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Controllable Self-Assembled Plasmonic Vesicle-Based Three-Dimensional SERS Platform for Picomolar Detection of Hydrophobic Contaminants

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Abstract

Hydrophobic contaminants in food and the environment seriously threaten human health. The ultrasensitive detection of these pollutants can minimize their damage. However, current ultrasensitive sensing strategies are limited to solid substrate-based surface-enhanced Raman spectroscopy (SERS) detection. Herein, we report a controllable and reproducible solution-based SERS detection platform for the direct and ultrasensitive detection of hydrophobic contaminants by using self-assembled three-dimensional plasmonic vesicles. To this end, amphiphilic gold nanoparticles (AuNPs) tethered with linear block copolymer (BCP) of polystyrene-*b*-poly(ethylene oxide) (PS-*b*-PEO) were designed, which display dual functions for improving detection sensitivity, including serving as building blocks for the construction of plasmonic vesicles to yield large numbers of hot-spots for SERS enhancement, and providing hydrophobic PS layers to enrich and concentrate target hydrophobic molecules for direct SERS detection with hydrophobic interaction. By modulating the AuNP size and the length of BCP chains, the ultrahigh detection sensitivity, down to the picomolar level, was obtained via using 80 nm AuNPs tethered with BCP of PEO₄₅-*b*-PS₉₀₀-SH. In addition, the proposed method exhibits excellent reproducibility, universality, practicability, as well as multiplexing detection capacity in actual contaminant-spiked soil samples. Briefly, the designed self-assembled plasmonic vesicle-based SERS platform provides an ideal generic methodology for the ultrasensitive detection of hydrophobic contaminants that can greatly accelerate on-site testing in food and environmental monitoring.

Graphic only for TOC

Keywords

plasmonic vesicle; self-assembly; surface-enhanced Raman spectroscopy (SERS); hydrophobic contaminants; on-site detection

Introduction

Hydrophobic contaminants in food and the environment seriously threaten human health. Biosensors for the ultrasensitive detection of these pollutants are urgently needed to minimize the damage. Surface-enhanced Raman spectroscopy (SERS), as a promising and powerful ultrasensitive analytical tool, has attracted extensive studies in the detection of trace amounts of chemical and biological analytes.^{1–7} Plasmonic nanomaterials such as gold nanoparticles (AuNPs) have been widely applied to design and fabricate various SERS-active substrates for ultrasensitive biosensing due to their unique localized surface plasmon resonance (LSPR) and intense local electromagnetic field in the hot-spots.^{8–14} These AuNP-based SERS-active substrates mainly involve rough AuNP surfaces,¹⁵ anisotropic AuNPs,¹⁶ AuNP oligomers,^{17, 18} core-shell nanomaterials,¹⁹ nanochains,²⁰ nanoaggregates,²¹ nanoclusters,^{22, 23} and nanoarrays.²⁴ By using these AuNP-based SERS-active substrates, high SERS enhancement factors (EFs) ranging from 10^4 to 10^9 have been obtained,⁶ which can potentially provide specific vibrational fingerprints of molecules even at ultralow concentrations. Nevertheless, the previously reported SERS-active substrates were mainly zero-dimensional (0D), one-dimensional (1D) or two dimensional (2D), of which the maximum number of SERS hot-spots is fairly limited.²⁵ In addition, the laser confocal volume for SERS detection is a three dimensional (3D) space, which suggests that the 0D, 1D, or 2D SERS-active substrates underutilized the active confocal volume although large-area SERS hot spots exist within planar geometry.^{26–28} Also, the incident laser is required to be tightly centred on the right plane to realize the maximum SERS enhancement, largely constraining the versatility of such low-dimensional substrates, especially for field detection.^{25, 28}

Recently, 3D plasmonic nanostructures have been used as SERS-active substrates to provide strong SERS enhancement.²⁸ Compared with conventional low dimensional plasmonic nanostructures, 3D plasmonic nanostructures provide many advantages as SERS-active substrates, including more SERS hot-spots for giant enhancement in all three dimensions, larger overall surface area, and higher tolerance to focus misalignment along the z-axis, thus improving the detection sensitivity.^{28, 29} However, traditional 3D SERS plasmonic nanostructures, such as round pillars, cylinders and columnar architectures, were mainly produced using techniques such as ion beam milling,³⁰ deep reactive ion etching,^{31, 32} optical lithography,³³ and electron beam lithography,³⁴ which are tedious processes and require expensive instruments. In contrast, controlled self-assembly of AuNPs has become a

promising alternative method for the construction of 3D SERS-active nanostructures, due to its simple and cheap preparation method.^{35–42} However, developing a simple and reproducible method to synthesize uniform SERS-active substrates with sufficiently high EFs, large numbers of hot-spots, and the capability to use the 3D laser confocal volume remains an enormous challenge.

