Adhesion Enhancements and Surface-Enhanced Raman Scattering Activity of Ag and Ag@SiO₂ Nanoparticle Decorated Ragweed Pollen Microparticle Sensor

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ABSTRACT: A simple solution processed layer-by-layer approach was used to immobilize metal nanoparticles (NPs) on the surface of ragweed pollen exine to obtain multifunctional particles with significant surface-enhanced Raman scattering (SERS), two-photon excited fluorescence, and enhanced adhesion properties. The rugged pollen exine was functionalized with an amine terminated silane and then treated with Ag or Ag@SiO₂ NPs that were electrostatically attached to the exterior of the pollen by incubation in an NP solution of the appropriate pH. Nanoparticle agglomeration on the pollen gives rise to broadband near infrared (NIR) (785–1064 nm) plasmonic activity, and strong SERS signals from benzenedithiol deposited on NP–pollen composite particles were



observed. In addition to SERS activity, the AgNP coating provides a twofold increase in the adhesive properties of the RW pollen exine on a silicon substrate, leading to a robust, adhesive, broadband NIR excitable SERS microparticle.

KEYWORDS: surface enhanced Raman scattering (SERS), biogenic sensor, core/shell nanoparticle, pollen adhesion, multifunctional pollen particle, composite microparticle

1. INTRODUCTION

Surface-enhanced Raman scattering (SERS) has become a very powerful tool across many scientific disciplines, including but not limited to chemical analysis,¹ single molecule detection,² electrochemistry,³ and in vivo biological sensing.⁴ Localized surface plasmon resonances (LSPR), produced by excitation of metallic nanostructures, result in an amplification of electromagnetic fields on or near the surface of the nanostructure with the strongest field enhancements in the gaps between closely interacting metal nanostructures known as "hot spots".⁵ SERS occurs when molecules interact with these extraordinarily enhanced electromagnetic fields. The wavelength and intensity of the LSPR depends on the nanoparticle composition, size, and shape of the nanostructure, and thus, a large array of SERS substrates has been fabricated and reviewed in the literature.⁶ There are two general types of SERS substrates: lithographically fabricated metal nanostructures and synthetically produced colloidal metal nanoparticles (NPs). Lithographically fashioned nanohole arrays,⁷ periodic nanoparticle arrays fabricated with ebeam lithography and template techniques,^{8,9} nanostructured gratings,¹⁰ and fabricated nanopillar arrays¹¹ have been used to develop SERS substrates with exquisite control of LSPR characteristics but require complex and expensive instrumentation to fabricate such nanostructures. Colloidal metal NPs can be synthesized in solution and can be formed in a variety of sizes and shapes to manipulate the LSPR.⁶ Although colloidal NPs are easily synthesized, they are subject to variations in size and shape and are not easily detectable by traditional optical microscopy techniques. Metal NPs have been immobilized on a variety of substrates, including microparticles and SERS active microparticle substrates, which offer a method to extend the nanoparticle LSPR properties to the microparticle size domain. Polymeric,¹² silica,¹³ polystyrene,¹⁴ and magnetic particles¹⁵ have been coated with a variety of metal nanostructures via electroless plating¹⁶ and electrostatic heterocoagulation to create SERS active composite particles.^{17,18} These types of 3D SERS substrates offer a high surface area-to-volume ratio and can be dispersed in aqueous solutions, making them effective platforms for potential biological or chemical sensing applications. Biological specimens have a transparency window in the near-infrared region (NIR) (700-1400 nm) where absorption and autofluorescence is minimized.¹⁹ Thus, the LSPR of the SERS substrate must be excitable in the NIR region to be applicable for biological samples.

