In Situ Creation of Surface-Enhanced Raman Scattering Active Au–AuOₓ Nanostructures through Electrochemical Process for Pigment Detection

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* Supporting Information

ABSTRACT: Roughing the metallic surface via oxidation–reduction cycles (ORC) to integrate the surface plasmon resonance and surface-enhanced Raman scattering (SERS) is predominant in developing sensor systems because of the facile preparation and uniform distribution of nanostructures. Herein, we proposed a distinctive ORC process: the forward potential passed through the oxidation of Au and reached the oxygen evolution reaction, and once the potential briefly remained at the vertex, the various reverse rates were employed to control the reduction state. The created hybrid Au–AuOₓ possessed electromagnetic and chemical enhancements concurrently, wherein the rough surface provided the strong local electromagnetic fields and significant interaction between AuOₓ and molecule to improve the charge transfer. The synergistic effects significantly amplified the intensity of Raman signal with an enhancement factor of 5.5 × 10⁶ under the optimal conditions. Furthermore, the prepared SERS substrate can simultaneously identify and quantify the mixed edible pigments, Brilliant Blue FCF and Indigo Carmine, individually. This result suggested that the development of SERS sensor based on the proposed SERS-activated methodology is feasible and reliable.

INTRODUCTION

Because the outbreak of food safety problems is putting the whole people on panic and alert, increasing attention has been paid to additives in food. To obtain information on the components, additives, and pathogens in food, numerous well-developed techniques have been employed, such as liquid chromatography—tandem mass spectrometry,†‡ gas chromatography—mass spectrometry, and enzyme-linked immunosorbent assay.³⁴ Nevertheless, the complex pretreatment, time-consuming measurement, and high cost of instruments greatly limit their practical applications. Especially, the long duration of data collection results in mass accumulation of pending text samples, thus not getting the information efficiently. Although electrochemical sensors have been developed to address this disadvantage, which performs sensitive and real-time detections, selectivity is a concern because of the severe conditions to the target analytes.⁵⁶

As a consequence, Raman spectroscopy can be advantageously utilized for developing efficient sensors because Raman spectroscopy can provide a fingerprint region that is unique to identify the individual materials and the quantitative analysis of target materials.⁷ It means that both the identification and quantification of analytes can be simultaneously performed. However, it is an extreme challenge to acquire a distinguishable Raman signal when the quantity of analytes is too low. Toward this end, surface-enhanced Raman scattering (SERS) has been demonstrated as an effective technique for enhancing Raman signal intensity by orders of magnitude to identify adsorbates on the surface. The mechanism of SERS consists of two major components, namely, the electromagnetic (EM) enhancement (approximately 10⁴–10¹²), resulting from the enhancement of local EM fields at the surface of metal that can offer a surface plasma resonance from electron oscillation (e.g., Ag and Au), and chemical (CHEM) enhancement (up to 10⁶), associated with a charge transfer between metal and adsorbate at atomic-scale roughness features.⁸–¹⁰ Thus, the major contribution to
the SERS enhancement can be attributed to the EM mechanism rather than the CHEM enhancement. Moreover, the distribution of electrochemical field was non-uniform around the plasmonic nanomaterials but highly localized in the relatively small radius of curvature, resulting in hotspots at the sharp edges.\(^\dagger\) Additionally, these hotspots are located within crevices from two closely spaced plasmonic nanoparticles.\(^\ddagger\)

Recently, numerous methodologies have been investigated for improving SERS performance. For instance, Tian et al. prepared a liquid-like golden droplet through a self-assembly at the chloroform/water interface as a quantitative liquid-state analyzer.\(^\ddagger\) Xu et al. prepared end, side, and satellite assemblies from Au nanoparticles and nanorods through the modification of nanorods with DNA oligomers.\(^\ddagger\) Besides, numerous materials and patterns were also designed to improve the SERS performance, including encoded Ag pyramid,\(^\ddagger\) highly organized supercrystals of Au nanorods, deposition of Pt atoms onto Ag nanocubes, and so forth.\(^\ddagger\)\(^\ddagger\) However, the prerequisite of most current studies is a high technical threshold regarding the fabrications of SERS substrates, and consequently, the practical applications of SERS are comparatively difficult. On the other hand, directly roughing the metal surface to create hotspot sites through electrochemical oxidation—reduction cycles (ORC), which cyclically scans between the potentials of metallic oxidation and reduction, is a promising manner owing to the advantages of facile preparation, green chemistry, and uniform surface.\(^\ddagger\)\(^\ddagger\)\(^\ddagger\) So far, the roughing process focuses on controlling the size and/or the shape of metallic nanoparticles for increasing local EM fields. Only a few studies, however, have been conducted for improving CHEM enhancement via ORC.

