation products are major concerns for regulatory agencies in the U.S. and the European Union. A maximum concentration limit (MCL) of $< 1 \mu\text{g/L}$ has been set for aromatic compounds in drinking water [14]. Quantitative dose-response curves of the SERS signals of some aromatic compound suggest a linear relationship with the log-scale concentration [15–17], and an EF of the SERS signals at 4× may improve limit of detection (LOD) by 3–4 orders. Our previous study demonstrated that the R6G Raman signal intensity from the nanomaterials is enhanced by up to 12× when compared to that of NPs-on-glass [18]. Therefore, the bioenabled nanomaterial is expected to improve the LOD of aromatic compounds by 3–4 orders. Previous study reported a LOD at 20 $\mu\text{g/L}$ of benzidine based on SERS detection with a portable Raman system [19]. In a proof-of-concept experiment, we have chosen 5, 5'-dithiobis (2-nitrobenzoic acid) (DTNB) as a model aromatic compound. The SERS spectra of DTNB were detected with LabRAM HR 800 Raman microscope by pipetting a small droplet of 1 $\mu\text{g/L}$ DTNB solution onto the bioenabled nanomaterials, as shown in Fig.3 (a). The optical image of a single diatom [Fig.3 (b)] and the SERS mapping results [Fig.3 (c)] with nearly exactly the same morphology confirm the enhanced SERS signals.

Sensitive SERS detection requires precision and expensive spectrometers such as confocal microscopes. In most cases, high-sensitivity SERS detection can be only achieved using bench-top equipment. Although portable Raman spectrometers are commercially available, the sensitivity is not comparable with Raman microscope. *In-situ* sensing with a portable Raman spectrometers requires highly sensitive sensor substrates that can be produced in cost-effective and eco-friendly fabrication techniques, which has not been provided yet. We demonstrated SERS sensing of DTNB solution in diatom-plasmonic SERS substrates via portable Raman spectrometer, ID Raman reader, as shown in Fig. 4. The LOD of DTNB with portable Raman spectrometer is 1 nM, which is comparable with that obtained by the confocal Raman spectroscopy with low numerical aperture objective lens.

5. CONCLUSION

In summary, we have successfully applied bioenabled SERS for melamine and aromatic compound detection, which exhibited excellent enhancement of the LOD that surpasses the food safety standard of WHO and EPA requirement. Such low cost, ultra-sensitive devices can be used as disposable optical sensors for food safety and drinking water monitoring.

Acknowledgments

Alan X. Wang would like to acknowledge the support of the National Institute of Health under grant No. 9R42ES024023 and 1R03EB018893, as well as the support of Oregon Nanoscience and Microtechnologies Institute (ONAMI).

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Fig.1.

SEM images of (a) EDTA-reduced Ag NPs with uniform size of 40–50nm; (b) a single diatom frustule with self-assembled Ag NPs; and (c) zoom view of Ag NPs and the fine structure of the diatom frustule with sub-micron pores.

Fig. 2.

SERS spectra of melamine with different concentrations on (a) Ag NPs on glass substrates (a), (b) Ag NPs on diatom frustules, and (c) comparison of the Raman peaks at 680 cm^{-1} .

Fig.3.

(a) SERS spectra of $1\mu\text{g/L}$ DTNB on the hybrid plasmonic-biosilica nano-probes; (b) Optical image of a single plasmonic-biosilica nano-probe; and (c) SERS mapping results of the DTNB band at 1331 cm^{-1} .

Fig. 4.

(a) SERS spectra of DTNB on Si substrates using the portable Raman spectrometer. (b) Optic image of the portable Raman spectrometer from Ocean Optics