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In situ reduction of Ag provided a route to metallization of the sporopollenin (ragweed (RW) and rye pollen) and has been used to identify Raman signals from compounds within the exine, which is intrinsic to the pollen.²⁰ The authors were able identify various SERS enhanced Raman spectra of the exine that provided insight into the prominent functional groups. In this paper, we report on the coating of RW pollen exine with preformed silver nanoparticles (AgNP) and silica coated silver nanoparticles (Ag@SiO2) NPs. The ragweed pollen exine is a chemically and mechanically robust microparticle and due to its "spiky" morphology; the RW pollen exhibits substantially higher surface area-to-volume than similarly sized spherical particles,^{21,22} which facilitates both the SERS signals and enhanced adhesion on hydroxylated substrates and is tunable. Additionally, ragweed pollen has been shown to exhibit two-photon excited fluorescence,²³ combined with the fact that AgNPs also exhibit two-photon emission themselves,^{24,25} and enhanced two-photon fluorescence in the presence of chromophores, which allows for detection of the tailored composite particles via two-photon microscopy.²⁶ Thus, the pollen exine provides adhesive microparticles and also allows for 2-photon fluorescence detection of particles with high surface area to volume ratio, which makes it an interesting template for the attachment of nanoparticles while maintaining the nanoparticle properties in the micrometer size domain, creating a multifunctional composite particle. Here, we show that after amine functionalization of the RW exine, negatively charged NPs can be electrostatically attached with a controllable NP surface coverage. With the NP coated RW pollen, the LSPR composite particle was excited over a wide range of NIR wavelengths, and strong SERS signals were observed following application of benzenedithiol (BDT) probe molecules. In addition to SERS activity, the adhesive properties of the composite particle were found to be enhanced by the presence of nanoparticle coatings.

2. EXPERIMENTAL SECTION

2.1. Materials. Native defatted ragweed (*Ambrosia artemisiifolia*) pollen grains (Greer Laboratories), silver nitrate (99.99999% trace metal basis), L-ascorbic acid (\geq 99.0%), sodium citrate, and toluene were purchased from Sigma-Aldrich. Dimethylamine (DMA, 40% w/w, Alfa Aesar) aqueous solution, (3-aminopropyl)triethoxysilane (APTES, Gelest Inc.), tetraethoxysilane (TEOS, 99.0+%, Gelest Inc.), potassium hydroxide (6 w/v%, EDM Millipore), orthophosphonic acid (85%, EDM Millipore), sulfuric acid (97% purity, BDH Chemicals Ltd.), hydrogen peroxide (30 wt %, BDH Chemicals Ltd.), polystyrene (MW = 100 000 g/mol, Avocado Research Chemicals), AFM cantilever (FORT-TL, Applied NanoStructures), Si substrates (Silicon, Inc.), silicone wellplates (Gace Bio-Laboratories), and epoxy resin (Epoxy Marine, Loctite) were used as received. Deionized water (DIW, 18.2 M Ω cm, pH 6.4) was prepared with a Barnstead E-Pure purification system.

2.2. Ag Nanoparticle Synthesis. Quasi-spherical silver nanoparticles were synthesized via a previously reported method.²⁷ In short, 12.5 mL of ascorbic acid (4.8 mM) and 8.825 mL of sodium citrate (1 w/w%) were added to 80 mL DIW. The reaction mixture was adjusted to pH 9.5 with 0.1 M citric acid and 0.1 M NaOH. The solution was placed in 30 °C hot bath, and 1 mL of aqueous solution of AgNO₃ (0.1 M) was injected into the reaction mixture, which was stirred at 1200 rpm for 15 min. The contents of the reaction flask were then transferred to an oil bath at 100 °C for 30 min without stirring and then placed in ice bath to quench the reaction.

2.3. Ag@SiO₂ Nanoparticle Synthesis. The silica coating of Ag nanoparticles was performed using a modified procedure from previous reports.^{28,29} Two milliliters of AgNP, filtered through 0.8 μ m Nylon filter to remove large or aggregated nanoparticles, was

added to 20 mL of 95/5% ethanol/water mixture in a 50 mL centrifuge tube. TEOS (600–1200 μ L, 10 mM in ethanol) was added, followed by the addition of 200 μ L of DMA. The reaction mixture was then shaken with vortex mixer for 2 h. Ag@SiO₂ NPs were collected using a centrifuge operating at 8000 rpm for 15 min. The resultant Ag@SiO₂ NPs were washed 3 times each with ethanol and water and then dispersed in 2 mL of ethanol.