In this work, we developed a 3D plasmonic vesicle as the SERS substrate to detect hydrophobic contaminants existing in food and the environment, in which target molecules were simultaneously encapsulated and enriched during the self-assembly process. In addition, we systematically investigated the effect of nanoparticle size and polymer length on the formation of 3D plasmonic vesicles and their SERS EFs. Our previous work indicated that AuNPs tethered with the amphiphilic block copolymer (BCP) of polystyrene-*b*-poly (ethylene oxide) (PS-*b*-PEO) were able to form plasmonic vesicles through the solvent-exchange method and film rehydration method due to their amphiphilic nature.^{43, 44} Herein, we prepared the plasmonic vesicles by using these amphiphilic BCP-tethered AuNPs through the oil-in-water emulsion method. To investigate the effect of nanoparticle size and length of BCP on the self-assembly process and the associated 3D plasmonic vesicle SERS EFs, we designed and synthesized three different AuNPs with sizes of 20, 50 and 80 nm, and three different BCPs with varying lengths of the PS block (with the repeating units of 50, 450, and 900, respectively) at the fixed PEO length (45 repeating units) (named P1–P3). We selected a common hydrophobic SERS probe, Nile red, as a model to study the effect of structural variations of SERS substrates on SERS performance. During the self-assembly of amphiphilic AuNPs into 3D plasmonic vesicles, the hydrophobic Nile red can be effectively enriched and concentrated into the hydrophobic PS layer because of hydrophobic interaction, thereby locating in the produced “hot-spots” between AuNPs that significantly enhanced the Raman signal of Nile red (Scheme 1). When BCP-tethered AuNPs with the long hydrophobic PS block (P3) and 80 nm Au core were used, the self-assembled nanostructures exhibited well-ordered plasmonic vesicles with the highest SERS EF of 1.87×10^8 , which was significantly higher than other obtained self-assembled nanostructures. Also, unlike these previously reported 3D nanostructures that require complicated synthesis producers, the direct self-assembly of the amphiphilic AuNPs into 3D plasmonic vesicles in solution is very simple and convenient, making this strategy well-suited for on-site rapid detection. Owing to the outstanding SERS EFs achieved and the versatility of the self-assembly process, we applied the self assembled plasmonic vesicles as 3D SERS substrates to detect hydrophobic contaminants existing in food and the environment with ultrahigh sensitivity down to picomolar levels.

Experimental section

Materials and instruments

Three different thiol-terminated block copolymers (BCPs) of thiol-terminated polystyrene-*b*-poly (ethylene oxide) HS-PS-*b*-PEO were synthesized and characterized according to our previous research (see Table S1).⁴⁴ Nile red, rhodamine 6G, crystal violet, benzo[a]pyrene (Bap), polychlorinated biphenyls (PCB 7, 77, and 209), tetrahydrofuran (THF), chloroform, acetone, N,N-dimethylformamide (DMF), gold(III) chloride trihydrate (HAuCl₄), sodium

citrate tribasic dihydrate, sodium dodecyl sulfate (SDS), and hydroquinone were purchased from Sigma-Aldrich. Standard dust, representing soil, was purchased from Powder Technology Inc. All chemicals and materials were employed without further purification.

The (UV-Vis absorption spectra were recorded *via* a Genesys 10s UV-Vis spectrophotometer. Dynamic light scattering (DLS) measurement was conducted on a scientific nanoparticle analyzer (SZ-100, Horiba). Transmission electron microscopy (TEM) images were recorded on a Tecnai TF30 transmission electron microscope (FEI, Hillsboro, OR) equipped with a Gatan Ultrascan 1000 CCD camera (Gatan, Pleasanton, CA). Scanning electron microscope (SEM) imaging was performed on a Hitachi SU-70 Schottky field emission gun scanning electron microscope. Special Raman spectra were recorded by using a small portable Raman spectrometer (BWS415, B&W Tek Inc.) with an excitation wavelength of 785 nm, a resolution of 5 cm⁻¹, and a beam diameter of 10 μm. The integration time for each case was typically 10 s.

Synthesis of different sized AuNPs

Three different AuNPs with sizes of 20, 50 and 80 nm were prepared by using a classical citrate reduction method and a seed-mediated growth method as reported, with slight modification.⁴⁵ The synthesis of 20 nm AuNP seeds was carried out by using a citrate reduction method as follows: 1 mL of 10 mg/mL HAuCl₄ solution was added to 99 mL of deionized water, and the mixture solution was heated to boiling. Later, 2.7 mL of 10 mg/mL sodium citrate solution was quickly injected into the boiled solution under strong stirring. The mixed solution was kept boiling for another 10 min. The resultant AuNP solutions were cooled at room temperature.

