Supporting Information

A microfluidic platform with digital readout and ultra-low detection limit for quantitative point-of-care diagnostics

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The supporting information includes:

1. Materials

SPR 220-7 was purchased from MicroChem Corp (Newton, MA, USA). MF-CD26 was obtained from Rohm and Haas Electronic Materials (Marlborough, MA, USA). Chloroplatinic acid hexahydrate ($H_2PtCl_6 \cdot 6H_2O$), sodium citrate, NH_4F, HF, HNO_3, (3-glycidoxypropyl) trimethoxysilane (3-GPS), toluene, ethanol, and centrifugal filter (molecular cutoff: 150,000 Da) were purchased from Sigma-Aldrich (St. Louis, MO, USA). DsRed-conjugated anti-rabbit secondary antibody were purchased from Abcam (Cambridge, UK). Tridecafluoro-1, 1, 2, 2-tetrahydrooctyl-1-trichlorosilane was purchased from Pfaltz and Bauer (Waterbury, CT, USA). Phosphate-buffered saline (PBS) (0.1 M, pH 7.4) was obtained from Lonza (Allendale, NJ, USA). Red ink was purchased from Fisher Scientific (Waltham, MA, USA). Polyvinyl chloride sealing tape was obtained from 3M (St. Paul, MN, USA). Amorphous diamond-coated drill bits (0.031 inch cutter diameter) were purchased from Harvey Tool (Rowley, MA, USA). Photomasks for the devices were designed using AutoCAD software and printed out as transparency photomasks by CAD/Art Services (Bandon, OR, USA) with resolution at 10 μm.

2. Methods

A. Microfluidic device fabrication

The pattern for the DV-chip was fabricated using a standard photolithography process, [1, 2] with the procedures as shown in Figure S1. In brief, glass slides (75×50×1 mm) were first spin-coated with a layer (~10 μm) of SPR220-7 photoresist, baked at 75°C for 3 min, and baked again at 110°C for 5 min. After cooling to room temperature, the photoresist-coated glass slides were exposed to UV light for 50 s through a photomask containing the DV-chip layout. Slides were then immersed in SPR developer solution (MF-CD26) for 3 min

to wash the photoresist off the exposed area. Slides were then thoroughly rinsed with Millipore water and dried with nitrogen gas to create the pattern. Before etching, the backs of the slides were protected with polyvinyl chloride sealing tape to prevent unintentional etching. The glass slides were then immersed in a glass-etching solution (1:0.5:0.75 mol/L $HF/NH_4F/HNO_3$) to etch the pattern. Etching was performed in a 35°C water bath to control the etching speed. After 45 min, slides were etched to a depth of ~50 μm. The sealing tape was removed and slides were rinsed with water and acetone and wiped with ethanol cloth. Finally, holes for sample inlets/outlets were formed with a 0.03-inch diamond drill. For surface modification, the glass slides were first treated with oxygen plasma followed by deposition of 10 μL tridecafluoro-1, 1, 2, 2-tetrahydrooctyl-1-trichlorosilane and drying in a vacuum drier for 2 h. Finally, the glass slides were wiped with ethanol cloth and dried with nitrogen gas.

B. Preparation and conjugation of platinum nanoparticles (PtNPs)

PtNPs were prepared using a method similar to previously reported methods. [3] One milliliter of 1% w/v H_2PtCl_6 aqueous solution was added to a 250-mL conical flask containing 100 mL water. The solution was heated to boiling, and 3 mL 1% w/v sodium citrate aqueous solution was added rapidly. The mixture was kept at a boil for ~30 min. PtNPs with average diameter 3.6 nm were synthesized. To produce larger PtNPs, we used 3 mL 1% w/v H_2PtCl_6 and added 0.33 mL 1% w/v sodium citrate into the boiled solution. After 30 min, sodium citrate (1% w/v, 2.67 mL) was added into the mixture solution, which was maintained at a boil for 1 h. PtNPs with average diameter 56.2 nm were produced. The size distribution of PtNPs was characterized by dynamic light scattering (Malvern Zetasizer Nano Series, Malvern, UK). The concentration of the PtNP stock solutions were 2.5 mg/mL

(~ 720 nM) for 3.6-nm PtNPs and 13.7 mg/mL (~ 1.44 nM) for 56.2-nm PtNPs. We compared the catalytic performance of these two PtNP populations (using 0.1 nM as the concentration for both of them) and found that the 56.2-nm PtNP catalyzed generation of O_2 from H_2O_2 is much more efficiently (Figure S3). Therefore, we used the 56.2-nm PtNPs in subsequent experiments.