2.4. Pollen Preparation. A sequential base/acid treatment was used to remove organic pollen kit from pollen exine.²¹ A 10 g batch of defatted RW was added to a 100 mL of potassium hydroxide (6 w/v%) solution for 6 h at room temperature. The basic solution was neutralized using HCl, centrifuged, and washed with boiling DIW and boiling ethanol three times, respectively. The pollen was then dried in a convection oven at 60 °C for 2 days. Dried base-treated pollens were then added to 200 mL of orthophosphonic acid at 50 °C for 7 days. The acidic solution was neutralized with NaOH, centrifuged, and washed three times each with boiling DIW, boiling acetone, and boiling ethanol. The base/acid treated pollen grains (BA-RW) were then dried in vacuum oven at 60 °C for 24 h.

BA-RW were then treated with APTES. Eighty milligrams of BA-RW was dispersed in 70 mL of anhydrous toluene and sealed in a round-bottom flask. Two milliliters of APTES was added, and the mixture was stirred for 24 h under nitrogen in a hot bath at 85 °C. The APTES treated pollen grains (APTES-RW) were then washed three times each with toluene, *n*-hexane, and ethanol. APTES-RW were then dispersed in 3 mL of ethanol.

2.5. NP–Pollen Composites. AgNPs or Ag@SiO₂ NPs (0.7–5.0 mL) were diluted with DIW to a total volume of 5.0 mL. The solution was then adjusted to a pH of 4 using 0.1 M citric acid and NaOH. Ten microliters of APTES-RW pollen was added to an NP solution and agitated with a nutating mixer overnight. The NP-RW were then washed three times with DIW and stored in DIW. For Raman measurements, pollen was drop-casted into 2 mm diameter silicone wells of a silicone well plate and allowed to dry under ambient conditions. Then, two sequential treatments with 5 μ L of BDT (0.1 mM in EtOH) were dropcasted and evaporated on a hot plate at 80 °C.

2.6. Adhesion Measurements. The adhesion forces were measured using atomic force microscopy (AFM, Veeco Dimension 3100) under ambient conditions (21 °C, relative humidity 38-40%) using an AFM colloidal probe technique.³⁰ Tipless rectangular cantilevers with nominal spring constants of 0.6-3.7 N/m (Applied NanoStructures, Inc., Santa Clara, CA) were used. Single pollen particles were glued to the tipless cantilevers with a small amount of epoxy resin using a procedure described in detail elsewhere.²¹ Si substrates were piranha-etched by sulfuric acid/hydrogen peroxide (3:1) mixture at 80 °C for 4 h. A PS thin film was blade cast on a Si wafer from a PS solution (10 wt % in toluene). The actual spring constants for the cantilevers with the attached pollen particles were determined directly by the methods of Burnham and Hutter et al.^{31,32} A series of 10 force-distance curves were measured for each combination of pollen tip and substrate surface. The measurements were performed on three separate substrate surfaces with randomly chosen areas on each substrate. For each pollen particle sample, 4 separate AFM tips with a bound pollen grain were measured with a low applied load of 2.5 nN for all force measurements. No obvious damage or deformation of pollen tips was observed by SEM imaging taken after all the force measurements.

