Fabrication of 3D nano-hemispherical cavity array plasmonic substrate for SERS applications

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ABSTRACT
Surface-enhanced Raman scattering (SERS) have numerous applications in areas such as analytical chemistry, biochemistry, and environmental science. However to manufacture SERS active substrates with good reproducibility and low cost is not easy, which hinder the SERS technology from being widespread in various applications. In this study, we developed a batch producible hot embossing 3D nanostructured SERS substrate technology for SERS applications. This study utilized the anodic aluminum oxide (AAO) self-assembled uniform nano-hemispherical array barrier layer as a template to create a durable nanostructured nickel mold. With the hot embossing technique and the durable nanostructured nickel mold, we were able to batch produce the 3D Nanostructured SERS chip with consistent quality and low cost. In addition, according to the SERS experiments, the 3D nano-hemispherical cavity array combined with sub-10-nm-gaps Au NPs showed distinct SERS signals in both Rhodamine 6G and Chlorpyrifos measurements. Therefore, the developed method is good to be used extensively in rapid chemical and biomolecular detection applications.

KEYWORDS
Surface-enhanced Raman scattering; SERS; hot embossing; batch production; label-free detection

1. Introduction
Surface-enhanced Raman scattering (SERS) is a promising technique for molecule recognition in analytical chemistry, biochemistry, and environmental science. Its high sensitivity and unique molecular “fingerprint” spectra makes it a powerful tool for rapid chemical and biomolecular detection applications. However, a major drawback of the SERS detection technique is that the measured spectra intensity varies from substrate to substrate, or even worse from points to points over the same sample substrate. It is mainly due to the failure of current fabrication methods to provide a good control of the nanostructure geometry and the nanoparticle distribution on SERS substrates. Therefore, the hot spots (Hot spots are of intense local field enhancement caused by local surface plasmon resonances [1]) are not uniformly distributed across the SERS substrate, and the reproducibility of the SERS substrates are not good. As a result, the SERS detection is not good for quantitative measurements. To overcome this drawback of the SERS detection, many studies used the sophisticated microfabrication process, in order to fabricate a substrate with uniform nanostructure and high density hot spots. For example, Schmidt [2] and his colleagues fabricated silver-coated silicon nanopillars array as a Raman active substrate by using reactive ion...
etching process to fabricate silicon nanopillars, and electron beam evaporation deposition to coat silver on the silicon nanopillars. Zhang\cite{3} and his colleague made nanoarrays of silver nanoparticles decorated silicon nanowires through wet etching and galvanic redox reaction processes. Kandjani\cite{4} and his colleagues synthesized ZnO/Ag nanorod arrays using hydrothermal technique and electroless plating method. Zhao\cite{5} et al. fabricated Au–graphene–Ag sandwiched hybrid structures through plasma sputtering Ag nanoparticles, wet-transferring CVD grown monolayer graphene film onto Ag nanoparticles, and another sputtering for Au film deposition. However, the relatively complicated microfabrication processes limit the capability of mass production, and leads to these SERS active substrates becoming economically unthroughible to be widely used.

In this work, we developed a batch producible hot embossing 3D nanostructured SERS chip with low cost and good reproducibility.\cite{6} We utilized the AAO self-assembled uniform nano-hemispherical array barrier layer as a template for electroforming a durable nanostructured nickel mold. With the hot embossing technique and the durable nanostructured nickel mold, we were able to mass-produce the 3D nanostructured polycarbonate (PC) substrates with consistent quality. Gold nanodots array were formed on the substrate with evenly spaced nano-gaps by the

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\caption{The fabrication process of the nano-hemispherical array structure. (a) The aluminum substrate was washed with water and dry with nitrogen. (b) A uniform nanotube array was obtained after anodic oxidation treatment. (c) After removing the anodic aluminum oxide layer, nano-hemispherical cavity array was created.}
\end{figure}
spontaneous separation and coalescence of the gold ultrathin film when deposited onto the 3D nanostructured PC substrate. The nano-gaps between gold nanodots provide a high spatial density of hot spots. Experiments were conducted to study the surface morphology and the performance of the fabricated SERS substrate. Rhodamine 6G (R6G) and pesticide Chlorpyrifos were used as probe molecules to evaluate the performance of the SERS substrates. The proposed 3D nanocomposite structured SERS active substrates were found to have high SERS enhancement factor (EF) and were able to detect a low concentration of 1 ppm Chlorpyrifos methanol solution.

