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Laser-Fabricated Plasmonic Nanostructures for Surface-Enhanced Raman Spectroscopy of Bacteria Quorum Sensing Molecules

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Abstract

We used a laser-directed fabrication to create silver nanostructures on glass cover slips via photo-reduction. The resulting silver films exhibited plasmonic properties which show promise in application towards surface enhanced Raman spectroscopy (SERS). The enhancement factor calculated for the deposits was approximately $\sim 10^6$ using the standard thiophenol, which is comparable to other SERS-active plasmonic nanostructures fabricated through more complex techniques, such as electron beam lithography. The silver nanostructures were then employed in the enhancement of Raman signals from *N*-butyryl-L-homoserine lactone, a signaling molecule relevant to bacteria quorum sensing. In particular, the work presented here shows that the laser-deposited plasmonic nanostructures are promising candidates for monitoring concentrations of signaling molecules within biofilms containing quorum sensing bacteria.

Keywords

biomaterial; optical properties; Raman spectroscopy

INTRODUCTION

Directed fabrication of mesoscopic plasmonic structures is a highly desirable asset for numerous areas in science and technology such as electronics, photonics, tissue engineering, and sensing. Recently, developments in the laser-directed fabrication of noble metal nanostructures has become a powerful tool to create a variety of directed assemblies that are promising for microelectronics[1–3] and Raman sensing [4–6]. Compared to other precise nanofabrication technique such as electron beam lithography [7], NanoSphere lithography [8], or dip-pen lithography [9], laser-deposition of metal nanostructures is more time and labor efficient, less costly, and offers more flexibility in the type of utilized substrates. Surface-enhanced Raman spectroscopy (SERS) has been widely studied using noble metal nanomaterials due to their remarkable plasmonic properties in the visible-near-infrared

region [10]. Until now, preparations of active SERS substrates mainly concentrated on chemical reaction in solution or vapor deposition, while the controlled and directed deposition of SERS-active structures on arbitrary substrates has presented a great challenge.

Quorum sensing bacteria produce and release signaling molecules (autoinducers) that increase in concentration as a function of cell density [11–14]. Gram-positive and Gram-negative bacteria use quorum sensing communication circuits to regulate a variety of physiological activities. Acyl-homoserine lactones (AHLs) are the most common autoinducer molecules used by Gram negative bacteria [15]. Several analytic techniques have been utilized to detect AHLs, including gas chromatography-mass spectrometry [16], thin-layer chromatography [12], biological assay [17], and the use of live bacterial sensors [18]. Recently, it has also been reported that noble metal nanoparticles can be used as effective SERS substrates to detect AHLs at very low concentrations [19, 20]. In our study of such possibilities, we fabricated silver nanostructures using the laser-deposition technique by a photoreduction reaction of noble metal ions from aqueous solution. The surface enhancement factor (EF) of the Raman scattering was evaluated for the laser-deposited silver nanostructures using thiophenol as the model molecule. These silver nanostructures were then used as a SERS sensor to detect the Raman signals from *N*-butyryl-L-homoserine lactone, a quorum sensing molecule in the family of AHLs.

EXPERIMENTAL

In a typical laser-deposition, AgNO₃ (2 mM, Sigma-Aldrich) was mixed with an aqueous solution of sodium citrate (NaCit, 0.1 M, Sigma-Aldrich) in a 1:1 molar ratio. A drop of this mixed solution was then placed onto a glass cover slip which was then mounted under a Leica TCS SP5 confocal laser scanning microscope. A 405 nm wavelength diode laser (7 mW) was employed to deposit silver nanostructures at the liquid-substrate interface using a 10× objective lens (NA=0.25). Parameters for the confocal microscope were tuned so that the deposition resulted in a 1mm×1mm square consisting of the silver nanostructures.

Morphology of the laser-deposited silver structures was characterized by using the Atomic Force Microscopy (AFM) mode in a Nearfield Scanning Optical Microscope (NSOM, Alpha300, Witec). The absorption spectra were acquired by an optical spectrometer (HR4000, Ocean Optics) using a high power Xenon lamp (HPX-2000, Ocean Optics). Raman spectra of thiophenol (Sigma-Aldrich) and *N*-butyryl-L-homoserine lactone (Cayman Chemical Company) were acquired through Raman mode in the NSOM utilizing a Nd-YAG laser (532 nm) and 20× objective lens (NA=0.4). The acquisition time was fixed to 10 s.

RESULTS AND DISCUSSION

The employed laser deposition technique was successfully used to produce noble metal wires, dots and patches, as shown in Figure 1. Our previous studies have also shown that this technique can be used to make micro-sized silver wires with manipulable conductivities [21]. In this work, we focus on producing continuous silver films with nanosized structures (Fig. 1c), which exhibits appreciable surface plasma resonance properties that are suitable for

SERS. Figure 2(a) shows the AFM image of a laser deposited silver film with nanosized structures. Its rough surface is favorable of forming the “hot-spots” ideal for SERS. While the wires and dots did not form a dense enough arrangement to obtain reliable optical spectra, the absorption spectrum shown in Figure 2(b) indicates that the silver patches possess a plasmonic resonance centered around 520 nm. Because the SERS intensity strongly depends on the excitation of local surface plasma resonance [10], these laser-deposited silver nanostructures are expected to exhibit optimal SERS effects in the following Raman characterization as the excitation laser was 532 nm, near the maximum of the structures SPR.

