Au Nanorods on Carbon-Based Nanomaterials as Nanohybrid Substrates for High-Efficiency Dynamic Surface-Enhanced Raman Scattering

Wen-Ru Chang,† Chun Hsiao,† Yan-Feng Chen, Chung-Feng Jeffrey Kuo, and Chih-Wei Chiu*

ABSTRACT: Gold nanorods (AuNRs) with different aspect ratios were prepared by the seed-mediated growth method and combined with three carbon-based nanomaterials of multiple dimensions (i.e., zero-dimensional (0D) carbon black (CB), one-dimensional (1D) carbon nanotubes (CNTs), and two-dimensional (2D) graphene oxide (GO)). The AuNR/carbon-based nanomaterial hybrids were utilized in dynamic surface-enhanced Raman scattering (D-SERS). First, cetyltrimethylammonium bromide (CTAB) was used to stabilize and coat the AuNRs, enabling them to be dispersed in water and conferring a positive charge to the surface. AuNR/carbon-based nanomaterial hybrids were then formed via electrostatic attraction with the negatively charged carbon-based nanomaterials. Subsequently, the AuNR/carbon-based nanomaterial hybrids were utilized as large-area and highly sensitive Raman spectroscopy substrates. The AuNR/GO hybrids afforded the best signal enhancement because the thickness of GO was less than 5 nm, which enabled the AuNRs adsorbed on GO to produce a good three-dimensional hotspot effect. The enhancement factor (EF) of the AuNR/GO hybrids for the dye molecule Rhodamine 6G (R6G) reached $1 \times 10^7$, where the limit of detection (LOD) was $10^{-8}$ M. The hybrids were further applied in D-SERS (detecting samples transitioning from the wet state to the dry state). During solvent evaporation, the system spontaneously formed many hotspots, which greatly enhanced the SERS signal. The final experimental results demonstrated that the AuNR/GO hybrids afforded the best D-SERS signal enhancement. The EF value for R6G reached $1.1 \times 10^8$ after 27 min, with a limit of detection of $10^{-9}$ M at 27 min. Therefore, the AuNR/GO nanohybrids have extremely high sensitivity as molecular sensing elements for SERS and are also very suitable for the rapid detection of single molecules in water quality and environmental management.

1. INTRODUCTION

Surface-enhanced Raman scattering (SERS) was first discovered by Fleischmann and colleagues. SERS is a powerful spectroscopic technique due to its unique fingerprint vibrations and single-molecule detection sensitivity. SERS enhances Raman signals by $10^6$ to $10^{14}$ times and resolves the limitation of the weakness of conventional Raman spectral signals. Gold and silver are the most widely used materials in SERS, among many metallic nanomaterials. In 1960, researchers used the vapor–liquid–solid (VLS) growth method to catalyze the deposition reaction to grow nanoscale metal particles, metal wires, semiconductor crystals, et cetera. In 1990, scholars represented a simple method of synthesizing colloidal metal nanoparticles (MNPs). In comparison with AgNPs, AuNPs have lower toxicity and chemical inertness and, hence, have higher environmental safety. Of the many anisotropic metal nanoparticles, gold nanorods (AuNRs) have received the most attention. AuNRs are thermodynamically unstable due to their shape. Specialized synthetic methods are required to...
prepare AuNRs, including electrochemical,\textsuperscript{12} photochemical,\textsuperscript{13} anodic alumina template deposition,\textsuperscript{14,15} and seed-mediated growth.\textsuperscript{16−18} Methods. AuNRs with tunable aspect ratios were synthesized via the most widely used seed-mediated growth method. The absorption wavelengths of the AuNRs correspond to both transverse surface plasma resonance (TSPR) and longitudinal surface plasma resonance (LSPR).\textsuperscript{19,20} The longitudinal absorption wavelength varies according to the aspect ratio of the AuNRs. The longitudinal absorption wavelength ranges from the visible to near-infrared spectral region, leading to strong light absorption, light scattering, and photothermal conversion properties. AuNRs also have broad applications in photothermal therapy\textsuperscript{21} and biomedical imaging.\textsuperscript{22−25} AuNRs are effective for hotspot generation in SERS applications.\textsuperscript{26,27} Therefore, AuNRs are recognized as materials with considerable development potential.

Among the various carbon-based nanomaterials, zero-dimensional carbon black (CB), also known as soot, is an amorphous carbon. CB is formed by the combustion of hydrocarbons in the absence of air to obtain an extremely fine black carbon powder, which is then separated from the exhaust gas. CB has a very loose, light, and thin appearance with a large surface area. Generally, the surface of carbon black possesses oxygen-containing functional groups after oxidation treatment, and these groups can be grafted or chemically modified.\textsuperscript{28−30}

As another carbon-based nanomaterial, one-dimensional carbon nanotubes (CNTs) are carbon isotopic isomers that were serendipitously discovered by Sumio Iijima during the preparation of carbon fibers using the arc method in the laboratory.\textsuperscript{31} Carbon nanotubes are hollow tubular structures made of carbon. The diameter of CNTs ranges from a few nanometers to tens of nanometers, with a length of approximately 1 μm. CNTs have excellent electrical conductivity, chemical stability, mechanical strength, and thermal conductivity properties.\textsuperscript{32−34} Although the density of CNTs is only one-sixth that of steel, their strength is hundreds of times that of steel. Graphene is a two-dimensional single-layer honeycomb lattice-like material composed of closely arranged carbon atoms.\textsuperscript{35} Graphene is the basic structure of all carbon materials. It has inherently high chemical stability due to its hexagonal benzene ring structure. The van der Waals forces between graphene layers are extremely strong, rendering it neither hydrophilic nor lipophilic. At the same time, graphene is difficult to dissolve in other media, and it is also prone to agglomeration. In order to overcome these shortcomings, surface modification is necessary. Through surface and functional group modifications, the processability and applicability of graphene can be improved. After modification, the interfacial bonding force of graphene was improved, enabling more uniform dispersion in the medium and polymer.\textsuperscript{36−39}

Graphene oxide (GO) is the most typical example of graphene modification. Graphene oxide is functionalized graphene with a large number of oxygen-containing functional groups at the boundary. GO has both hydrophilic and lipophilic amphiphiles and, hence, easily forms a good dispersion slurry with different composite materials. The application of GO in functional composite materials also fully demonstrates its utility.\textsuperscript{40−43}