2. Methodology

The proposed SERS active substrates fabrication process including: (a) Utilizing the anodic aluminum oxide’s (AAO) self-assembly property to create a uniform nano-hemispherical array barrier layer. (b) The nano-hemispherical array’s AAO barrier layer was used as a template for the electroforming of a durable nanostructured nickel mold. After electroforming, the AAO template was etched away and a nickel mold with nano-hemispherical array nanostructure was obtained. The 3D nano-hemispherical cavity mold was then imprinted on the PC substrate with nano-hot embossing technique. Finally, the gold nanoparticles were deposited uniformly on the 3D nanostructured substrate by controlled sputtering and utilizing the spontaneous separation and coalescence of gold ultrathin film. The details of each step are described in following sections:

2.1. Nano-hemispherical cavity arrays fabrication

A number of nano-hemispherical cavity arrays with different sizes were fabricated by self-assembly of anodic aluminum oxide.[7] The fabrication process of the nano-hemispherical cavity array of the aluminum substrate included the following steps: electrolytic polishing, anodic treatment, removal of anodic aluminum oxide. Detailed steps are described as follows:

1. First, a 99.999% purity aluminum substrate was cleansed using acetone then rinsed with deionized water and dry in air. The cleansed aluminum substrate was then placed in a polishing solution mixed with perchloric acid and anhydrous alcohol in a volume ratio of 1:3.5 and applied with a voltage of 25 V for 2 min. After polish, the aluminum substrate was washed with water and dried with nitrogen, as shown in Figure 1(a).

2. The polished aluminum substrate was put into 0.3M oxalic acid solution in a cooling water tank at 0 °C and applied 50 V power was applied for 2 h for anodic oxidation treatment. After anodic oxidation treatment, a uniform nanotube array with a pore size of about 100 nm and a tube length of about 50 µm was obtained as shown in Figure 1(b).
3. The nanotube array was placed in a 5 wt% phosphoric acid solution at a temperature of 35°C to remove the anodic aluminum oxide layer, for about 1 h. After removing the anodic aluminum oxide layer, an aluminum template with a nano-hemispherical cavity array was created. The diameter of each nano-hemispherical cavity was about 100 nm as shown in Figure 1(c).

4. Higher anodic oxidation voltage resulted in greater diameter of the cavity, so adjusting the voltage at the anodic oxidation process, and repeating steps 1 to 3, one can get different sizes of three-dimensional nano-hemispherical cavity array structure.

2.2. Electroforming nanostructured nickel mold

The Schematics of the electroforming process of the nickel mold with nano-hemispherical array structure is shown in Figure 2(a). To electroform the nanostructured nickel mold, nickel sulfamate was used as the electroforming solution, the nickel palladium material was used as the anode, and the nanostructured aluminum substrate was used as the working electrode. The electroforming time depends on the desired nickel mold thickness. After the electroforming was finished, pure aluminum was etched away by the CuCl₂ and HCl mixture solution. The nickel mold with nano-hemispherical array structure was obtained as shown in Figure 2(b). Through this electroforming process, we were able to produce a large nickel mold with the size of 120 x 80 mm. Considering that an SERS chip requires only 3 x 3 mm nanostructured substrate, with this large surface nickel mold, we are able to batch produce one thousand pieces of the nanostructured substrate in one hot embossing process. Typically, a nickel mold can perform at least 5000 x of hot embossing processes before it wears out. Therefore, a nickel mold can be used to produce at least 5,000,000 pieces of SERS substrates.

2.3. Hot embossing of 3D nanostructure

The formed 3D nano-hemispherical cavity nickel mold was used in a replica molding of a polycarbonate (PC) nanosubstrate through hot embossing. The nano-hemispherical cavity structure was imprinted on a polycarbonate (PC) plastic substrate using a hot-press molding machine for heating PC to above its glass transition temperature (150°C) and pressing the 3D nano-nickel mold on PC surface for 10 min. After cooling down to room temperature, the PC substrate (as shown in Figure 3) was released from the master mold. Using this method, we were able to batch produce a large number of low-cost 3D nano-structured PC substrate with the same quality. The large surface area of the nano-hemispherical cavity array structure can significantly increase the number of attached analytes and hence, can enhance the performance of a SERS substrate, which
makes the 3D nano-structured PC substrate well suitable for chemical and biomedical sensing applications.