Herein, we propose a novel electrochemical process to rough the Au surface, which is similar to the conventional ORC, in which the forward potential reaches the occurrence of oxygen evolution reaction (OER) and the reverse potential stops at the reduction potential with various scan rates. By adjusting these parameters in commercial screen-printed Au electrode (SPE-Au), we can construct a series of hybrid Au—\(\text{AuO}_x\) substrates. In addition to increasing EM enhancement contributed from the rough surface, the unique structure of hybrid Au—\(\text{AuO}_x\) reveals that this hybrid structure can considerably improve the CHEM enhancement.

**RESULTS AND DISCUSSION**

**Surface Morphologies of Prepared SPE-Au Substrates.** To rough the surface of electrode, the self-electrodepositions of SPE-Au substrates were performed by fabricating using linear sweep voltammetry with various forward rates from −0.3 to 1.3 V and maintaining at the first vertex for 3 s before reversing to −0.3 V, in which the forward scan and the maintained potential at the vertex produced the dissolved Au ions for subsequent electrodepositions (Figure 1). The roughness and the condition of Au could be optimized by tuning the reverse rate, while the dissolving process was also occurring during the reverse scan once the scan passed through the oxidation region.

Comparing with the primary SPE-Au substrate, the treated SPE-Au substrates were obviously distinguishable from the untreated one by the obvious change of color (dark brown as illustrated in Figure 1 inset). To reveal the surface change of SPE-Au substrates induced by the treatment process, the scanning electron microscopy (SEM) images indicated that the surface of primary SPE-Au substrate was constructed by stacking Au sheets (Figure 2a). Subsequently, only a small amount of the stacking structure was oxidized to generate Au ions during the forward process and was redeposited on the surface by rapid reverse with a scan rate of 200 mV/s (SPE-Au200) (Figure 2b). Especially, the sharp edge with relatively high surface energy of sheets was deformed into a blunt condition. This result was in accordance with the observed image by optical microscopy, in which the structural patterns had no significant change (Figure S1a,b). Accompanying the formation of porous nanostructures, most native stacking sheets disappeared at a reverse rate of 100 mV/s (SPE-Au100) (Figure 2c and Figure S1c). Afterward, the slower reverse with scan rates of 50 to 5 mV/s (SPE-Au50, SPE-Au25, and SPE-Au5) drove the formation of nanoparticles followed by a remarkable fusion to form microspheres (Figure 2d–f). Also, these phenomena correspond to the presented images by optical microscopy, in which the surface feature turned denser followed by an aggregation to generate bulk Au that displayed the gold area from SPE-Au5 (Figure S1d–f).

**Estimation of Surface Area and Surface Roughness.** The surface area corresponding to the result of SEM was calculated through the measurement of electrochemical surface area (ECSA) that was represented by an electrochemical double layer, in which ECSA was obtained from cyclic voltammetry (CV) curves at various scan rates within a faradaic silent region of 0.25 to 0.3 V (Figure 3a and Figure S2). The slope of SPE-Au200 sample was 0.50 mF; this value was 3.1 times higher than that of bare SPE-Au. It should be noticed that, nonetheless, the surface of primary SPE-Au substrate was initially roughed and the surface area could be further increased because of the formation of porous structure as demonstrated in Figure 3b. Subsequently, the surface area increased gently with decreasing reverse rate and exhibited the highest value at a scan rate of 50 mV/s. However, this tendency changed once applying a much slower reverse rate, because the neighboring nanoparticles would fuse into bigger particles as observed in Figure 2f and Figure S1f. The surface roughness that was suggested to be positively correlated to its surface area was evaluated by atomic force microscopy (AFM). The three-dimensional surface of SPE-Au substrate showed a flat surface with a mean roughness of 0.168 \(\mu\)m (Figure 3c, Figures S3a and S4), whereas the mean roughness also increased with decreasing reverse rates, which was consistent
Figure 2. SEM images of SPE-Au substrates corresponding to the various reverse rates: (a) SPE-Au, (b) SPE-Au200, (c) SPE-Au100, (d) SPE-Au50, (e) SPE-Au25, and (f) SPE-Au5.