AuNPs with sizes of 50 and 80 nm were prepared by using a seed-mediated growth method. In brief, 50 nm AuNPs were prepared by adding 1.0 mL of 20 nm AuNP seed solution and 600 μL of 10 mg/mL HAuCl₄ solution to 100 mL deionized water under vigorous stirring. Subsequently, 200 μL of 10 mg/mL sodium citrate solution and 100 μL of 30 mM hydroquinone solutions were added every 10 min for 10 times. Additionally, 80 nm AuNPs were prepared by adding 1.0 mL of 20 nm AuNP seed solution and 2 mL of 10 mg/mL HAuCl₄ solution to 100 mL deionized water. Under vigorous stirring, 400 μL of 10 mg/mL sodium citrate solution and 200 μL of 30 mM hydroquinone solutions were added every 10 min for 12 times. After another 1 h of reaction, the as-prepared 50 nm and 80 nm AuNPs were centrifuged and re-suspended in 20 mL of 0.2 mg/mL sodium citrate solution. These resultant AuNP solutions were stored at 4 °C for further use. The concentrations of as-prepared 20, 50 and 80 nm AuNP solutions were calculated to be 6, 0.3, and 0.3 nM, respectively, according to a previous report.⁴⁶

Surface modification of AuNPs with BCPs

AuNPs were modified by BCPs through a ligand exchange method with minor modification.⁴⁷ Briefly, 5 mL of the AuNP solution obtained above was centrifuged, and the precipitates were re-dissolved in 10 mL of DMF containing 4.5 mg of BCPs under sonication. The resulting solution mixture was kept stirring overnight at room temperature. The resultant BCP-tethered AuNPs were centrifuged and washed twice with THF solution to remove the

unreacted BCP. The final BCP-tethered AuNPs were dissolved in THF with a concentration of 0.3 nM.

Self-assembly of BCP-tethered AuNPs

Chloroform solution (350 μL) containing 0.3 nM BCP-tethered AuNPs was added to 3 mL of 2.5% (w/v) SDS solution, followed by vigorous shaking for emulsification at room temperature. The resulting emulsion was kept stirring at room temperature until the complete evaporation of chloroform. During the evaporation, the plasmonic assemblies were formed.

SERS analysis of analytes using plasmonic vesicles

Nile red (10^{-4} g/mL), rhodamine 6G (10^{-4} g/mL), crystal violet (10^{-4} g/mL), Bap (10^{-4} g/mL), PCB 7 (10^{-4} g/mL), PCB 77 (10^{-4} g/mL), and PCB 209 (10^{-4} g/mL) were prepared in chloroform solution. A series of desired concentrations ranging from 10^{-5} to 10^{-13} g/mL were obtained by 10-fold dilution with chloroform solution. The resultant analyte solutions (50 μL) were first mixed with 300 μL of BCP-tethered AuNPs in chloroform solution, and the mixed solution was added to 3 mL of 2.5% (w/v) SDS solution to form the emulsion by vortex stirring for the production of self-assembled plasmonic vesicles. Then, the solution containing the plasmonic vesicle solution was directly analyzed with the portable Raman spectrometer.

Sample preparation

PCB-spiked real samples were prepared as described previously with some modifications.⁴⁸ Standard dust with a size of 20 μm served as a representative of soil was obtained from Powder Technology Inc. (Burnsville, USA), and 0.1 g of dust was first spiked with PCB 7 at a desired concentration. To extract the PCB 7 contaminants, 0.1 g of spiked soil was re-suspended with 2 mL of acetone. With the precipitation of the soil, 1 mL of acetone supernatants including the released PCB 7 were evaporated and dissolved in 1 mL of chloroform solution. Then, 50 μL of the prepared chloroform solution with PCB 7 was directly applied for SERS analysis as described earlier. The polluted sample including Bap, PCB 7, 77, and 209 for individual or simultaneous detection was prepared by using the same method.

3 Results and discussion

Characterization of self-assembled plasmonic vesicles

The self-assembly of amphiphilic BCP-tethered AuNPs into 3D plasmonic vesicles was achieved *via* oil-in-water emulsion in the current study. Since the self-assembly is mainly determined by the hydrophilic/hydrophobic balance of BCPs coated on the AuNP surface and size of AuNPs,⁴⁹ different assembled nanostructures can be produced by modulating the AuNP size and the length of the PS block with a fixed length of PEO (P1-P3, Table S1, ESI[†]). First, three different-sized AuNPs were prepared according to our previously reported method, with slight modification.⁴⁵ The morphology and size of the obtained nanostructures were characterized by TEM and DLS analysis. TEM images in Figure S1A (ESI[†]) indicated that these as-prepared AuNPs displayed regularly spherical nanoparticles with sizes of

