Beyond the SERS: Raman Enhancement of Small Molecules Using Nanofluidic Channels with Localized Surface Plasmon Resonance

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Surface-enhanced Raman spectroscopy (SERS) has been extensively used to identify the fingerprints of biological molecules at low concentration as a label-free and nondestructive optical detection. The SERS with localized surface plasmon resonance (LSPR) can even allow biomolecular detection at attomolar levels. Diverse nanostructures with LSPR near an excitation wavelength induce strong local-field enhancement at electromagnetic hot spots, where target biomolecules manifest an extraordinary SERS enhancement factor. However, the spatial confinement of biomolecules near hot spots for intense SERS is still challenging. Surface-binding methods can be involved to attach biomolecules on metal surfaces, but they are time-consuming and limited to certain target molecules; moreover they even hinder the label-free detection, which is a major advantage of SERS. Small molecules include cell-signaling molecules, pharmaceuticals, metabolites, and many other natural or artificial organic compounds. They play key roles in miscellaneous cellular functions and treating diseases. For example, neurotransmitters transmit signals from neurons to target cells, thus affecting the activities of neural networks that are crucial to the motion, memory, muscle contraction, emotional response, and a number of other bodily functions. In addition, most drugs across cell membranes or the blood-brain barrier are made of small molecules. Consequently, the detection and identification of small molecules has become an emerging issue. SERS is a promising candidate for identifying small molecules as a label-free method. However, most small molecules show very weak signals, thus requiring high excitation power and long exposure time. Therefore, the spatial confinement of small molecules near hot spots is essential for the SERS detection of small molecules at low concentration.

Nanofluidics offers promising prospects for controlling biomolecular transport in nanoscale. Nanofluidic mass transport has unique features in the strong interaction between fluids and channel walls due to the large surface-to-volume ratio. The infinitesimal nature provides distinct fluid dynamic behaviors induced by surface charge. Additionally, nanofluid has physicochemical peculiarities originating from the intrinsic tiny nanospace inside the channel for trapping and separating the molecules. The recent developments have been driven by the spatial confinement of biomolecules with electrokinetic, hydrodynamic, or optical methods. Most preconcentration techniques are coupled with fluorescence detection to obtain high sensitivity and signal-to-noise ratio. However, previous work has not taken full benefit of both manipulating and detecting label-free biomolecules at the nanoscale, even though nanofluidic methods can facilitate the spatial confinement of biomolecules at nanoscale detection sites.

This work presents the SERS enhancement of small molecules spatially entrapped near electromagnetic hot spots by using nanofluidic channels with plasmonic nanostructures. A monolayer of silver film over silica nanospheres (AgFONs) is prepared on a glass substrate and then simply covered with a polydimethylsiloxane (PDMS) slab (Figure 1). The interstitial nanogaps between the AgFONs and PDMS feature nanofluidic channels. A small-molecule analyte at low concentration is introduced into the nanofluidic channels by capillary force. A stagnation point, i.e., point of zero velocity, has been widely used to trap and concentrate nanoparticles or biomolecules in electrokinetic, hydrodynamic, thermophoretic, and optoelectrofluidic devices. The nanofluidic channel has an assortment of stagnation points near the cuspidal points between contiguous nanospheres, where the diffused small molecules are spatially confined and concentrated (Figure 1). The concurrence between nanofluid stagnation points and hot spots with plasmon resonance substantially contributes to high enhancement of the SERS signals. This work describes the plasmon tuning of AgFONs, the nanofluidic confinement of small molecules near hot spots, and the ultrahigh SERS enhancement of major neurotransmitters.

The resonance wavelength of metal film over nanosphere ensembles can be tuned by metal composition, shape, or environment. Four different shapes of AgFONs were obtained by an oblique angle deposition (OAD), i.e., tilting.
the substrate orientation by 0°, 25°, 50°, and 75° under the electron beam evaporation of a 20 nm thick silver film (Figure 2a). The AgFONs show strong asymmetry because silver vapors are blocked by neighboring nanospheres during the evaporation, so that it enables wide-range tuning of the LSPR wavelength in the visible range of 450 to 700 nm. The dark-field backscattering was also measured for the AgFONs in distilled water. The results show a strong blue-shift as the deposition angle increases. In other words, the LSPR wavelengths are 642, 607, 547, and 490 nm at the deposition angle of 0°, 25°, 50°, and 75°, respectively.

Figure 1. SERS enhancement of small molecules spatially entrapped near electromagnetic hot spots by using nanofluidic channels with localized surface plasmon resonance (LSPR). A monolayer of silver film over silica nanospheres (AgFONs) is prepared on a glass substrate and covered by a polydimethylsiloxane (PDMS) slab. Small molecules are introduced into the interstitial nanogaps between AgFONs and PDMS, which serve as ‘nanofluidic channels.’ The concurrence between nanofluid stagnation points and electromagnetic hot spots significantly increases the SERS signals due to a large number of small molecules placed near hot spots.

