Supporting Information

Bioinspired multistructured paper microfluidics for POCT

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Experiment Section

Materials

All monodisperse SiO_2 NPs (diameters of 255, 305 and 347 nm) were obtained from Nanjing Nanorainbow Biotechnology Co., Ltd. HF and PBS were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). NC was obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Fluorescein isothiocyanate (FITC), polysorbate-20 (TWEEN-20), acetone, dimethyl formamide (DMF), ethanol, bovine serum albumin (BSA), acrylic acid (AA), acrylamide (AAm), methyl methacrylate (MMA), n-butyl acrylate (BA), sodium p-styrene sulfonate (NaPSS), potassium chloride (KCl), ammonium persulfate (APS), ethylene glycol dimethacrylate (EGDMA), hydrochloric acid, potassium hydroxide (KOH), and 2-morpholinoethanesulfonic acid (MES) was obtained from Solon, USA. All the other chemicals, including 1-ethyl-3-(3-dimethylaminepropyl) carbodiimide (EDC) and N-hydroxysuccinimide (NHS), were purchased from Chemical Reagent Co., Ltd. (Shanghai, China). All reagents were used as received without further purification. Human serum samples were obtained from Nanjing Yifu Hospital. All chemicals were used as received. Deionized water with a resistivity of 18.2 MΩ•cm with ultraviolet sterilization was used for the preparation of all aqueous solutions.

All mouse monoclonal AFP, CA125 and CEA capture antibodies, HRP-labeled AFP, CA125 and CEA signal antibodies were obtained from Linc-Bio Science Co. Ltd. (Shanghai, China), and standard AFP, CA125, and CEA solutions were all purchased from Santa Cruz Biotechnology (Shanghai) Co., Ltd. Human cTnI, CKMB, Myo, Cy3-conjugated goat anti-human cTnI antibody, anti-human CKMB antibody and anti-human Myo antibody were obtained from Bioss. (Beijing, China). The luminol-p-iodophenol-H_2O_2 solution used as the HRP CL substrate was supplied by Autobio Diagnostics Co. Ltd. The coupling buffer for antibody immobilization was 0.01 mol L^{-1} pH 7.4 PBS. The blocking buffer for the residual reactive sites on the antibody-immobilized paper was PBS containing 0.5% bovine serum albumin and 0.5% casein. Tween-20 (0.05%) was spiked into 0.01 mol L^{-1} pH 7.4 PBS as wash buffer (PBST) to minimize nonspecific adsorption.

Mouse fibroblast 3T3 cells (NIH 3T3) were supplied by China Type Culture Collection. Other related reagents for cell culture were purchased from GIBCO Corporation. For cell staining, calcein acetoxymethyl ester (Calcein AM)/propidium iodide (PI) was obtained from Dojindo (Japan).

The artificial urine solution contained 1.1 mM lactic acid, 2.0 mM citric acid, 25 mM sodium bicarbonate, 170 mM urea, 2.5 mM calcium chloride, 90 mM sodium chloride, 2.0 mM magnesium sulfate, 10 mM sodium sulfate, 7.0 mM potassium dihydrogen phosphate, 7.0 mM dipotassium hydrogen phosphate, and 25 mM ammonium chloride all mixed in Millipore-purified water. The pH of the solution was

adjusted to 6.0 by addition of 1.0 M hydrochloric acid. All reagents were obtained from Sigma-Aldrich.

All human serum samples were obtained from commercial provider (InnoReagents, LLC, Huzhou, China). The serum samples were collected at the commercial provider so no human participants were involved during the research.

Experimental procedures.

1. Fabrication of NC MSPs: The fabrication of MSPs is shown in Figure 1. Silicon molds with various pillar patterns were prepared by photolithography with photomasks of each pattern. PDMS molds were prepared by replicating a Si master mold with patterns using a PDMS base polymer (Sylgard 184, Dow Corning Korea, Korea) and curing agent (10 wt%). To obtain the SiO_2 PCs for NC MSPs, a 0.50 μL aliquot of a water suspension containing 30% (w/v) monodisperse SiO_2 nanoparticles, 13% (v/v) ethanol and 0.050% Tween-20 was drop-cast onto the substrate. A smooth blade of hard rubber or an automatic coater was used for blade-coating of the suspension on the patterned substrate. Due to the hydrophobicity of the toner printed on the substrate, the suspension was selectively filled into the hydrophilic hollow channels formed between two adjacent toner layers. After complete drying at a room temperature of 25 °C, patterned PCs of SiO_2 nanoparticles were obtained. To obtain the photonic NC MSPs, an acetone and DMF (1:1 v/v) solution containing 7.5% (w/v) NC was prepared. After stirring the solution at room temperature (25 °C) for 12 h, the solution was then drop-cast onto the pattered SiO_2 PC. After baking at 60 °C on a hot plate for 6 h, an aqueous solution containing 4.0% HF was introduced to etch the SiO_2 nanoparticles. Finally, the substrate was thoroughly washed with deionized water and then dried to obtain the photonic NC membrane with an inverse-opal structure.