approximately 20, 50 and 80 nm, which were further confirmed by DLS analysis (Figure S1B, ESI†). Then, three kinds of thiolated amphiphilic BCPs (P1-P3, Table S1, ESI†) were grafted onto the AuNP surface with a ligand exchange method as described earlier.⁴⁹ The BCP tethered AuNPs exhibited a slight red-shift of absorption peak compared with the corresponding original AuNPs, suggesting the successful conjugation of amphiphilic BCPs onto the surface of AuNPs through covalent S-Au bonds (Figure S2, ESI†). Later, the self-assembly of such BCP-tethered AuNPs was performed through the emulsion method. Briefly, a solution of BCP-tethered AuNPs in chloroform was first added to the SDS solution, followed by vigorous shaking to emulsify. These self-assembled nanostructures were formed after the evaporation of chloroform, and were then characterized by scanning electron microscopy (SEM), TEM, DLS, and UV-Vis absorption spectra, respectively. TEM images showed the successful formation of 3D plasmonic vesicles from 20, 50, and 80 nm AuNPs tethered with P2 and P3 (Figure 1A and 1D; see ESI† for more images), and the average diameters of these assembled vesicles along with their yields are summarized in Table 1. However, no 3D plasmonic vesicles were formed from 20, 50 and 80 nm AuNPs tethered with P1. Instead, only some irregular and unordered nanostructures, including nanochains, nanoclusters or nanoaggregates, were observed combined with numerous individual nanoparticles (Figure S3, ESI†). The formation of 3D plasmonic nanostructures was further confirmed by DLS analysis. The hydrodynamic diameter of the original AuNPs was 85 nm, whereas the hydrodynamic size of nanoassemblies increased to 620 nm after the formation of vesicles by using 80 nm AuNPs tethered with P3 (Figure 1C), which is in accordance with the SEM and TEM measurements (Figure 1B and 1E, see ESI† for more images). Similarly, a significant increase in hydrodynamic size with the formation of plasmonic vesicles for 50 nm AuNPs tethered with P2 was observed (Figure S4, ESI†), whereas a smaller increase in hydrodynamic diameter was seen by using 20, 50, and 80 nm AuNPs tethered with P1 (Figure S4, ESI†). The possible reason for the failure to form 3D plasmonic vesicle using P1 tethered AuNPs is that the length of the PS block in P1 is too short to provide enough deformability for the formation of 3D plasmonic vesicles.⁴⁹ Additionally, SEM images were further applied to characterize these assembled nanoarchitectures (Figure 1B and 1E, see ESI† for more images) and to determine the interparticle distance between AuNPs in the assembled nanostructures. The vesicles made from 80 nm AuNPs tethered with P3 have densely packed AuNPs in the membrane, in which the interparticle distance between AuNP was only 0.5 ± 0.2 nm, causing strong plasmonic coupling between adjacent AuNPs and an obvious red-shift in the UV-Vis absorption spectra from 540 nm to 750 nm (Figure 1F). For other plasmonic vesicles, the maximum absorption peaks of SPR also demonstrated an obvious red-shift compared with the corresponding original AuNPs, whereas a slight red-shift was found when 20, 50, and 80 nm AuNPs tethered with P1 were assembled into the nanostructures of 1D nanochains, 3D nanoclusters or nanoaggregates (Figure S5, ESI†). The large red-shift in the SPR absorption peak in plasmonic vesicles indicated the strong plasmonic coupling between adjacent AuNPs, which can lead to increased electromagnetic enhancement from large numbers of hot-spots existing in the assembled nanostructures for SERS enhancement.

Characterization of the relationship between nanoassembly structures and SERS enhancement performance

To investigate the relationship between the structural variations of nanoassemblies and their ability to enhance the SERS signals of hydrophobic molecules, we summarized the SERS signals of different nanoassemblies and their corresponding dominant assembly morphologies with the variation of AuNP sizes and BCP chain lengths in Figure 2.