Figure 3. (a) CVs in the potential range of faradaic silence with various scan rates for SPE-Au substrates. (b) Measured capacitive currents of all prepared SPE-Au at various scan rates. (c) The mean roughness of prepared SPE-Au substrates through AFM measurement.

Figure 4. (a) XRD patterns and (b) crystallite size derived from the (111) plane for SPE-Au substrates with various reverse rates.
with the result of ECSA except for the scan rates of 25 and 5 mV/s (Figure 3c, Figures S3 and S4). Besides, an obvious separation of two peaks in the histogram of SPE-Au5 substrate revealed a coexistence of both large and small particles (Figure S4f) and further confirmed the observation in SEM.

**Crystallite Size of Prepared SPE-Au Substrates.** The corresponding X-ray diffraction (XRD) patterns of the SPE-Au substrates with/without treatment are depicted in Figure 4. The diffraction peaks at 38.5, 44.7, and 66.7° can be referred to the crystal planes (111), (200), and (220) of face-centered cubic crystallographic structure of gold (JCPDS Card No. 65-2870), respectively. The strong diffraction at 38.5° suggested a preferred growth orientation along the (111) direction, whereas the diffraction peaks ascribed to the bare ceramic substrate were present in all obtained results. The peak intensity of Au evidently declined with decreasing reverse rate and reached the lowest intensity at a scan rate of 25 mV/s, indicating that the crystallinity was destroyed during the electrochemical process. Note that the intensity of peaks reverted to become intense in Au5 sample because of the formation of bulk Au as illustrated in Figure S1f. Furthermore, the correlation between the crystallite size and the scan rate of the reverse process was investigated according to the Scherrer equation; the calculated results verified that the variations of crystallite size were within 1 nm and further suggested no strong correlation in between (Figure 4b).

**SERS Performance of Probe Molecule Adsorbed on Prepared SPE-Au Substrates.** The corresponding SERS effects of the prepared samples were verified by a model probe of Rhodamine 6G (R6G, 10^{-6} M), wherein the significantly different surface morphologies would lead to their specific performance. As shown in Figure 5a, the peaks at 609, 771, 1180, 1308/1569, and 1359/1506/1647 cm^{-1} were attributed to the C–C–C ring in-plane vibrational mode, C–H out-of-plane bending mode, C–H in-plane bending mode, N–H in-plane bending modes, and C–C stretching modes, respectively. All peak intensities of 1359 cm^{-1} were 1496, 2065, 58668, 28283, 33907, and 18821 cps based on SPE-Au, SPE-Au25, SPE-Au100, SPE-Au50, SPE-Au25, and SPE-Au5 samples, respectively. Mindfully, the Raman signal of R6G was undetectable on the bare ceramic substrate until the concentration was raised to 0.1 M; even so, the intensity was still rather weak (Figure S5). Contrarily, a faint Raman signal was observed from SPE-Au substrate adsorbing 10^{-6} M R6G, meaning the edge of stacking Au sheets on SPE-Au substrate showed a slightly SERS activity. Notably, this feature could be further enhanced by the electrochemically treated process developed in the present study. The intensity of Raman signal increased with a decrease of reverse rate, which could be ascribed to the enhancement of surface area as well as roughness except for SPE-Au25 and SPE-Au5 samples. It could be expected that the SPE-Au5 would exhibit a lower performance of SERS than SPE-Au50 and SPE-Au25 substrates because of the presence of both big Au particles and bulk Au. The enhancement factor (EF) of all substrates based on the peak intensity at 1359 cm^{-1} is shown in Figure 5b; the highest EF of 5.5 × 10^6 from SPE-Au25 substrate was higher than 3.2 × 10^6 from Au@AuAg yolk–shell nanostructures, 5.6 × 10^6 from gold nanotriangles, 1.0 × 10^6 from Au/ZnO nanohybrid films, and 0.47 × 10^5 from Ag@Au nanodisks. Moreover, the SERS mapping was performed with a Raman peak of 1359 cm^{-1} in a region of 20 × 20 μm^2 to evaluate the distribution of the nanostructure on the substrate. The result showed a relatively uniform Raman feature over the entire region, indicating the generated SERS-activated
structures were well-distributed regarding the nanostructure (Figure 5c–e).