2. Fabrication of copolymer nanoparticles and EC MSPs: EC NPs with controlled sizes are fabricated as previously reported.[1] Briefly, by introducing the soft component into the colloids through a one-step emulsifier-free emulsion polymerization synthesis method as we previously reported, the copolymer nanoparticles were synthesized by emulsifier-free emulsion polymerization. In a typical synthesis, a few monomers, including 3 mL of MMA, 3 mL of BA, 50 μL of EGDMA, 0.2 mL of AA and 0.2 g of AAm, were first mixed with 60 mL of deionized water in a 100 mL three-necked flask. After stirring in a nitrogen atmosphere for 0.5 h, the mixture was heated to 90 °C. Then, 4 mL of an aqueous solution containing 180 mg of APS and the desired amounts of KCl and NaPSS was added into the mixture. The reaction lasted for 10 h. The products were collected by centrifugation and washed three times with deionized water and then redispersed in 20 mL of deionized water for further use.

To obtain the EC MSPs, the suspension containing 15 wt% copolymer nanoparticles of different sizes was cast onto PDMS molds (with or without patterns) and dried at room temperature. Then, the copolymer film was peeled off from the PDMS molds to obtain freestanding MSPs.

3. Immunoassay on NC MSP microfluidic chips: For the immunoassay, the photonic NC membrane was immersed in 0.10 M PBS. Next, a 2.0 μL aliquot of 0.50 mg/mL capture antibody (i.e., goat anti-human cTnI antibody, anti-human CKMB antibody and anti-human Myo antibody) was drop-cast onto the detection zone of the NC MSPs.

After 1 h at room temperature (25 °C), the detection zone was washed 3 times with 0.10 M PBS solution (pH 7.4) containing 0.20% TWEEN-20 to remove the free antibody, and then the membrane was immersed in a 0.10 M PBS solution (pH 7.4) containing 5.0% BSA for 1 h to block the remaining active spots. Finally, the membrane was washed with 0.10 M PBS solution (pH 7.4) containing 0.20% TWEEN-20. The analytical device was stored at 4 °C before use.

To use the analytical chip for the immunoassay, 20 μL samples containing analytes were first incubated with the mixture of Cy3-conjugated goat anti-human cTnI antibody, anti-human CKMB antibody and anti-human Myo antibody for 30 min and dropped on sampling spot. After the sample wicking through the chip and reacted for 10 min, the absorbent paper was used for removal of redundant components, and then fluorescence microscopy was used to obtain the fluorescent image of the detection zone. For quantitative analysis, the image was imported into Adobe Photoshop CS6, and the color intensity was obtained from a histogram of each reservoir.

4. Immunoassay on EC MSP microfluidic chips: For the immunoassay, the photonic MSPs were incubated with MES, EDC and NHS for 30 min and washed with PBS. The different sensing areas were incubated with the respective antibodies targeting cancer markers at 4 °C for 12 h and then with BSA for 30 min. Finally, the membrane was washed with 0.10 M PBS solution. For detection, 10 μL of sample solutions containing different concentrations of antigen in PBS were added to the sampling area and incubated. Then, 10 μL of 25 $\mu g \cdot mL^{-1}$ HRP-labeled signal antibodies were added to the sampling areas and allowed to incubate. Finally, the CL reaction was performed by dropping the luminol-p-iodophenol-H_2O_2 solution on the sampling area, and then a microscope with a CCD camera was used to obtain the CL image of the detection zone. The CL intensities were measured by an F-4600 fluorescence spectrophotometer (Hitachi, Japan) when the lamp was off.