Hydrophobic Nile red was selected as a model SERS probe, which showed a typical peak with the Raman shift at 591 cm^{-1} . During the self-assembly of BCP-tethered AuNPs, the SERS probe in chloroform was effectively enriched and concentrated into the hydrophobic PS layer due to hydrophobic interaction, thus resulting in the SERS probe being located in the hot-spots between adjacent AuNPs originating from the self-assembled nanostructures. Several things can be concluded from the data in Figure 2A and 2B. First, the AuNPs with smaller size are more amenable to the formation of 3D plasmonic vesicles. This is reflected in the yield of vesicles formed. The 20 nm AuNPs tethered with P2 and P3 formed 3D plasmonic vesicle with yields over 98%, whereas the 50 nm AuNPs tethered with P2 and P3 formed 3D plasmonic vesicles with a yield of 80% and 90%, respectively, and the 80 nm AuNPs tethered with P2 formed 3D plasmonic vesicle with a yield of 65%. Increasing the BCP length also favours the formation of 3D plasmonic vesicles. As shown in Figure S3 (ESI[†]) and Figure 2B, the self-assembly of P3 tethered AuNPs of 50 nm and 80 nm resulted in vesicle yields of above 90%, which were higher than those of P2 tethered AuNPs of 50 nm and 80 nm with vesicle yield of about 80% and 65%, respectively. Meanwhile, the hydrodynamic size of the formed assemblies increased with the BCP length (Figure S4, ESI[†]). The size of 3D plasmonic vesicles also increased somewhat when the BCP length increased (see Table 1).

The coupling of plasmonic fields between adjacent AuNPs, and hence their ability to enhance Raman signal, is influenced by the AuNP sizes and their interparticle distance. To determine the interparticle distances in various nanoassemblies, SEM images were further applied to characterize these assembled nanoarchitectures (Figure 1B and 1E, see ESI[†] for more images) and to determine the interparticle distance between AuNPs in the assembled nanostructures; the results are summarized in Table 1. Generally, the interparticle distance in the nanoassemblies formed from 20 nm AuNPs is larger than that for the 50 nm and 80 nm AuNPs. As shown in Table 1, the interparticle distance in the corresponding nanostructures made from AuNPs tethered with P1 are $3.5 \pm 0.4\text{ nm}$ for 20 nm AuNPs, $2.5 \pm 0.4\text{ nm}$ for 50 nm AuNPs, and $2.1 \pm 0.2\text{ nm}$ for 80 nm, respectively. In this group, the SERS signals of the nanoassemblies made from larger AuNPs were stronger, which could be explained by the stronger plasmonic coupling between the larger AuNPs and their relatively smaller interparticle distances. This phenomenon is consistent with the earlier study, which showed that the plasmonic coupling between two nanospheres is related to the ratio of the gap distance to the particle radius.⁵⁰ However, all the SERS signals for these three systems remained rather low. As the length of BCP increased, the interparticle distance in the nanoassemblies formed from 20 nm AuNPs increased, but the interparticle distance decreased for the nanoassemblies formed from 50 nm and 80 nm AuNPs. The decrease in the interparticle distance found in the nanoassemblies formed from 50 nm or 80 nm AuNPs tethered with P2 and P3 as compared with 20 nm AuNPs could be related to the different

grafting densities of BCPs ligands on AuNPs achieved at different surface curvature.⁵¹ When longer BCPs were attached to AuNPs, a lower grafting density was achieved on the AuNP surface. During vesicle formation, redistribution of BCP chains on the AuNP surface occurred, leading to a smaller interparticle distance between the adjacent AuNPs.⁴⁴ On the other hand, the use of short BCP with the decreased deformation ability of the polymer chain ligands achieved a higher grafting density on AuNP surfaces, which resulted in irregular assemblies with larger interparticle distances.

The 3D plasmonic vesicles formed from 20 nm AuNPs tethered with P2 and P3 showed modest gains in SERS signals when compared with the nanostructures formed from 20 nm AuNPs tethered with P1, despite the fact that the interparticle distance in the P1 system (3.5 ± 0.4 nm) is much smaller than in P2 and P3 (8.5 ± 3.5 nm and 11.6 ± 2.1 nm, respectively). One may attribute the modest gains in SERS signals in P2 and P3 systems to the additional coupling gained from the 3D arrangements of these AuNPs in vesicle form. Significant gains in SERS signals were seen from the 3D plasmonic vesicles formed from 50 nm AuNPs and 80 nm AuNPs tethered with P2 and P3, especially the 80 nm AuNPs tethered with P3. The significant gain in SERS seen in these systems could be traced to the extremely small interparticle distance (less than 1.0 nm) that gave rise to the strong plasmonic coupling between these AuNPs in 3D vesicle forms. Additionally, the length of the hydrophobic PS layer is also an important factor to influence the EFs. Increasing the length of the PS layer facilitates the formation of 3D plasmonic vesicles and allows for the encapsulation of hydrophobic SERS probes (or hydrophobic analytes) into hot-spots on the 3D plasmonic vesicles. Taken together, we speculate that well-ordered 3D plasmonic vesicles made from AuNPs tethered with longer PS layers of BCPs can provide more hot-spots with stronger local electromagnetic fields due to the short interparticle distance between adjacent AuNPs, leading to higher SERS signals than those of irregular and unordered self-assembled nanostructures.