In recent years, studies have focused on dynamic Raman scattering (D-SERS), where the biggest difference between D-SERS and SERS is that D-SERS collects Raman signals during the process of gradually evaporating the molecule’s solvent to be tested. The generation of stronger SERS signals is required for application in rapid screening detection in daily life. According to the literature, the distance between nanomaterials is the key to determining the intensity of the SERS signal.\textsuperscript{44−47}

As the solvent evaporates, the nanoarrays approach each other very closely. At the same time, particle–particle interactions occur via van der Waals forces and electrostatic repulsion. Through these interactions, the particles can be fixed on both sides of the 2D carbon material, and many hotspots with 3D geometries can be spontaneously formed. Therefore, through D-SERS, the ideal low limit of detection and high enhancement factor can be achieved, and practical application can be realized.\textsuperscript{48−50} In this study, AuNRs with tunable aspect ratios are prepared by the seed-mediated growth method. Carbon-based nanomaterials of different dimensions (one-dimensional modified carbon black (OCB), two-dimensional modified carbon nanotubes (CNT-COOH), and three-dimensional graphene oxide (GO)), as substrates, are combined with the AuNRs by electrostatic attraction. The resulting nanohybrids are used for SERS measurements. The nanohybrid materials have good optical penetration, increase the contact area with the target analyte, and generate hotspots on the z axis.\textsuperscript{51−53} When further applied in D-SERS, this method affords fast, stable, and more strongly enhanced Raman signals. Finally, the sensitivity of the fabricated SERS substrate to Rhodamine 6G (R6G), a dye molecule with fluorescent properties and high photostability, is analyzed, where the single molecule is rapidly detected, enabling water quality and environmental monitoring.\textsuperscript{54}

2. EXPERIMENTAL SECTION

2.1. Materials. Tetrachloroauric acid (HAuCl₄, 99.9%), cetyltrimethylammonium bromide (CTAB, 99%), silver nitrate (AgNO₃, 99.85%), sodium borohydride (NaBH₄, 99%), ascorbic acid (AA, 99%), and Rhodamine 6G (R6G) were procured from Echo Chemical Co., Taiwan. Oxidatively modified carbon black (OCB, 99% purity) was sourced from TIMCAL, Switzerland. Oxidatively modified carbon nanotubes (CNT-COOH, 95% purity) were purchased from ConJutek, Taiwan. Graphene oxide (GO, 97% purity) was purchased from E WAY Technology Co., Taiwan.

2.2. Preparation of Gold Nanorods with Different Aspect Ratios. All the reagents were freshly prepared before starting the synthesis process. The AuNRs were prepared by the seed-mediated growth method proposed by Murphy and co-workers.\textsuperscript{16−18} HAuCl₄ (0.1 mL, 0.024 M) and CTAB (7.5 mL, 0.1 M) were placed in a sample bottle and stirred evenly until the solution turned orange. Thereafter, NaBH₄ (0.6 mL, 0.01 M) was slowly added in an ice bath to form gold particles smaller than 2.5 nm in size. At this point, the color of the solution slowly changed from orange to brown. DI water (1.8 mL) was added and mixed well to complete the seed-mediated growth solution. HAuCl₄ (0.2 mL, 0.024 M) and CTAB (10 mL, 0.1 M) were added to another sample vial. The two solutions were stirred evenly until the solution turned orange, after which AgNO₃ (0−0.10 mL, 0.01 M) was added. The aspect ratio of the AuNRs was controlled by adding silver nitrate. At this point, AA (0.08 mL, 0.1 M) was added for reduction. When the reduction was complete, the solution turned colorless. Finally, the growth solution was prepared by adding H₂SO₄ (0.2 mL, 0.5 M) to adjust the pH. Subsequently, 0.024 mL of the seed crystal solution was added to the growth solution, mixed uniformly, and allowed to stand for 12 h to obtain the AuNR solution.
2.3. Preparation of Gold Nanorods/Carbon-Based Nanohybrids. The AuNRs provided a better Raman-enhanced signal than the gold nanoparticles (AuNPs), but the Raman signal strength of the AuNRs is unstable because the AuNRs tend to remain in a state with low surface energy, which results in agglomeration or uneven distribution. Therefore, herein, the AuNRs were adsorbed on zero-dimensional modified OCB through electrostatic attraction self-assembly to form AuNR/OCB nanohybrids. The pre-synthesized AuNR solution was centrifuged at 6000 rpm for 1 h. After centrifugation, the supernatant was discarded and redispersed in DI water. Next, different amounts of AuNR solutions (five weight ratios: 5/1, 10/1, 15/1, 20/1, and 25/1) were added to the same amount of OCB. In addition, the weight of AuNR was calculated based on the redispersed solution. Finally, the AuNR/OCB nanohybrids were obtained by magnetic stirring for 5 h. Likewise, AuNRs were adsorbed on one-dimensional modified CNT-COOH and two-dimensional GO. After preparing the AuNR/CNT-COOH and AuNR/GO nanohybrids with different weight ratios, the preparation of the SERS substrates was completed. Therefore, the final products were then ready to be applied to SERS detection.

2.4. Characterization and Instruments. UV–visible spectrophotometric measurements were performed using a Jasco (V-670) instrument. After diluting the synthesized nanocomposite solution, reduction of the gold rods was monitored by the absorption of specific wavelengths in the UV–visible range. X-ray diffraction data were acquired using a PSAXS-USAXS-WAXS-002 system. The diffraction angle (2θ) of the XRD scan ranged from 5 to 90°, and the interval was 0.05° with a scan time of 0.5 s for each point. The sample solution (1
3. RESULTS AND DISCUSSION