To prepare antibody-conjugated PtNPs, 2 μL 1 mg/mL detection antibody was mixed with 200 μL 1 mg/mL PtNPs in PBS and maintained at 4ºC overnight. Antibodies bonded to the surface of PtNPs (Figure S4) by the cleavage of the sulfur-sulfur bond in disulfides through oxidation.[4] Then bovine serum albumin (BSA) was added to final concentration of 1% w/v to block the surface of the PtNPs, and the unconjugated antibody was removed using a centrifugal filter. Finally, the antibody-conjugated PtNPs were suspended in 200 μL 10 mM PBS, pH 7.4, containing 0.1% v/v Triton X-100, 5% w/v sucrose, and 1% w/v BSA.[5]

C. Evaluation of the DV-chip

A series of PtNP solutions to serve as standards, or controls, was prepared by diluting the stock solution. Then, 10 μL of each control PtNP solution (5, 8, 10, 20, 30, and 60 pM) was loaded into the wells of the uppermost lane and 10 μL PtNP solution of known concentration (3.5 pM) was loaded in the wells of the lowermost lane as experimental sample. Hydrogen peroxide (H_2O_2, 35% w/v, 10 μL) was loaded into the second lanes from top and bottom. Red ink (10 μL) was loaded into the central lane. To generate a dynamic bar chart over time, the readout was recorded every 6 s. For other evaluation experiments, the readout was recorded in 5 min after initiation of the reaction by sliding of the top plate.

D. Silane treatment of the surface

Before the ELISA assay, the surface of the bottom plate must be treated with silane. Briefly, wells of the first lane from the top and bottom ends were cleaned with piranha solution for 1 h, rinsed with Millipore water, and dried with nitrogen gas. Next, 2 μL 10% v/v 3-GPS diluted in toluene was added to each well followed by incubation for 1 h, and wells were washed with fresh toluene to remove excess 3-GPS. The glass slides were then dried with nitrogen gas and baked at 120°C for 30 min. Finally, epoxy groups were covalently bonded to the surface of each well. [2]

E. Test of antibody coating efficiency on the DV-chip

Before the biomarker test, the coating efficiency of capture antibody on the wet-etched glass surface was tested. After silane treatment, the wet-etched well was coated with rabbit monoclonal antibody to CEA at 4ºC overnight and then blocked with BSA at room temperature for 1 h. DsRed-conjugated anti-rabbit IgG secondary antibody was then added to the well and incubated for 1 h at room temperature. After thorough washing with PBS, the fluorescence image of the well surface was obtained using an EVOS® FL Auto inverted microscopy and a 40× objective (Life Technologies, Grand Island, NY, USA).

References

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[3] M. Huang, Y. Jin, H. Jiang, X. Sun, H. Chen, B. Liu, E. Wang, S. Dong, *J. Phys. Chem. B* **2005**, *109*, 15264-15271.

[4] L. H. Dubois, R. G. Nuzzo, Annu. *Rev. Phys. Chem.* **1992**. *43*, 437-463.

[5] H. C. Lin, I. L. Wang, H. P. Lin, T. C. Chang, Y. C. Lin, *Sensor Actuat B-Chem* **2011**, *154*, 185-190.

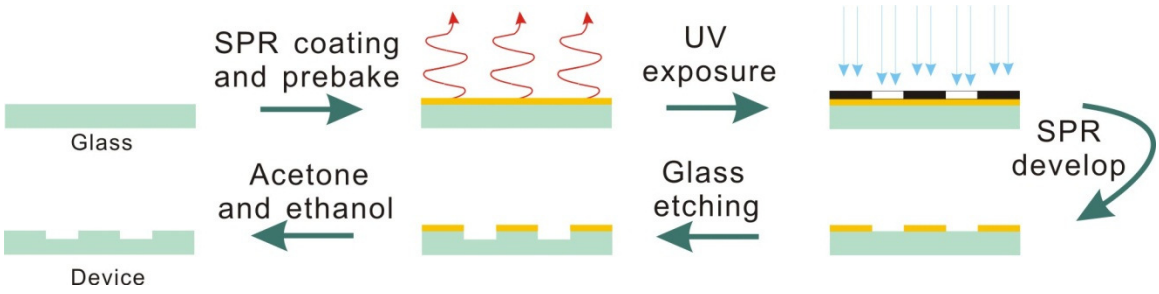

Figure S1 Fabrication of the DV-chip.

