Clean and modified substrates for direct detection of living cells by surface-enhanced Raman spectroscopy

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Supporting information

1. Apparatus

Raman spectra and images were obtained using a confocal microprobe Raman system (Invia, Renishaw). A He-Ne laser (632.8 nm) was used for the Raman measurement. The laser power at the sample was ~1 mW and typical exposure time for cell measurement was 10 s unless otherwise stated. The laser beam was focused on the sample and the Raman scattering signals were collected via a 100× glass-slide-correction microscope objective (with a NA of 0.8). The laser spot was about 1 μm in diameter in a spot-focus mode, which was used for detecting the SERS behavior of the substrates. In the living cell study, a line-focus mode that can produce a spot with an area of about 5×15 μm^2. To further reduce the laser power density, the laser spot was defocused by about 4 μm away from the sample following our reported method[1].

The electrochemical desorption measurements were performed on a CHI631B electrochemical workstation (CH Instruments, Shanghai, China) in a conventional three-electrode system with a Pt sheet as the auxiliary electrode, saturated calomel electrode (SCE) as the reference electrode, and the assembled SERS substrate as the working electrode. Scanning electron microscope (SEM) images were taken with a field emission microscope (S-4800, HITACHI) operated at an accelerating voltage of 10 kV.

2. Preparation of uniform SERS-active substrates

In order to obtain uniform and clean SERS substrates, 60 nm Au nanoparticles synthesized using the hydroxylamine seed-mediated growing method[2], were assembled on a 3-aminopropyltrimethoxysilane (APTMS)-functionalized surfaces of indium tin oxide (ITO), which

has been cleaned following the reported protocol[3]. Fig. S1a shows the morphology of the assembled substrate, and the inset is the image obtained at a high magnification, from which we can find that the size and shape distribution of Au nanoparticles are fairly uniform on the ITO surface. For characterizing the uniformity of the SERS signals over the solution, a Raman mapping experiment was carried out after the surface was adsorbed with pyridine. Fig. S1b shows the Raman mapping of the substrate using the most intense band of pyridine at 1007 cm^{-1} as the mapping signal. The scan area was 25×25 μm^2, the step size was 2 μm, and the acquisition time at each point was 1 s. The signal deviation at the strongest point (Fig. S1b-p) to the weakest point (Fig. S1b-q) is about 10%, indicating the substrate can give a quite uniform SERS enhancement over the whole surface.

