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Three-Dimensional (3D) Plasmonic Hot Spots for Label-Free Sensing and Effective Photothermal Killing of Multiple Drug Resistant Superbugs

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Abstract

Drug resistant superbug infection is one of the foremost threats to human health. Plasmonic nanoparticles can be used for ultrasensitive bio-imaging and photothermal killing by amplification of electromagnetic fields at nanoscale “hot spots”. One of the main challenges to plasmonic imaging and photothermal killing is design of a plasmonic substrate with a large number of “hot spots”. Driven by this need, this article reports design of a three-dimensional (3D) plasmonic “hot spot”-based substrate using gold nanoparticle attached hybrid graphene oxide (GO), free from the traditional 2D limitations. Experimental results show that the 3D substrate has capability for highly sensitive label-free sensing and generates high photothermal heat. Reported data using p-aminothiophenol conjugated 3D substrate show that the surface enhanced Raman spectroscopy (SERS) enhancement factor for the 3D “hot spot”-based substrate is more than two orders of magnitude greater than that for the two-dimensional (2D) substrate and five orders of magnitude greater than that for the zero-dimensional (0D) p-aminothiophenol conjugated gold nanoparticle. 3D-Finite-Difference Time-Domain (3D-FDTD) simulation calculations indicate that the SERS enhancement factor can be greater than 10^4 because of the bent assembly structure in the 3D substrate. Results demonstrate that the 3D-substrate-based SERS can be used for fingerprint identification of several multi-drug resistant superbugs with detection limits of 5 colony forming units/mL. Experimental data show that 785 nm near infrared (NIR) light generates around two times more photothermal heat for the 3D substrate with respect to the 2D substrate, and allows rapid and effective killing of 100% of the multi-drug resistant superbugs within 5 minutes.

Graphical Abstract

Three-dimensional plasmonic “hot spots” for label-free sensing and photothermal killing of superbugs

Introduction

According to the World Health Organization (WHO), drug resistant superbug infection is one of the foremost threats to human health, responsible for 50,000 deaths each year in Europe and the US alone ^{1,2}. The emergence of superbugs compounded by lack of availability of new antibiotics make early detection the key for survival from superbug infection ^{3–6}. Also there is an urgent need for development of alternate approaches for killing drug resistant superbugs ^{3–6}.

As a result of localized surface plasmon resonance (LSPR), plasmonic nanoparticles can be used for bio-imaging via optical near-field enhancement at the nanoscale and for photothermal killing via pronounced heat generation in the presence of light ^{7–16}. The most important factor for sensitive sensing and effective photothermal killing is amplification of electromagnetic fields at nanoscale “hot spots” ^{17–26}. However, design of a reproducible plasmonic substrate with a large number of “hot spots” has proven to be a challenge ^{23–32}. This study reports the design of a three-dimensional (3D) plasmonic “hot spot”-based substrate, which facilitates highly sensitive label-free sensing and effective photothermal killing of drug resistant superbugs through amplification of electromagnetic fields at nanoscale “hot spots”.

Surface enhanced Raman spectroscopy (SERS) was used for label-free fingerprint sensing ^{15–24}. In SERS, the LSPR of metallic nanoparticles helps confine the incident light into sub-wavelength volumes in “hot spots”, which generate highly intense local electromagnetic fields between two nanoparticles and enhance Raman signals enormously ^{21–32}. As the plasmonic hot spot predominantly determines the SERS sensitivity ^{17–26}, this study reports development of a three-dimensional (3D) plasmonic “hot spot”-based surface enhanced Raman spectroscopy (SERS) substrate in 3D space, free from the traditional 2D limitation and exhibiting label-free ultrasensitive detection capability. The SERS technique was made more versatile by extending the “hot spot” formation into the third dimension. To attain extremely high sensitivity for fingerprint detection, a SERS probe