It was well known that the SERS efficiency was mainly governed by the hotspots, which could exhibit a strongly intense EM field, and the hotspot region with a strong local field enhancement generated by the surface plasmon resonances commonly occurred within interstitial crevices of metal nanostructures. In other words, a large surface area or a highly rough surface could generate more interstitial crevices and further perform high SERS efficiency. Nevertheless, by comparing the intensity of Raman signal with both ECSA and mean roughness, the SPE-Au25 substrate could perform the strongest intensity with the absence of the largest surface area as well as the highest mean roughness, indicating that additional effects should be considered as well. Additionally, the performance of SERS was related to the crystallite size, in which a larger crystallite size could lead to a higher scattering, thereby enhancing the SERS efficiency.23,24 Note that the variations of crystallite sizes in all prepared SERS substrates were less than 1 nm, suggesting that the remarkable enhancements based on SPE-Au50, SPE-Au25, and SPE-Au5 substrates were not ascribed to the effect of crystallite size only. All of the abovementioned results reveal the fact that SERS enhancement in the present study might be attributed to some unexpected effects, which was generated during the reverse process in addition to the contribution of interstitial crevice from particle aggregation. To reveal this unexpected substance that was generated during the whole electrochemical process, its chemical information was investigated by Raman spectroscopy due to its rapid and sensitive nature in comparison with other measuring techniques (Figure 6).25–31 In the condition that reverse rate was higher than forward rate, it could be clarified that the Au halide, which was produced from the dissolved Au in a KCl electrolyte, was unable to be completely reduced. This incomplete Au halide deposition/reduction could be revealed by observing a characteristic peak at 310 cm$^{-1}$, which was assigned to the stretching vibration of Au–Cl ($\nu_{\text{Au-Cl}}$) on the surface of both SPE-Au200 and SPE-Au100 samples.32 Although the presence of anion induced the reorientation of adsorbed R6G and increased the junction site,23,33 the performance of SERS was still much poor than those of other SERS-active SPE-Au substrates. Most interestingly, the structure of Au–Cl further disappeared with the generation of two peaks at 508 and 822 cm$^{-1}$, which suggested the formation of new phase from the reduction of Au halide. Because a previous study affirmed that the $\nu_{\text{Au–OH}}$ lies at 635 to 677 cm$^{-1}$, these two peaks can be defined as the stretching vibrations of AuO$_x$ (Au–O) and hydroperoxy species (Au–OOH), respectively.34,35 This phenomenon is expected because the formation of hydroperoxy species (M–OOH) that had been recognized as a key intermediate commonly occurred in both OER and oxygen reduction reaction (ORR).34,35 In contrast to the conventional ORR, in which the oxidation process was carried out until the oxidation potential of Au, the forward process passed through the oxidation potential of Au as well as the potential of OER in this study. Notably, for OER, the formation of Au–O and Au–OOH would not occur at the forward scan with a rapid scan rate of 100 mV/s for oxidizing Au and a short duration of 3 s at the vertex. Consequently, the formation of Au–O and Au–OOH was dominated in the reverse scan. The reverse process involved the OER, ORR, and Au reduction. The rapid reverse process with a scan rate above 100 mV/s was unable to form AuO$_x$ and Au–OOH, whereas the case of below 50 mV/s could offer sufficient time to complete the formation in both OER and ORR. Nevertheless, the reduction of Au–OOH also occurred at the slowest scan rate of 5 mV/s, resulting in the disappearance of Au–OOH feature in the Raman spectrum. By comparing the correlation between the intensity of R6G signal and the feature of SERS substrate in the Raman spectra, the relatively lower intensity in the presence of Au–Cl might be attributed to a lower roughness surface. Furthermore, the intensity failed to vary significantly with and without Au–OOH once the scan rate was conducted between 50 and 5 mV/s; this observation could reveal that the enhancement species in the Raman signal was Au–O. It has been demonstrated that the mechanism of SERS consists of two abovementioned components (i.e., EM and CHEM enhancements); the EM enhancement was attributed to the enhancement of local EM field on the metal surface, which possessed the surface plasma resonance, and the CHEM enhancement was referred to the specific interaction between metal surface and molecule involved in the variation of charge transfer. On the basis of the results of Raman spectra (Figure 6), it could evidently indicate that the EF of Raman spectroscopy contributed to the synergistic effect of both EM and CHEM enhancements. The CHEM enhancement was attributed to the formation of interaction