Theoretical simulation studies of our developed 3D plasmonic vesicles

To test our hypothesis about 3D plasmonic vesicles, we also performed theoretical studies using the Discrete Dipole Approximation (DDA) method implemented in DDSCAT7.3 to calculate the extinction spectra and electromagnetic field enhancement for 3D plasmonic vesicles. The simulated system had 154 of 80 nm spherical AuNPs assembled onto the 550 nm hollow nanovesicle surface (Figure 3A). DDSCAT7.3 was then used to calculate the extinction spectra and electric field enhancement. As shown in Figure 3B, the calculated extinction spectrum exhibits a broad peak centred at 640 nm, which is attributed to the sum of absorption and scattering from the assembled AuNPs in the plasmonic vesicle. The 3D “hot-spots” and near-field distribution of our developed vesicle was measured under the incident wavelength of 785 nm. The visualization of hot-spots seen from 3D plasmonic vesicles is presented in Figure 3C. A significant number of hot-spots were clearly observed when the AuNPs were arranged in 3D vesicle form. Further, the near-field intensity of our vesicles was obtained with a 2D-slice cut through the origin in the x-y plane, as shown in Figure 3D. The results demonstrate a significant electromagnetic field enhancement between these assembled AuNPs in the formed vesicles. The above findings indicate that our

developed 3D plasmonic vesicle can serve as potential SERS substrates to provide numerous hot-spots for improved electromagnetic field enhancement.

To further confirm the enhanced Raman signal resulting from the assembled 3D plasmonic vesicles, we disassembled the nanoassemblies in THF and the Raman signal was significantly reduced (Figure S6A, ESI†). The addition of THF triggered the disassembly of the formed 3D plasmonic vesicles into individual AuNPs or small aggregates since THF is a good solvent for the BCPs. The disassembly of vesicles in THF was also confirmed by DLS and UV-Vis absorption spectra (Figure S6B and S6C, ESI†). With the disassembly of the nanoassemblies, the number of hot-spots between particles was reduced, which then led to a weak SERS enhancement.

We also carried out an approximate estimation for SERS EFs of our developed SERS substrates according to the most common calculation method.⁴² The detailed calculation can be found in ESI†. Table 1 summarizes the calculated SERS EFs for each developed SERS substrate. The highest EF of 1.87×10^8 was obtained in vesicles made from 80 nm AuNPs tethered with P3, which is much higher than those of other plasmonic substrates (Table 1). The ultrahigh EF will benefit the development of highly sensitive SERS platform for target detection. Therefore, 80 nm AuNPs tethered with P3 were selected as the best building blocks to fabricate plasmonic vesicles as 3D SERS substrates in subsequent SERS analysis.

Evaluation performance of the plasmonic vesicle-based 3D SERS platform

Under the optimal conditions, we first evaluated the detection ability by applying plasmonic vesicles for the SERS detection of several hydrophobic dye molecules with different concentrations of 10^{-5} to 10^{-13} g mL⁻¹. Our developed SERS platform presented good linear relationships between Raman intensity and target concentrations in the individual detection of these three target analytes (Figure 4A). The detection sensitivities for crystal violet, rhodamine 6G and Nile red, reached up to 10^{-10} , 10^{-11} , and 10^{-12} g mL⁻¹, respectively. The high detection sensitivity of our SERS platform is superior or comparable to most 3D SERS nanostructures reported recently (Table S2, ESI†). Moreover, we performed the simultaneous detection of multiplex targets by encapsulating three types of Raman molecules at the same time using our designed SERS platform. The resulting multiplex SERS spectra showed three typical Raman shift signals at 591, 1511 and 1619 cm⁻¹, which correspond to Nile red, rhodamine 6G, and crystal violet, respectively (Figure 4B). These results indicate that the designed approach possessed superior differentiation capacity for different targets in one analysis according to the specific Raman fingerprint information of each target, which can be used to rapidly determine the potential elements present in analytes, and meanwhile quantify the actual concentration of each analyte in the sample. To further examine the reproducibility of the 3D plasmonic vesicles as SERS substrates, the variation of the SERS signal intensity at 591 cm⁻¹ of Nile red with the same concentration of 10^{-10} g mL⁻¹ were recorded from ten randomly different-batched self-assemblies of 80 nm AuNPs coated with P3. All Raman intensities at 591 cm⁻¹ from those ten Raman spectra were described as the histogram in Figure 4C, and the mean intensity was 1827 ± 113 . The low relative standard deviation (RSD) of 6.2% demonstrated the superior reproducibility of our as-prepared SERS platform. A possible explanation for the small RSD is that our

proposed plasmonic vesicle is highly reproducible among different self-assembled batches, and only the presence of SERS probes in the hot-spots between adjacent AuNPs can produce an amplified and detectable Raman signal. However, this highly reproducible self-assembled SERS nanostructure with reproducible signal enhancement ability has always been a major obstacle in conventional solution based direct target SERS detection. Collectively, the usage of plasmonic vesicles as SERS substrates for target detection exhibited high sensitivity, high reproducibility, as well as outstanding multiplexing capacity for simultaneous detection, especially for on-site quantitative detection.