3.1. Synthesis and Identification of Gold Nanorods with Different Aspect Ratios. First, a certain amount of seed crystal solution was added to the growth solution. Concurrently, under the stabilization of the protective agent CTAB, the content of silver nitrate was regulated because the silver ions in silver nitrate react with the bromide ions in CTAB to form silver bromide (AgBr), which is adsorbed on the Au(100) crystal plane. Therefore, by this method, gold grows along the Au(111) crystal plane to form the AuNR morphology. The aspect ratio of the AuNRs can be altered by adding silver nitrate. Figure 1a shows the AuNRs synthesized by adding different weight ratios of silver nitrate. The optical properties of the AuNRs differ based on the aspect ratio. The AuNRs have absorption wavelengths corresponding to both the horizontal and vertical axes of the rods. The absorption wavelength of the vertical axis was gradually redshifted as the aspect ratio of the AuNRs became longer. The inset images of Figure 1a show AuNRs with different aspect ratios. The color of the AuNR solution changed from purple-red to blue-green, green, and orange. When no silver nitrate is added to form gold nanoparticles, the solution showed a red color. Figure 1b shows the plot of the absorption peaks of the vertical axis for the AuNRs, corresponding to the different aspect ratios. There was a linear relationship between the absorption and the aspect ratio, where the $R^2$ value reached 0.9989. The XRD patterns in Figure 1c show diffraction peaks of the AuNRs corresponding to the Au(111), Au(200), Au(220), and Au(311) planes at respective 2θ positions of 38.2, 44.4, 64.6, and 77.4°, consistent with the JCPDS data for face-centered cubic gold nanocrystals (JCPDS file: 04-0784). Figure 1d presents the TEM images of the AuNRs with different aspect ratios, showing the rod-like shape and aspect ratios. The localized surface plasmon resonance (LSPR) of nanoscale metallic structures depends on environmental factors such as the shape, size, spacing, and orientation of the nanoparticles and the properties of the medium surrounding the electromagnetic field. AuNRs provide a better LSPR effect than that of AuNPs, and the SERS properties are strongly dependent on the LSPR effect. Furthermore, it was concluded that AuNRs provide a stronger SERS signal. According to the finite-difference time-domain (FDTD) simulations in the literature, the electromagnetic field around the tip of the AuNRs is significantly enhanced. This phenomenon is caused by LSPR and also shows that the LSPR effect plays a major role in SERS enhancement. Figure 1e shows the SERS signals of the AuNRs with different aspect ratios for the detection of R6G dye molecules at a laser wavelength of 633 nm. The SERS-enhanced signal of the AuNRs was better than that of the AuNPs (AR = 1.06). Based on the discussion of dispersion and endpoint distance, it was inferred that an appropriate aspect ratio would produce the greatest signal enhancement effect. As

### Table 1. Synthesis of Gold Nanorods and Gold Nanorod/Carbon-Based Nanomaterials and SERS Detection of R6G Dye Molecules

| sample name | AuNR/carbon-based nanomaterials(weight ratio) | zeta potential (mV) | SERS intensity$^b$ | SERS EF values$^c$ |
|-------------|-----------------------------------------------|---------------------|---------------------|---------------------|
| AuNRs$^a$  | +38.7                                         | 1.81 × 10$^4$       | 3.0 × 10$^4$        |
| OCB         | −36.2                                         |                     |                     |
| AuNR/OCB    | 5/1                                          | +20.2               | 1.24 × 10$^4$       |
|             | 10/1                                         | +24.5               | 1.45 × 10$^4$       |
|             | 15/1                                         | +28.6               | 1.66 × 10$^4$       |
|             | 20/1                                         | +31.1               | 1.88 × 10$^4$       |
|             | 25/1                                         | +33.4               | 1.70 × 10$^4$       |
| CNT-COOH    | −37.5                                         |                     |                     |
| AuNR/CNT-COOH | 5/1                                         | +19.4               | 1.30 × 10$^4$       |
|             | 10/1                                         | +25.0               | 1.51 × 10$^4$       |
|             | 15/1                                         | +31.4               | 1.94 × 10$^4$       |
|             | 20/1                                         | +34.8               | 1.72 × 10$^4$       |
|             | 25/1                                         | +37.1               | 1.70 × 10$^4$       |
| GO          | −38.2                                         |                     |                     |
| AuNR/GO     | 10/1                                         | +23.5               | 1.40 × 10$^4$       |
|             | 20/1                                         | +29.9               | 1.79 × 10$^4$       |
|             | 30/1                                         | +33.5               | 2.39 × 10$^4$       |
|             | 40/1                                         | +35.1               | 2.01 × 10$^4$       |
|             | 50/1                                         | +39.4               | 1.96 × 10$^4$       |

$^a$Experimental results for AuNRs with AR = 4.45. $^b$Integrated intensity in the wavelength range of 600–625 cm$^{-1}$. $^c$Formula for calculating EF value: EF = ($I_{SERS}/N_{SERS}$)/($I_{Raman}/N_{bulk}$).

mL) was dropped onto the glass substrate and completely dried, after which the diffraction pattern was obtained. The XRD data were used to determine the structure or morphology of the sample components and the arrangement of molecules or atoms in the samples. Zeta potential analysis was performed using a Nano-Zs90 instrument for detection. The diluted sample was loaded into a zeta cuvette to derive the surface potential of the charged particles. A JEOL JEM-2100 transmission electron microscope was used for imaging. The AuNRs were redispersed in water after centrifugation at 6000 rpm for 1 h. At this point, 10 μL drops were drawn and dried on carbon-coated copper mesh. After preparation of the test specimen, the TEM images were acquired. Micro-Raman spectra were obtained using a Horiba iHR550 system. A 633 nm band laser was utilized for detection, and the measurement range was set as 200–2000 cm$^{-1}$. The sample solution (10 μL) was dropped an aluminum sheet, and 10 μL of the analyte (R6G) was added after drying. After the test specimen was completely dried, the Raman spectrum was analyzed, and the change in the Raman peak at 611 cm$^{-1}$ (the characteristic peak of R6G) was observed simultaneously.