2.7. Particle Characterization. AgNPs and Ag@SiO₂ NPs were drop-casted on carbon coated copper transmission electron microscopy (TEM) grids (Electron Microscopy Science). TEM images were collected on a JEOL 100CX II TEM with a 100 keV accelerating voltage. ζ -Potential measurements were obtained with a Malvern Zetasizer Nano-ZS. NP-RW composites were drop-casted on copper tape mounted on a SEM stub, and images were collected with a ZEISS ULTRA 60 FE SEM. Micro-Raman spectra were collected with a Thermo Nicolet Almega XR Dispersive Raman Spectrometer with an excitation wavelength of 785 nm and power of 1.0 mW. A long working distance 50× objective was used to focus the laser on the pollen specimen, and the scattered light was collected using a



Figure 1. UV/vis spectra of plasmonic absorption of AgNPs and Ag@SiO₂ NPs (left) and TEM image of Ag@SiO₂ NPs (right).

backscattering geometry. Ten scans were averaged for each spectrum. Bulk Raman spectra were collected with a Bruker MultiRAM FT-Spectrometer equipped with a Nd:YAG laser with a excitation wavelength of 1064 nm and power of 10 mW. Two-photon excited fluorescence images were collected with a Zeiss LSM780 with a 63× oil immersion objective, an excitation wavelength of 790 nm, a power of 0.56 ± 0.1 mW, signal collection over 415-740 nm, gain of 700, X-Y-resolution of $26.9 \times 26.9 \ \mu m$ (0.05 $\ \mu m$ /pixel), and a Z-resolution of 0.8 $\ \mu m$ /layer. All samples were dispersed in immersion oil to reduce scattering and sandwiched between two coverslips.

3. RESULTS AND DISCUSSION

3.1. Particle Synthesis and Characterization. AgNPs were synthesized via the reduction of silver nitrate by ascorbic acid in the presence of trisodium citrate capping agent.²⁷ The pH of the reaction media controls the reactivity of ascorbic acid and thus can be used to tune the size of the NPs. An elevated pH was used to promote the reactivity of ascorbic acid, favoring nucleation over growth, resulting in a smaller particle size. After 15 min, the reaction was completed, and the flask was moved to a 100 °C hot bath for 30 min, resulting in more homogenized spherical particles due to intraparticle ripening. At pH 9.5, the resultant AgNPs were 34.1 ± 4.6 nm (averaged over 100 measurements) in diameter with an absorption maximum of 404 nm (Figure 1).

AgNPs were coated with SiO₂ via a modified Stober method.²⁸ The silica shell was utilized to prevent probe molecules from selectively binding or adsorbing to the nanoparticle surface, and can be easily functionalized through silane chemistry. This shell, however, must be thin to allow effective transfer of the electromagnetic field from the AgNP core to the probed surface. The shell thickness can be tuned from several nanometers to greater than 50 nm by controlling the concentration of TEOS. For this study, a 300 μ M concentration of TEOS was used to produce a 9.0 ± 1.4 nmthick silica shell. Due to the change in the dielectric environment surrounding the particle, Ag@SiO₂ NPs showed a red-shifted UV/vis spectrum compared to that of the AgNPs (Figure 1) with an absorption maxima of 425 nm.

A sequential base/acid treatment was used to remove the pollen kit and proteins from the pollen exine as well as to remove the pollen's internal cellular contents. The resulting cleaned ragweed (BA-RW) consists only of the pollen exine, which is composed of sporopollenin, a robust cross-linked polymer containing lipid and phenolic groups. To prepare the BA-RW pollen for nanoparticle decoration, APTES was used to functionalize the pollen surface to create an amine terminated surface (APTES-RW). ζ -Potential measurements (Figure 2) of



Figure 2. ζ -Potential measurements of BA-RW, APTES-RW, AgNP, and Ag@SiO₂ NP under various pH conditions.

BA-RW, AgNPs, and Ag@SiO₂ NPs showed that all three exhibit negative surface charge over the pH range investigated, pH 4–10. In contrast, APTES-RW showed a transition from negative to positive surface charge at pH ~7 with decreasing pH. The positive surface charge of APTES-RW at pH 4 was utilized to electrostatically attach negatively charged AgNPs and Ag@SiO₂ NPs to the surface of the pollen.