Practical application of the plasmonic vesicle-based 3D SERS platform

To further investigate the universality and practicability of this plasmonic vesicle-based 3D SERS platform, we performed the SERS analysis for hydrophobic contaminants that exist extensively in food and environmental samples (e.g. water and soil). Among these pollutants, benzo(a)pyrene (Bap) and polychlorinated biphenyl (PCB), as a class of highly toxic persistent organic pollutants, have attracted increasing attention because of their adverse health effects of carcinogenicity, neurotoxicity, dermatological and pulmonary diseases, as well as endocrine and developmental disorders.^{52–55} Conventional analytical methods for the measurement of these hydrophobic organic target analytes with a trace contamination level mainly depend on chromatographic analysis and mass spectrometry,^{56, 57} which suffer from obvious limitations including the requirement of complicated instruments, tedious sample preparation, as well as being labour-intensive and time-consuming. In this work, we employed the designed self-assembled 3D SERS platform to measure these two model target analytes. Due to their strong hydrophobicity, both are easily loaded into the hydrophobic PS layer coated onto the AuNP surface during the self-assembly process to obtain an enhanced SERS signal intensity, thereby enabling the analysis of targets. Standard dust was applied as a representative of real soil for the preparation of target-spiked samples. Briefly, the contaminated soil was first mixed with acetone solution, and then the hydrophobic target molecules were completely released into acetone solution from the soil. After the precipitation of soil, acetone supernatants containing the released target contaminants were evaporated and re-suspended into chloroform solution for further SERS detection (Figure 5A). Figure 5B and 5C indicate the SERS spectra of Bap and PCB under different concentrations of 10^{-7} to 10^{-12} g/mL. The characteristic Raman shift peaks at 1384 and 1607 cm^{-1} are significantly distinguishable even with the ultralow contaminant level of 10^{-12} g mL^{-1} for both Bap and PCB 7. Meanwhile, the excellent linear SERS responses were observed for these two targets at the concentrations ranging from 10^{-11} to 10^{-7} g mL^{-1} for Bap, and from 10^{-12} to 10^{-7} g mL^{-1} for PCB 7 (Figure 5D). Later, a correlation analysis of our 3D SERS platform with a standard high-performance liquid chromatography (HPLC) method⁵⁸ for Bap detection was completed to estimate the acceptability and reliability through addition and recovery analysis. Standard dust samples (Bap-free) were spiked with four different concentrations of Bap at 10, 1, 0.1 and 0.01 ng/g, respectively. All Bap-contaminated soil samples were simultaneously tested with these two methods. As shown in Table S3, an excellent correlation was observed between these two methods, indicating that the proposed plasmonic vesicle-based 3D SERS technology can be applied for reliable Bap detection in complex real samples. Besides, we also investigated the multiplexing detection ability of this SERS method for the simultaneous detection of

multiple analytes in real samples. The spiked soil samples with three different PCB contaminants of 7, 77, and 209 at the contaminated concentration of 10^{-9} g mL⁻¹ were used for multiple SERS analysis. As shown in Figure S7 (ESI[†]), the results indicated that these typical Raman shift peaks for PCB 7, 77 and 209 could be clearly observed in multiple SERS spectra in spite of their similar molecular geometries. Also, we found there is an obvious overlap for some Raman shift peaks in multiple SERS spectra, but the quantitative analysis of individual targets could be achieved by decoupling individual Raman signals for each target according to the previously reported Gaussian deconvolution method.⁴⁸ These findings demonstrate that the plasmonic vesicle-based 3D SERS platform can be employed for the individual or multiplex analysis of hydrophobic contaminants with ultrahigh detection sensitivity in real samples.