5. Cell Culture: Before cell culture, all MSPs were thoroughly washed with sanitized water, completely dried and then placed under an ultraviolet lamp in an aseptic console for 2 h. 3T3 cells were first cultured inside a humidified incubator (37 °C and 5% CO_2, HERACELL 150i, Thermo Scientific). 3T3 cells with a density of 2×10^5 cell/cm^2 were then seeded on the culture area of the MSPs.

Characterization

Scanning electron microscopy (SEM) images were taken using a field emission scanning electron microscope (FESEM, Zeiss Ultra Plus). Transmission electron microscopy (TEM, JEM-2100EX) was used to observe the structure of the copolymer nanoparticles. Reflection spectra were obtained by using a spectrophotometer (Ocean Optics, QE65000). The fluorescence intensities were detected by a fluorescence microscope (DM2000, Leica). The CL intensities were measured by an F-4600 fluorescence spectrophotometer (Hitachi, Japan) when the lamp was off.

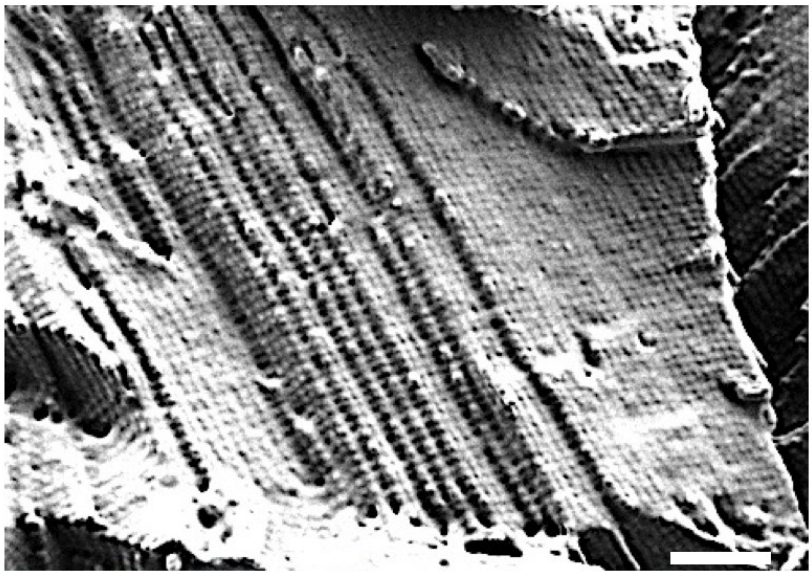

Figure S1. SEM image of the cross-section of the EC MSPs. Scale bar: 2 μm.