should have the capability to enhance SERS signal via strong electromagnetic and chemical enhancement (CM) simultaneously^{24–32}. SERS signal enhancement can only be two to three orders of magnitude because of the CM factor^{25,27–32}, but in this study a plasmonic gold nanoparticle attached hybrid 3D GO was used to enhance the SERS signal by more than 11 orders of magnitude. Experiments using p-aminothiophenol show that the SERS enhancement factor for 3D “hot spot”-based SERS is about two orders of magnitude higher than the 2D substrate and six orders of magnitude higher than zero-dimensional (0D) p-aminothiophenol conjugated gold nanoparticle only. To demonstrate the practical use of the 3D plasmonic hot spot-based SERS sensor, the label-free fingerprint superbug identification capability is demonstrated at trace levels of multi-drug resistant (MDR) superbugs such as carbapenem-resistant enterobacteriaceae (CRE) and *Klebsiella pneumonia* (KP). As the 3D SERS substrate has the capability to enhance the SERS signal intensity tremendously via the CM and EM enhancement mechanism, the reported experimental data demonstrated that the detection limit for superbugs can be 5 colony forming units (CFU) superbugs/mL.

As coherent oscillation of the electrons within the nanostructures allows huge intense absorption of incident light by 3D plasmonic “hot spots”, the absorbed photon energy is converted to heat^{7–14} and the collective heat produced in 3D plasmonic “hot spots” is very high. This is because the heat fluxes from individual nanoparticles combine to yield a high collective temperature which is ultimately transferred to the surrounding medium to kill drug resistant superbugs. This study reports enhanced photothermal effects using 3D plasmonic “hot spots” for killing of superbugs. Reported experimental data show that 785 nm near infrared (NIR) light generates around two times more photothermal heat for 3D substrate with respect to 2D substrate. also It is also demonstrated that 3D plasmonic “hot spots” cause rapid and effective killing of 100% of multi-drug resistant superbugs within 5 min of 785 nm NIR light exposure.

Results and Discussion

Development and characterization of 3D “hot spot” substrate

A plasmonic gold nanoparticle decorated 3D hybrid graphene oxide-based substrate was prepared from 2D graphene oxide (Figure 1A). Initially, 2D graphene oxide was prepared from graphite using a modified Hummers approach, as the present authors and others have reported before^{25,27,31} (Figure 1A). The details of the synthesis are reported in the experimental section. Next a homogeneous suspension of GO was mixed with $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ and NaBH_4 in water, and the mixture was heated at 160°C for 90 minutes to produce gold nanoparticle decorated hybrid 2D graphene oxide. NaBH_4 acted as a reducing agent, to reduce Au^{3+} to Au (0) by hydride transfer, and also helped to stabilize the gold nanoparticle core using BH_4^- and/or H^- via bond formation. High-resolution JEM-2100F transmission electron microscopy (TEM), Hitachi 5500 ultra high-resolution scanning electron microscopy (SEM), and energy-dispersive X-ray spectroscopy (EDX) analysis were used to characterize all the developed materials (Figure 1). Figure 1B is an SEM image of the 2D hybrid graphene oxide, clearly showing that gold nanoparticles are well decorated on the graphene oxide. The inset to Figure 1B shows EDX mapping data indicating the presence of C, O, N and Au in the 2D substrate. In the next step, gold nanoparticle decorated

3D graphene oxide-based SERS substrate was developed from the gold nanoparticle decorated hybrid 2D graphene oxide using amine-functionalized poly(ethylene glycol) (PEG) as a cross-linking agent (Figure 1A).

Amine-functionalized PEG was used to develop a 3D porous architecture by interconnecting the gold nanoparticle decorated 2D graphene oxide sheets via amine groups. Coupling chemistry between the $-\text{CO}_2\text{H}$ group of 2D graphene oxide and the $-\text{NH}_2$ group poly(ethylene glycol) (PEG) was used to form a 3D material-based SERS substrate. Figure 1C shows the SEM characterization of the SERS substrate, highlighting an interconnected 3D network with a pore size of 1–2 μm . The inset to Figure 1C shows EDX mapping data indicating the presence of Au, C and O in the 3D SERS substrate. Using Brunauer–Emmett–Teller (BET) nitrogen adsorption analysis, the specific surface area for the 3D SERS substrate was found to be $660 \text{ m}^2 \text{ g}^{-1}$, and the pore volume was $0.650 \text{ cm}^3 \text{ g}^{-1}$. The average pore diameter for the 3D SERS substrate is about 1.6 μm , as determined by BET analysis. Figure 1D shows the Raman spectra of the 3D SERS substrate, clearly indicating a strong D-band 1345 cm^{-1} and a G-band $\sim 1625 \text{ cm}^{-1}$.^{25,27–32} The presence of the strong D band in SERS spectra implies that extent of surface modification of the graphene oxide is very high.

