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Graphene laminated gold bipyramids as sensitive detection platforms of antibiotic molecules

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Abstract

Atomic layers of graphene were optomechanically laminated onto gold bipyramids (length of $\sim 95 \pm 3$ nm and sharp tips radius is less than 10 nm) using laser induced shock pressure. Fabricated graphene-gold bipyramid hybrids were employed as surface enhanced Raman scattering (SERS)-active substrates for the detection of tetracycline, an antibiotic, at very low concentration.

Tetracycline residues in milk products, has the potential to cause allergies and therefore require careful monitoring to conform to the safety standards. Tetracycline, is an antibiotic, and is currently detected by microbiological inhibition tests, immunoassays, spectrophotometric, chromatographic, and fluorimetric methods^{1–7}. These tests require complex steps and are nonspecific for tetracycline, whereas immunoassays are expensive. Localized surface plasmon resonances (LSPR) of plasmonic nanostructures have strong extinction, scattering, and absorption properties are dimension tunable^{8–12} and could serve as potential sensor substrates for label-free sensing of a range of targets. It has also been shown that near-field enhancements at the sharp plasmonic edges and strengthening of inter-particle optical coupling, critical for signal enhancement can be observed due to LSPR¹³. LSPR has been exploited to develop highly sensitive biological assays, molecular imaging, photodynamic therapy, drug delivery, and surface-enhanced Raman spectroscopy (SERS).^{13–16} In SERS, upon enhancement, the Raman scattering signal can be amplified

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several folds due to the localized electromagnetic field to obtain molecular fingerprints at single molecule sensitivity for the detection of trace level of analytes. Of the existing sensing technologies, SERS is one of the promising approaches as it exhibits a unique blend of advantages, such as (a) low cost, (b) non-invasive probing, (c) compatibility with aqueous solution, (d) observation at low frequency, (e) minimal sample preparation, and (f) label-free monitoring. Plasmonic nanoparticles with sharp tips such as the gold bipyramids deposited on glass has the potential to serve as sensitive molecular sensors. In our previous study, gold bipyramids has been shown to exhibit a high electromagnetic field enhancement¹⁷ due to the refractive index change of their surroundings, which is higher than other type of gold nanoparticles such as sphere and nanorods.^{14,18} Further, gold bipyramids on glass substrates exhibited higher sensor response in a real immunoassay based on LSPR.¹⁴ The characteristics of graphene that are compatible with the LSPR bipyramid substrates to develop unique sensor technology platforms are due to its: (a) excellent thermal conductivity, (b) adequate dielectric strength that confines the plasmonic field and (c) transparency to laser light and localized plasmonic fields.^{19–26} Therefore, tight integration of atomically thin graphene sheets to sharp plasmonic nanostructures could result in a seamless fabrication of SERS substrates with highly enhanced local fields.

Laser induced pressure in the GPa range was used to laminate Au nanostructures of various shapes, in particular gold bipyramids (Au BP) with sharp tips (< 10 nm) with transparent atomic sheets of graphene. Graphene wrapped bipyramids were then used to detect the antibiotic tetracycline at the ppb levels by SERS because tetracycline with 4 ring chemical structures can adsorb onto graphene through the π - π interaction which can then be detected by SERS. Plasmonic nanostructures were characterized by microscopy (FESEM, AFM, TEM) and their plasmon peaks were confirmed by UV-VIS-NIR absorption spectroscopy. Enhanced Raman scattering signals of tetracycline were then recorded from these graphene laminated Au bipyramids to test the feasibility of graphene-Au bipyramids as SERS-active substrates.

Au nanostructures comprising of Au spheres (NS), Au nanorods (NRs) and Au bipyramids (BPs) were investigated as potential substrates to develop the graphene wrapped SERS platform. Au BPs have been shown to exhibit superior plasmonic properties due to their sharp tips. Synthesis of Au BPs with penta-twinned crystalline structure has been reported earlier.¹¹ It should be noted that higher crystallinity of nanostructures is essential to achieve a strong localization of the plasmon field. Based on the seed-mediated method, the aspect ratio of Au BPs can conveniently be tuned depending on the volume ratio of the seed solution. Au BPs were coated in a film form on glass substrates as previously reported.¹⁴ The synthesized Au BPs has similar dimensions and uniform coating as evidenced from the large area SEM image (Fig. 1(a)), and AFM image (Fig. 1(b)). As observed in the AFM image shown in Fig. 1(b), one tip of the gold bipyramids lie on the glass substrate with the other tip elevated and their topography exhibits an uneven contrast in which the brighter location represents the elevated tip. TEM image of Au BPs (Fig. 1(c)) shows Au BPs with sharp tips (radius of curvature less than 10 nm). UV-VIS-NIR absorption spectrum for Au BPs, as shown in Fig. 1(d) shows the plasmon resonance at 543 nm and 785 nm corresponding to the transverse and longitudinal peak, respectively. It should be noted that the physical geometries and their orientations of Au BPs help in establishing a very strong

plasmonic field enhancement at the sharp tips as evidenced from the narrow FWHM (~75 nm) of the longitudinal plasmon resonance.