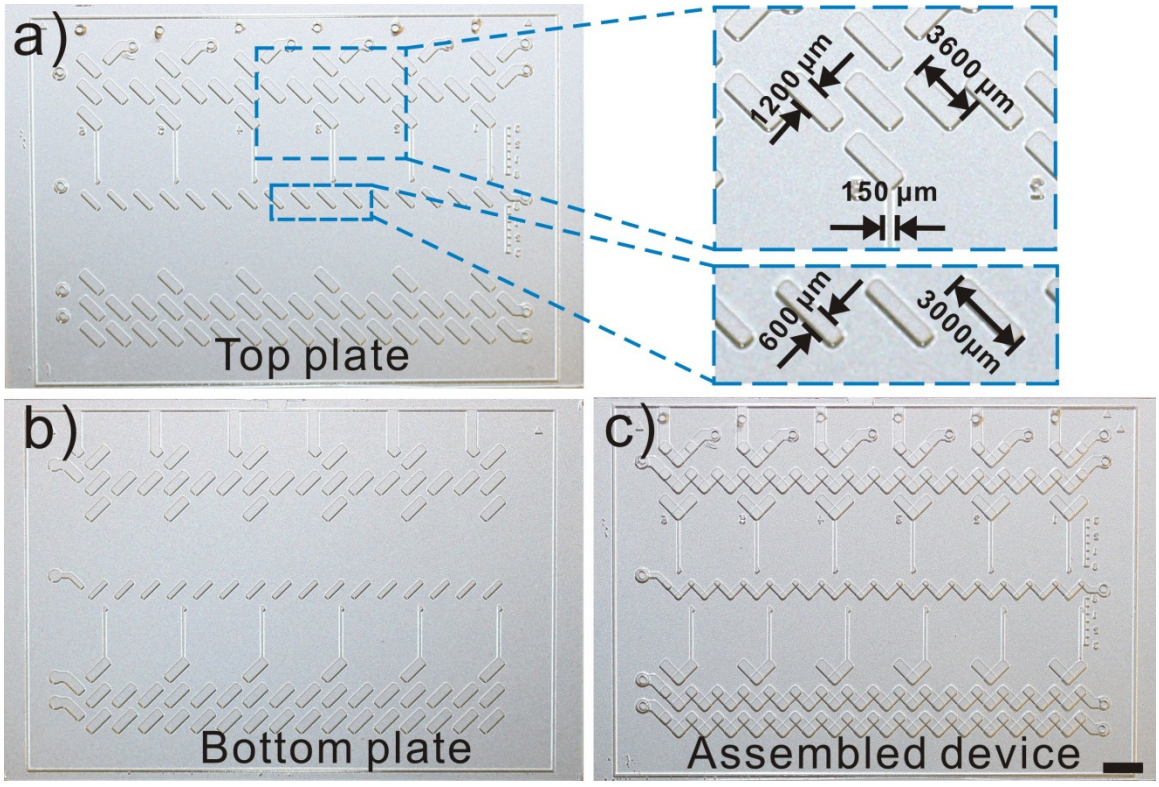

Figure S2 Components of the DV-Chip. (a) Top plate of the device. The enlarged image shows the size of the wells and channels. (b) Bottom plate of the device. (c) The assembled device. Scale bar, 0.5 cm for (a-c).

