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Superwettable Nanodendritic Gold Substrate for Directly miRNA SERS Detection

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Keywords: SERS sensors, superwettable microchip, superhydrophilic array, nanodendritic gold, mi-RNA detection

Materials and instruments

The oligonucleotides sequences were synthesized from Sangon Biotech (Shanghai, China). The sequences were as follows:

Probe DNA: 5'-SH-(CH_2)6-AAA AAC CAG AAT TAA AAA AAT AAC ACT GTC TGG-Rox-3'

Placeholder DNA：5'-CCA TCT TTA CCA GAC AGT GTT A-3'

The target miRNA was obtained from GenePharma.

Target miRNA-141: 5'-UAA CAC UGU CUG GUA AAG AUG G-3'

Indium tin oxide (ITO) (resistivity of ca. 10-20 Ω/cm) glass was purchased from Asahi Glass (Japan). Sulfuric acid (H_2SO_4, 98%, AR), acetone (>99.5%, AR), ethanol (>99.8%, GR) and Rhodamine6G were purchased from Sigma-Aldrich. The dodecylmercaptan was obtained from J&K Chemical and hydrogen tetrachloroaurate (III) trihydrate ($HAuCl_4 \cdot 3H_2O$) was purchased from Alfa Aesar. The photomask with array was custom made from Beijing Zhongjingkeyi Technology Co., Ltd, China. All chemicals were used without any further purification and prepared by dilution using ultrapure water (Milli-Q, 18.2 MΩ•cm) otherwise specified.

All experiments were carried out at room temperature (25 °C) except for special instructions. Electrochemical deposition of gold nanostructure was carried out by a homemade device connected with a CHI-660D electrochemical workstation (CHI instruments, shanghai, China). The morphologies of dendritic gold nanostructures were observed by Scanning electron microscope (SEM, JSM-6700F, Japan). The characterization of Water contact angles (CA) on the superhydrophobic substrate was by an OCA20 system (Data-Physics, Germany). The average CA was calculated by the measuring of 10 different positions on the same microchip. The DT-03 plasma processor (Suzhou OPS Plasma Technology Co., Ltd., China) was used for O_2 plasma treatment to obtain the superhydrophilic array. The Micro Raman Spectroscopy system (InVia-Reflex, Renishaw, England) connected to a CCD detector was used to detect the R6G and miRNA-141. Before the detection, the equipment was calibrated with standard Raman signal from Si at 520 cm^{-1}.

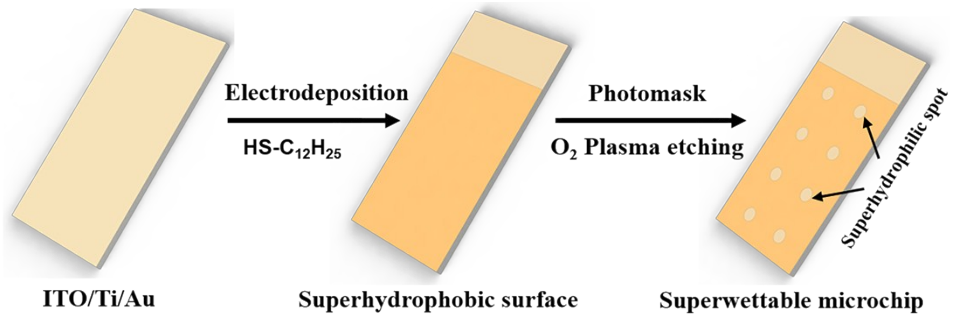
Fabrication of the superwettable SERS biosensor