The surface coverage on the APTES-RW was controlled by varying the relative concentrations of the NPs and pollen. The nanoparticle surface coverage was determined using highcontrast SEM images. The area of the pollen surface was measured using ImageJ software. The contrast threshold was adjusted to select the area that was coated by the nanoparticles. The nanoparticle coverage was determined by comparing the NP-coated area to the entire area of the pollen with averaged measurements derived from five different pollens. The NP-RW composites were synthesized using either 0.7, 1.0, or 1.3 mL aliquots of AgNP, which yielded AgNP coverages of 19.7 \pm 2.23, 53.1 \pm 5.54, and \approx 100%. Additionally, for solutions with 1, 3, or 5 mL, we obtained Ag@SiO₂NP coverages of 20.6 \pm 4.44, 53.1 \pm 7.22, and \approx 100%, respectively (Figure 3). For clarity, we refer to these samples as 20, 55, and 100% AgNP-RW or Ag@SiO₂-RW.

3.2. Optical Characteristics of Metallized Pollen. The metallized pollen with AgNP-RW or Ag@SiO₂NP-RW shows a rather strong dependence of the plasmon spectra on the



Figure 3. SEM images of (a) BA-RW, (b) APTES-RW, and NP-RW composites with various NP coverages of (c) 20% AgNP-RW, (d) 55% AgNP-RW, (e) 100% AgNP-RW, (f) 20% Ag@SiO₂ NP-RW, (g) 55% Ag@SiO₂ NP-RW, and (h) 100% Ag@SiO₂ NP-RW. All scale bars are 1 µm.



Figure 4. Coverage dependence of the LSPR for the AgNP-RW or $Ag@SiO_2NP$ -RW nanoparticles. (Left) Extinction spectra of the AgNP-RW with varying NP coverage. (Right) Extinction spectra of the $Ag@SiO_2NP$ -RW.

nanoparticle surface density. Nanoparticle aggregation generally leads to interparticle coupling which results in a broadening and red shift in the LSPR.³³ The aggregation that occurs upon NP coating of the APTES-RW resulted in broadband LSPR activity that extends across from ~400 nm to beyond 1100 nm (Figure 4). The silica coating on the Ag core reduces not only the strength of the LSPR at the surface of the particle but also the interparticle coupling, which leads to a reduction in the redshift in the plasmonic resonance of the Ag@SiO₂NP-RW particles as compared to that of the AgNP-RW (Figure 4).

3.3. Nanoparticle Decorated Pollens as a Micro-SERS Platform. To investigate the potential of NP-RW as microscale SERS substrates, Raman spectra were collected from single particles using a Raman microscope (Figure 5) and from a collection of particles using a Fourier transform (FT)-Raman spectrometer (Figure 6). BDT was used as the probe molecule in this study because it has been well-characterized in the literature.^{34,35} To evaluate the SERS activity of the AgNP and Ag@SiO₂ NP decorated pollen, the particles were embedded in a solid BDT matrix, and BA-RW without NPs were used as a control. Micro-Raman spectra were collected with an excitation wavelength of 785 nm with a spot size of 3.14 μ m², focused directly on the pollen surface. The BA-RW without nanoparticles resulted in no detectable Raman signal. Four



Figure 5. Micro-Raman spectra at 785 nm and 1 mW: (top) AgNP-RW and (bottom) $Ag@SiO_2$ NP-RW; each of the samples was treated with 0.1 mM BDT via drop-casting. 100% NP coverage, 55% NP coverage, 25% NP coverage, and BA-RW. The spectra in the figure represent the average of 10 particles.



Figure 6. Raman spectra, 1064 nm (10 mW), of NP-RW embedded in the BDT matrix.

characteristic signals of BDT^{34,35} (1555, 1180, 1065, and 729 cm⁻¹) were observed in each of the NP-RW spectra. As nanoparticle surface coverage increases from 20 to 100%, the SERS signal increased by an order of magnitude (Figure 5) due to the increase in strength of the LSPR at the excitation wavelength and the formation of high intensity hotspots located in small gaps between nanoparticles. The silica coating on the Ag core not only dampens the strength of the LSPR at the surface of the particle but also reduces the interparticle coupling, which leads to a reduction of the LSPR strength at

785 nm and limits the formation of hotspots, resulting in a peak SERS signal that is \approx 50% of their Ag counterparts. Although the enhancement was reduced, the silica coating serves to protect the silver surface from contamination and provides a surface that can be further functionalized through silane coupling chemistry.