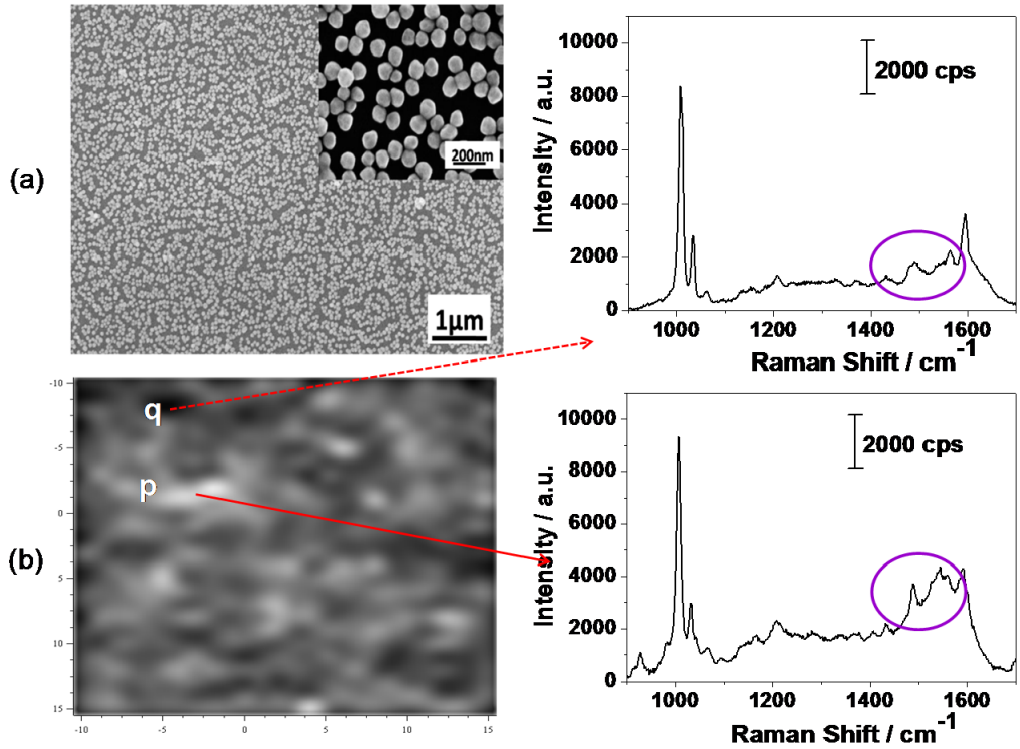
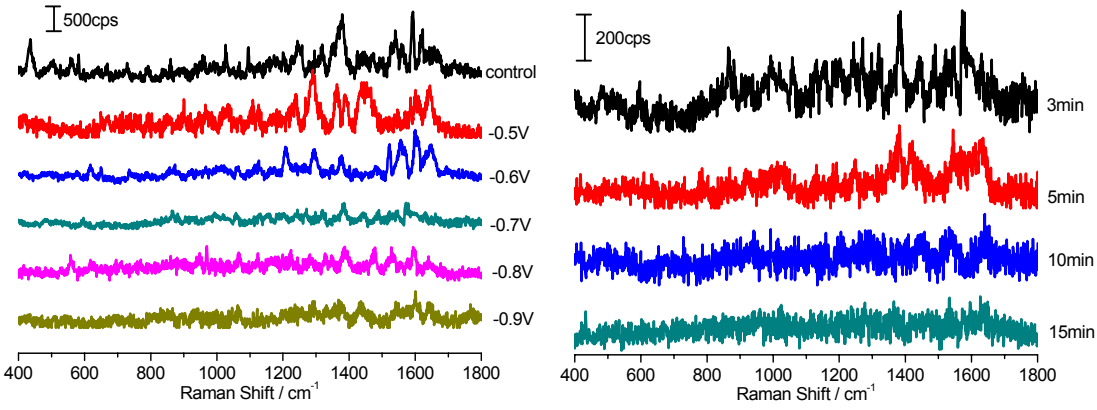


Figure S1 (a) SEM image of the assembled Au nanoparticles on the ITO and the inset is the image obtained at a higher magnification. (b) SERS mapping of the substrate using pyridine as the probe molecule: spectrum obtained at point *p* shows the strongest signal and that at point *q* shows the weakest signal. The collection time was 1 s, and the laser power at the sample was 0.6 mW. Solution was 1×10^{-2} mol·L^{-1} pyridine. The purple ring marks the bands of impurities.

3. Cleaning procedures of the negative-potential desorption

The assembled Au/ITO substrates were immersed in 0.1 mol·L^{-1} $NaClO_4$ solution and were

used as the working electrode. Then the electrode was electrochemically reduced at desired negative potentials for 3 min to remove the adsorbed impurities. To prevent re-adsorption of the impurities, the electrode was remained at a negative potential and rinsed with a fresh $NaClO_4$ solution while the Au/ITO substrate was taken out from the $NaClO_4$ solution. Finally, the substrate was washed with ultrapure water for 3 times. Fig. S2a shows the SERS spectra obtained at different positions of the Au/ITO substrate in the absence of probe molecules. It can be seen from the figure that the bands of impurities decrease obviously at potentials more negative than -0.6 V. But the impurities could not be completely removed even at -0.9 V. In order to solve this problem, we prolonged the time of potential treatment. Fig. S2b shows SERS spectra obtained at -0.7 V but at different holding time. From Fig. S2b, it is clear that almost all of the impurities have been eliminated when the time is longer than 5 min. An optimal parameter is to hold the electrode at -0.7 V for 10 min and then the electrode was pulled out of the solution while being rinsed with the electrolyte solution with a potential remained at -0.7 V.