Figure 2. Plasmon tuning and SERS enhancement of AgFONs with LSPR at the excitation wavelength of 488 nm: a) SEM images of a silica nanosphere monolayer on a glass substrate and silver thin films on nanospheres evaporated at different angles (0°, 25°, 50°, and 75°) to tune the plasmonic resonance near 488 nm. b) Depending on the evaporation angle, the backward scattering spectra measured in a medium of distilled water, and SERS signals of adenosine as a reference molecule (laser power: 10 mW, detection time: 1 s). The scattering spectra are blue-shifted as the evaporation angle increases. A silver evaporation with a tilting angle of 75° provides the plasmon resonance at 490 nm, which is near the SERS excitation wavelength. The maximum SERS signals are clearly shown when the excitation wavelength fits with the plasmon resonance. The peak height at 735 cm⁻¹ increases about 80 times compared to a bare silver film (20 nm).
angles of 0°, 25°, 50°, and 75°, respectively. The smaller aspect ratio from the higher deposition angle gives a blue-shifted resonance. The SERS measurement was performed with adenosine at an excitation of 488 nm. Note that the LSPR wavelength of AgFONs with a 75° deposition angle is 490 nm, which is close to the SERS excitation wavelength of 488 nm. Due to the LSPR matching, the AgFONs clearly show the maximum enhancement of the SERS peak height at 735 cm⁻¹, roughly 80 times higher than that of a bare silver film (Figure 2b).

The nanofluidic confinement of small molecules near hot spots was demonstrated by using fluorescent dyes with confocal laser scanning microscopy (CLSM) (Figure 3). A monolayer of AgFONs is covered by a PDMS slab that provides both the spacing for nanofluidic channels and the prevention of evaporation. The cross-sectional view of the AgFONs shows the interstitial nanogaps between AgFONs evaporated at 75° (Figure 3a,b). The upper cross-sectional channel area with silver structures is about 1100 nm², which corresponds to the hydraulic diameter of 14.7 nm. Fluorescent dyes (Rhodamine 6G) were introduced into the nanofluidic channels by strong capillary force due to the hydrophilicity of silica nanoparticles. Using a CLSM with an excitation wavelength of 488 nm, a laser-induced fluorescence image was obtained for 1 μm Rhodamine 6G (R6G) dyes at the interface area (180 μm × 180 μm) between the reservoir and the nanofluidic channels with plasmon resonance (Figure 3c). Note that the reservoir has AgFONs with no nanofluidic channels. The fluorescence intensities show clear difference between the reservoir and the nanofluidic channels. Apparently, the enhanced fluorescence intensity states the increment of local R6G concentration near hot spots inside nanofluidic channels. Figure 3d shows a histogram of the fluorescence intensities measured in each pixel of the fluorescence image. The data were extracted from the 310 μm² area of the nanochannel and the reservoir, and then each area was divided into 2500 pixels. The number of pixels with the same fluorescence intensity at the nanochannel is clearly higher at high intensity, compared to those at the reservoir. These results indicate that the nanofluidic channels increase the probability that molecules can be confined near the hot spots between AgFONs.

The local confinement of biomolecules can be explained by the following mechanisms: during the early stage of capillary action, a fluid wets the nanochannel wall, which promotes the physisorption of molecules onto silver surfaces by van der Waals force. After filling the interstitial nanogaps between nanoparticles, the nanofluid forms stagnation points at the cursoidal points between the AgFONs that corresponds to the electromagnetic hot spots. The subsequent capillary flow continues to transport molecules through the nanochannel. Due to the minuscule nanospace, the molecules can be rapidly diffused and trapped into the stagnation points, where the molecules would only show highly restricted motion. Therefore, the considerable increase of the number of molecules at the stagnation points, i.e., the hot spots between AgFONs, eventually improves the fluorescence intensities.

The population of molecules near hot spots can also be utilized for explaining SERS enhancement by the nanofluidic channels with LSPR. From the previous report, ‘hottest sites’ of the Raman enhancement factor over 10⁹ account for 24% of the total SERS intensity. Referring to the value, the number of ‘hottest sites’ can be estimated from the fluorescence measurement data. The SERS intensity is roughly...
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proportional to the fourth power of the electric field, while the fluorescence intensity is related to the square. The fluorescence intensities were squared to be considered as indicators of SERS intensities and then the number of ‘hottest sites’ was calculated from the number of pixels that contributes to 24% of the total squared intensity. The number of ‘hottest sites’ calculated from the nanofluidic channels with plasmon resonance (enhancement factor > 10^4) turns out to be 112 out of a total 2500 pixels. In contrast, the number at the reservoir is only 12 out of the total number of pixels. The calculated values suggest 9.3-fold enhancements of the number of molecules near hot spots inside the nanofluidic channels, compared to that of the reservoir.