The peak enhancement factor (EF) of 10^6 was calculated for the 100% AgNP-RW substrate. The normal Raman spectra were collected on a 6 μ m thin melt processed BDT film, and the SERS spectra were collected on a monolayer of BDT on 100% AgNP-RW.

$$EF = \frac{\frac{I_{sers}}{P_{sers}N_{surf}}}{\frac{I_{rs}}{P_{rs}N_{vol}}} = \frac{\frac{I_{sers}}{P_{ser}\mu_{tr}\mu_{s}A_{M}}}{\frac{I_{rs}}{P_{rs}N_{vol}}}$$
(1)

The enhancement factor was calculated using eq 1, where $I_{\text{sers}}/P_{\text{sers}}$ and $I_{\text{rs}}/P_{\text{rs}}$ are the intensity and power of the SERS and normal Raman spectra, respectively, N_{surf} is the number of adsorbed molecules on the surface of SERS substrate, N_{vol} is the number of molecules in the normal Raman excitation volume, μ_{m} is the number of nanostructures on the substrate, A_{m} is the surface area of the nanostructure, and μ_{s} is the surface density of molecules on the surface.³⁶ The EF was calculated with an estimated molecular footprint of 0.19 nm² and a density of 1 g/ cm³.

To investigate the potential of the NP-RW as bulk SERS substrates, the particles were dispersed in a solid BDT matrix, and Raman spectra were collected using a FT-Raman spectrometer. APTES-RW without NPs showed no detectable BDT Raman signal at 10 mW at 1064 nm. The AgNP-RW showed strong SERS activity (Figure 6) that is relatively independent of NP coverage. The 75% Ag@SiO2NP-RW and 25% Ag@SiO2NP-RW show an 80 and 60% reduction in their SERS signal,s respectively, compared to those of their AgNP-RW counterparts. As mentioned above, the silica shell isolates the NPs from one another, resulting in reduced interparticle coupling that lowers the LSPR at 1064 nm. The aggregation of NP that occurs during the coating process makes the NP-RW an effective broadband (785-1064 nm) SERS substrate platform which can be utilized as a single particle or bulk sensor.

3.4. Two-Photon Excited Fluorescence. We also investigated the two-photon excited fluorescence from the pollen exine (Figure 7), the 100% AgNP-RW, and the 100% $Ag@SiO_2$ -RWparticles. The pollen exine produced measurable but weak fluorescence, whereas the 100% AgNP-RW showed comparatively strong two-photon excited fluorescence emission as expected due to the presence of the aggregated AgNPs. The 100% Ag@SiO_2-RW showed diminished two-photon fluorescence relative to their AgNP counterparts due to the reduced intensity of the hotspots associated with the SiO₂ coating of the AgNP.

3.5. Adhesion Properties of Composite Particles. Two different substrates were used to examine the effect of surface chemistry on the adhesion forces of each pollen particle. Polystyrene (PS) was chosen as a model hydrophobic polymer substrate with a water static contact angle $\theta_w \approx 104^\circ$, while piranha-etched silicon (Si) was used as a model inorganic, hydrophilic, and protic surface with $\theta_w \approx 14^\circ$. Both substrate surfaces are relatively smooth with both the mean (R_a) and root-mean-square (rms) surface roughness of ~0.3 and 2.0 nm for Si and PS, respectively, which were obtained from



Figure 7. Two-photon excited fluorescence images of RW and nanoparticle coated RW pollen.

topography scans of 3 randomly chosen $10 \times 10 \ \mu m$ areas on each substrate surface by using atomic force microscopy.