The Indium tin oxide (ITO) coated glass was cut into 3×1.5 cm pieces for suiting the stipulation of photomask and convenient for electrochemical deposition. The ITO glasses were immersed in piranha solution(98%H_2SO_4:30%H_2O_2, V/V=3:1) for 1 h. Then ultrasonic in ethanol, acetone and ultrapure water for 30 min respectively to remove the organic impurities and blown desiccative with nitrogen gas. For the convenience to electrodeposition and enhancing the adhesion of nanodendritic gold, the layer of titanium and gold were sputtered on the conductive side of ITO substrate by the magnetic shielding method. The ITO substrate was then assembled in a three-electrode system and soaked half in the solution. The ITO glass was regarded as the working electrode, the reference electrode was an Ag/AgCl wire and the counter electrode was a Pt wire. The nanodendritic gold was electrodeposited at −1.8 V for 1800 s from an electrolyte composed of aqueous solution of sulfuric acid (0.5 M) and $HAuCl_4$ (1 mg/mL). After electrochemical deposition, the nanodendritic gold was washed 3 times with ultrapure water and dried with nitrogen. Then the microchip was immersed in the dodecylmercaptan solution (10 vol. % in ethanol) and sealed for 24 h. Transferred the microchip into ethanol to remove unmodified dodecylmercaptan and dried naturally in the fume hood. The superhydrophobic substrate was obtained. The photomask (2×1.5 cm) which has eight arrays of 0.5 mm diameter holes was covered on the superhydrophobic substrate carefully. Then clamp the photomask and superhydrophobic substrate by binder clips for oxygen plasma irradiation at 100 W for 120 s. The modified dodecylmercaptan on the unoccupied substrate (eight holes) was decomposed and exposed nanodendritic gold microwells were superhydrophilic. Thus we fabricated the superwettable SERS microchip.

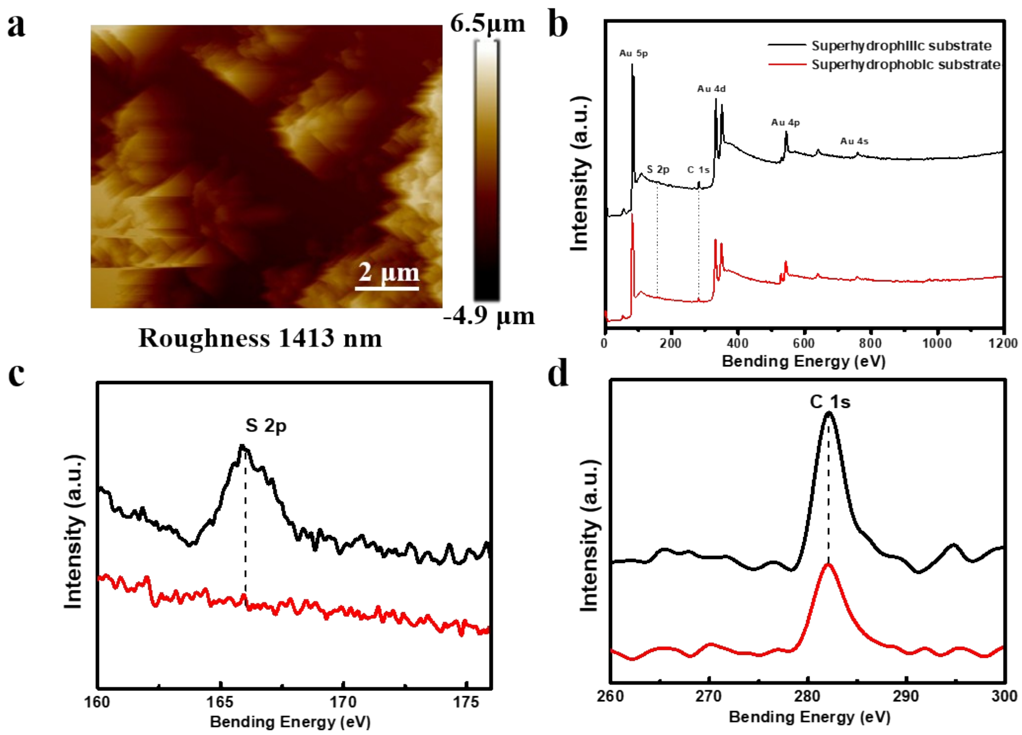
The miRNA-141 detection process

The eight superhydrophilic microwells (diameter: 1 mm) in the same superwettable microchip were dropped the 2μL PBS droplets with 10 μM probe DNA respectively. The droplets were incubated at 37 ℃ one hour for the probe DNA self-assembled on the nanodendritic gold surface adequately. Immersed the microchip in the ultrapure water for removing the unfixed DNA. Subsequently, added 2 μL placeholder DNA solution (10 μM) in microwells at saturated humidity overnight for hybridizing with probe DNA and washed thoroughly for the following miRNA-141 detection. Next, added the unified volume (2 μL) different concentration (10^{-5} M, 10^{-6} M, 10^{-7} M, 10^{-8} M, 10^{-9} M, 10^{-10} M, 10^{-11} M and 10^{-12} M) miRNA-141 solution in the microwells and incubated 2 hours at saturated humidity for the placeholder DNA displacing completely and probe DNA ringing on the nanodendritic gold surface automatically. The eight concentration miRNA-141 SERS spectrums were interrogated by the 632 nm excitation laser source.