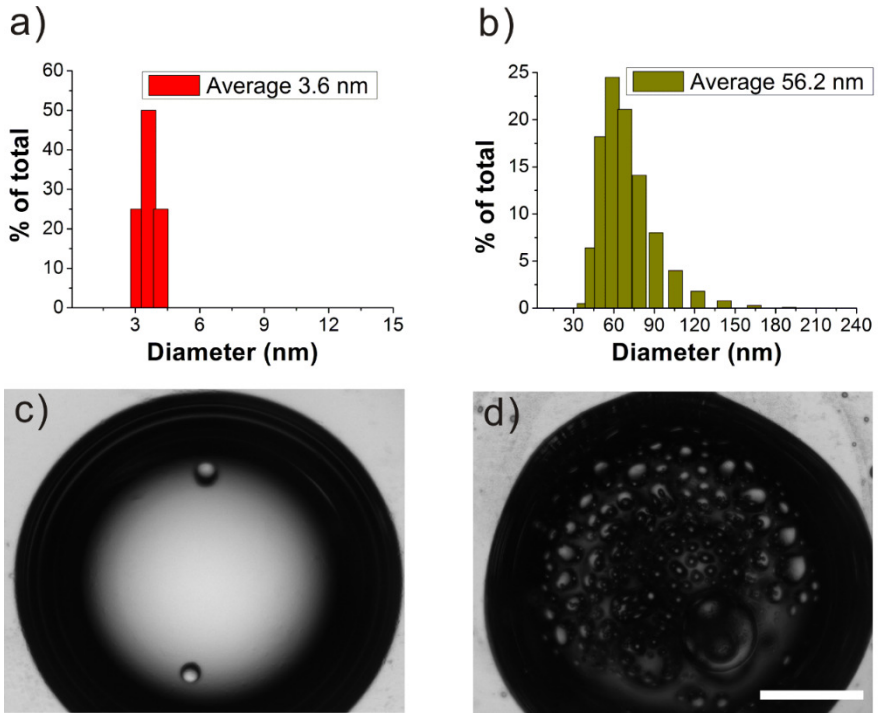

Figure S3 Comparison of catalytic performance of PtNPs of different sizes. (a, b) Size distribution of PtNPs synthesized with average diameters of 3.6 nm (a) and 56.2 nm (b). (c, d) Bubbles induced by oxygen generated in 20 seconds after addition of 2 μL PtNPs of diameter 3.6 nm (c) and 56.2 nm (d) to 2 μL H_2O_2 (35 % w/v). The efficiency of oxygen generation of the 56.2-nm PtNPs was much higher than that of the 3.6-nm PtNPs. The concentration used for both of the two PtNPs is 0.1 nM. Scale bar, 0.5 mm for (c) and (d).

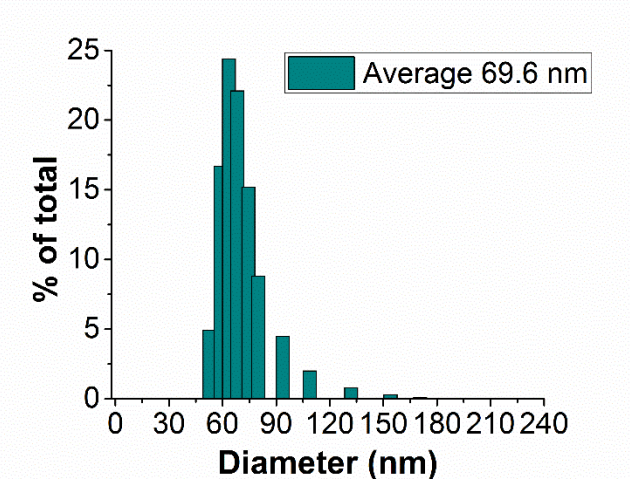

Figure S4 Size distribution of antibody-conjugated PtNPs. The average diameter is 69.6 nm, which is 13.4 nm larger than the original PtNPs with size of 56.2 nm. The antibody used here is mouse CEA monoclonal antibody. This result confirmed that the antibodies have been conjugated to the PtNPS.