between AuO$_x$ and molecule, resulting in a charge transfer from Au to molecule.37 Thus, the SPE-Au25 exhibited the superior SERS performance of EF compared to the literature.19–22 The SPE-Au25 sample possessed a higher performance of SERS than SPE-Au50 because of a stronger peak intensity of AuO$_x$, which corresponds to the amount of produced AuO$_x$ on the surface. Additionally, the lowest SERS performance of SPE-Au5 sample among SPE-Au50, SPE-Au25, and SPE-Au5 substrates could be caused by the formation of bulk Au. Furthermore, to assess the reproducibility of SPE-Au25 substrate which exhibited the SERS performance through the contributions of the conformation and the component of nanostructures. Meanwhile, the commercial SPE-Au substrate and the fixed electrochemical parameters provided the stable process for reproducibly constructing geometric surfaces (Figure S6). Also, Raman spectra indicated that the SERS-active AuO$_x$ species could be created in three different batches (Figure S7). By examining $10^{-6}$ M R6G through the three SPE-Au25 substrates, the peak intensities at 1359 cm$^{-1}$ were 35794, 33126, and 34970 with an RSD of 4.6%, demonstrating that the SPE-Au substrate with the SERS-active Au–AuO$_x$ was reproducible (Figure S8).
Application of SPE-Au25 Substrate in the Detection of Pigments. To achieve brightly colored food that can reflect its delicacy and commonly cause appetite, food additives are used to improve the appearance of articles, such as beverages and processed food products. However, these additives, which include some pigments, are harmful to health even if they are edible. For instance, Brilliant Blue FCF (BBF) is recognized as a nontoxic blue additive, which is widely used in soft drinks and ice cream. BBF has been revealed to inhibit intimal hyperplasia during vein graft preparation and suppress amyloid fibrillogenesis of lysozyme. Recent studies, nevertheless, also demonstrated that BBF was genotoxic and cytotoxic. With the aim of proving the concept of this study, Figure 7a displays the SERS spectra onto the SPE-Au25 substrate with a concentration of BBF from $10^{-8}$ to $10^{-3}$ M. The main peaks at 910, 1177, 1224, and 1621 cm$^{-1}$ were defined as in-plane C$\equiv$H swing on the benzene ring, C$\equiv$C/C$\equiv$H nonsurface swing, C$\equiv$N asymmetric telescope, and C$\equiv$N nonplane swing, respectively. This SERS sensor in the detection of BBF manifested a linear range of $10^{-8}$ to $10^{-3}$ M with an RSD of 3.6$\text{--}$8.8% based on the calculated intensity at 1621 cm$^{-1}$ (Figure 7b). Basically, the lowest detection concentration ($10^{-8}$ M) of the obtained calibration curve toward BBF was sufficient for quantifying the BBF level in practical applications because BBF would lose its coloring function, becoming nearly colorless, once the concentration was lower than $10^{-6}$ M. Also, the repeatability was performed by measuring BBF after removing and reinjecting the BBF solution of $10^{-6}$ M into a cell; meanwhile, the position of measured spot was fixed. The obtained mean intensity at 1621 cm$^{-1}$ was 3914.0 with an RSD value of 3.9%, meaning the SPE-Au25 substrate was repeatable (Figure S9). The stability of SPE-Au25 substrate in ambient conditions was evaluated by measuring a BBF solution of $10^{-6}$ M (Figure S10). It could be observed that the fluctuation of peak intensity at 1621 cm$^{-1}$ remained steady obviously with an RSD of 4.7% on the 10th day. Furthermore, the characteristic peaks of produced SERS-active Au$\cdots$AuO$_x$ was still present in the Raman spectrum (Figure S11), demonstrating that the active site was not destroyed. These two phenomena revealed the SPE-Au25 substrate was highly stable. Additionally, another blue pigment, Indigo Carmine (IC), was also examined by SPE-Au25 substrate, and the result is shown in Figure 7c. Similarly, some characteristic peaks in Raman spectra were revealed to be in-plane C$\equiv$H/C$\equiv$O bending at 1244 cm$^{-1}$, $\nu_{C\equiv N}$ at 1296 cm$^{-1}$, in-plane C$\equiv$C/H$\equiv$N$\equiv$H bending at 1352 cm$^{-1}$, asymmetrical $\nu_{C\equiv N C}$ at 1582 cm$^{-1}$, and $\nu_{C\equiv O}$ of pyrrolidone ring at 1631 and 1703 cm$^{-1}$. The peak intensity at 1582 cm$^{-1}$ could exhibit a linear correlation with the concentration of IC, with a range of $10^{-7}$ to $10^{-3}$ M and RSD values of 5.6$\text{--}$8.9% (Figure 7d). Notably, once the BBF and IC were mixed together, their corresponding features were distinguishable through the SERS spectrum (Figure 8). This result is extremely important in the development of a reliable and accurate sensor because an analyte can be identified even if it exists in real samples. The individual concentrations of BBF and IC in the mixture were calculated by applying their corresponding linear equations regarding the intensities at 1621 cm$^{-1}$ for BBF and at 1582 cm$^{-1}$ for IC, in which a variation of 6.7% that was caused by the band overlapping was revealed. As a consequence, the above results indicate that the