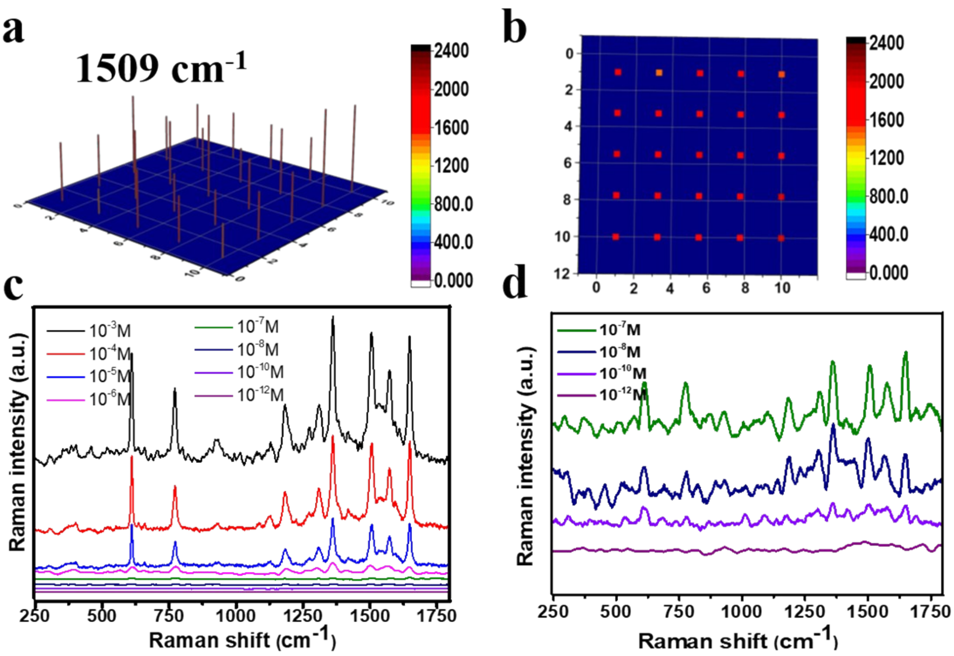
Supporting Figures



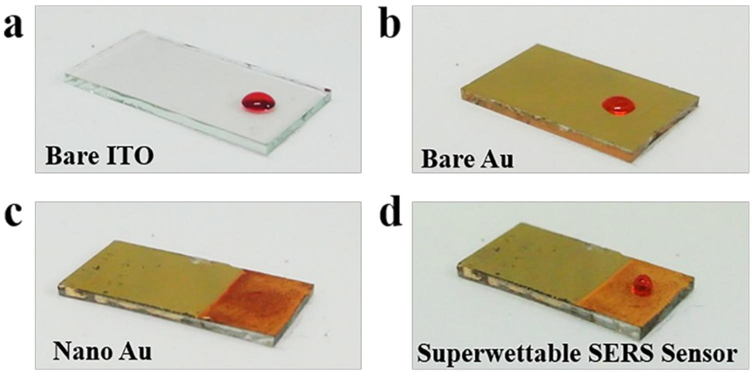
SI Fig. 1 Fabrication of the superwettable SERS sensor based on Au nanodendritic substrates, following by sputtering the layer of Ti and Au on an ITO glass, electrodeposition of Au nanodendritic structure, superhydrophobic modification, and O_2 plasma etching.