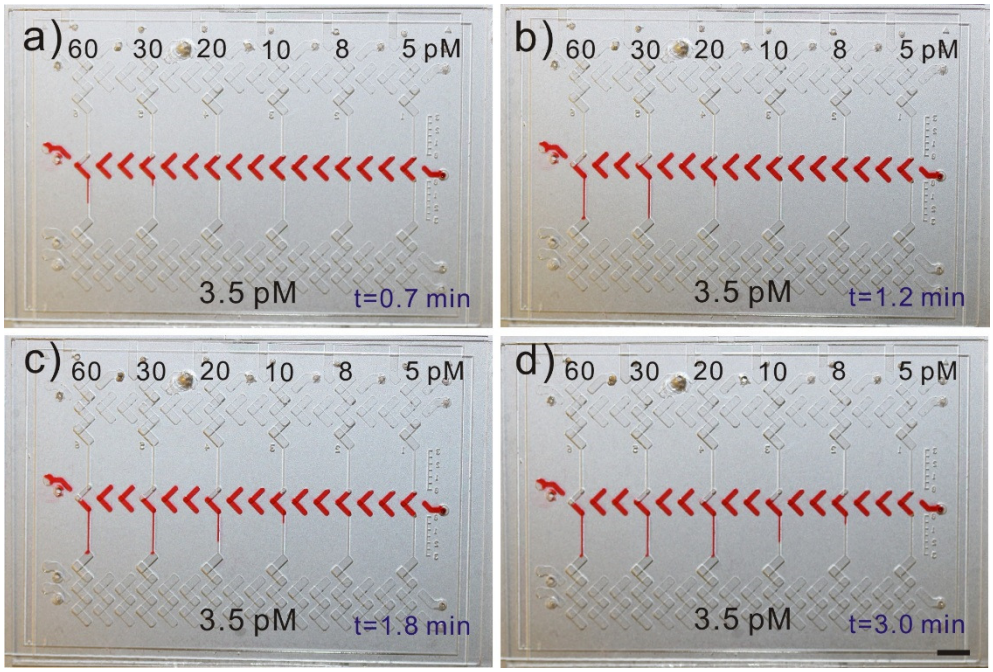

Figure S5 Formation of downward bar charts with time at constant PtNP concentration. (a-d) Readout after reaction of the indicated control (upper labels) and experimental (lower labels) PtNP samples at t (time) =0.7 min (two downward bars) (a), at t=1.2 min (three bars) (b), at t=1.8 min (four bars) (c), and at t=3.0 min (five bars) (d). Downward bars were generated because in all cases, control PtNP concentration was greater than experimental PtNP concentration, resulting in greater O_2 production by controls and propulsion of ink farther away from the control reaction site. Scale bar, 0.5 cm for (a–d).