Graphene, when laser shock laminated onto Au BPs makes a very unique combination of material with significant potential for biosensing. First, graphene is transparent to light and therefore allows laser light to pass through and exposes the Au BPs layer underneath it²⁵. Second, graphene with a dielectric constant different from air helps in the distribution of the plasmonic field. Third, graphene inhibits or dissipates the localized heat at the sharp tips of Au BPs, which prevents carbonization of the test molecule. We expect these physical properties of graphene would help exploit the Au BPs wrapped graphene hybrids to achieve unprecedented sensitivity as a biosensor. However, wrinkles formed at the graphene-Au BPs interface compromises the seamless integration of these materials. Therefore, laser shock lamination of Au BPs by thin graphene layer presents a unique route to custom design 3D graphene hybrid structures. It should be noted that laser shock with few GPa pressure can help in the seamless nanoshaping of atomic sheets of graphene onto plasmonic structures as demonstrated in our prior study.^{27,28} Enhancement of Raman fingerprints of graphene is an excellent indicator of the integrity of graphene wrapped BPs. The laser shock lamination strategy is shown in Fig 2 (a). Fig. 2 (b) shows AFM images of bare Au BPs substrate and graphene transferred on Au BPs before/after laser shock. Cross-sectional line profiles of samples at each stage of the process show the uniqueness of laser shock lamination in terms of tight integration of graphene and Au BPs. We would henceforth quantify the interfacial quality by the SERS signal. Fig. 2 (c) shows the Raman spectra of graphene transferred on to the Au BP films before and after the laser shock lamination. Compared to the pristine graphene sheet on glass and graphene on bipyramids on glass prior to laser shock lamination, the Raman spectrum of laser shock laminated graphene shows a highly intense peak. Raman intensity of the G-peak of graphene increased over 3 times upon laser shock lamination and about 15 times higher than bare graphene, as summarized in Fig. 2(d). Since the mPEG-SH surrounding Au BPs can influence the Raman signature of tetracycline molecules attached to Au BPs, we investigated the Raman spectra of Au BPs before and after plasma cleaning (see supplementary information for details).

Detection of tetracycline with plasmonic gold nanoparticles by Raman spectroscopy has been investigated.⁶ The SERS-active substrate based on gold hollow spheres show a detection limit of 0.1×10^{-6} g/L. However, the concentration dependent SERS signals on plasmonic nanoparticles were not sufficient to show a specific distinction. Here, we investigate the SERS signals for various concentration of tetracycline to demonstrate the outstanding performance of the fabricated plasmonic hybrid SERS-active graphene wrapped substrates. Aqueous tetracycline solution at different concentrations, namely 10 μ M, 5 μ M, 500 nM, and 100 nM were used in our experiment as shown in Fig. 3. The measured intensity at 1060 cm^{-1} with standard deviation are presented as: 1142.4 ± 346.4 (10 μ M); 461.0 ± 117.0 (5 μ M), 374.1 ± 97.2 (500 nM); and 199.5 ± 80.6 (100 nM). The dominant SERS peaks observed was also achieved on the hybrid sample and their peak intensity decreased with concentration. The average and standard deviation of notable peaks for each concentration are displayed in the Supplementary Information, Table S1. The intrinsic peaks of graphene as noted are at 1248 and 1565 cm^{-1} (shown in blue and green strips). The intensities of the overall SERS signals of tetracycline in Fig. 3 establishes the molecular

detection limit with some of the notable peaks, to detect at a limit of detection (LOD) of 100 nM. A LOD in the pM range can easily be accomplished by single molecule fluorescence methods or by SERS and is published extensively^{29–33}. But most of these methods use labels. We propose the laser shock laminated graphene-Au BPs hybrid structures to serve as a viable SERS platform for label-free chemical fingerprinting of target molecules. Here, the scattered intensity is enhanced due to the localized electric field by plasmonic confinement at the sharp features of the gold bipyramids. Upon laser excitation, a substantial plasmon enhancement could be realized at the bipyramid tips, which is also wrapped with graphene by laser shock pressure. The concentration dependence of the relative peak intensity at 1278 cm^{-1} is shown in Fig. 3 (c). Raman fingerprints of the analyte on general SERS platform at below the threshold concentration are often very weak and hard to resolve. The chemical fingerprint signal intensity of the analyte (tetracycline) at various concentrations determines that threshold value. It should be pointed out that while optimizing the LOD, one has to work below the carbonization limit of the incident laser intensity. We have observed that the threshold concentration of the present SERS platform can be extended to the sub- μM level. The role of the atomically thin graphene layer (transparent to incident laser as well as the localized plasmon field) in plasmon confinement and the dissipation of unwanted heat could play a significant role in the design of these hybrid structures and needs to be standardized.