The SERS spectra of small molecules have also been measured with two major neurotransmitters, namely dopamine and gamma-aminobutyric acids (GABA). Dopamine and GABA solutions of different concentrations were prepared in distilled water. The SERS signals at the nanofluidic channels was measured with different concentrations and compared with those at the reservoir (Figure 4). The results show that the SERS signals increase with the concentrations of both neurotransmitters. They are also significantly enhanced at the LSPR nanofluidic channels. The SERS peak height of dopamine at 1605 cm⁻¹ from the nanofluidic channel is about 8 times, and the peak of GABA at 1370 cm⁻¹ from nanofluidic channel is about 20 times higher than that from each reservoir. The difference of the signal enhancement factor for small molecules might originate from the molecular weight. The molecular weight of the dopamine hydrochloride and GABA is 189.64 and 103.12 g mol⁻¹, respectively. The lower molecular weight of the GABA would give a smaller effective diameter and a higher diffusion coefficient, which may provide a higher flux toward the stagnation points. All the experiments have been conducted under an excitation wavelength of 488 nm, and the major SERS fingerprints from both neurotransmitters were observed at 10 nm. This result supports that the LSPR nanofluidic channel enables high-sensitive SERS detection by trapping neurotransmitters near hot spots. Note that the LSPR-matched nanoensembles increases SERS intensities by more than one order of magnitude compared to rough silver films and the LSPR nanofluidic channel also increases the signals by about another order of magnitude. It turns out that the nanofluidic channel with LSPR can enhance the SERS signals by a total of two orders of magnitude or more than a simple SERS-active substrate.

To conclude, this work demonstrates the SERS enhancement of small molecules by employing nanofluidics and nanoplasmonics. The concurrence between nanofluid stagnation points and electromagnetic hot spots increases the probability that small molecules can be entrapped near hot spots by one order of magnitude. Consequently, this method provides the SERS enhancement by approximately a factor of ten or more higher than that of the substrate only with plasmon resonance so that it allows the label-free detection of two major neurotransmitters at nanomolar level. This work opens many possibilities for further improvements and applications. For example, the LSPR of AgFON can be tuned by changing the thickness of silver and gold composites without changing the film shape, other than the OAD to increase the number of hot spots between the AgFONs. In addition, this method can also be integrated with diverse techniques of nanofluidic manipulation for advanced high-throughput small-molecular bioassays.

**Experimental Section**

**Sample Preparation:** Colloidal silica (∼100 nm) was purchased from Nissan Chemical and diluted with isopropyl alcohol (IPA). Dopamine hydrochloride, gamma-aminobutyric acid (GABA), adenosine and R6G powder were purchased from Sigma-Aldrich. R6G have been used as fluorescence active dye, and other chemicals are neurotransmitters as representative small molecules. The liquid samples have been prepared by mixing powder and triply distilled water. The sample solutions have been poured into the reservoir and flowed into the nanofluidic channel by capillary force.

**Substrate Fabrication:** Silica nanoparticle monolayers have been made by spin-coating, (3% silica solution, spin time 5 s at 500 rpm and 30 s at 3000 rpm) and thin silver layers (20 nm) have been deposited onto the nanoparticle layer by electron beam evaporator. LSPR wavelength has been tuned by OAD.
Optical and Fluid Simulations: Simulation results for schematic in Figure 1 are based on the electric field and fluid simulation by commercialized software COMSOL Multiphysics. To simulate electric field distribution, we defined the geometry of AgFONs as 2D nanoensembles. The 2D nanoensembles present in the water between the Pyrex and the PDMS with transverse magnetic (TM) plane wave. The wave passes through from bottom to top. And fluid velocity (based on incompressible Navier-Stokes equation) within AgFONs has also been simulated. Experimentally, fluid velocity (from reservoir to nanochannel) was about 10–50 μm/s. Under these conditions, the fluid velocity approaches to zero near the hot spots in the simulations.

Optical Measurements: The microscopy system consisted of an inverted microscope (Carl Zeiss Axiovert 200M) equipped with a spectrometer, a laser, a dark-field lens and a dark-field adapter cube. The LSPR scattering spectra of AgFONs and SERS spectra were recorded with a MicroSpec 2300i spectrometer equipped with a charge-coupled device (CCD) camera (Princeton Instruments, Model PIXIS: 400BR). The LSPR scattering spectra of AgFONs were measured by using 50x dark-field lens (NA 0.8) and an adapter cube. A cover slip was placed over a drop of distilled water, and light (halogen lamp) illuminated the substrates. Subsequently, scattered light was collected. The SERS and fluorescence spectra were measured using a common 50x objective lens (NA 0.5). The samples were excited by 488 nm line of an argon laser (Modu-Laser, Model Stellar-Pro Select 150) for SERS (the laser power of 10 mW, and the detection time of 1 s), and the fluorescence intensities were measured using confocal mode with its built-in 488 nm laser. The height of the sectioning slice and pixel time were 1 μm and 1.6 μs, respectively. No significant quenching or autofluorescence was observed. The SERS and fluorescence signals were measured about 3 min after pouring sample solutions into a reservoir, and SERS signals were measured from the nanochannel region, approximately 500 μm away from the edge of the reservoir. The experimental conditions were set to compare the SERS signals with varying sample concentrations.

Acknowledgements

This work was supported by KRIIB (Korea Research Institute of Bioscience & Biotechnology) and the National Research Foundation of Korea (NRF) grant funded by the Korean government (MEST) (No. 2010–0017693).

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Received: August 7, 2010
Revised: August 27, 2010
Published online: November 22, 2010