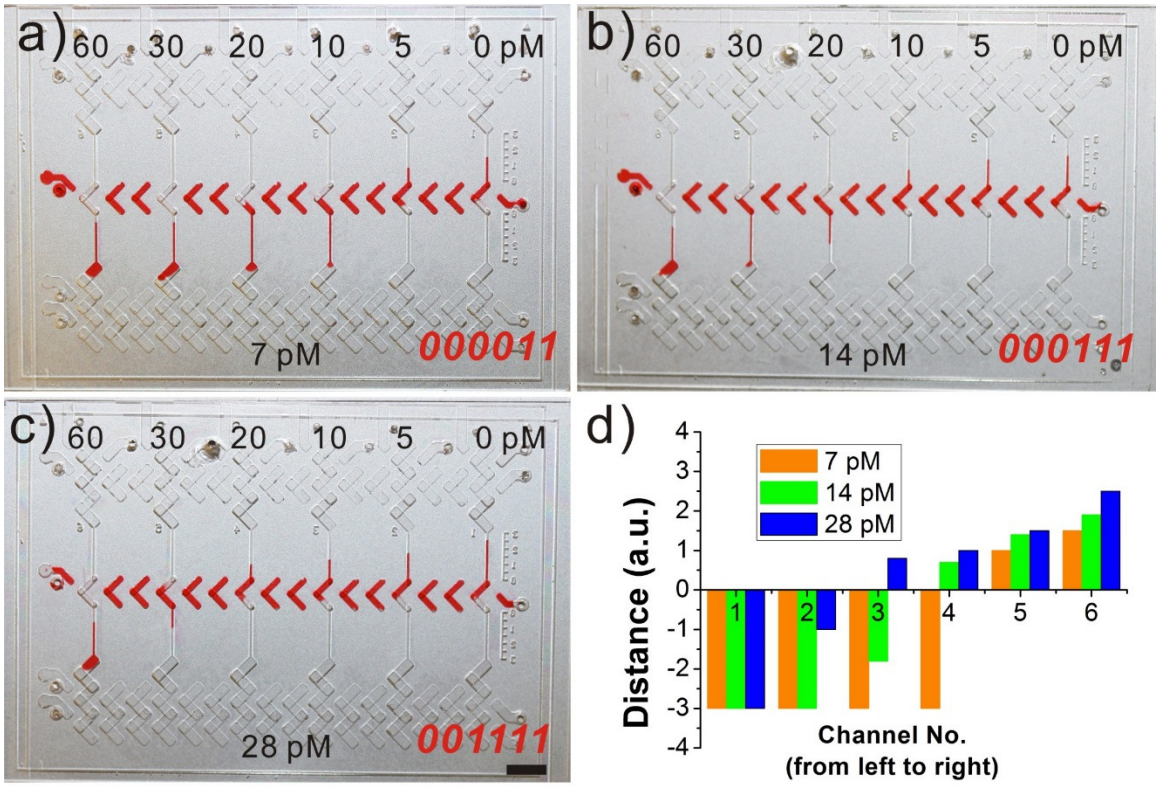

Figure S6 Test of bar-chart formation with selected PtNP concentrations as samples. (a-c) Bar-chart formation with 7 pM (a), 14 pM (b), and 28 pM (c) samples and indicated controls of 0–60 pM PtNPs. Downward bars form when the concentration of the control is greater than that of the experimental sample; upward bars form when the concentration of the experimental sample is greater than that of the control sample. (d) Quantitation of the bar-charts in (a-c). Distances traveled by ink bars exceeding the channel length were designated '3'. a.u., arbitrary units. Scale bar, 0.5 cm for (a-c).

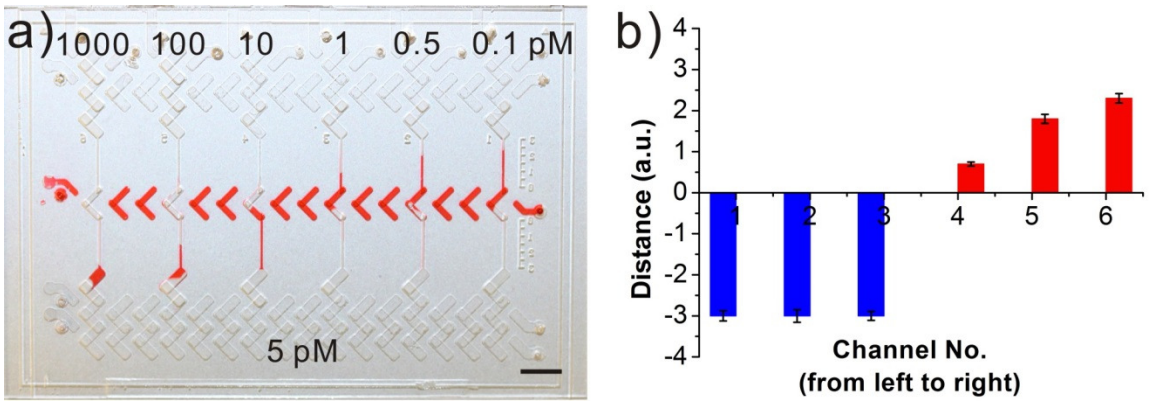

Figure S7 Test of DV-chip dynamic range. Six PtNP solutions with concentration range from 0.1 to 1000 pM were loaded at the top end as controls. Bar chart formation with control (upper labels) and experimental (lower label) PtNP solutions (a) and quantitation (b). Distances traveled by ink bars exceeding the channel length were designated '3'. Values are mean and standard deviation of three independent experiments. a.u., arbitrary units. Scale bar, 0.5 cm for (a). Results demonstrate that DV-chip can be used to detect samples with a wide dynamic range only by changing the concentration range of the controls.