SERS enhancement efficiency, stability and reproducibility using 3D “hot spot” substrates

SERS enhancement efficiency is the most important parameter for an SERS substrate designed to be used for highly efficient label-free detection^{20–29}. To determine the Raman enhancement efficiency, a p-aminothiophenol conjugated gold nanoparticle attached hybrid graphene oxide-based SERS substrate was used. After preparation of a gold nanoparticle attached 2D hybrid graphene oxide, p-aminothiophenol was conjugated to the gold nanoparticle via Au-S linkage. The p-aminothiophenol conjugated gold nanoparticle attached hybrid graphene oxide-based 3D SERS substrate was then prepared using PEG, as previously described. The SERS enhancement factors were evaluated using 785 nm excitation laser light. To ensure that the D and G bands from hybrid graphene oxide did not interfere with the Raman bands from p-aminothiophenol, the Raman spectra of p-aminothiophenol attached SERS substrate were subtracted from SERS spectrum for SERS substrate only without p-aminothiophenol. Hence, all the observed Raman bands, as shown in Figure 2, can be attributed to vibration bands for p-aminothiophenol¹⁹. The SERS spectra from p-aminothiophenol are dominated by a_1 vibrational mode peaks, such as $\nu(\text{CC} + \text{NH}_2 \text{ bend})$ at $\sim 1590 \text{ cm}^{-1}$ and $\nu(\text{CS})$ at $\sim 1078 \text{ cm}^{-1}$. Also observed are vibrational peaks attributed to b_2 modes located at $\sim 1435 \text{ cm}^{-1}$ from the CC str in Ph ring + NH_2 rock vibration and $\sim 1170 \text{ cm}^{-1}$ from CH bend vibration.

To determine how the Raman enhancement factor varies from 3D to 2D to 0D SERS substrates, SERS enhancement for “G” for p-aminothiophenol was measured by direct comparison of Raman intensity of $\nu(\text{CC} + \text{NH}_2 \text{ bend})$ at $\sim 1590 \text{ cm}^{-1}$ in the presence and absence of SERS substrate using the following equation^{16–24}.

$$G = [I_{3\text{D SERS}}] / [I_{\text{bulk}}] \times [M_{\text{bulk}}] / [M_{\text{ads}}] \quad (1)$$

where I_{3DSERS} is the intensity of a 1590 cm^{-1} vibrational mode from p-aminothiophenol in the presence of 3D SERS substrate and I_{bulk} is the intensity of the 1590 cm^{-1} vibrational mode in the bulk without SERS substrate from p-aminothiophenol only. M_{bulk} is the number of p-aminothiophenol used in the bulk, and M_{ads} is the number of p-aminothiophenol used for the SERS experiment using 3D SERS substrate. From the experimental data reported in Figure 2C, it is estimated that the enhancement factor is approximately 3.6×10^{12} for 3D SERS substrate, whereas the enhancement factor is approximately 1.1×10^{10} for 2D SERS substrate.

The observed two orders of magnitude higher Raman enhancement by 3D substrate is mainly a result of formation of “hot spots” in the third dimension, producing distinct plasmon fields in the first, second and third dimensions of the interior and exterior surfaces. For further analysis, the Raman enhancement factor for p-aminothiophenol in the presence of 0D SERS substrate was measured using p-aminothiophenol conjugated gold nanoparticles. From the experimental data reported in Figure 2B, 2C, the enhancement factor was estimated as approximately 3.6×10^{12} for 3D SERS substrate, but only approximately 1.3×10^7 for the 40 nm size gold nanoparticles. The five orders of magnitude SERS enhancement for the 3D hybrid structure compared with the nanoparticles only, occurs because in the 3D SERS substrate GO chemically enhances the Raman signal and the gold nanoparticles enhance the Raman signal by plasmon enhancement. In the presence of p-aminothiophenol, the aromatic rings and active oxygen on the surface of GO enhance the local electric field via a charge transfer mechanism, which can enhance the SERS signal by two to three orders of magnitude. As shown in Figure 1C, the formation of hotspots in x, y and z directions is very high in the 3D substrate, and as a result, plasmon enhancement by the gold nanoparticles in the aggregated assembly structure is very high. As the plasmon field of each gold nanoparticle undergoes coupling in a hotspot, the effective plasmon field generated by the gold 3D substrate will be much more intense than the individual AuNP plasmon field intensity.