### Table 1. Synthesis of Gold Nanorods and Gold Nanorod/Carbon-Based Nanomaterials and SERS Detection of R6G Dye Molecules

| sample name | AuNR/carbon-based nanomaterials(weight ratio) | zeta potential (mV) | SERS intensity$^b$ | SERS EF values$^c$ |
|-------------|-----------------------------------------------|---------------------|---------------------|---------------------|
| AuNRs$^a$  | +38.7                                         | 1.81 × 10$^4$       | 3.0 × 10$^4$        |
| OCB         | −36.2                                         |                     |                     |
| AuNR/OCB    | 5/1                                          | +20.2               | 1.24 × 10$^4$       |
|             | 10/1                                         | +24.5               | 1.45 × 10$^4$       |
|             | 15/1                                         | +28.6               | 1.66 × 10$^4$       |
|             | 20/1                                         | +31.1               | 1.88 × 10$^4$       |
|             | 25/1                                         | +33.4               | 1.70 × 10$^4$       |
| CNT-COOH    | −37.5                                         |                     |                     |
| AuNR/CNT-COOH | 5/1                                         | +19.4               | 1.30 × 10$^4$       |
|             | 10/1                                         | +25.0               | 1.51 × 10$^4$       |
|             | 15/1                                         | +31.4               | 1.94 × 10$^4$       |
|             | 20/1                                         | +34.8               | 1.72 × 10$^4$       |
|             | 25/1                                         | +37.1               | 1.70 × 10$^4$       |
| GO          | −38.2                                         |                     |                     |
| AuNR/GO     | 10/1                                         | +23.5               | 1.40 × 10$^4$       |
|             | 20/1                                         | +29.9               | 1.79 × 10$^4$       |
|             | 30/1                                         | +33.5               | 2.39 × 10$^4$       |
|             | 40/1                                         | +35.1               | 2.01 × 10$^4$       |
|             | 50/1                                         | +39.4               | 1.96 × 10$^4$       |

$^a$Experimental results for AuNRs with AR = 4.45. $^b$Integrated intensity in the wavelength range of 600–625 cm$^{-1}$. $^c$Formula for calculating EF value: EF = ($I_{SERS}/N_{SERS}$)/($I_{Raman}/N_{bulk}$).

 mL) was dropped onto the glass substrate and completely dried, after which the diffraction pattern was obtained. The XRD data were used to determine the structure or morphology of the sample components and the arrangement of molecules or atoms in the samples. Zeta potential analysis was performed using a Nano-Zs90 instrument for detection. The diluted sample was loaded into a zeta cuvette to derive the surface potential of the charged particles. A JEOL JEM-2100 transmission electron microscope was used for imaging. The AuNRs were redispersed in water after centrifugation at 6000 rpm for 1 h. At this point, 10 μL drops were drawn and dried on carbon-coated copper mesh. After preparation of the test specimen, the TEM images were acquired. Micro-Raman spectra were obtained using a Horiba iHR550 system. A 633 nm band laser was utilized for detection, and the measurement range was set as 200–2000 cm$^{-1}$. The sample solution (10 μL) was dropped an aluminum sheet, and 10 μL of the analyte (R6G) was added after drying. After the test specimen was completely dried, the Raman spectrum was analyzed, and the change in the Raman peak at 611 cm$^{-1}$ (the characteristic peak of R6G) was observed simultaneously.
shown in Figure 1f, we calculated the SERS signal intensities in the integrated wavelength range of 600–25 cm$^{-1}$ and plotted the data for comparison. The AuNRs had the best signal enhancement at AR = 4.45. Thus, the AuNRs with AR = 4.45 were used in the ensuing experiments to determine the effect of adding carbon materials of different dimensions. In addition to testing the sensitivity of the AuNRs to the SERS signal of R6G, the detection limit of the SERS signal of R6G was also determined. Figure 1g shows that the limit of detection of the AuNRs was 10$^{-7}$ M. The sensitivity of the SERS substrate for each kind of backplane was quantified based on the enhancement factor (EF). The formula for calculating the EF value is as follows: 

$$EF = \frac{I_{\text{SERS}}}{N_{\text{SERS}}} / \frac{I_{\text{Raman}}}{N_{\text{bulk}}}$$

$N_{\text{bulk}}$ indicates the number of objects to be tested (R6G) in the laser focal length range in the general Raman spectrum; $N_{\text{SERS}}$ is the number of objects to be tested (R6G) within the laser detection range on the SERS substrate; $I_{\text{Raman}}$ is the normal Raman spectral intensity; $I_{\text{SERS}}$ is the signal intensity for SERS. The characteristic peak of R6G at 611 cm$^{-1}$ was used for the calculation. In this equation, $N_{\text{bulk}}$ (0.1 M) and $N_{\text{SERS}}$ represent the limit of concentrations of R6G on the AuNR/carbon hybrid substrate, whereas $I_{\text{SERS}}$ and $I_{\text{Raman}}$ represent the signal intensities obtained from R6G on the AuNR/carbon and aluminum substrates, respectively; the calculated EF was 3 × 10$^{5}$, and the results are summarized in Table 1.

### 3.2. Synthesis and Structural Identification of AuNR/Carbon-Based Nanomaterial Hybrids

The AuNR/OCB composite is formed by the adsorption of the AuNRs on OCB. When CTAB is dissolved in water, it dissociates into $(C_{16}H_{33})(CH_{3})_{3}N^+$ and a bromide ion (Br$^-$). The periphery of the AuNRs is covered by $(C_{16}H_{33})(CH_{3})_{3}N^+$ as a protective layer, which confers a positive charge to the AuNRs. The zeta potential plot is shown in Figure S1 of the Supporting Information. The surface of OCB has many oxygen-containing functional groups, such as hydroxyl (-OH) and carboxyl (-COOH). When OCB is dispersed in water, the surface is negatively charged. Therefore, the positively charged AuNRs would be adsorbed on the negatively charged OCB via electrostatic attraction. A schematic of the formation of the AuNR/OCB nanohybrids is shown in Figure 2a. Figure 2b shows the UV spectra of the AuNR/OCB nanohybrids. The main peak absorption of OCB occurred at 242 nm, while the absorption peak of the AuNRs corresponding to the horizontal axis appeared at 518 nm and that of the vertical axis at 844 nm. For the AuNR/OCB nanohybrids, the absorption peak corresponding to the vertical axis was blueshifted to 835 nm and broadened. The change in the absorption peak corresponding to the vertical axis of the AuNRs is attributed to
the change in the local dielectric environment of the AuNRs, which indicates that the AuNRs were modified on the OCB. The XRD pattern in Figure 2c shows diffraction peaks of the Au(111), Au(200), Au(220), and Au(311) planes of the AuNRs at 2θ values of 38.2, 44.4, 64.6, and 77.4°, respectively, consistent with face-centered cubic gold nanocrystals, according to the JCPDS database (JCPDS file: 04-0784). However, for AuNR/OCB, an additional C(002) diffraction peak appeared at 2θ = 25.8°. The XRD and UV data confirm that there was a strong interaction between the AuNRs and OCB, as further confirmed by zeta potential analysis. The data in Figure 2d demonstrate that the surface of the AuNRs was positively charged, whereas the surface of OCB was negatively charged. Due to the different surface potentials of the AuNRs and OCB, the AuNRs can be stably adsorbed. The AuNR/OCB nanohybrids were formulated at different weight ratios to determine the optimum composition for maximizing the SERS enhancement. The structure of the AuNR/OCBs with different weight ratios and the attachment of the AuNRs on OCB were observed by TEM (Figure 2e).