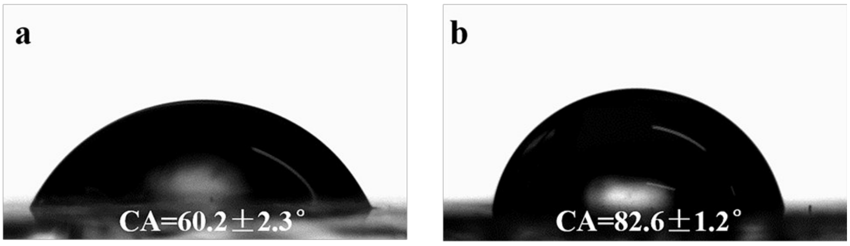
SI Figure 2. a) AFM image of nanodendritic gold substrate (roughness: 1413 nm). b) The XPS spectra of superhydrophobic and superhydrophilic substrate. c) The magnified of S 2p peak. d) The magnified of C 1s peak.



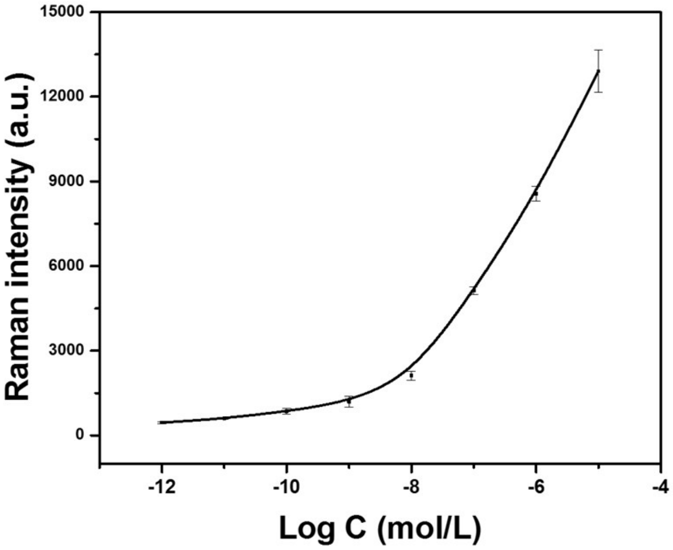
SI Figure 3. a) 3D image and b) 2D color image for demonstrating the SERS peak after subtracting noise from microwell in the microchip at wavenumber of 1509 cm^{-1}. c) SERS spectra of R6G-containing droplets (from top:10^{-3} M, 10^{-4} M, 10^{-5} M,10^{-6} M,10^{-7} M,10^{-8} M,10^{-10} M and 10^{-12} M) in the superhydrophilic microwells on the same microchip. d) The magnified SERS spectra of low concentrations (10^{-9}, 10^{-10}, 10^{-11} and 10^{-1}2 M) of R6G.



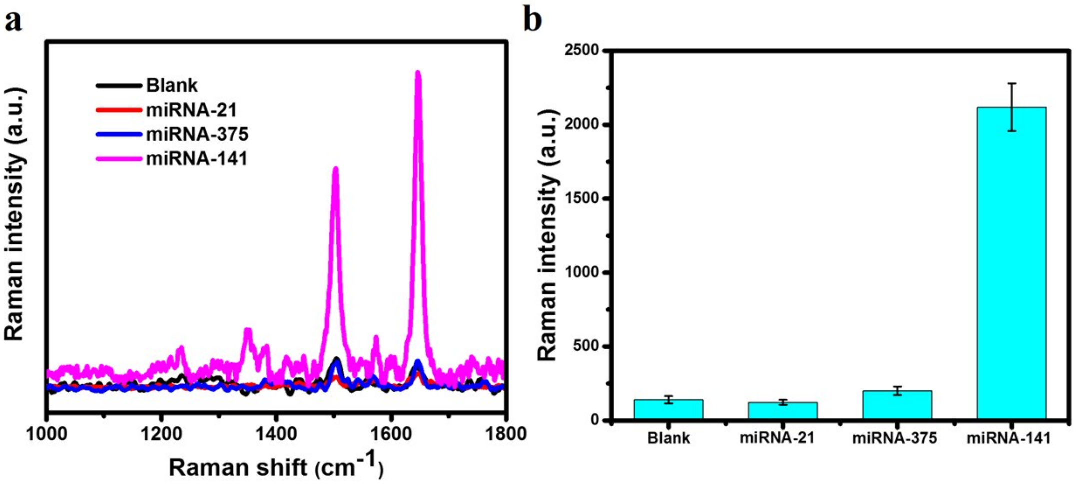
SI Figure 4. Comparison of diffusion status of droplets on different substrate: a) Bare ITO, b) Bare Au, c) Nano Au and d) Superwettable SERS sensor.