To understand the possible mechanism for very high sensitivity at “hot sites”, three-dimensional finite-difference time-domain (3D-FDTD) simulation was used for full-field electromagnetic wave calculations, as previously reported^{33–35}. 3D-FDTD simulation uses Maxwell’s equations for numerical calculations which can be used to understand plasmon-coupling in nanoparticle aggregates, responsible for the huge EM enhancement mechanism in SERS. For calculation in the present study, electric field intensities were simulated using gold nanoparticles of diameter 40 nm decorated on graphene oxide, as observed experimentally. An incident laser wavelength of 671 nm was used for calculation. For the simulation calculation, the entire process was performed at 0.001 nm mesh resolution and 4000 fs time. As shown in Figure 2D, FDTD calculations indicate that the field enhancement for a gold nanoparticle assembly consisting of three particles can be around two orders of magnitude higher than that for the individual AuNP, potentially increasing the Raman enhancement by around four orders of magnitude. FDTD simulation shows that the bent structure of three-dimensional optically active materials allows the nanoparticles to be closer in different dimensions, significantly increasing the plasmonic field enhancement.

As reproducibility and stability of SERS signals from a 3D SERS-active substrate are important criteria for practical applications, the reproducibility of the SERS signal was monitored from p-aminothiophenol attached 3D substrate made in different batches. Experimental data (Figure 2E) show very good reproducibility, implying that the homogeneity of the developed 3D substrate is good. The intense band at 1590 cm^{-1} , corresponding to the $\nu(\text{CC}+\text{NH}_2\text{ bend})$ band, was used to calculate the relative standard deviation of the SERS signal intensity from superbugs at various locations in 3D substrate. The relative standard deviation was about 4.8%.

Use of 3D “hot spot” substrate for label-free fingerprint sensing of superbugs

To demonstrate the practical use of 3D plasmonic hot spot-based SERS sensors, the label-free fingerprint superbug sensing capability was demonstrated at trace levels of multi-drug resistant superbugs. Varying concentrations (CFU/mL) of multi-drug resistant carbapenem-resistant enterobacteriaceae (CRE) and multi-drug resistant *Klebsiella pneumoniae* (KP) were added to the 3D substrate. The Raman fingerprint spectra and SERS enhancement factors were evaluated using 785 nm excitation laser light. As shown in Figure 1D, Raman spectra from the 3D SERS substrate exhibit the strong D-band $\sim 1345\text{ cm}^{-1}$ and a G-band $\sim 1625\text{ cm}^{-1}$. To ensure that the D and G bands from the hybrid graphene oxide did not interfere with the Raman bands from the superbugs, the Raman spectra of MDR with SERS substrate were subtracted from the spectrum for SERS substrate only. As a result, all the observed Raman bands, as shown in Figure 3, can be assigned to vibration bands for MDR-CRE and MDR-KP ^{25–27,630,36–37}.

As shown in Figure 3, MDR-KP exhibits unique SERS bands at 1820 , 1710 and 1670 cm^{-1} , which correspond to $-\text{C}=\text{O}$, $-\text{C}=\text{C}$ and $-\text{C}=\text{N}$ stretching for lipids and nucleic acids. However, as shown in Figure 3A, the Raman spectrum for MDR-CRE exhibits strong amide I, II and III bands at 1640 , 1507 and 1301 cm^{-1} , bands which do not appear prominently in MDR-KP. Similarly, the Raman bands at 1195 cm^{-1} for tyrosine and 1120 cm^{-1} for lipid C-C deformation are unique for MDR-CRE, and also the Raman band at 610 cm^{-1} for phenylalanine is strong and unique for MDR-CRE. These results indicate that the 3D substrate-based SERS sensor can be used for fingerprint label-free sensing of superbugs MDR-KP and MDR-CRE. Concentration-dependent SERS data (Figure 4B) clearly indicate that the 3D substrate-based SERS sensor can be used for fingerprint detection of MDR-CRE even at a concentration of 5 CFU/mL.