Subsequently, the AuNR/CNT-COOH composite was formed by adsorbing the AuNRs on CNT-COOH. The surface of CNT-COOH has many oxygen-containing functional groups, such as carboxyl groups (-COOH). When CNT-COOH is dissociated in water, the surface is negatively charged. Therefore, the positively charged AuNRs were electrostatically attracted to the negatively charged CNT-COOH. A schematic of the formation of the AuNR/CNT-COOH nanohybrids is shown in Figure 3a. Figure 3b shows the UV spectra for identification of the AuNR/CNT-COOH nanohybrids. The main peak of CNT-COOH appeared at 240 nm. The absorption peak corresponding to the horizontal axis of the AuNRs appeared at 518 nm, and the absorption peak corresponding to the vertical axis appeared at 844 nm. For the AuNR/CNT-COOH composite, the absorption peak of the vertical axis was blueshifted to 842 nm and broadened. The change in the absorption peak of the vertical axis is attributed to the change in the local dielectric environment of the AuNRs, which indicates that the AuNRs were modified on CNT-COOH. The XRD pattern (Figure 3c) shows diffraction peaks of the Au(111), Au(200), Au(220), and Au(311) planes of the AuNRs at 2θ = 38.2, 44.4, 64.6, and 77.4°, respectively, consistent with face-centered cubic gold nanocrystals, according to the JCPDS database (JCPDS file: 04-0784). For the AuNR/CNT-COOH, an additional C(002) diffraction peak was observed at 2θ = 25.8°. Interaction between the AuNRs and CNT-COOH was thus confirmed by the XRD and UV analyses and corroborated by zeta potential analysis. From Figure 3d and Table 1, it was observed that the surface of the AuNRs was positively charged, while the surface of CNT-COOH was negatively charged. Due to the different surface potentials of the AuNRs and CNT-COOH, the AuNRs can be...
stably adsorbed. AuNR/CNT-COOH nanohybrids were formulated in different weight ratios to test the optimum composition for maximizing the SERS enhancement. The structures of AuNR/CNT-COOH with different weight ratios and the attachment of the AuNRs on CNT-COOH were observed by TEM, as shown in Figure 3e.

Finally, AuNR/GO nanohybrids were formed by adsorbing the AuNRs on GO. GO has many oxygen-containing functional groups on the surface, such as hydroxyl (-OH) and carboxyl (-COOH). When GO is dispersed in water, the surface is negatively charged. Therefore, the positively charged AuNRs would be electrostatically attracted to the negatively charged GO. A schematic of the formation of the AuNR/GO nanohybrids is shown in Figure 4a. Figure 4b shows the UV spectra for identifying the AuNR/GO nanohybrids. The main peak of GO appeared at 234 nm; the absorption peak corresponding to the horizontal axis of the AuNRs appeared at 512 nm, and that corresponding to the vertical axis appeared at 830 nm. For the AuNR/GO composite, the absorption peak of the longitudinal axis was blueshifted to 485 nm and broadened. The change in the absorption peak of the vertical axis is attributed to the change in the local dielectric environment of the AuNRs, which indicates that the AuNRs were modified on GO. The XRD pattern in Figure 4c shows diffraction peaks of the Au(111), Au(200), Au(220), and Au(311) planes of the AuNRs at $2\theta = 38.2$, $44.4$, $64.6$, and $77.4^\circ$, consistent with face-centered cubic gold nanocrystals, according to the JCPDS database (JCPDS file: 04-0784). An additional peak was observed at $2\theta = 10.7^\circ$, corresponding to the C(001) diffraction of the AuNR/GO. Therefore, the XRD and UV analyses confirmed that there was a special interaction between the AuNRs and GO, as further validated by zeta potential analysis. The surface of the AuNRs was positively charged, while the surface of GO was negatively charged (Figure 4d). Due to the different surface potentials of the AuNRs and GO, the AuNRs can be stably adsorbed. AuNR/GO nanohybrids were formulated in different weight ratios to determine the optimum composition for maximizing the SERS enhancement. The structure of the AuNR/GO with different weight ratios and the attachment of the AuNRs on GO were observed by TEM, as shown in Figure 4e.

3.3. SERS Application and Discussion of AuNR/Carbon-Based Nanomaterial Hybrids. Because carbon black, carbon nanotubes, and graphene oxide contain the resonance benzene ring structure, charge transfer can be generated between the molecules. Therefore, the Raman signal can be enhanced by the chemical enhancement effect. Therefore, the component ratio of the AuNR/OCB nanohybrids, AuNR/CNT-COOH nanohybrids, and AuNR/GO nanohybrids were varied for SERS detection. Figure 5a shows the SERS profiles of the three nanohybrids. The integral wavelength in the range of $600 - 625 \text{ cm}^{-1}$ was calculated, and the intensity is compared in Figure S2 of the Supporting Information and Table 1. The optimal weight ratios of the
AuNR/OCB, AuNR/CNT-COOH, and AuNR/GO nano-hybrids were 20/1, 15/1, and 30/1, respectively. Furthermore, 50 positions of detection signal enhancement were randomly selected, where it was found that the results have good reproducibility. As shown in Figure 5b, the RSDs for the three nanohybrids were 10.1, 8.6, and 7.1% in the aforementioned sequence. The integrated intensity and the analyte concentration were plotted on a logarithmic scale (Figure 5c), illustrating a good linear relationship between log intensity and log concentration, with $R^2 > 0.99$. In addition to the detection

Figure 5. (a) SERS profile of (1) AuNR/OCB, (2) AuNR/CNT-COOH, and (3) AuNR/GO nanohybrids with different weight ratios. (b) Fifty randomly selected Raman signals for detection on (1) AuNR/OCB = 20/1, (2) AuNR/CNT-COOH = 15/1, and (3) AuNR/GO = 30/1 composite substrates. (c) Logarithmic plot of intensity versus R6G concentration for (1) AuNR/OCB = 20/1, (2) AuNR/CNT-COOH = 15/1, and (3) AuNR/GO = 30/1 nanohybrids.