(a) (b)

Figure S2 SERS spectra obtained from the Au/ITO substrates in the absence of probe molecules after being treated at (a) different potentials for 3 min and (b) -0.7 V potential at different immersion time in a 0.1 $mol \cdot L^{-1}$ $NaClO_4$ solution. The collection time was 10 s, and the laser power was 0.2 mW.

4. Cleaning procedures of the competitive adsorption of iodide

The assembled Au/ITO substrates were immersed in 1×10^{-3} $mol \cdot L^{-1}$ KI solution for 10 min, and then washed with ultrapure water for 3 times. Fig. S3 shows the SERS spectra obtained from different positions of the Au/ITO substrate in the absence of probe molecules. A strong band at

158 cm^{-1} is from the Au-I vibration, meaning the formation of an adsorbed iodide layer on the substrate.[3] The strong band at 127 cm^{-1} is a result of the cut-off edge of the edge filter of the Raman instrument. A relatively weak band at 1450 cm^{-1} may be polyvinylpyrrolidone (PVP) which is enhanced after formation of PVP-iodine complex.

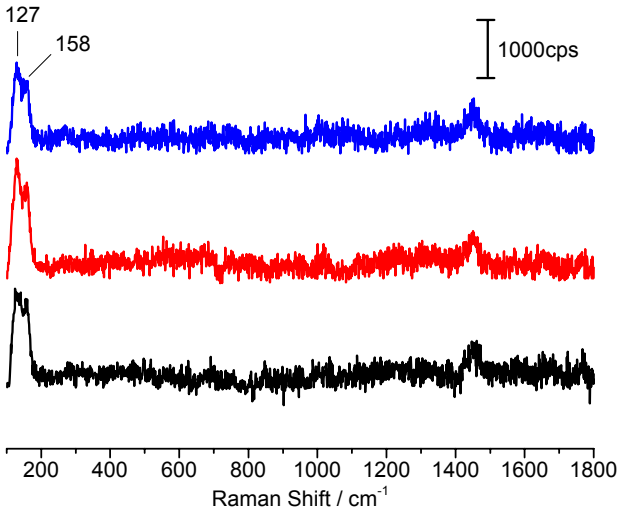


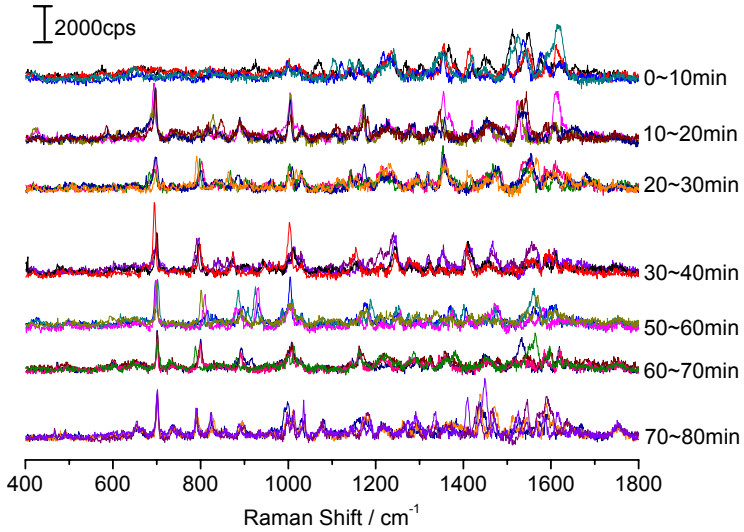
Figure S3 SERS spectra obtained from different positions of the Au/ITO substrate in the absence of probe molecules after being treated with 1×10^{-3} $mol\cdot L^{-1}$ KI for 10 min. The collection time was 10 s, and the laser power was 0.2 mW.