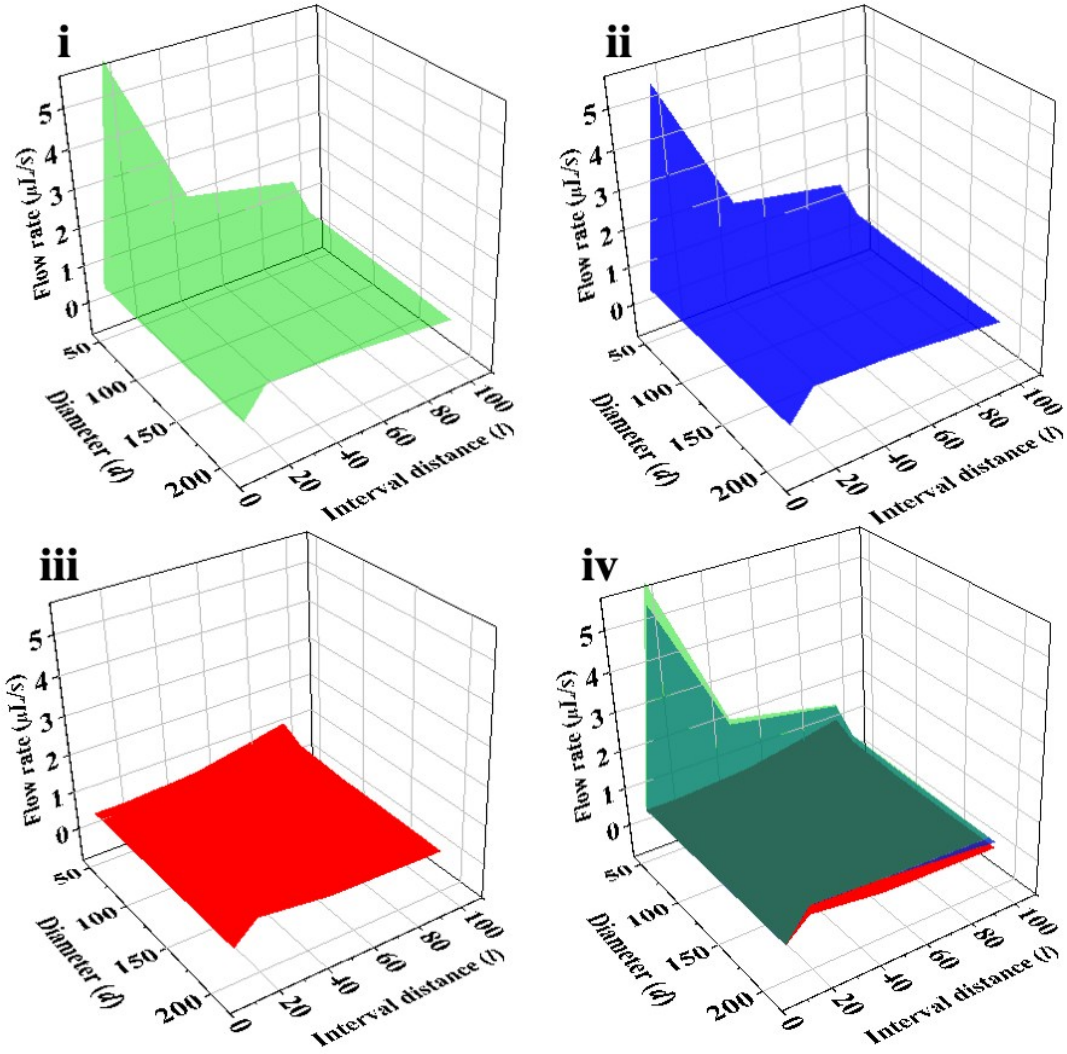

Figure S2. Pumpless liquid flow rate (μL/s) of 5 μL of water (i), artificial human urine (ii) and artificial human serum (iii) through NC MSP strips with different

diameters (*d*=30, 50, 100, 200 μm) and intervals (*l*-*d*=10, 20, 50, 100 μm). (iv) is the merged image of (i), (ii) and (iii).

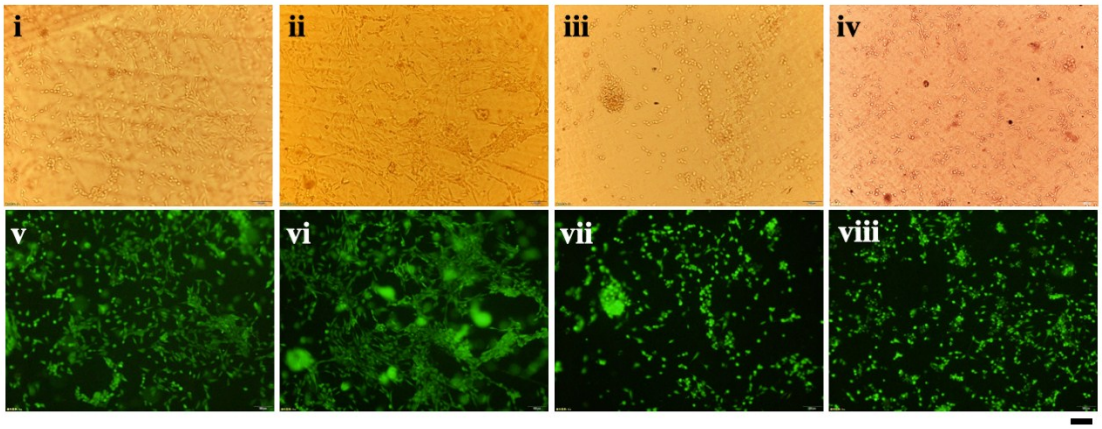

Figure S3. Bright optical images (i-iv) and fluorescent images (v-viii) of fluorescent staining (Calcein-AM) of 3T3 cell culture on NC MSPs (v, vi) and EC MSPs (vii, viii) for 1 day (v, vii) and 3 days (vi, viii), scale bar: 100 μm.

References:

1. J. Liao, C. Zhu, B. Gao, Z. Zhao, X. Liu, L. Tian, Y. Zeng, X. Zhou, Z. Xie and Z. Gu, Advanced Functional Materials, 2019, 1902954.