Instead of directly using Anodic alumina as an SERS substrate, in this paper we used hot embossed nanostructured polycarbonate plate as our SERS substrate. The good thing of the hot embossed nanostructured polycarbonate substrate is that it can be produced quickly (30 min for one replication, which can be faster if roll-to-roll embossing technology is used) and the quality is stable. With our large surface nickel mold, we were able to batch produce 1000 pieces of the nanostructured substrate in a single hot embossing process. Therefore, it can further reduce the cost per SERS substrate.

2.4. Deposition of gold nanoparticles array

The gold nanoparticles were formed uniformly on the 3D nanostructured substrate by carefully controlled sputtering and utilizing the spontaneous separation and coalescence of the gold ultra-thin film (thickness ~100 Å).[9] A sputter (Cressington sputter coater 108) was used for all gold nanoparticles sputtering processes. The sputtering current was set to 10 mA, Argon pressure was 0.5 bar, and sputtering duration was 40 s.

2.5. Experiments

Experiments were conducted to study the surface morphology of the fabricated SERS substrate, which included scanning electron microscope (SEM, JEOL JSM-7800F) and atomic force microscope (AFM, Bruker Dimension Icon). For the Raman spectra measurements, 5 µL of various concentrations of R6G aqueous solutions and Chlorpyrifos methanol solutions were dropped onto the fabricated SERS substrates separately, and dried at room temperature. SERS spectra were measured by a microscope Raman spectrometer (Tokyo Instruments, Nanofinder 30) with a 632.8 nm HeNe laser excitation, where the effective power of the laser source was set to 0.1 mW. The focused laser spot on the sample surface was about 2 μm in diameter. The integration time
was 10 s for each measurement. Since we used a thin coating of gold nanoparticles to enhance the analyte SERS signal, the substrate background signal may have been enhanced (SERS and luminescence). To solve this issue, the SERS signals were measured before and after the analyte was placed on the substrate in all of our experiments. The actual analyte signal was obtained by subtracting the substrate background signal from the measured SERS signal. SERS substrate performance was then characterized by calculating the SERS enhancement factor.

3. Results and discussion

3.1. Nanostructured substrate replication results

The AFM image in Figure 4 indicates that the diameter of these nano-hemispherical cavities in replicated PC is about 100 nm, and the depth of each cavity is about 35 nm. The diameter of each nano-hemispherical cavity in the replicated PC substrate is about the same as it is of the nickel mold. However, the depth of the nano-hemispherical cavity in the replicated PC substrate is slightly lower than that of the nickel mold. The main reason is that during hot-embossing the semi-molten PC substrate may not completely fill the nanostructure of the nickel mold due to its relatively high viscosity. The slightly lowered depth of the nano-hemispherical cavity should not largely affect the consistency of the PC substrate duplication. Figure 5 shows the SEM images to further demonstrate the morphology of the hot embossed PC substrate with nano-hemispherical cavity array structure on the PC surface. From the SEM image, we can see that the nano-hemispherical cavities were successfully transferred to the PC substrate.

3.2. Gold nanoparticles deposition results

Under the same pressure and argon injection rate, the morphology of the deposited gold nanoparticles was determined by sputtering parameters including the applied current and the sputtering duration. Figures 6(a–c) are SEM images of the gold nanoparticles deposited with an applied current 10 mA and duration of 20, 40, and 60 s, respectively. The sputtering result shown in Figure 6(b) reveals that an applied current 10 mA and sputtering duration of 40 s were able to form the evenly distributed gold nanoparticles on the 3D nanostructured PC substrate. We can see that a very good uniformity of the gold nanoparticles was obtained under this condition. The size of these gold nanoparticles was about 10 nm, and the gap between gold nanoparticles was less than 10 nm, which can form numerously hot spots in between gold nanoparticles.
Figure 6. SEM images of the gold nanoparticles deposited on 3D nano-hemispherical cavity array with an applied current 10 mA and duration of (a) 20 s, (b) 40 s, and (c) 60 s, respectively. Picture (b) shows the uniform distributed gold nanoparticles on the nano-hemispherical cavity array structure. Picture (d) is the detailed view of the red squared area in the picture (b).