Figure 7. SERS spectra under various concentrations of (a) BBF and (c) IC onto the SPE-Au25 substrates. The quantitative relationship of (b) BBF and (d) IC and their respective values of RSD.
system developed in this study was feasible to simultaneously characterize the food additive and quantify its amount.

**CONCLUSIONS**

In summary, the hybrid Au−AuOx produced on the SPE-Au substrata via a novel ORC process significantly improved SERS performance with the presence of the synergistic effects of EM and CHEM enhancements. Both the preparation of SERS substrate and the detection of pigment were carried out in the electrochemistry−Raman spectroscopy combined system. It is worth noting that this procedure exhibited the advantages of low technical threshold, simple preparation, environmentally friendly process, low cost, and reliable detection, indicating that it has high potential for the mass manufacture of SERS substrates. Furthermore, the SERS enhancement through the metal−metallic oxide interaction might provide a new strategy for catalyst design in electrocatalytic reaction.

**EXPERIMENTAL SECTION**

**Chemicals and Materials.** Potassium chloride (KCl), R6G, BBF, and IC were purchased from Sigma-Aldrich. Ethanol was obtained from Tedia. SPE-Au with a series number of 220AT was purchased from DropSens, where the working (4 mm diameter), counter-, and reference electrodes are made of Au, Au, and silver, respectively. Deionized (DI) water was used in all experiments.

**Preparation of Rough SPE-Au Electrodes.** The surface of primitive SPE-Au was roughed in 0.1 M KCl via electrochemical process by a BioLogic VSP. KCl aqueous solution (500 μL) was dropped on a SPE-Au covering the three electrodes. The forward potential was from −0.3 to 1.3 V with a scan rate of 100 mV/s. The potential was maintained at the first vertex for 3 s and then reversed to −0.3 V with scan rates of 200, 100, 50, 25, and 5 mV/s, which were named SPE-Au200, SPE-Au100, SPE-Au50, SPE-Au25, and SPE-Au5, respectively. Furthermore, the forward was immediately followed by reverse without maintaining the potential at the second vertex. After repeating for 10 cycles, the roughed SPE-Au was washed with DI water and ethanol for the following test.

**Raman Spectrum Measurement.** Raman spectra were recorded by UniNano UNIDRON—a Raman microscopy system, employing a diode laser at 532 nm. A 50× objective lens was used to focus the laser on the sample, in which the size of laser spot is 1 μm. The SERS measurement was performed under an exposure time of 3 s and an accumulation number of 5 times by illuminating 15 mW laser power. The Raman mapping was carried out with a computer-controlled three-axis encoded (XYZ) motorized stage with a minimum step of 0.1 μm. The area of measurement was 21 × 21 μm with an exposure time of 1 s and an accumulation number of 1 time.

**Equipment and Measurements.** SEM for examining the morphology of prepared SERS substrate was performed by using a JEM-6700F (JEOL) scanning electron microscope. The measurement of surface roughness was performed by a MultiView 2000 system (NANONICS IMAGING Ltd.), and XRD patterns were obtained by a Bruker D2 phaser.

**ASSOCIATED CONTENT**

3 Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.8b02677.

Images of optical microscopy of SPE-Au substrates, CVs in the potential range of faradaic silence with various scan rates, AFM images of SPE-Au corresponding to different reverse rates, distribution of particle size, Raman spectra of ceramic substrate, and examinations of repeatability, stability, and reproducibility of SPE-Au25 substrate (PDF)

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**Notes**
The authors declare no competing financial interest.

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