Contact mode AFM force measurements were used to evaluate the short-range adhesion of six different NP-RW with varied NP surface coverage to the Si and PS substrates. The average values of the adhesion forces for each particle and substrate combination are shown in (Figure 8). Note that each



Figure 8. Adhesion data for various NP coated pollens on silicon and polystyrene. NP coverage given in parentheses.

average value was obtained from 40 measurements consisting of 10 analyses for each of 4 similar particle/cantilever probes. Because the scale of pollen particle surface microtopography is relatively larger than the surface roughness of Si and PS substrates (0.3 and 2.0 nm respectively), the effect of these small differences in roughness is likely to be negligible. For cleaned BA-RW, it was demonstrated previously that the adhesion forces on Si and PS substrates were governed by van der Waals (VDW) interactions.²¹ Thus, no appreciable differences were observed in the adhesion forces of BA-RW on Si and PS surfaces. In contrast, for AgNP-RW or Ag@ SiO₂NP-RW, the adhesion forces were dependent on substrate composition, and the force was stronger on Si than on PS. Furthermore, an appreciable enhancement of the adhesion force was observed with increasing NP surface coverage for AgNP-RW and Ag@SiO₂ NP-RW. The average adhesion force values for 100% AgNP-RW and Ag@SiO2NP-RW were 25% higher on PS and 100% higher on Si compared to that of BA-RW.

Typically, the adhesion forces of particles on a substrate can be controlled and tuned by the surface morphology and chemistry, feature size, and coating materials. In this study, the short-range adhesion on the PS substrate is expected to be governed by the VDW force. Therefore, the dependence of VDW-based adhesion on the pollen ornamentations should be approximated well by the Hamaker equation of the sphere– plane model:³⁷

$$F_{\rm VDW} = \frac{A_{123}R}{6D^2}$$
(2)

where, A_{132} is the nonretarded Hamaker constant of materials 1 and 2 interacting across a medium 3 (air), *R* is the contact radius, and *D* is the cutoff separation distance for the VDW interaction (≈ 0.165 nm). With the assumption that $A_{33} = 0$, A_{132} can be approximated as

$$A_{123} = \sqrt{A_{11}A_{22}} \tag{3}$$

Thus, A_{132} values for ragweed pollen particles coated with AgNPs and Ag@SiO₂NPs on both Si and PS substrates (Table 1) were calculated from eq 2 using Hamaker constants of Ag,

Table 1. Average Measured Values of Adhesion Force (F_{tot} , nN), Calculated Hamaker Constants (A_{132} , ×10⁻²⁰ J), Calculated Contact Radii (R, nm), Calculated VDW Force (F_{vdw} , nN), and Calculated non-VDW Force (F_{nvdw} , nN)

		RW	AgNP- RW (25%)	Ag@SiO2 NP-RW (100%)	AgNP- RW (25%)	Ag@SiO ₂ NP-RW (100%)
PS	A_{132}	8.4	16	16	6.4	6.4
	$F_{\rm tot}$	27 ± 1	26 ± 1	35 ± 1	29 ± 1	35 ± 2
	R	52	27	35	74	89
Si	A_{132}	8.5	16	16	6.3	6.3
	$F_{\rm tot}$	27 ± 1	40 ± 2	51 ± 2	39 ± 1	55 ± 2
	$F_{\rm vdw}$	27 ± 1	27 ± 2	34 ± 2	28 ± 1	34 ± 2
	$F_{\rm nvdw}$	0	13 ± 2	17 ± 2	11 ± 1	21 ± 2

SiO₂, and PS available in the literature $(40 \times 10^{-20}, 6.3 \times 10^{-20}, and 6.5 \times 10^{-20}$ J, respectively).³⁷ Approximate A_{132} values for BA-RW on the Si and PS substrates were calculated from appropriate A_{11} and A_{22} values available in the literature.^{21,37} Therefore, according to eq 1 using the measured average values of adhesion force along with the calculated A_{132} constants, the predicted values of contact radii for each pollen particle with varied coatings on the PS surface were calculated (Table 1) to be ~52 nm (BA-RW), ~27–36 nm (AgNP-RW), and ~74–89