5. Cell culture and sample preparation

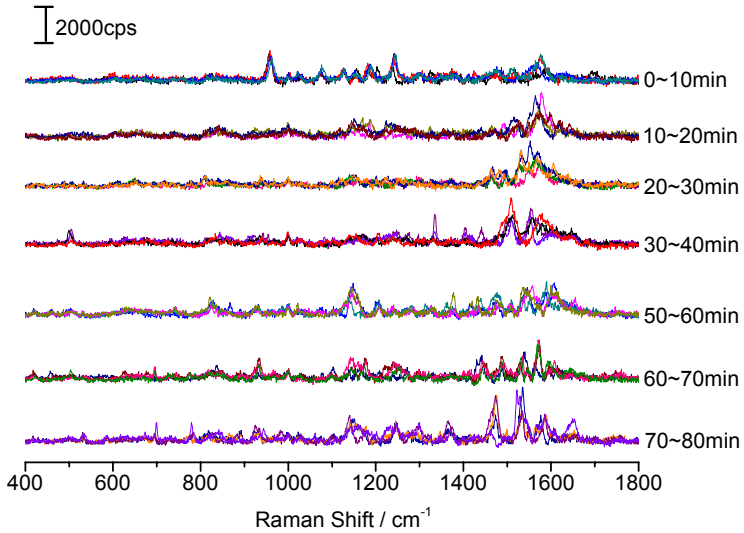
Human cervical cancer cells (CaSki cell line) were cultured in Dulbecco's modified Eagle's medium (DMEM, Hyclone Co.) supplemented with 10% fetal bovine serum (Hyclone Co.) and 1% penicillin/streptomycin (Hyclone Co.) and incubated at 37 □ and in 5% CO_2 atmosphere. After 72 hrs, the cells were collected after separated from the medium by centrifugation at 1000 rpm for 10 min and washed 3 times with the sterile PBS (pH 7.4). The sediment was re-suspended in a fresh supplemented DMEM to obtain a homogeneous suspended cell solution with a final concentration of 3×10^{6} $cells\cdot ml^{-1}$. The cells were dropped on a clean substrate and then cultured in the fresh supplemented DMEM until they were attached to the surface of the substrate well. Finally, the medium was discarded and the cells were washed 3 times with PBS and ready for the following Raman measurement.

Fig. S4 shows the time dependent SERS spectra of cell membrane obtained at a same spot of a same cell attached to the KI or negative potential treated substrates. Fig. S5 displays the SERS

spectra of cell membranes obtained at different cells on KI treated substrates.



(a)



(b)

Figure S4 SERS spectra of cell membranes obtained at same spot of a same cell from substrates cleaned (a) in 1×10^{-3} mol·L^{-1} KI and (b) at -0.7 V. The collection time was 10 s, and the laser power was 0.2 mW.

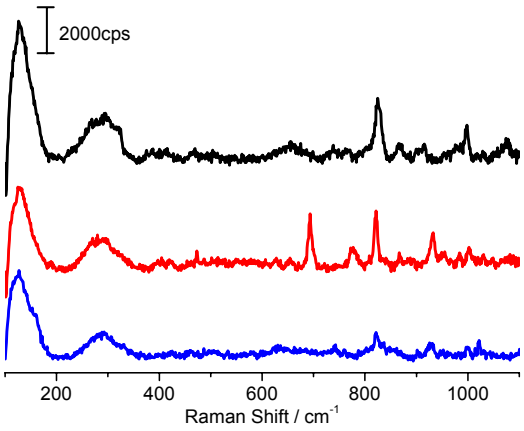


Figure S5 SERS spectra of cell membranes obtained at different cells on a KI treated substrate. The collection time was 10 s, and the laser power was 0.2 mW.

References:

1. Y. F. Huang, H. P. Zhu, G. K. Liu, D. Y. Wu, B. Ren and Z. Q. Tian, *J. Am. Chem. Soc.*, 2010, **132**, 9244-9246.
2. P.-P. Fang, J.-F. Li, Z.-L. Yang, L.-M. Li, B. Ren and Z.-Q. Tian, *J. Raman Spectrosc.*, 2008, **39**, 1679-1687.
3. M. D. Li, Y. Cui, M. X. Gao, J. Luo, B. Ren and Z. Q. Tian, *Anal. Chem.*, 2008, **80**, 5118-5125.