Supporting Information

Implementation of Molecularly Imprinted Polymer Beads for Surface Enhanced Raman Detection

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Materials

Acetone, acetonitrile (99.7%), azobisisobutyronitrile (AIBN, 98%) were purchased from Merck (Darmstadt, Germany). Methacrylic acid (MAA, 98.5%) was purchased from ACROS (Geel, Belgium). Propranolol hydrochloride (99%) supplied by Fluka (Dorset, UK) was converted into free base form before use. Nicotine, ethylene glycol dimethacrylate (EGDMA, 98%), *N*-(1-pyrenyl)maleimide (NPM), Rhodamine B (≥95%), *N*,*N*-dimethylformamide (DMF) and cumyl dithiobenzoate (CDB, 99%) were purchased from Sigma-Aldrich. L-[*N*-methyl-^{3}H]-nicotine (specific activity of 80.4 Ci mmol^{-1}, 12.4 mM in ethanol) was obtained from NEN Life Science Products, Inc. (Boston, MA). The scintillation liquid (Ecoscint A) was bought from National Diagnostics (Atlanta, GA). AIBN was recrystallized from methanol before use. The other chemicals were analytical grade and were used as received from commercial suppliers. All the aqueous solutions were prepared in ultra-pure water (18 MΩ cm). A 1-inch n-doped Si wafer with phosphorous as dopant material was used as the supporting substrate. The Si wafer was coated with 200 nm Au coating using 20 nm Ti as an adhesion layer. Gold colloids (20 nm, citrate stabilized and suspended in ethanol) were purchased from Sigma-Aldrich.

Synthesis of nicotine imprinted polymer microspheres bearing terminal thiol groups (MIP-SH)

Nicotine imprinted polymer microspheres bearing terminal thiol groups were synthesized using RAFT precipitation polymerization following a protocol reported in a previous publication.[1] Briefly, nicotine (80 mL, 0.5 mmol), MAA (111 mL, 1.3 mmol), EGDMA (1200 mL, 6.4 mmol), AIBN (25 mg) and CDB (60 mg) were dissolved in 40 mL acetonitrile. The solution was added into a one neck round bottom flask (100 mL) and purged with nitrogen for 30 minutes. The flask was then attached to the rotor-arm of a rotary evaporator. The polymerization was initiated at 60 °C and continued for 24 h while the flask was rotated at a speed of 30 rpm. After polymerization

the solid particles were collected by centrifugation and washed with methanol for two times. To remove the template the microspheres were washed with methanol containing 10% acetic acid until no template could be detected from the washing solvent. Finally, the polymer beads were washed with methanol for two times and acetonitrile for three times before they were dried in a vacuum chamber. The dithioester end groups on the particles were converted into terminal thiols by treatment with organic amines. The polymer microspheres (200 mg), ethylene diamine (110 μL) and triethylamine (230 μL) were mixed in DMF (3.3 mL) in a 10-mL one-neck round bottom flask. After being purged with nitrogen for 5 minutes, the reaction mixture was stirred at 50 °C for 16 h. After the aminolysis reaction, the particle suspension was acidified to ~ pH 5 by adding 0.1 M HCl. The particles were finally washed with water until the pH of the supernatant became neutral. In this way the dithioester groups on the surface of particles were transformed into thiol groups. The obtained polymer was named as MIP-SH.

Synthesis of non-imprinted polymer microspheres bearing terminal thiol groups (NIP-SH)

For comparison, a non-imprinted polymer (NIP-SH) was synthesized in the same way as used to synthesize the MIP-SH except that the nicotine template was omitted in the pre-polymerization solution. The terminal thiol groups on the polymer beads were confirmed by labelling the beads with the fluorogenic reagent NPM, and specific nicotine binding to MIP-SH beads was verified by radioligand binding analysis.[1]

Apparatus

Sputter coating of Au and Ti was performed using a high vacuum thermal evaporator Pfeiffer Classic 500. The pressure was maintained at 5×10^{-6} mbar for the deposition of both titanium and gold. Raman measurements were carried out using an iRaman system from B&W Tek Inc. equipped with an excitation laser at 785 nm and source power of 325 mW. The fiber optics probe

was maintained at a distance of 6 mm from the sample surface during the measurement. Raman spectra were collected in the range 200 - 2000 cm^{-1} with a spectral resolution of 3 cm^{-1}. For each spectrum 10 scans were measured, and each scan took 1 s. The spectra were recorded using the B&Wspec software and analyzed using Origin Pro 9.0.

