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Supporting Information

Tuning the surface chemistry of iPDMS for improved protein microarray performance

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Experiemntal details.

Materials. Oligo (ethylene glycol) methacrylate (OEGMA, M_n=526, 98%) was purchased from Aldrich and used as received. Poly (dimethylsiloxane) (Sylgard 184) was obtained from Dow Corning. The vinyl-terminated initiator (undec-10-enyl 2-bromo-2-methylpropanoate) was purchased from HZDW (99%, Hangzhou, China). The goat IgG and rabbit anti-goat IgG/HRP were purchased from Beijing Zhong Shan-Golden Bridge Biological Technology Co, Ltd, China. All other antibodies, antigens and reagents for the multiplexed ELISA assays were gifts from HealthDigit (Shanghi, China). Serum samples were collected from local hospitals, with consent from all patients. The SuperSignal Chemiluminescent Substrates were purchased from Thermo Fisher Scientific Inc. All other reagents were from Sinopharm Chemical Reagent Co, Ltd.

Buffer/other solutions. Phosphate buffered saline (PBS, 0.1 M, pH~7.4); Tris-buffered saline (TBS, 0.1M, pH~7.6); Tween-Tris-buffered saline (TTBS, 0.1 M. add 0.02% (w/v) Tween 20 to TBS, pH~7.6); EDC/NHS activation solution (an aqueous solution containing 0.1 M (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide) and 0.1 M (N-hydroxysuccinimide)); Blocking solution (5% BSA (w/v) and 4% Sucrose (w/v) in PBS); Array Protein solution (One capture antibody was added as needed, 0.2% Glycerol (w/v) to PBS) ; Target protein solution (One or several kinds of antigens or unknown samples were added, 5% BSA (w/v) and 2% Sucrose (w/v) to PBS); Detection solution (Detection antibodies were diluted with guardian peroxidase conjugate stabilizer/diluents).

Preparation of iPDMS sheets. The vinyl-terminated initiator (C) was first mixed well with the PDMS precursor (A) and curing agent (B) at a ratio of 10:1:0.1 (A:B:C), which could react with hydrosilane hydrogens in the presence of Pt catalysts. This mixture was then poured into a 6.6 × 7.7 cm^2 mold and cured at 80^oC for 2 hours, resulting in a transparent elastomer. The resulting iPDMS was then subjected to surface modification via surface initiated polymerization (SIP). The SIP reaction mixture had a mole ratio of OEGMA526/$CuCl_2$/Bipy/AscA =100/1/2/1, with a feed [$CuCl_2$] at 2.76 mM. SIP was carried out under argon protection and continued for 2 hours at ~25^oC. When iPDMS was removed from the solution, polymerization quenched. Samples were thoroughly rinsed with methanol and Milli-Q water, and dried under flowing nitrogen.

Surface characterizations. (1) X-ray Photoelectron Spectroscopy (XPS). XPS (AXIS Ultra by Kratos Analytical, UK) was used to determine the surface composition of the iPDMS sheets. Monochromatic Al Kα X-rays (1486.7 eV) were employed. The X-ray source was 2 mm nominal X-ray spot size operating at 15 kV, 10 mA for both survey and high resolution spectra. Survey spectra, from 0 to 1100 eV binding energy (BE), were recorded at 160 eV pass energy with an energy step of 1.0 eV and a dwell time of 100 ms. High-resolution spectra were recorded at 40 eV pass energy with an energy step of 0.1 eV and a dwell time of 1.2 s, with a typical average of 12 scans. The operating pressure of the spectrometer was typically ~10^{-9} mbar. For quantitative XPS measurements, a survey scan was first taken at an angle of 90°, defined as the angle between the collection axis of the photoelectron analyzer and sample plane. All data were collected and analyzed using software provided by the manufacturer. **(2) Contact Angle.** Contact angles were measured on a Dataphysics OCA20 contact angle system at room temperature. **(3)**

Scanning Electron Microscopy (SEM). A Quanta 400 FEG SEM (FEI) was used to observe the morphology of the partially modified iPDMS. Before observation, all samples were sputter-coated with a gold coating about 10 nm thick to decrease the charge effect.