Photothermal efficiency of 3D “hot spot” substrate and its use in photothermal killing of superbugs

To determine the photothermal capability of the 3D plasmonic “hot spots” substrate, a photothermal experiment was performed using 785 nm NIR light at 1.0 W/cm^2 at different time intervals with a 785 nm OEM laser. To follow how the temperature increased during the experiment, thermal imaging was performed at 1 min intervals for 2D and 3D substrates using a Micro-Shot camera.

As shown in Figure 4A, the temperature increased to more than 60°C after 6 minutes of light exposure when the 3D plasmonic “hot spot” substrate was exposed to 785 nm NIR light. For

the 2D substrate, the temperature increased to about 45°C after 6 minutes of exposure to 785 nm NIR light. There is a high collective temperature for the 3D nanoassembly because the heat fluxes from each individual nanoparticles in the highly dense ensemble of nanoparticles add up³⁸. In the 3D plasmonic substrate, the gold nanoparticles serve as a photothermal source through nonradiative decay and 3D “hot spots” act as local nanoantennae to collect heat reflux from individual particles in all three dimensions. The extended “hot spot” formation into the third dimension means that the heating efficiency is higher for the 3D platform than the 2D platform. Similar experiments were performed with gold nanoparticles only. As plasmonic gold nanoparticles do not have absorption at 785 nm light, the temperature increased to about 32°C after 6 minutes of exposure to 785 nm NIR light.

To understand whether 3D plasmonic “hot spots” cause rapid and effective killing of multi-drug resistant superbugs using biological-I window light, a superbug-specific antibody attached to a 3D plasmonic substrate was developed. MDR-superbugs were incubated with 3D plasmonic substrate for 6 hours in the absence of 785 nm light to identify any cytotoxicity. As shown in Figure 4B, almost 100% MDR-superbug viability was observed, clearly indicating that 3D plasmonic “hot spots” are not cytotoxic to superbugs in the absence of external 785 nm NIR light. On the other hand, as shown in Figure 4B, 100% of MDR-CRE were dead with 3D substrate after just 5 min of 785 nm light exposure. In comparison, 80% of MDR-CRE were dead after 10 min of 785 nm light exposure when 2D substrate was used. Figure 4C shows time-dependent photothermal killing efficiency of 3D substrate for MDR-CRE and MDR-KP, with 100% killing of MDR-superbugs possible just after 5 min of 785 nm light exposure.

Conclusion

In conclusion, this study reports development of a plasmonic “hot spot”-based substrate in 3D space, free from the traditional limitations of 2D and exhibiting highly sensitive label-free sensing capability and high efficiency for effective photothermal killing of drug resistant superbugs. Experimental data show that use of the 3D substrate can result in SERS enhancement of more than 11 orders of magnitude resulting from formation of “hot sites” in all three dimensions. Reported data using p-aminothiophenol conjugated nanoparticles show that the SERS enhancement factor for 3D “hot spot”-based SERS is about two orders of magnitude higher than that of the 2D hybrid graphene oxide substrate and five orders of magnitude higher than that of 0D p-aminothiophenol conjugated gold nanoparticles. Three dimensional finite-difference time-domain (3D-FDTD) simulation data indicate that the SERS enhancement factor can be greater than 10^4 because of the bend assembly structure of the 3D substrate. The 3D SERS substrate has label-free fingerprint sensing capability for multi-drug resistant superbugs such as carbapenem-resistant enterobacteriaceae (CRE) and *Klebsiella pneumonia* (KP). The 3D SERS substrate-based sensor can be used for label-free superbug identification even at the 5 CFU/mL level. Photothermal experimental data using 785 nm NIR biological window light show that the 3D plasmonic “hot spot”-based substrate generates around two times more photothermal heat than the 2D substrate. The plasmonic “hot spot”-based 3D substrate also causes rapid and effective killing of 100% of multi-drug resistant superbugs within 5 minutes of 785 nm NIR light exposure.