Sputtering Au onto the immobilized polymer microspheres was performed using an AJA Orion Sputterer with a DC power of 75 W. The sputtering lasted for 20-30 s for 5 nm, 50 s for 10 nm and 90 s for 20 nm Au coating.

To study the roughness and homogeneity of the prepared surfaces tapping-mode AFM was carried out in ambient environment using an instrument by Veeco (New York, USA). The n-doped silicon cantilever tip obtained from Veeco (Plainview, New York, USA) has a typical curvature radius of 10 nm, a force constant ≈ 10-130 N/m (PPP-NCHR-20) and a resonance frequency of 204-497 kHz. The AFM images were analyzed using the Nanoscope software by Digital Instruments.

SEM characterization of the SERS substrates was carried out using a SEM LEO 1560 microscope (Zeiss, Oberkochen, Germany) operated at a voltage of 10 kV. The SEM analysis was carried out directly on the samples without prior metal coating.

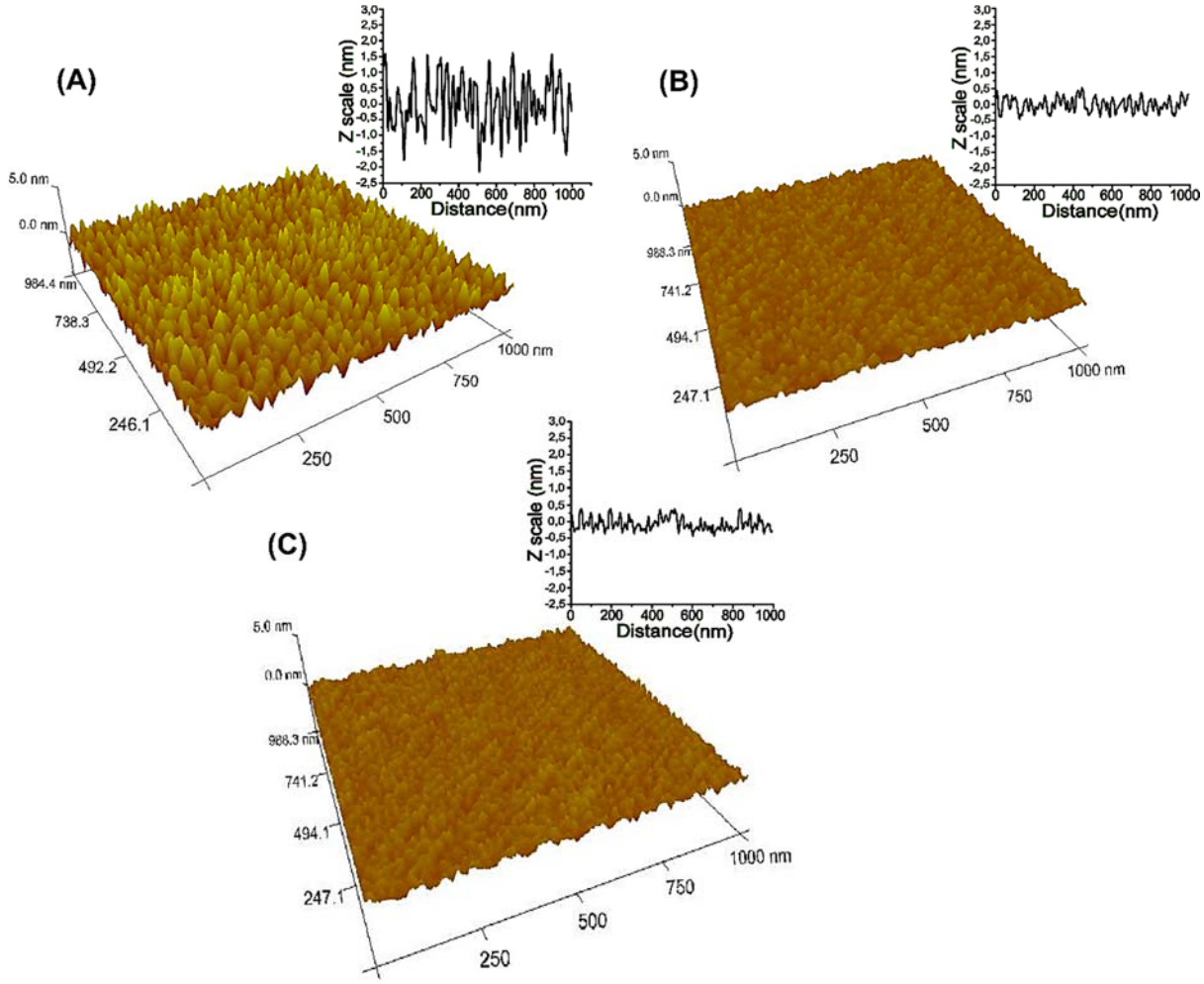

Figure S1. AFM images of silicon wafer sputter-coated with (A) 5 nm Au, (B) 10 nm Au, (C) 20 nm Au.

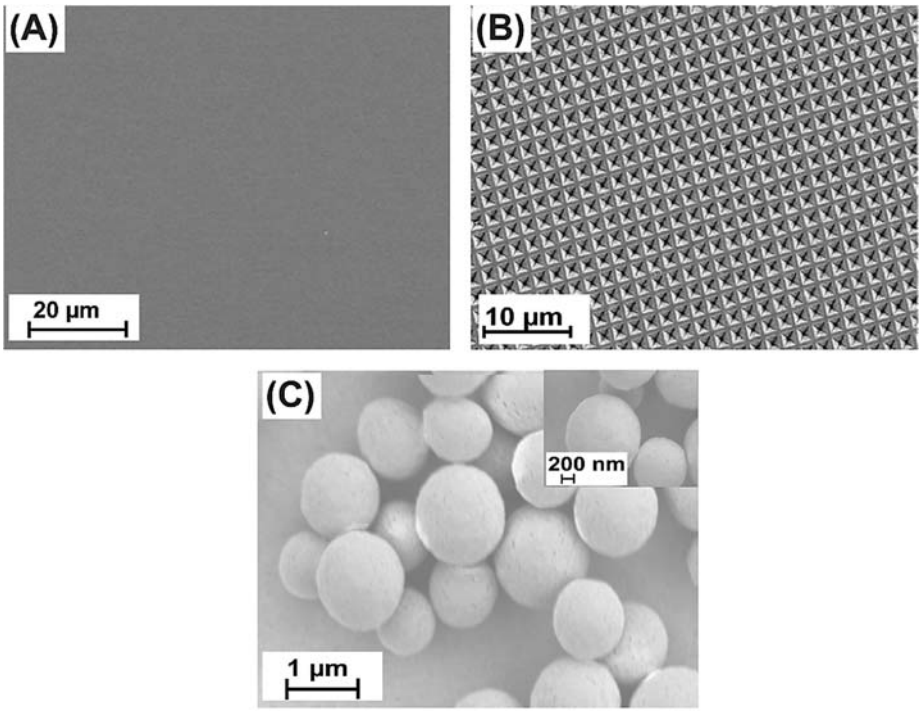

Figure S2. SEM images of (A) a clean Au surface on silicon wafer, (B) an empty Klarite surface, (C) MIP-SH microspheres immobilized on Au-coated silicon wafer, after sputter-coating of 20 nm Au.

Reference

(1) Zhou, T. C.; Jorgensen, L.; Mattebjerg, M. A.; Chronakis, I. S.; Ye, L. *RSC Adv.* **2014**, *4*, 30292-30299.