Supplementary Material (ESI) for Lab on a Chip

Electronic Supplementary Information

Gel-pad Microarrays Templated by Patterned Porous Silicon for Dual-mode Detection of Proteins

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1 Materials

*N*α,*N*α-bis(carboxymethyl)-L-lysine hydrate (ANTA) was purchased from Fluka. Poly (ethylene glycol) methacrylate (PEGMA) was purchased from SIGMA-ALDRICH, typical Mn: 360g/mol. Succinic anhydride, 4-dimethylaminopyridine (DMAP), N-hydroxysuccinimide (NHS), N,N'-dicyclohexylcarbodiimide (DCC), N,N,N'N'-tetramethylenediamine (TEMED), CuBr, $CuBr_2$, and $NiSO_4$ were purchased from Sinopharm Chemical Reagent Co. Ltd. Dichloromethane and other reagents were reagent grade or higher and used as received unless otherwise specified. Silicon wafers (<100>, p-type, 5.0-8.0 Ω· cm resistivity) were obtained from GuangWei Microelectronics (China). MILLI Q water (18 MΩ) was used for all experiments. The histidine-tagged proteins, thioredoxin-urodilatin (His-tagged Trx-urodilatin) and FITC-labelled His-tagged Trx-urodilatin, were prepared in one of our labs according to literature 1.

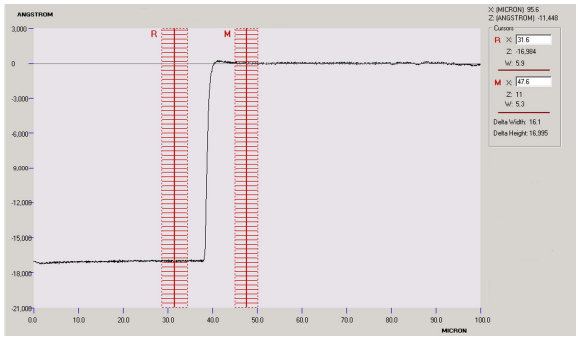
2 Preparation of FITC-labelled His-tagged Trx-urodilatin

The fusion protein, His-tagged Trx-urodilatin, was expressed and purified as reported in a previous publication.[1] For FITC labelling, His-tagged Trx-urodilatin was dissolved in 100 mM carbonate solution (pH = 9.0) at a concentration of 1 mg/ml. A 50 µl DMSO solution containing 10 mg/ml fluorescein isothiocyanate (FITC, Sigma) was added to the protein solution and stirred at 4 °C in dark for 4 hours. Then, 1 mL 200 mM NH_4Cl was added to the mixture to stop the reaction. After 2 h, 200 µl glycol was added and the mixture was applied to a sephadex G25 column. Elution was performed with 50 mM PBS buffer (pH = 7.4). The yellow solution which was eluted from the column firstly was collected, desalted, and lyophilised. And the molar concentration of His-tagged Trx-urodilatin or FITC-labelled one was estimated with His-tagged Trx-urodilatin's molecular weight 20.5 kDa from its MALDI mass spectrum. The protein was typically dissolved in a Tris buffer (20 mM Tris–HCl, 0.1 M NaCl, pH 7.4) for microarray assay.

3 Preparation of the photoresist microarray

Prior to the preparation procedure, the wafers were cleaned with 3:1 (v/v) H_2SO_4-H_2O_2 for 30 min (Notice: piranha solution reacts violently with organic materials and should be handled with extreme care), rinsed with copious amounts of water, and blown dry under nitrogen gas. The wafer was placed

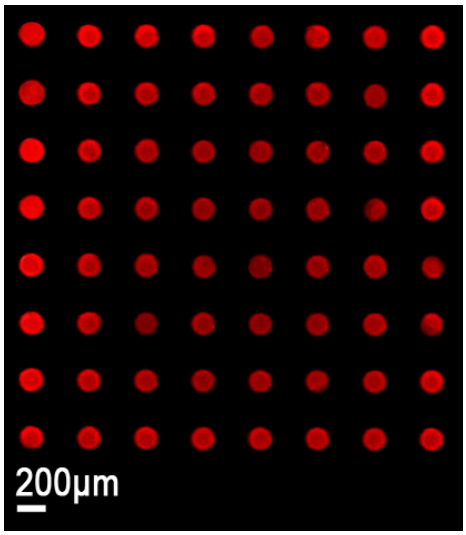
on a heating plate at 100 °C for 5 min to evaporate water completely. An AZ2700 positive photoresist (Jiangyin Jianghua Micro-electronics Materials Co., Ltd. China) was spun-coated onto the dry silicon wafer at 1500 rpm for 5 seconds and at 5000 rpm for 30 seconds and soft-baked at 100 °C for 5 min, resulting in a photoresist film about 1.7 μm thick (see S-Fig. 1). Then a photomask pattern was transferred onto the substrate by exposure to UV (λ= 360 nm, 100 mJ/cm^2) irradiation. The photo-exposed wafers were immersed in a ZX-238 developing solution for 15 seconds, washed with water and hard-baked at 100 °C for 15 min (before hard-baking the patterns were always checked by optical microscope). All preparation steps were carried out in clean room. The radius of patches is 100 μm and the patch-patch distance is 600 μm. By using different photomasks, patterns with different periodicities and sizes can be produced.