Figure 6. (a) SERS signals of AuNR/OCB nanohybrids with a weight ratio of 20/1 with different concentrations of R6G. (b) SERS signals of AuNR/CNT-COOH nanohybrids with a weight ratio of 15/1 with different concentrations of R6G. (c) SERS signals of AuNR/GO nanohybrids with a weight ratio of 30/1 with different concentrations of R6G.
sensitivity and reproducibility of the SERS signal of R6G, the limit of detection and EF value of the SERS signal for R6G were also calculated.

Figure 6 shows the SERS profiles of the three substrates (AuNR/OCB, AuNR/CNT-COOH, and AuNR/GO) at the optimal ratios. When the ratio of the AuNR/OCB composite was 20/1, the LOD was $10^{-8}$ M, and the EF value was $5.1 \times 10^6$. When the weight ratio of the AuNR/CNT-COOH composite was 15/1, the LOD was $10^{-8}$ M, and the EF value was $5.6 \times 10^6$. When the weight ratio of the AuNR/GO composite was 30/1, the LOD was $10^{-8}$ M, and the EF value was $1 \times 10^7$. Figure S3 of the Supporting Information presents a comparison of the integrated intensities of the SERS signals of the three substrates at different concentrations of R6G, wherein AuNR/GO had the best SERS substrate efficiency. Compared with zero-dimensional carbon black and one-dimensional carbon tubes, two-dimensional GO can provide a large specific surface area. It can be adopted to stabilize AuNRs and become excellent carriers. GO enhanced the SERS signal of the original metal nanoparticles and reduced the

Figure 7. (a) Schematic of dynamic reaction process and Raman enhancement of D-SERS. (b) D-SERS spectral signal detected per minute at R6G of $10^{-4}$ M when (1) AuNR/OCB = 20/1, (2) AuNR/CNT-COOH = 15/1, and (3) AuNR/GO = 30/1. (c) D-SERS signal of (1) AuNR/OCB = 20/1, (2) AuNR/CNT-COOH = 15/1, (3) AuNR/GO = 30/1 composites with different concentrations of R6G. (d) Logarithmic plot of signal intensity of (1) AuNR/OCB = 20/1, (2) AuNR/CNT-COOH = 15/1, and (3) AuNR/GO = 30/1 nanohybrids versus R6G concentration.

ACS Omega 2022, 7, 41815–41826
influence of fluorescence on the signal. Moreover, GO has sufficient reactive oxygen sites. Therefore, GO can significantly enhance the binding with metals/molecules. The benzene ring resonance structure of GO can also enable charge transfer between GO and the molecules, which in turn enhances the Raman signal.38

3.4. D-SERS Effect and Discussion of AuNR/Carbon-Based Nanomaterial Hybrids. An ideal SERS substrate should have excellent sensitivity, good reproducibility, and a sufficiently high EF value. According to previous studies, the efficient generation of hotspot structures leads to better SERS signals, especially the 3D hotspot effect.37 D-SERS collects Raman signals during transition of the SERS substrate from the wet state to the dry state. During the process, solvent evaporation drives the nanoarrays into closer proximity. D-SERS spontaneously generates many 3D hotspots through van der Waals interaction forces and electrostatic repulsion between particles. A schematic of the significant enhancement of the Raman signal using D-SERS is shown in Figure 7a. D-SERS detection was performed with the AuNR/OCB, AuNR/CNT-COOH, and AuNR/GO substrates at weight ratios of 20/1, 15/1, and 30/1, respectively. During detection, samples are gradually dried at room temperature (25 °C). Then, we recorded the change in the spectral signal at 1 min intervals. The integration wavelength was set to 600–625 cm⁻¹ for comparison of the spectral intensity per minute. As demonstrated in Figure 7b and Table S1 and Video S1 of the Supporting Information, the D-SERS signal gradually increased with time during the process. The peaks of the three backbone planes were observed after 27–28 min, as shown in Figure S4 of the Supporting Information. After 27–28 min, the signal started to decline. As the solvent is completely evaporated and dried, the nanostructure will return to its original position, making the 3D hotspot disappear.39 Other than discussing the detection sensitivity of the D-SERS signal of R6G, we also calculated the limit of detection and EF value of the D-SERS signal of R6G. The D-SERS signals of the three substrates at different concentrations of R6G are shown in Figure 7c. When the weight ratio of the AuNR/OCB composite was 20/1, the LOD was approximately 10⁻⁹ M, and the EF value was 2.7 × 10⁻⁹. When the weight ratio of the AuNR/CNT-COOH composite was 15/1, the LOD was ∼10⁻⁹ M, and the EF value was 3.8 × 10⁻⁹. When the weight ratio of the AuNR/GO composite was 30/1, the LOD was ∼10⁻⁹ M, and the EF value was 1.1 × 10⁻⁹. The integrated intensity versus the analyte concentration was plotted logarithmically. As shown in Figure 7d, there was a good linear relationship between log intensity and log concentration, with R² > 0.99. Due to the unit volume, the hotspot area generated by D-SERS was higher than that of SERS. Therefore, based on the above results, it was determined that the effect of 3D D-SERS was better than that of 2D SERS. The data also show that the AuNR/GO nanohybrids have the highest sensitivity as the molecular sensor of SERS. Finally, we also compared recent reports of gold nanorod hybrids in the detection of R6G. The results are shown in Table S2 of the Supporting Information.