To compare the SERS efficiency of the laser-deposited silver nanostructures to those of other relevant substrates, we evaluated the Enhancement Factor (EF) using thiophenol as the model molecule. Thiophenol has been widely used to evaluate the EF of noble metal nanomaterials due to its simple chemical structures and strong affinity to noble metal surfaces [22–25]. This affinity enables the formation of a stable self-assembled monolayer advantageous to the precise measurement of the surface’s EF. Before SERS measurements, a 0.1 mM solution of thiophenol in ethanol was prepared and dropped onto laser-deposited silver nanostructures. After 2 h soaking, the substrate was washed thoroughly with ethanol and dried. The EF was estimated using the following equation:

$$EF = \frac{I_{SERS}}{N_{SERS}} / \frac{I_{Raman}}{N_{Raman}} \quad (1)$$

where I_{SERS} and I_{Raman} are the measured Raman intensities for SERS and normal Raman spectroscopy, respectively. N_{SERS} is the number of thiophenol molecules in the detection volume, which can be calculated as:

$$N_{SERS} = N_A \times \sigma_{surf} \times S \quad (2)$$

where N_A is Avogadro’s number, S is the area of collection, and σ_{surf} is the surface coverage of thiophenol which is approximately 0.544 nmol/cm² [26].

N_{Raman} in Equation (1) can be calculated as:

$$N_{Raman} = N_A \times C \times V \quad (3)$$

where C is the concentration of thiophenol (1 M), and V is the scattering volume which was calculated based on a method reported previously [27]. Specifically, the diameter of laser spot was about 4 μm and the collection depth was approximately 140 μm. Assuming that the illuminated cross-sectional area remains the same along the focal spot, the total effective detection volume was approximately 1.76 pL.

The strongest signature stretching mode at 1005 cm⁻¹ was used in the calculation of the EF, as shown in Figure 3. With an SERS intensity 36 times higher than that of the normal

intensity, the EF was calculated to be 9.27×10^5 . Although this result seems minimal when compared to EFs reported using gold nanodisks [25] or nanopillar arrays [24], these fabrication techniques can be expensive and time-consuming. Alternatively, the laser deposition technique offers a straightforward and cost-effective method to produce noble metal nanostructures with useful SERS efficiencies.

Figure 4 shows the SERS spectrum of *N*-butyryl-L-homoserine lactone (0.1 mM) once placed on the laser-deposited silver microstructures. The amide I (1653 cm^{-1}), amide II (1508 cm^{-1}), amide III (1285 cm^{-1}), and amide IV (795 cm^{-1}) bands are clearly visible as compared to those in its' normal Raman spectrum (Fig. 4 inset). Previous studies have shown that colloidal silver nanoparticles can be applied to measure quorum sensing molecules with detection limits down to 10^{-9} M [19, 20]. In our study, because the molecules' Raman signals are only enhanced when the close enough to the surface of silver films, the sensitivity of SERS may be lower than those of using nanoparticle suspensions. However, one key advantage that the laser-deposited structures offer is reusability of both the analyte and the SERS substrate. This is particularly crucial for continuous and reproducible Raman measurements. Detailed detection limit investigations of the laser-deposited silver nanostructures on *N*-butyryl-L-homoserine lactone and other molecules in the family of AHLs will be carried out in our future studies.

CONCLUSION

A laser-deposition technique was utilized to fabricate silver nanostructures which exhibit characteristic surface plasma resonance. These structures can be used as effective substrates for surface enhanced Raman spectroscopy. The enhancement factor was determined as $\sim 10^6$, which is comparable to those metal nanostructures fabricated by complex techniques such as electron beam lithography. The applicability of laser-deposited silver nanostructures for enhancing Raman signals of *N*-butyryl-L-homoserine lactone, a quorum sensing molecule, was demonstrated. These results suggest that the laser-fabricated noble metal nanostructures are promising candidates for sensitive detection of bacteria quorum sensing molecules based on SERS.

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Figure 1. Optical images of various silver deposits. The silver micro-wires (a) and dots (b) did not exhibit strong SPR absorption, while the patches (c) did.

Figure 2.
(a) AFM image and (b) absorption spectrum of laser-deposited continuous silver nanofilms.

Figure 3. SERS spectrum of thiophenol on a laser-deposited silver nanostructure. The inset shows normal Raman spectrum of thiophenol (1 M), where the molecule was not attached to a metal deposit.

Figure 4. SERS spectrum of *N*-butyryl-L-homoserine lactone on a laser-deposited silver nanostructure. The inset shows normal Raman spectrum of *N*-butyryl-L-homoserine lactone (0.1 mM) , where the molecule was not attached to a metal deposit.