Fabrication of Protein Microarray. Before protein microarray fabrication, the iPDMS sheets were activated by immersion into an EDC/NHS activation solution for 30 min. Then, the sheets were washed three times with Milli-Q water, dried under flowing nitrogen and used immediately. For nitrocellulose (NC) film, there was no activation step. An HD-2003A noncontact arrayer (Health Digit, Shanghai, China) was utilized to array capture antibodies on NC films or iPDMS sheets at 25 °C and 60% humidity. After arraying, the iPDMS sheets (with capture antibody arrays) were fixed at room temperature overnight. It was then placed on the base plate and covered with a cover plate, which was pressed to form a 48-well cassette (each well had a single, isolated array). This complete cassette was then vacuum-packed for future use. Different from iPDMS, after assembling into 48-well cassettes, the surface of the NC film needed further blocking with the blocking solution for 1 h. Then, the film was washed with PBS, and after complete drying, the NC cassette was vacuum-packed as well.

ELISA. Using a freshly opened iPDMS cassette, 0.3 mL of TTBS were added to each well, and incubated for 5 min to achieve re-hydration.

For a sandwich ELISA protocol, 100 μL of target protein solution were added into each well and incubated at 37 °C for 30 min. Then, the wells were rinsed 6 times with TTBS. Next, detection solutions (with detection antibodies, 100 μL/well) were added and incubated at 37 °C for 30 min, followed by another 6 rinses with TTBS. After removal of the cover plate, Super Signal Chemiluminescent Substrate was uniformly added to the surface. Images were taken by a HD-2001 Chip Reader (Health Digit, Shanghai, China) or LAS-4000 imaging system (Fujifilm, Japan). Data were read out and analyzed by Bioca (Version 5.0, Health Digit). For a direct ELISA protocol, detection solutions were directly added. The contents of the target protein solutions and detection solutions were adjusted according to experimental needs.

For NC, the protocol was similar to iPDMS, except that after each incubation step, the wells were immersed in TTBS for 4 times for 8 min each with shaking, and rinsed thoroughly with TTBS to reduce NPA.

A Roche Diagnostics Elecsys 2010 system (Roche, USA) was used to calibrate the specific tumor marker concentration in human serum samples. All the serums were directly analyzed without any pretreatments.

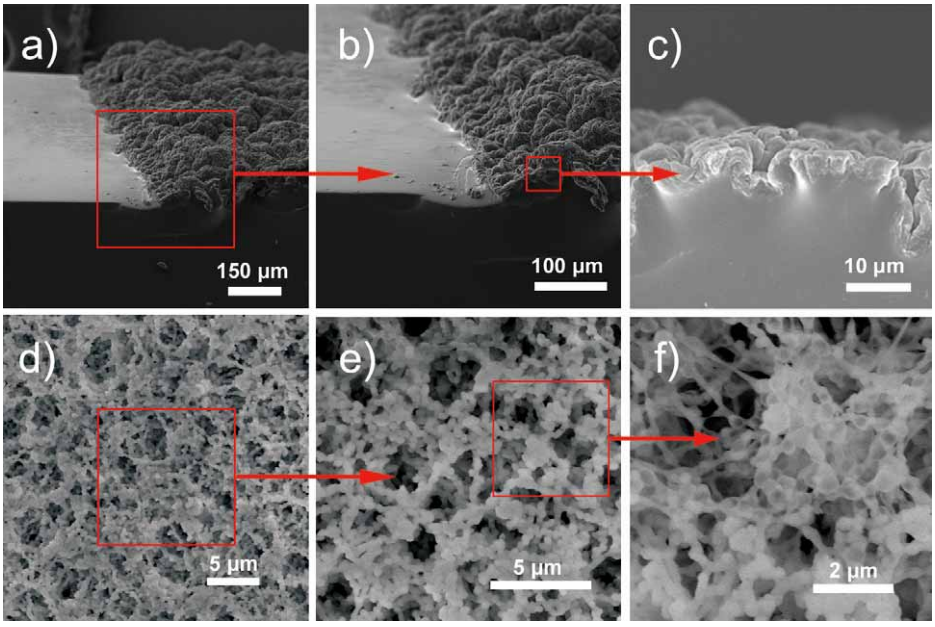

Figure S1. SEM result of partially modified iPDMS (a, b, c) and NC film (d, e, f).