Despite reported advances, development of a robust platform for diagnostics and photothermal killing of superbugs in clinics is in its infancy. It remains to be determined how the SERS detection and photothermal killing efficiency varies as pathogens vary. The superbug diagnostic assay must be validated in the presence of different types of pathogens in complex clinical samples, in which Raman signals from other biomolecules can interfere with the selectivity and sensitivity of the assay, before the assay can be used in point-of-care applications. For photothermal killing applications in clinical settings, various technical and practical problems such as possible adverse toxicological effects of the SERS substrate, aggregation in human blood serum, formation of protein corona, and low-efficient renal clearance represent potential obstacles

Experimental

All the chemicals, including gold chloride, NaBH_4 , poly(ethylene glycol) (PEG), doxorubicin, KMnO_4 , graphite, gold nitrate, NaBH_4 , citric acid, and nitric acid, were purchased from Fisher Scientific and Sigma-Aldrich. The superbugs multi-drug resistant carbapenem-resistant enterobacteriaceae (CRE), *Acinetobacter baumannii* (AB) and *Klebsiella pneumonia* (KP), and growth media were purchased from the American Type Culture Collection (ATCC, Rockville, MD).

Development of 3D SERS substrate

1.00 g of graphite powder and 1.00 g of NaNO_3 were mixed with 45 mL of H_2SO_4 . Then, 3.00 g of KMnO_4 were added slowly to the mixture and the reaction continued for 30 minutes in a water bath with continuous stirring. Next, 150 mL of water were added and the reaction continued for a further 30 minutes. 0.5 mL of homogeneous suspension of GO (5 mg/mL) was mixed with 0.025g $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ and 0.10g NaBH_4 , 10mL water.

The mixture was then heated at 160°C for 90 minutes in an oil bath. At the end, the gold nanoparticle decorated hybrid 2D graphene oxide was centrifuged at 8000 rpm for 10 minutes and washed three times to separate unwanted product and un-reacted reactants. The gold nanoparticle decorated 3D graphene oxide-based SERS substrate was developed from gold nanoparticle decorated hybrid 2D graphene oxide using amine-functionalized poly(ethylene glycol) (PEG) as a cross-linking agent, as shown in Figure 1. For this purpose, amine-functionalized PEG was used to develop a 3D porous architecture by interconnecting the gold nanoparticle decorated 2D graphene oxide sheets via amine groups. Coupling chemistry between the $-\text{CO}_2\text{H}$ group of the gold nanoparticle decorated 2D graphene oxide and the $-\text{NH}_2$ group of poly(ethylene glycol) (PEG) resulted in formation of a 3D material-based SERS substrate, using EDC {1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide} as a cross-linking agent. The coupling efficiency was close to 90%, as determined by measuring the absorbance before and after coupling. Next, the gold nanoparticle decorated 3D graphene oxide was spin-casted to develop a 3D SERS substrate with size $5 \times 5 \text{ cm}^2$.

Developing 3D SERS substrate-based portable SERS probe

A versatile portable SERS probe was developed for fingerprint detection of MDR-CRE and MDR-KP, with SERS sensor configured to maximize sensor sensitivity (Figure 5). For the

SERS experiments, a continuous wavelength DPSS laser with 2 mW of power at 785 nm light was used as the excitation light source. The fiber optics probe were designed for optimum signal collection with effective filtering of the excitation laser wavelength. The design used a single optical fiber to guide the excitation laser light to the sample, which has the capability to collect SERS scattered light and feed into the spectrometer. For collection of SERS data from superbugs adsorbed on 3D substrate, a miniaturized QE65000 spectrometer from Ocean optics was used. For SERS data collection from superbugs, 10-second acquisition time and 5-scan averaging was used to obtain a very good signal-to-noise ratio.