Figure 7. The electric field distribution around the gold nanoparticles on a concaved PC surface.

Figure 6(a,c) present SEM of 20 s and 60 s sputtering duration, respectively. From Figure 6(a), it can be observed that there are fewer gold nanoparticles formed on PC substrate, and uniformity is relatively low compared to Figure 6(b). Figure 6(c) shows that a longer sputtering duration will result in the gold nanoparticles contacting each other and eventually forming a gold film layer on the PC substrate. Therefore, the sputtering conditions were set to apply current of 10 mA and duration of 40 s for the fabrication of gold nanoparticles decorated 3D nano-hemispherical cavity SERS substrate.
3.3. Electric field simulation

A simulation was conducted to further understand the effect of the gold nanoparticles decorated 3D nanostructured substrate. We calculated the local electric field of the Au nanoparticles decorated nano-hemispherical cavity structure using the commercial finite element method (FEM) software (COMSOL Multiphysics). We set the diameter of Au nanoparticles as 10 nm and pitch between nanoparticles as 12 nm. Figure 7 shows the x–z views of the electric field distribution on

![Figure 7](image1.png)

**Figure 7**. SERS substrate background signal and the 1E-6M R6G signal, before and after subtracting the substrate background signal.

![Figure 8](image2.png)

**Figure 8**. SERS substrate background signal and the 1E-6M R6G signal, before and after subtracting the substrate background signal.

![Figure 9](image3.png)

**Figure 9**. SERS spectra of $1 \times 10^{-6}$ M R6G aqueous solution dried on SERS substrates fabricated with different sputtering durations (20 s, 40 s, and 60 s, respectively). The red line indicated SERS spectrum of the R6G with 40 s sputtering duration substrate, which shows the most prominent R6G characteristic peaks.

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the nano-hemispherical cavity with an incident light wavelength of 633 nm. It clearly shows that strong electric field formed around the gold nanoparticles, especially in between nanoparticles. The concaved surface helped gold nanoparticles to form closely packed, strong hot spots.

### 3.4. SERS measurement results

The SERS substrate background signal was measured prior to placing the analyte on the substrate and compared to the measured SERS signal after placing the analyte on the substrate. Figure 8 shows the SERS substrate background signal and the 1E-6M R6G SERS signal before and after subtracting the substrate background signal. From the figure, we can see that the measured substrate background signal is relatively small compared to the actual signals.

SERS measurements of substrates fabricated with different sputtering durations were conducted for comparison. Substrates with three different sputtering durations (20 s, 40 s, and 60 s) were tested with the same concentration of R6G solutions. 5 μL of 1 × 10⁻⁶ M R6G solutions were dropped onto these SERS substrates separately and were dried at room temperature. Finally, SERS spectra of each SERS substrate were measured. Figure 9 shows the resulting SERS spectra of the R6G solution with different sputtering durations. The spectrum of R6G with a sputtering duration of 40 s shows most prominent characteristic peaks. These results are consistent with previous SEM images in Figure 6, where the 40 s sputtering duration shows the most uniform distribution of the gold nanoparticles on the 3D nanostructured PC substrate compared to the 20 s and 60 s durations. Therefore, the rest of the experiments used a 40 s sputtering duration.

Figure 10 illustrates the measured SERS spectra for the R6G solution (with various concentration) on the SERS substrate, the 1 × 10⁻⁴ M R6G on bare PC substrate, and a bare PC substrate, respectively. The characteristic peaks of R6G at 621, 1199, 1283, 1360, 1510 and 1651 cm⁻¹ are clearly observed from the spectrum. The spectra show well-resolved vibrational patterns of R6G when it is deposited on the SERS substrate. While it is deposited on a bare PC substrate, it shows almost no R6G characteristic peaks but only the PC substrate signal. These spectra demonstrating
the good enhancement of the developed SERS substrate. The integration time was 10 s for each measurement. The SERS substrate enhancement factors (EF) were calculated using the commonly used definition of EF\textsuperscript{[10]} as Equation (1):

$$EF = \frac{I_{SERS}}{I_{RS}} \frac{N_{SERS}}{N_{RS}},$$

where $I_{SERS}$ and $I_{RS}$ are the peak intensity of SERS spectra and normal Raman spectra, respectively. $N_{SERS}$ and $N_{RS}$ represent the numbers of molecules on the substrate within the laser spot,
respectively. The intensity of the most prominent 1510 cm\(^{-1}\) peak of R6G molecules from the SERS and Raman measurements was used for EF calculation. The EF was calculated to be about \(1 \times 10^8\), which is very good for a gold-based SERS substrate.\(^{[11]}\)