S-Figure 1 The height of photoresist membrane (1.7 μm) measured by XP-2 Stylus Profilometer (Ambios Technology Inc.).

4 Etching procedure for porous silicon (PSi)

The above photoresist microarray substrates used for electrochemical etching to produce porous silicon microarrays. Porous silicon micropatches were fabricated in ethanolic HF solution (2:1 (v/v) 40% HF/DMF) at a constant current density of 4 mA/cm^2 for 3 min in the absence of light. A Pt-ring was used as a counter-electrode and the backside of the wafer was contacted with aluminum foil before mounting in a Teflon etching cell. After etching, the wafers were removed from the cell, rinsed with copious amounts of EtOH. The remaining photoresist was stripped off using acetone, leading to a Si-H terminated porous silicon microarray. The freshly etched porous silicon has a well-known photoluminescence phenomenon reported by Canham Leigh.[2] Therefore we can easily detect a red fluorescence microarray shown in S-Fig. 2 at 640 nm with an excitation laser at 520 nm.

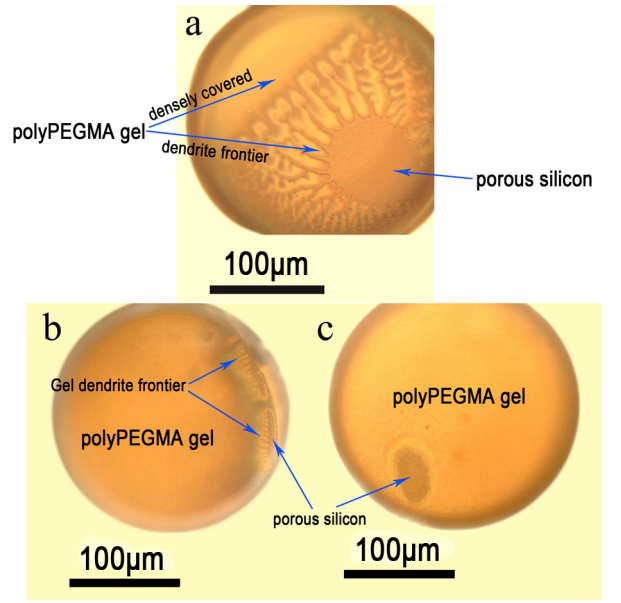


S-Figure 2 Fluorescence image of porous silicon microarray etched at a constant current density of 4 mA/cm^2 for 3 min in the absence of light. The radius of patches is 100 μm and the patch-patch distance is 600 μm. The excitation laser is at 520 nm and the fluorescent emission signal was obtained using a 640 nm wavelength filter.

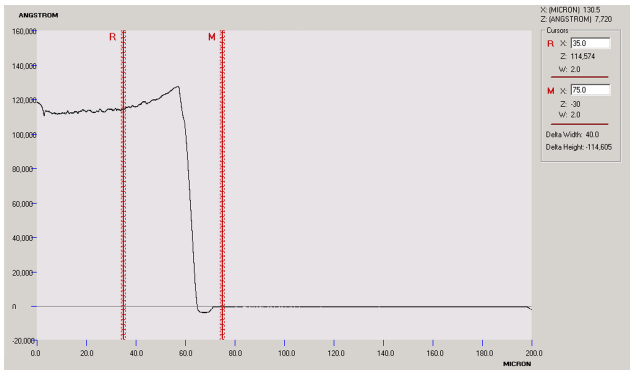
5 Surface modification

The polymerisation was performed by immersing the PSi microarray chip in 6 mL aqueous solution containing PEGMA (1 mL), CuBr (0.015 g, 0.036 mmol), $CuBr_2$ (0.008 g, 0.011 mmol), TEMED (50 μL) and water (5 mL) at room temperature (26 °C) for 3 min. Then the sample was washed with copious water and ethanol.

In order to investigate the polymerisation process, we decreased both concentrations of monomer and catalysts (0.5 mL PEGMA, 0.0064 g CuBr, 0.0024 g $CuBr_2$, 25μL TEMED, and 5 mL water). Therefore we can follow the morphology evolution of gel-growth with optical microscopy. S-Figs 3a is an image taken after 3 min reaction and S-Figs 3b and c are images after 6 min's incubation. S-Fig. 3a demonstrated that the gel followed the well-known dendritic growth under diffusion control, while S-Figs 3b and c illustrated the ending stages of gel-pads. The gel-pads almost ended close to the opposite edge of the starting site. The growth frontier is the dendrite, and followed by filling the dendrite gaps with further polymer growth. The thickness of gel membrane was measured as around 10 μm higher above the silicon wafer in S-Fig. 4.



S-Figure 3 Gel-growth evolution on the porous silicon templates. Optical microscopy images after 3 min (a) and 6 min's (b and c) incubations of a chip with 8×8 porous silicon dots in a solution of 0.5 mL PEGMA, 0.0064 g CuBr, 0.0024 g $CuBr_2$, 25μL TEMED, and 5 mL water.