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nm (Ag@SiO2 NP-RW), respectively. For the BA-RW, the calculated contact radius $(52 \pm 1 \text{ nm})$ is similar to the average spine tip radius (52 ± 5 nm) obtained from SEM analysis; that is consistent with a previous report that the VDW-based adhesion force for this pollen particle was a single spine tip contact to a given substrate.²¹ However, the calculated contact radii for the AgNP-RW (~27-35 nm) and Ag@SiO2NP-RW $(\sim 74-89 \text{ nm})$ were significantly smaller than the average spine tip radii of these particles (80 \pm 17 and 100 \pm 20 nm for AgNP-RW and Ag@SiO2 NP-RW, respectively) measured by SEM analyses. Instead, the calculated contact radii for these pollen particles were in good agreement with the average NP crystallite size (35-40 nm for AgNP and 45-50 nm for Ag@ SiO₂ NP) of these particles obtained from TEM analyses. These results indicated that VDW-based adhesion forces of the pollen with NP coatings were consistent with the contact between one or two NPs located at the end of spine tips with the substrates.

Comparing the adhesion forces of NP-RW particles on Si and PS substrates, it is clear that the hydrophilic Si surface exhibited appreciably stronger adhesion to NP-RW. This is attributed to the additional plausible non-VDW interactions (F_{nvdw}) , for example, the hydrogen bonding interactions between the oxygen and hydroxyl functional groups of the Si substrate and NP surfaces. Because both substrates are relatively flat and smooth, the contact radii R of those particles on the Si substrate are considered as the same as those on PS substrate. Then, the VDW-based adhesion force, F_{vdw} , for those pollen particles on the Si surface can be back calculated by using eq 2 with the calculated A_{132} constants and radii R, as listed in (Table 1). Furthermore, the non-VDW forces were obtained by $F_{nvdw} = F_{tot} - F_{vdw}$, where F_{tot} is the experimentally measured force as shown in (Table 1), this F_{nvdw} is also dependent on both the contact radii R and properties of the NP materials. Pollen particles with higher coverage of AgNP and Ag@SiO₂NP exhibited higher adhesion forces for both VDW and non-VDW interactions on Si substrates as compared to PS. The adhesion forces of pollen with similar coverages of AgNP and Ag@SiO2NP were comparable even though the latter showed much higher contact radii (27-35 vs 74-89 nm). This is attributed to the much higher Hamaker constant of AgNP compared to Ag@SiO_2 NP (40 \times 10⁻²⁰ and 6.3 \times 10⁻²⁰ J, respectively).

4. CONCLUSION

Here, we demonstrated the ability to coat RW pollen exine with nanoparticles with precise control over the nanoparticle average surface coverage. Negatively charged nanoparticles were immobilized on the surface through electrostatic attraction to create an adhesive SERS substrate. Strong Raman signals were realized from AgNP and Ag@SiO2NP coated pollen dispersed in a BDT matrix with broadband NIR (785-1064 nm) LSPR response. In addition to SERS activity, the adhesive NP-pollen microparticles display tunable VDW and non-VDW forces (i.e., hydrogen bonding). The adhesion forces were found to exhibit short-range (<5 nm) VDW-based and non-VDW adhesion governed by the NP size and composition at the pollen tip. A maximum of 100% increase in the adhesion force for Ag@ SiO₂NP-RW on a Si substrate was realized. Two-photon excited fluorescence measurements demonstrate the multimodal detection ability of composite particles through both SERS and two-photon confocal microscopy. This work demonstrates the tailoring of the adhesive properties of the

nanoparticle coated pollen exine structures enabled by the spiky pollen morphology, the composition of the NP-pollen constructs, and the nature of the substrate. These adhesive NP-pollen constructs also exhibit strong SERS activity with broadband NIR (785–1064 nm) LSPR. The development of biologically derived microparticles with large surface area to volume ratio, strong optical signatures, and enhanced adhesion properties can have applications in security features, biosensing, and chemical agent detection.

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Notes

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