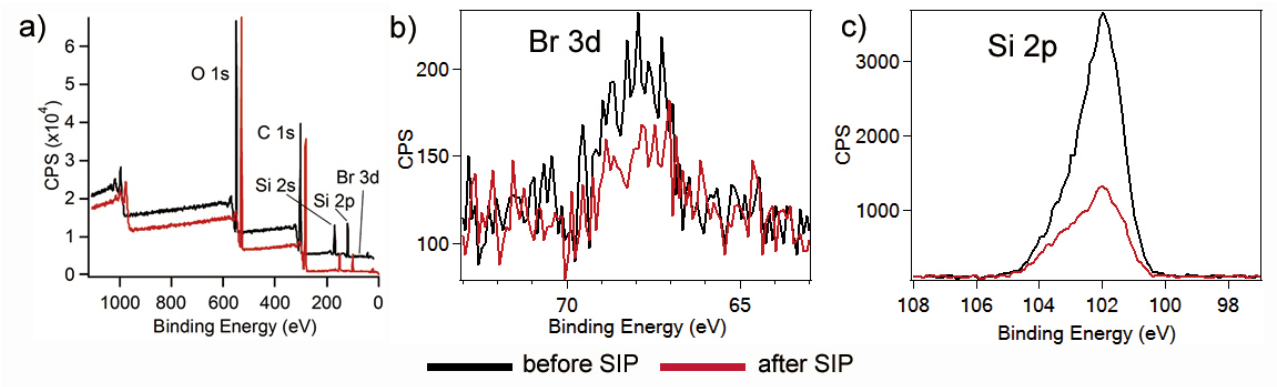

Figure S2. The surface modification was further confirmed by the XPS results. (a) In the survey scan, before (black line) and after (red line) SIP, one observed the disappearance of the Br peak and the weakening of the Si peak, both due to the addition of a polymer coating. Details were shown in the core scan of (b) Br 3d and (c) Si 2p.

Table S1. The surface tension of array buffers and array protein solutions.

Buffer/Array protein solution	Surface tension (mN/m)
PB (pH 7.6)	30.47
Carbonate Buffer (pH 9.6)	32.69
Citrate Buffer (pH 4.0)	29.76
Acetate Buffer (pH 4.6)	32.83
H IgG[1] array protein solution (0.1 mg/mL)	30.20
H IgG array protein solution (0.05 mg/mL)	30.26
R IgG[2] array protein solution (0.05 mg/mL)	30.22
G IgG[3] array protein solution (0.05 mg/mL)	30.20

[1] Human IgG; [2] Rabbit IgG; [3] Goat IgG. All the array protein solutions were composed of IgG, PB and a wetting agent. 0.1 mg/mL means one mL array protein solution that contains 0.1 mg IgG (this is the same going forward).

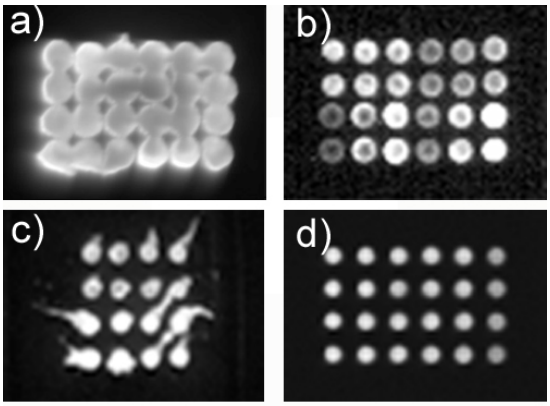

Figure S3. The Chemiluminesence result images show most common array defects. a) exosmosis, b) coffee rings, and c) overflow. d) A Chemiluminesence result images of no defect microarray.