S-Figure 4 The height of the gel membrane is 11.5 μm thick from the silicon surface measured by XP-2 Stylus Profilometer (Ambios Technology Inc.).

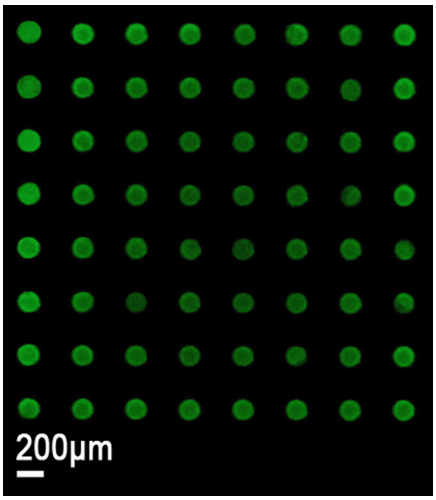
The polyPEGMA microarray chip was placed in a dry flask. A solution of 10 ml CH_2Cl_2 containing succinic anhydride (0.030 g) and 4-dimethylaminopyridine (0.010 g) was added in and the flask was kept at 27 °C overnight, then the chip was washed with copious EtOH and dried with nitrogen.

The –COOH functionalised chip was then activated by immersion in a solution of NHS (0.092 g, 0.8 mmol) and DCC (0.165g, 0.8 mmol) in 5 mL of 1,4-dioxane at room temperature for 3 h. After reaction, the NHS ester-activated sample was washed with copious EtOH and dried under nitrogen.

In the next step, the chip was exposed to an aqueous solution of 100 mM ANTA in K_2CO_3 buffer (pH = 8.5) for 1 h. The amino group of ANTA reacts with NHS-ester to form an amide linkage. Excess ANTA was removed by washing with copious water.

The NTA-functionalised chip was then immersed in 100 mM $NiSO_4$ solution for 30 min to ligate the Ni^{2+} ion on via the three carboxylates and the tertiary amine of NTA.

For the dynamic assay of protein microarrays, the chip was incubated in 200 μL at higher concentrations (≥ 1 μM) or 500 μL at lower concentrations (< 1 μM) of FITC-labelled His-tagged Trx-urodilatin Tris buffer for 1 h. The chips for IR and MALDI mass analyses were prepared by incubation in a 0.2 mg/mL (10 μM) His-tagged Trx-urodilatin Tris buffer. After incubation, the chip was rinsed with buffer and copious amounts of water, and finally dried under a mild stream of nitrogen. S-Fig. 5 clearly shows an 8×8 dots microarray by fluorescence scanning.



S-Figure 5 Fluorescence image of an 8×8 dots gel-pad microarray by incubation in a 0.2 mg/mL (10 μM) FITC-labelled His-tagged Trx-urodilatin Tris buffer. TECAN-LS300 Scanner was used to obtain the image with a photomultiplier tube (PMT) gain 125. The excitation was with an argon ion laser at 488 nm, and the fluorescent emission signal was obtained using a 535 nm wavelength filter.

6 FTIR measurements

Transmission infrared Fourier-transform (FTIR) spectra were recorded with a Bruker IFS66/S spectrometer at 4 cm^{-1} resolution. The samples were mounted in a dry-air purged chamber. Typically 32 scans were acquired per spectrum. Piranha solution treated Si (100) wafers were used as reference samples.

7 Scanning electron microscopy

Scanning electron micrographs were recorded with Hitachi S-4800 (Japan) SEM at an accelerating voltage of 15.0 kV.

8 Microarray scanner

TECAN-LS300 Scanner was used to scan the protein microarray with different photomultiplier tube (PMT) gains according to fluorescence intensities. The excitation was with an argon ion laser at 488 nm, and the fluorescent emission signal was obtained using a 535 nm wavelength filter. The fluorescence intensities were analyzed with SpotDataT Pro V3.0 (CapitalBio, Beijing). The fluorescence intensities of each sample were obtained after subtraction of the background. The normalisation of fluorescence intensities at different gains was according to the method suggested by Church et al.[3]

9 MALDI-TOF-MS

The mass measurements were performed in a Bruker Reflex II MALDI-TOF mass spectrometer. This instrument is equipped with a delayed ion extraction device with 100 ns delay time and a pulsed nitrogen laser operated at 337 nm. The analyte ions were accelerated at 20 kV. All spectra were recorded in the linear and positive ion mode for each spot separately. Spectra were averaged over 100 discrete laser shots. The laser power was adjusted slightly above the threshold for the desorption/ionisation process. All data were processed using Bruker Daltonics FlexAnalysis 2.4.

Reference:

1 Z. Sun, W. Lu, Y. Tang, J. Zhang, J. Chen, H. Deng, X. Li, J.-N. Liu, *Protein Expr. Purif.* 2007, **55**, 312.

2 L.T. Canham *Appl. Phys. Lett.* 1990, **57**, 1046-1048.

3 A. M. Dudley, J. Aach, M. A. Steffen, and G. M. Church, *PNAS*, 2002, **99**, 7554-7559.