Conclusions

In summary, we successfully developed a controllable and reproducible method to produce plasmonic vesicles as 3D SERS-active substrates to construct an ultrasensitive SERS biosensing platform for the direct detection of hydrophobic contaminants including toxins and chemical pollutants in food and environment. The designed plasmonic vesicles were achieved by using the self-assembly of amphiphilic BCP-tethered AuNPs, which exhibited dual roles for enhancing the target detection sensitivity. On the one hand, the amphiphilic BCP-tethered AuNPs can serve as the building blocks for the fabrication of plasmonic vesicles to provide a large number of hot-spots for SERS enhancement; on the other hand, the amphiphilic BCP-tethered AuNPs provide hydrophobic PS layers for the enrichment and concentration of target molecules with hydrophobic interaction. Through modulating the AuNP size and the length of the PS block, the optimized plasmonic vesicles for SERS substrates were obtained with 80 nm AuNPs tethered with P3, which exhibited an ultrahigh sensitivity of 10^{-12} g mL⁻¹ for Nile red with an SERS EF of 1.87×10^8 , which is superior or comparable to most of the other reported 3D SERS nanostructures. In addition, the proposed method displays high reproducibility and excellent multiplexing detection capacity. The universality and practicability of our designed method were further evaluated by using actual contaminant-spiked soil samples. Therefore, our designed plasmonic vesicles can serve as a universal 3D SERS platform for the ultrasensitive and reproducible analysis of various hydrophobic contaminants, especially for field detection in food safety analysis and environmental monitoring.

Supplementary Material

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Scheme 1.

Schematic illustration of our designed plasmonic vesicle-based SERS platform for the direct detection of hydrophobic contaminants.

Figure 1. Characterization of the assembled plasmonic vesicles with 80 nm AuNPs tethered with BCPs of P3 (Table S1). (A and D) Representative TEM images, (B and E) representative SEM images of the vesicles, (C) DLS analysis, and (F) UV-Vis spectra before and after the AuNP assembly.

Figure 2.

(A) Normalized Raman intensity from various assembled nanostructures, and (B) the corresponding self-assembled “product diagram” with varying AuNP sizes and the PS lengths of BCPs. ◇, nanochains + single nanoparticle; ○, nanoaggregates or clusters; ☆, vesicles + nanoaggregates or clusters; and ▽, vesicles.

Figure 3.

Theoretical simulation of the extinction spectrum and the near-field distribution of our designed vesicle by using the DDA method. (A) An illustrated 3D model for the plasmonic vesicle used for calculation. (B) The calculated extinction spectrum of the vesicle. (C) The simulated 3D distribution of the hot-spots of this vesicle at the incident wavelength of 785 nm. (D) Near-field intensity distribution in the x-y plane close to the centre of the vesicle at the incident wavelength of 785 nm.

Figure 4.

(A) Linear relationship of Raman intensity with different concentrations of target molecules, including Nile red, rhodamine 6G, and crystal violet, in their individual detection. (B) Multiplexed Raman spectra for triple, double as well as individual detections of Nile red, rhodamine 6G, and crystal violet. (C) The reproducibility of the Raman intensity of Nile red at 591 cm^{-1} from ten randomly different-batched self-assemblies of 80 nm AuNPs coated with P3 with the concentration of 10^{-10} g mL^{-1} .

Figure 5.

(A) Schematic illustration of the experimental procedure for target molecules extracted from the soil particulates by using acetone as the dispersion liquid. The released molecules were evaporated and re-suspended into chloroform solution for subsequent SERS analysis. (B) and (C) Raman spectra for individual detection of Bap and PCB 7 released from contaminated soil. (D) The corresponding linear response of Raman intensity against different concentrations of Bap and PCB 7 in their individual detection.

Table 1.

Summary of the enhancement factors (EFs) of SERS for Nile red from different assembled nanostructures by using different combinations of AuNP sizes and polymer lengths.

AuNP diameter (nm)	Polymer type	Formed nanostructure for SERS substrates (Vesicle yield (%))	Size (nm) from TEM (n = 100)	Estimated AuNP numbers per nanostructure ^b	Interparticle distance (nm, n= 200)	EFs ^c
20	P1	Nanochains + single nanoparticle	NA ^a	NA	3.5 ± 0.4	7.8×10 ³
20	P2	Vesicles (98%)	660 ± 159	2345	8.5 ± 3.5	1.9×10 ⁵
20	P3	Vesicles (98%)	718 ± 114	3036	11.6 ± 2.1	7.0×10 ⁴
50	P1	Nanoaggregates or clusters	NA	NA	2.5 ± 0.4	2.9×10 ⁵
50	P2	Nanoaggregates or clusters + Vesicles (80%)	400 ± 107	219	0.8 ± 0.2	1.3×10 ⁷
50	P3	Vesicles (90%)	521 ± 145	481	1.0 ± 0.1	2.6×10 ⁷
80	P1	Nanoaggregates or clusters	NA	NA	2.1 ± 0.2	3.0×10 ⁶
80	P2	Nanoaggregates or clusters + Vesicles (65%)	450 ± 71	96	0.7 ± 0.1	3.9×10 ⁷
80	P3	Vesicles (90%)	550 ± 119	154	0.5 ± 0.2	1.9×10 ⁸

^aNA means “Not available”.

The details for the calculation of b and c can be found in ESI†.