4. CONCLUSIONS

AuNRs with tunable aspect ratios were successfully synthesized by seed-mediated growth, and their SERS enhancement effect was compared. The enhancement effect was maximized when the aspect ratio (AR) of the AuNRs was 4.45. Carbon materials of different dimensions were added to the solution of AuNRs with an aspect ratio of 4.45. The AuNRs were adsorbed on the carbon materials through electrostatic attraction, leading to more uniform dispersion of the metal particles and more effective enhancement of the SERS signals. The SERS and D-SERS signal enhancement effects of the nanohybrids of the different carbon materials and AuNRs were compared. The AuNR/GO nanocomposite had the best SERS enhancement effect. TEM imaging confirmed that GO leads to better dispersion of the AuNRs. Because GO is a two-dimensional nanomaterial with a thickness of less than 5 nm, the AuNRs were adsorbed on both sides of GO and produced a hotspot effect along the z axis. In the SERS detection, the EF value of the AuNR/GO nanocomposite for the R6G dye molecule was 1 × 10⁵, and the limit of detection was 10⁻⁹ M. The AuNR/GO nanocomposite was further applied in D-SERS. The EF value of the AuNR/GO nanocomposite for R6G was 1.1 × 10⁷, and the limit of detection was 10⁻⁹ M. Therefore, the AuNR/GO nanohybrids have extremely high sensitivity as the molecular sensing element of SERS and are believed to be highly suitable for the rapid detection of single molecules in water quality and environmental monitoring.

ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.2c06485.

(A Figure S1) Zeta potential spectra of AuNRs with different aspect ratios; (Figure S2) signal intensities of different weight ratios of AuNR/OCB nanohybrids, AuNR/CNT-COOH nanohybrids, and AuNR/GO nanohybrids that have undergone surface-enhanced Raman scattering; (Figure S3) comparison of the integrated SERS signal intensities of AuNR/OCB nanohybrid with a weight ratio of 20/1, AuNR/CNT-COOH nanohybrid with a weight ratio of 15/1, and AuNR/GO nanohybrid with a weight ratio of 30/1 in different concentrations of R6G (the integrated intensity range of wavelength was 600–625 cm⁻¹); (Figure S4) Intensity of the D-SERS spectral signals detected per minute and comparison of the integrated D-SERS intensities in different concentrations of R6G; (Table S1) gold nanorod/carbon-based nanomaterials to D-SERS detection of R6G dye molecules; (Table S2) summary of various AuNR-related nanohybrid substrates for SERS (PDF)

(Video S1) Demonstration of rapid detection of R6G by 3D hot-junctions of Raman-enhancing AuNR/GO nanohybrid substrate (MP4)

AUTHOR INFORMATION

Corresponding Author
Chih-Wei Chiu — Department of Materials Science and Engineering, National Taiwan University of Science and Technology, Taipei 10607, Taiwan; orcid.org/0000-0003-2258-2454; Phone: +886-2-2737-6521; Email: cwchiu@mail.ntust.edu.tw

Authors
Wen-Ru Chang — Department of Materials Science and Engineering, National Taiwan University of Science and Technology, Taipei 10607, Taiwan
Chun Hsiao — Department of Materials Science and Engineering, National Taiwan University of Science and Technology, Taipei 10607, Taiwan
Yan-Feng Chen — Department of Materials Science and Engineering, National Taiwan University of Science and Technology, Taipei 10607, Taiwan
Chung-Feng Jeffrey Kuo — Department of Materials Science and Engineering, National Taiwan University of Science and Technology, Taipei 10607, Taiwan

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.2c06485

Author Contributions
\*W.-R.C. and C.H. contributed equally to this work.

Author Contributions

The manuscript was written through contributions from all the authors. Data curation: W.-R.C., C.H., and C.-W.C.; formal analysis: W.-R.C., C.H., and Y.-F.C.; supervision, C.-W.C.; writing - original draft: W.-R.C., C.H., and C.-W.C.; writing - review and editing: W.-R.C., C.H., Y.-F.C., C.-F.J.K., and C.-W.C. All authors have read and agreed to the published version of the manuscript.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This research was funded by the Ministry of Science and Technology (MOST 108-2221-E-011-042-MY2, MOST 111-2628-E-011-009-MY3, and MOST 111-2622-8-011-009-TE2) of Taiwan.