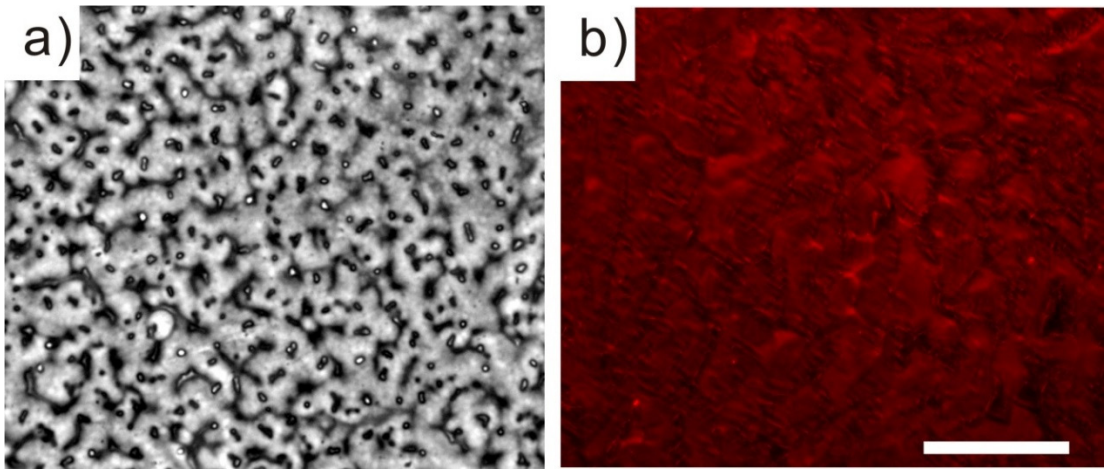

Figure S8 Assessment of efficiency of antibody coating on wet-etched glass surface. (a) Bright field micrograph of wet-etched glass surface. (b) Fluorescence image of rabbit monoclonal anti-CEA antibody coated on wet-etched well and probed with DsRed-conjugated anti-rabbit IgG secondary antibody. Scale bar, 100 μm for (a) and (b).

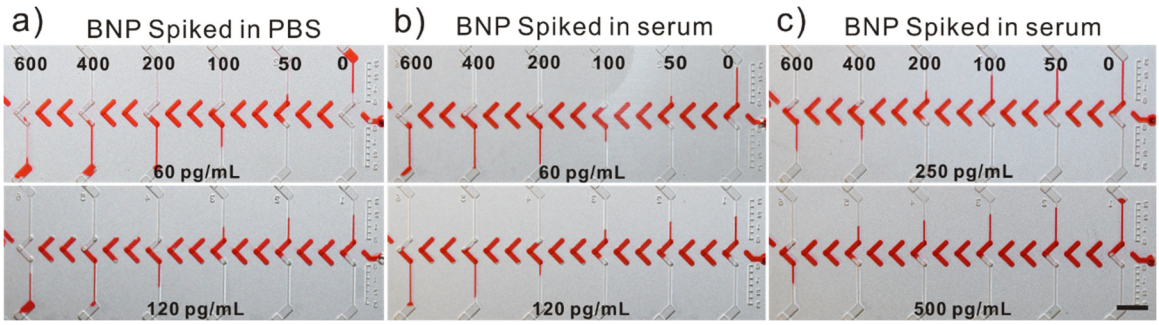

Figure S9 Bar charts for samples of B-type natriuretic peptide (BNP) in PBS and serum. (a, b) Samples of 60 pg/mL (upper) and 120 pg/mL (lower) BNP in PBS (a) and serum (b). The lengths of ink bars for BNP in serum are a little shorter than those for BNP in PBS. (c) Samples of 250 pg/mL (upper) and 500 pg/mL (lower) BNP in serum. Concentrations of control BNP samples are indicated at top. Scale bar, 0.5 cm for (a-c).

Table S1 Clinical information for the HF patients.

Pt. #	Gender	Age (years)	Clinical result (BNP, pg/mL)	Diagnosis result	Pt. #	Gender	Age (years)	Clinical result (BNP, pg/mL)	Diagnosis result
1	M	34	16	Palpitations	11	F	46	23	Hypertenstion
2	M	53	90	Heart failure	12	M	51	850	Heart failure
3	M	62	133	Heart failure	13	F	75	119	Acute respiratory failure
4	M	77	234	Heart failure	14	F	86	530	Malaise, hypertension
5	F	95	481	Osteoporosis	15	F	55	20	Unstable angina
6	M	87	1402	Heart failure	16	M	84	176	Malignant hypertension
7	F	74	64	Hypertension	17	M	40	66	Heart failure
8	F	21	876	Hyperkalemia, renal failure	18	M	53	283	Heart failure
9	F	68	151	Heart failure	19	M	83	420	Small bowel obstruction
10	F	34	226	Heart failure	20	M	23	69	Kaposi sarcoma

HF, heart failure; Pt, patient; BNP, B-type natriuretic peptide; M, male; F, female.