In order to see if there is a difference between the master sample and subsequent replicas, an aluminum master sample, the first 10 PC replicas, and 10 replicas after 100 times of replication were sputtered with gold nanoparticles and tested with \(1 \times 10^{-6}\) M R6G solutions. The SERS spectra of \(1 \times 10^{-6}\) M R6G from the master sample and subsequent replicas are shown in Figure 11. From the figure, we can see that the SERS signal of the master sample and subsequent replicas are almost the same. The only difference between the master sample and replicas is the master sample obtained a distinct peak at 776 cm\(^{-1}\), which is not obvious in replicas. This indicates that different base materials may have slightly different effects on the resulting SERS spectrum.\(^{[12]}\)

The SERS spectra between the first 10 replicas and those after 100 times of replication did not show a significant difference, which means that the replicas are of very consistent quality.

The proposed SERS substrates were also tested with low concentration Chlorpyrifos solution. The SERS substrates were found to be able to detect a low concentration (1 ppm) Chlorpyrifos methanol solution. Figure 12 displays the measured SERS spectrum for 200 ppm, 100 ppm, 20 ppm, 1 ppm, 0.5 ppm, and 0.2 ppm Chlorpyrifos on the SERS substrate, respectively. The characteristic peaks of Chlorpyrifos at 576, 644, 669, 1098, 1286, 1333, and 1463 cm\(^{-1}\) are clearly observed from the spectrum. The Limit of detection (LOD) in this paper is defined as the signal required to produce a signal-to-noise ratio (S/N) of 3. The 1333 cm\(^{-1}\) peak of chlorpyrifos was chosen for quantification because of its relative insensitivity to molecular orientation on an Au surface and is a strong peak. The noise for our SERS substrate was determined by measuring the peak-to-peak noise in the 1333 cm\(^{-1}\) region of the chlorpyrifos spectrum for 10 different spectra, and the average noise is 106 counts. The average peak intensity of 0.2 ppm chlorpyrifos spectrum at 1333 cm\(^{-1}\) is 358 counts, for which the S/N is 3.4. Therefore, for our SERS substrate, the LOD of chlorpyrifos is slightly below 0.2 ppm. In Figure 12, most of the characteristic peaks of Chlorpyrifos still can be observed at a low concentration of 0.2 ppm, which is consistent with the calculated LOD of chlorpyrifos. According to FDA policy, different food has a different tolerance for chlorpyrifos residues. For example, the chlorpyrifos residue in cherry has a tolerance of 1 ppm and the kiwifruit has a tolerance of 2 ppm. The LOD of our SERS substrate should be enough for detecting chlorpyrifos residues of cherry, kiwi fruit, and many other foods. Figure 13 shows the Raman intensity of chlorpyrifos’ peak at 1333 cm\(^{-1}\) as a function of the chlorpyrifos concentration.
concentration (plotted in a log–log scale). The value of the coefficient of determination (R²) reaches 0.961 indicating the good linear relation between peak intensity and chlorpyrifos concentration in the 0.2 ppm to 200 ppm region.

4. Conclusion

In conclusion, we have presented an effective method for the synthesis of 3D nanostructured SERS substrates. The main advantage of the batch producible hot embossing 3D nanostructured SERS substrate is the capability of mass production, which reduces the cost of production of SERS substrates. In addition, the concaved 3D surface helped gold nanoparticles closely packed to each other and increasing the number of nanoparticles per unit area. The SEM pictures showed that sub-10 nm inter-nanoparticle-gaps were obtained by simply controlling the sputtering parameters, and therefore the high density and uniform hot spots were formed on the substrate. According to the SERS experiments, the 3D nano-hemispherical cavity array combined with sub-10-nm-gaps Au NPs showed distinct SERS signals in both R6G and Chlorpyrifos measurements, indicating their good potential for biomedical and chemical analysis applications. Our further study will focus on complex samples with multiple analytes once we complete several different single analyte tests and create a calibration database.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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