■ REFERENCES

(1) Yang, B.; Jin, S.; Guo, S.; Park, Y.; Chen, L.; Zhao, B.; Jung, Y. M. Recent Development of SERS Technology: Semiconductor-Based Study. ACS Omega 2019, 4, 20101−20108.
(2) Liang, A.; Li, C.; Wang, X.; Luo, Y.; Wen, G.; Jiang, Z. Immunocontrolling Graphene Oxide Catalytic Nanogold Reaction and Its Application to SERS Quantitative Analysis. ACS Omega 2017, 2, 7349−7358.
(3) Pan, C.; Li, X.; Sun, J.; Li, Z.; Zhang, L.; Qian, W.; Wang, P.; Dong, J. A Multiplexed SERS-Active Microneedle for Simultaneous Redox Potential and pH Measurements in Rat Joints. ACS Appl. Bio Mater. 2019, 2, 2102−2108.
(4) Sinha, S. P.; Jones, S. P.; Pramanik, A.; Ray, P. C. Nanoarchitecture Based SERS for Biomolecular Fingerprinting and Label-Free Disease Markers Diagnosis. Acc. Chem. Res. 2016, 49, 2725−2735.
(5) Blanco-Formoso, M.; Pazos-Perez, N.; Alvarez-Puebla, R. A. Fabrication of Plasmonic Supercrystals and Their SERS Enhancing Properties. ACS Omega 2020, 5, 25485−25492.
(6) Panariello, L.; To, K. C.; Khan, Z.; Wu, G.; Gkogkos, G.; Damilos, S.; Parkin, I. P.; Gavrillidis, A. Kinetics-based Design of a Flow Platform for Highly Reproducible on Demand Synthesis of Gold Nanoparticles with Controlled Size Between 50 and 150 nm and Their Application in SERS and PIERS Sensing. Chem. Eng. J. 2021, 423, 129069.
(7) Li, S.; Lin, Y. C.; Zhao, W.; Wu, J.; Wang, Z.; Hu, Z.; Shary, Y.; Tang, D. M.; Wang, J.; Zhang, Q.; Zhu, H.; Chu, L.; Zhao, W.; Liu, C.; Sun, Z.; Taniguchi, T.; Osada, M.; Chen, W.; Xu, Q. H.; Andrew, T. S. W.; Suenage, K.; Ding, F.; Eda, G. Vapour−Liquid−Solid Growth of Monolayer MoS2 Nanoribbons. Nat. Materials. 2018, 17, 535−542.
(8) Lee, J. W.; Choi, S. R.; Heo, J. H. Simultaneous Stabilization and Functionalization of Gold Nanoparticles via Biomolecule Conjugation: Progress and Perspectives. ACS Appl. Mater. Interfaces 2021, 13, 42311−42328.
(9) Loza, K.; Heggen, M.; Epple, M. Synthesis, Structure, Properties, and Applications of Bimetallic Nanoparticles of Noble Metals. Adv. Funct. Mater. 2020, 30, 1909260.
(10) Su, M. T.; Joseph, M. M.; Karunakaran, V.; Saranya, G.; Adukkadan, R. N.; Shamjith, S.; Thomas, R.; Nair, J. B.; Swathi, R. R.; Maiti, K. K. Biogenic Cluster-Encased Gold Nanorods as a Targeted Three-in-One Theranostic Nanoenvelope for SERS-Guided Photothermal Therapy Against Metastatic Melanoma. ACS Appl. Bio Mater. 2019, 2, 588−600.
(11) Wei, M. Z.; Deng, T. S.; Zang, Q.; Cheng, Z.; Li, S. Seed-Mediated Synthesis of Gold Nanorods at Low Concentrations of CTAB. ACS Omega 2021, 6, 9188−9195.
(12) Su, Q.; Ma, X.; Dong, J.; Jiang, C.; Qian, W. A Reproducible SERS Substrate Based on Electrostatically Assisted APTES-Functionalized Surface-Assembly of Gold Nanostars. ACS Appl. Mater. Interfaces 2011, 3, 1873−1879.
(13) Jara, N.; Milan, N. S.; Rahman, A.; Mouheb, L.; Boffito, D. C.; Jeffreys, C.; Dahoumane, S. A. Photochemical Synthesis of Gold and Silver Nanoparticles—A Review. Molecules 2021, 26, 4585.
(14) Brumlik, C. J.; Martin, C. R. Template Synthesis of Metal Microtubes. J. Am. Chem. Soc. 1991, 113, 3174−3175.
(15) Wang, H. H.; Liu, C. Y.; Wu, S. B.; Liu, N. W.; Peng, C. Y.; Chan, T. H.; Hsu, C. F.; Wang, J. K.; Wang, Y. L. Highly Raman-Enhancing Substrates Based on Silver Nanoparticle Arrays with Tunable Sub-10nm Gaps. Adv. Mater. 2006, 18, 491−495.
(16) Burrows, N. D.; Harvey, S.; Iedesis, F. A.; Murphy, C. J. Understanding the Seed-mediated Growth of Gold Nanorods Through a Fractional Factorial Design of Experiments. Langmuir 2017, 33, 1891−1907.
(17) Jenkins, J. A.; Wax, T. J.; Zhao, J. Seed-Mediated Synthesis of Gold Nanoparticles of Controlled Sizes to Demonstrate the Impact of Size on Optical Properties. J. Chem. Educ. 2017, 94, 1090−1093.
(18) Chen, H.; Shao, L.; Lia, Q.; Wang, J. Gold Nanorods and Their Plasmonic Properties. Chem. Soc. Rev. 2013, 42, 2679−2724.
(19) Aruwa, C. E.; Olatope, S. O. A. Characterization of Bacillus Species from Convenience Foods with Conventional and API Kit Method: A Comparative Analysis. J. Appl. Life Sci Int. 2015, 3, 42−48.
(20) Alsammarie, F. K.; Lin, M. Using Standing Gold Nanorod Arrays as Surface-Enhanced Raman Spectroscopy (SERS) Substrates for Detection of Carcubary Residues in Fruit Juice and Milk. J. Agric. Food Chem. 2017, 65, 666−674.
(21) Li, W.; Zamani, R.; Rivera Gil, P.; Pelaz, B.; Ibanez, M.; Cadavid, D.; Shavel, A.; Alvarez-Puebla, R. A.; Parak, W. J.; Arbiol, J.; Cabot, A. CuTe Nanocrystals: Shape and Size Control, Plasmonic Properties, and Use as SERS Probes and Photothermal Agents. J. Am. Chem. Soc. 2013, 135, 7098−7101.
(22) Elahi, N.; Kamali, M.; Baghersad, M. H. Recent Biomedical Applications of Gold Nanoparticles: A Review. Talanta 2018, 184, 537−556.
(23) Ankri, R.; Fixler, D. Gold Nanorods Based Diffusion Reflection Measurements: Current Status and Perspectives for Clinical Applications. NANO 2017, 6, 1031−1042.
(24) Fernandez-Lodeiro, A.; Djafari, J.; Fernandez-Lodeiro, J.; Duarte, M. P.; Muchagato Mauricio, E.; Capelo-Martinez, J. L.; Lodeiro, C. Synthesis of Mesoporous Silica Coated Gold Nanorods Loaded with Methylene Blue and Its Potentials in Antibacterial Applications. Nanomaterials 2021, 11, 1338.
(25) Zheng, X. S.; Jahn, I. J.; Weber, K.; Cialla-May, D.; Popp, J. Label-Free SERS in Biological and Biomedical Applications: Recent Progress, Current Challenges and Opportunities. Spectrochem. Acta. Part A 2018, 197, 56−77.
(26) Wu, H.; Luo, Y.; Hou, C.; Hsiao, D.; Wang, W.; Zhao, J.; Lei, Y. Rapid and Fingerprinted Monitoring of Pesticide Methyl Parathion on the Surface of Fruits/Leaves as Well as in Surface Water Enabled by Gold Nanorods Based Casting-and-Sensing SERS Platform. Talanta 2019, 200, 84−90.
(27) Jiang, Y.; Sun, D. W.; Pu, H.; Wei, Q. Surface Enhanced Raman Spectroscopy (SERS): A Novel Reliable Technique for Rapid