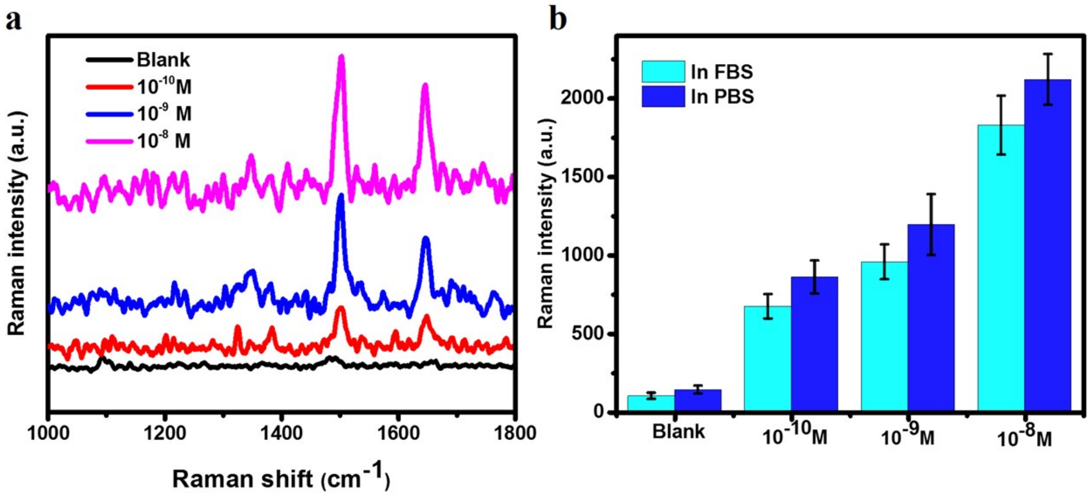
SI Figure 5. Water contact angle of bare ITO (left) and bare Au (right) substrate.



SI Figure 6. The SERS intensity at 1644 cm^{-1} for different miRNA-141 concentrations ranging from 10^{-5} to 10^{-12} M.



SI Figure 7. The selectivity of the superwettable nanodendritic gold SERS sensors. a) The SERS spectra and b) SERS intensity of the signal at 1644 cm^{-1} in the presence of buffer, miRNA-21, miRNA-375 and miRNA-141.



SI Figure 8. a) SERS spectra of miRNA-141-containing FBS droplets (blank, 10^{-10}, 10^{-9} and 10^{-8} M) in the superhydrophilic spots. b) The SERS signals at 1644 cm^{-1} comparison of different concentration miRNA-141 droplets (blank, 10^{-10}, 10^{-9} and 10^{-8} M) in PBS and FBS.

Table 1 The sensing applications comparison of superwettable nanodendritic gold and other substrates.

Substrate	**Analytes**	**Signal Output**	**LOD$_S$**	**Reference**
Carbon fiber	Glucose	Electrochemical	36 µA/mM	1
Mesoporous silicon nanowire arrays	Glucose	Electrochemical	25 µM	2
Au /SPCE	Sialic acid	Electrochemical	0.4 pM	3
TiO2 coating	miRNA-141	Fluorescent	88 pM	4
TiO2 coating	Free PSA,	Fluorescent	10 fg/mL	5
PCs dots	miRNAs	Fluorescent	0.01 nM	6
PCs dots	Tetracycline	Colorimetric	2 nM	7
SiNPs coated with OTMS and AEPTMS	Urea Glucose	Visual	100 nM 1 nM	8
AgNPs coated ZnO nanorods array	Adenine	SERS	1 µM	9
Assembly of Ag nanocubes	R6G	SERS	0.1 fM	10
AgNPs diatom biosilica nanopores PC microchip	Trinitrotoluene	SERS	0.1 nM	11
Nanodendritic gold	**miRNA-141**	**SERS**	**1 pM**	**Our work**

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