Plasmonic features with dimensions larger than the wavelength of light, has multipole signal contributions which reduces the chances of Raman scattering event, while at a feature size much smaller than the wavelength of light, the scattered signal mostly has dipolar contributions, which participates in plasmon oscillations. Patterned plasmonic structures extending to longer lengths often lead to propagating plasmons while, the sharp tips result in localized plasmons. For molecular detection in particular, localized plasmon would be ideal. There are many determining factors contributing to the Raman enhancement in our experimental investigations apart from the graphene veil, these are: (a) concentration of Au BPs, (b) orientation of Au BPs, (c) distribution of Au BP on the glass substrate, (d) concentration of the target analyte, tetracycline, and (e) proximity of the target molecule to the Au BPs tips. Since the thickness of graphene is at the atomic level; this makes it an excellent candidate for single layer lamination of plasmonic structures to develop hybrid SERS substrates. A graphene veil would help in the anchoring of selected target organic molecules through π - π interactions for label-free detection by Raman chemical fingerprinting.

The left-shift of the G peak of graphene wrapped Au BPs at 1560 cm^{-1} compared to the usual position at 1580 cm^{-1} signifies the strong interaction between graphene and plasmonic Au BPs. Tetracycline in particular, has a broad-spectrum and can strongly bind to the graphene surface by π - π interaction due to its 4 ring structures, as evidenced by the shift in the G-peak of graphene when tetracycline is in its vicinity, due to a charge transfer effect which will add to the electromagnetic contribution of the SERS spectrum. Thus in our system of investigation, namely the tetracycline/graphene/Au BP, strong interaction occurs at the interface, namely the tetracycline/graphene and graphene/Au BP. Graphene, as it couples well with the surface plasmon, would help in localizing the plasmon field and also in retaining it^{20,25,34–36}. Moreover, coupling will be significant when they are in tight

contact. Therefore, the role of laser shock lamination is crucial in the present experiment for direct and sensitive fingerprinting of target molecules.

As shown in Fig. 3, the SERS spectra exhibits additional peaks compared to the normal Raman spectra of tetracycline in Figure S1(b)(iv). This occurs, because the crystallographic texture of the SERS substrate and its interaction with the target molecule would determine the manner in which molecules to be analyzed would bind with the surface and which in turn determines the symmetry of molecules.³⁷ The Raman enhancement upon laser shock lamination provides clear evidence of improved interfacial quality between graphene and Au BPs. The role of graphene in providing a platform for binding through π - π interaction is highly desirable. It should be noted that the adsorption of target molecules onto graphene and the localized enhancement field of the sharp end of the Au BPs are vital to achieving the LOD by SERS. Further, the instantaneous heat removal enabled by graphene wrapping (due to the high thermal conductivity) from the Au BP tips prevents carbonization of tetracycline molecules upon laser exposure at the focused spot, a critical factor in signal stability. Graphene is a very unique material because it functions as an excellent anchor of biomolecules and yet is transparent to laser light as well as to the localized electric field, it supports plasmons and its high thermal conductivity prevents heat localization, when utilizing sharp plasmonic structures, that have intense enhancements and temperature fields. The developed SERS platform can be used for solid as well as in an aqueous phase to detect targets at a high level of sensitivity due to efficient signal enhancement and signal stability. We expect our laser-shock approach to facilitate the design and development of a range of plasmonic sensors that are stable with a high signal to noise ratio.

For the first time we have shown that laser shock lamination of graphene onto gold bipyramids can be used as efficient SERS substrates. Laser shock lamination is fast, scalable, reproducible and yields tight atomic scale integration of graphene layers on to gold nanostructures of various shapes and sizes. The Graphene-Au BP hybrid nanomaterial attained by this unique approach was employed as SERS substrate to directly detect tetracycline at the ppb level from their Raman fingerprint. The label-free SERS platform described in this article can be extended to detect metabolites in plants and organisms. The graphene/AuBP SERS technology is simple, fast, inexpensive, sensitive and can be used to detect analytes in solution or on a solid surface and therefore emerges as a robust technology for chemical fingerprinting of biomolecules.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1. Characterization of Au BPs. (a) Large area SEM, (b) AFM, (c) TEM images and (d) UV-VIS-NIR absorption spectra of glass substrate coated with Au BPs.

Fig. 2.

(a) Schematic depicting the preparation of graphene-Au BPs hybrid based SERS platform. (b) AFM images of Au BP, Graphene/Au BPs/glass before and after laser shock lamination, corresponding cross-sectional line profiles, and representative molecular dynamics simulations. (c) Raman spectra of Graphene/glass, Graphene/Au BPs/glass before and after laser shock lamination (from 1 to 3). (Inset: Relative Raman intensity of laser shock induced graphene wrapped samples compared to samples before wrapping.)

Fig. 3. SERS spectra of tetracycline on graphene-Au BP SERS platform achieved by laser shock integration at different concentrations of the analyte: (i) 10 μM , (b) 5 μM , (iii) 500 nM, and (iv) 100 nM. (Inset: relative Raman intensity vs. Concentration of tetracycline.).