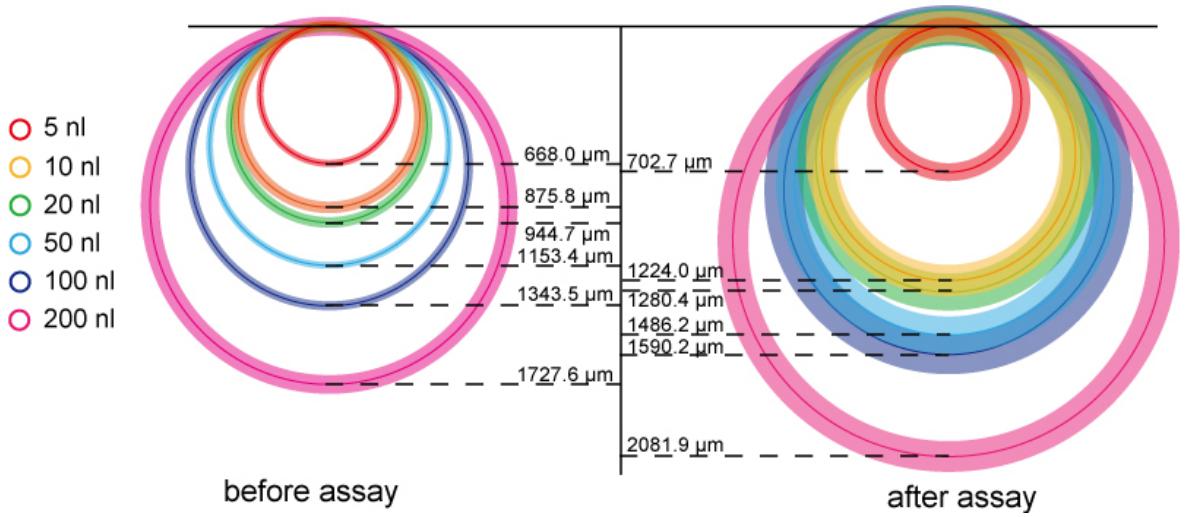

Figure S4. The relationship between array volume and spot diameter. Different colors means different spotting volume, the solid line ring represent the average diameter of spot with each volume, and the semitransparent ring illustrate the standard deviation of average diameter.

Patient Serum Assay Validation

The accuracy of iPDMS based microarray platform was verified in clinical diagnosis, comparing with Roch Diagnostics Elecsys 2010 system. Dozens of human serum samples were randomly selected and calibrated by a Roche Diagnostics Elecsys 2010 system. We chose 25 samples with only one tumor marker (CA199, CA125, CEA, AFP or CK19) around the clinical cut off value (i.e., the critical concentration value to differentiate the normal person and patient). In this experiment, we first test an unknow serum with Roche Method. If one of its biomarker values is close to the clinical cut off value, we continued to test this serum with protein microarray on iPDMS and NC. Such experimental design was to highlight the excellent low-end sensitivity of iPDMS based protein microarray.[1] The results showed that the linear correlation coefficient of 0.98 between the Roche value and the iPDMS value (Fig. S4a) far outclassed the coefficient of 0.89 between the Roche value and the NC value (Fig. S4b), which indicated the protein microarray based on the iPDMS sheet had an excellent low-end sensitivity.

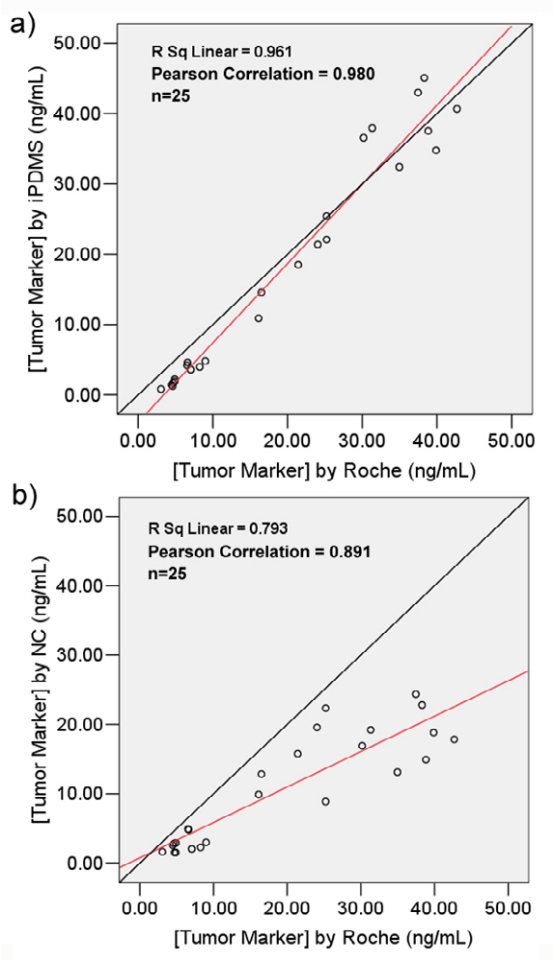

Figure S5. Linear regression and spearman correlation analysis for a) iPDMS vs. Roche; b) NC vs. Roche. (All the serum samples were randomly selected, with tumor markers including CA199, CA125, CEA, AFP and CK19)

References

(1) Ma H, Wu Y, Yang X, et al. *Anal. Chem.* 2010; **82**: 6338-6342.