Three Dimensional Finite Difference Time Domain (3D-FDTD) Simulation for full-field electromagnetic wave calculations

Three dimensional finite-difference time-domain (3D-FDTD) simulation was used for full-field electromagnetic wave calculations, as previously reported^{25,32–34} and discussed above.

MDR-CRE and MDR-KP sample preparation

MDR-CRE and MDR-KP were purchased from the ATCC and then cultured according to the ATCC protocol. The bacteria were grown to 10^8 CFU/mL for use in SERS experiments. From the stock solution of MDR bacteria, dilutions were made to vary the concentration of MDR bacteria from 5 to 10^5 CFU/mL. After that, the MDR-bacteria were immersed in the 3D SERS substrate at room temperature before obtaining the SERS spectra.

3D plasmonic “hot spot”-based photothermal killing of superbugs and determining photothermal killing efficiency

In the superbug photothermal therapy experiment using 3D plasmonic “hot spots”, the excitation light source was 785 nm light generated by a portable laser diode. For MDR bacteria photothermal killing experiments, 1 W/cm² power from the diode laser was used. After performing photothermal killing experiments, a colony counting plate was used to determine photothermal killing efficiency. The colony numbers for the plates were counted with a colony counter (Bantex, Model 920A) to identify the number of live superbugs.

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Figure 1.

A) Schematic representation of synthetic procedure used to develop 2D and 3D “hot spot” substrates. B) High-resolution SEM picture of the morphology of gold nanoparticle attached hybrid 2D graphene oxide. Inset, EDX data clearly show the presence of C, O, N and Au. C) High-resolution SEM picture of the morphology of gold nanoparticle attached hybrid 3D graphene oxide-based 3D “hot spot” substrate. Inserted EDX mapping clearly show the presence of C, O and Au. SEM view of 3D morphology clearly shows the formation of assembly structure by gold nanoparticle on graphene oxide surface. D) SERS spectra from 3D hybrid graphene oxide indicate the presence of D and G bands.

Figure 2.

A) SERS spectrum shows Raman signal from p-aminothiophenol in the presence of 2D SERS substrate compared with p-aminothiophenol conjugated gold nanoparticles. To remove the D and G band contributions from hybrid graphene oxide, the Raman spectra of p-aminothiophenol with 2D SERS substrate was subtracted from the spectrum for SERS substrate only. B) SERS spectrum shows Raman signal from p-aminothiophenol in the presence of 3D and 2D SERS substrates separately. C) 3D-FDTD simulated electric field enhancement profiles (arb. unit) for three gold nanoparticles, in bent assembly structure and in linear assembly structure. The FDTD simulation used gold nanoparticles of 40 nm size with separation distance maintained at 5 nm. D) SERS spectra of p-aminothiophenol attached 3D SERS substrate, made in eight different batches.

Figure 3.

A) SERS spectra show Raman signals from MDR-CRE and MDR-KP, highlighting that SERS can be used for fingerprint identification of superbugs. To remove the contribution of D and G bands, the Raman spectra of MDR was subtracted from the SERS substrate Raman spectra. B) SERS spectra show Raman signal from MDR-CRE at different concentrations in the presence of 3D SERS substrate. Inset SEM image shows MDR-CRE has been captured by 3D substrate.

Figure 4.

A) Temperature evolution profile for 2D and 3D substrates on irradiation of 785 nm laser ($1\text{W}/\text{cm}^2$ power) at different time intervals. B) Colonies of MDRB-CRE. B1) Colonies show that the MDR-CRE viability is 100% in the absence of 785 nm light even after 6 h of incubation in the presence of a 2D substrate. B2) Colonies show about 80% MDRB-CRE after exposure of 785 nm NIR light for 10 minutes in the presence of 2D substrate. B3) Colonies show that the MDR-CRE viability is 100% in the absence of 785 nm light even after 6 h of incubation in the presence of a 3D substrate. B4) Colonies show about 100% MDRB-CRE after exposure of 785 nm light for 4 minutes in the presence of 3D substrate. C) Plot demonstrating the time-dependent photothermal killing efficiency for MDR-CRE and MDR-KP.

Figure 5. Schematic representation showing the portable SERS probe configuration used to maximize sensor sensitivity.