Supporting Information

An Automated and Portable Microfluidic Chemiluminescent Immunoassay for Quantitative Detection of Biomarkers

Binfeng Hu[a,b], Juanjuan Li[a], Lei Mou[a,b], Yong Liu[a,b], Jinqi Deng[a,b], Wei Qian[a,b], Jiashu Sun*[a,b], Ruitao Cha*[a], Xingyu Jiang*[a,b]

[a]CAS Key Laboratory for Biological Effects of Nanomaterials and Nanosafety, CAS Key Laboratory of Standardization and Measurement for Nanotechnology, CAS Center for Excellence in Nanoscience, National Center for Nanoscience and Technology, Beijing 100190, P. R. China

[b]University of Chinese Academy of Sciences, Beijing 100049, P. R. China

E-mail address: xingyujiang@nanoctr.cn; sunjs@nanoctr.cn; chart@nanoctr.cn.

Tel:+86-10-82545558; Fax: +86-10-82545631

Storage stability of patterned antibodies/antigens on tinfoil layer

To fabricate the fully integrated microfluidic device before on-site use, stable storage of pre-patterned antibodies/antigens on tinfoil layer is a prerequisite. In this work, we test the storage time of patterned CRP-Ab_1. After patterning CRP-Ab_1 antibodies (25.0 μg/mL) onto tinfoil and drying at room temperature, the tinfoil is cut into six pieces (each piece containing three identical stripes of CRP-Ab_1 antibodies), which are stored in sealed plastic bags before test (experimental details in "Preparation of the tinfoil layer with patterned antibodies/antigens" at "Materials and Methods" section). The activity of patterned CRP-Ab_1 antibodies is assessed by detecting standard CRP samples (100 ng/mL) inside microfluidic devices for a period of six consecutive days (one piece of tinfoil per day). For each piece of tinfoil with three patterned identical stripes of CRP-Ab_1, nine signal dots are generated in one microfluidic chip after chemiluminescent immunoassay of 100 ng/mL of CRP. These signal dots are used for determination of intra-assay relative standard deviations (RSDs) and inter-assay RSDs. Experimental results indicate that patterned CRP-Ab_1 antibodies could effectively detect CRP samples after six days, while remaining the low intra-assay RSDs (< 12 %) and inter-assay RSDs (7 %) (Table S4).

Simultaneous detection of CRP and IL-6

We perform the simultaneous detection of CRP and interleukin-6 (IL-6) within the single microfluidic device. We first prepare the tinfoil layer with two stripes of patterned antibodies: one stripe for CRP-Ab_1 (25.0 μg/mL), and the other stripe for IL-6-Ab_1 (52 μg/mL) using the methods described in "Preparation of the tinfoil layer

with patterned antibodies/antigens" at "Materials and Methods" section. After assembly of microfluidic device, 60 μL of 3 % BSA solution, 60 μL of mixture sample of CRP (200 ng/mL, 30 μL) and IL-6 (3 ng/mL, 30 μL), 60 μL of 0.5% PBST solution, 60 μL of mixture of CRP-Ab_2 (2.5 μg/mL, 30 μL) and IL-6-Ab_2 (3 μg/mL, 30 μL), 60 μL of 0.5% PBST solution, and 60 μL of CL substrate are preloaded into the inlet reservoirs 1 to 6, respectively. The automated detection of CRP and IL-6 within the single microfluidic device is performed by the customized instrument. Five microfluidic devices are tested, all of which enable simultaneous detection of CRP and IL-6 by chemiluminescent immunoassay (Figure S5). Intra-assay RSDs for CRP or IL-6 detection are within 15 %, and inter-assay RSDs are less than 14 % (Table S5).

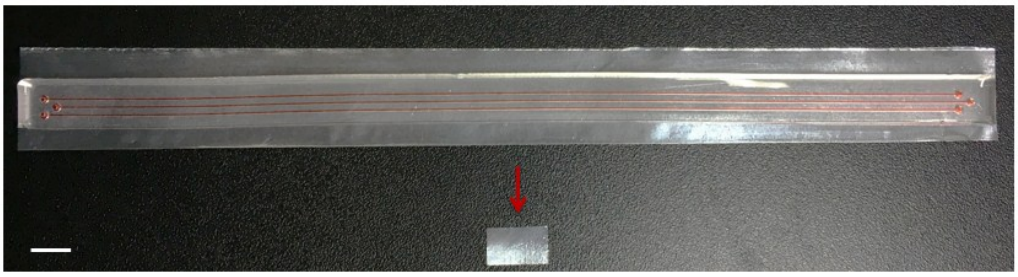

Fig. S1 The process to prepare the middle tinfoil layer with patterned CRP-Ab_1 antibodies/T-BSA antigens stripes using a polydimethylsiloxane (PDMS) chip with three embedded parallel microchannels. There three channels are 500 μm wide, 210 mm long, and 500 μm high, the distance between two adjacent channels is 2.0 mm. Two holes (1.2 mm in diameter) are punched at each end of the channel for injection of CRP-Ab_1 antibodies or T-BSA antigens solution into channel. The process to prepare the middle tinfoil layer includes five steps: (1) placing a PDMS chip onto a cleaned long tinfoil band; (2) injecting about 52 μL CRP-Ab_1 antibodies (25.0 μg/mL) or T-BSA antigens (30.0 μg/mL) solution into each channel and incubating the long tinfoil for 60 min; (3) washing the long tinfoil with 0.01 mol/mL PBS solution; (4) peeling off the PDMS chip; and (5) cutting the long tinfoil with three patterned stripes (each stripe is 500 μm wide and 210 mm long) to obtain the middle tinfoil layer (10 mm wide, 15 mm long, and 50 μm thick with three patterned stripes). Each stripe on the middle tinfoil layer is 15 mm long and 500 μm wide, and the distance between two adjacent stripes is 2.0 mm. Scale bar is 8 mm.

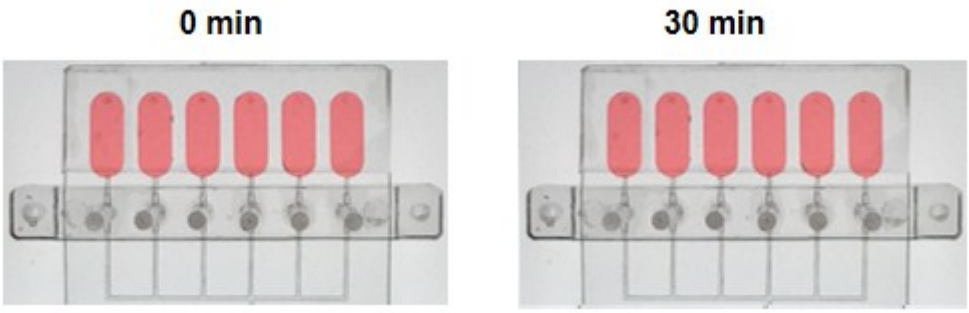

Fig. S2 Baking experiment to test sealing effect of on-chip valves at initial closed state. The baking experiment contains three steps: (1) injecting the red-colored liquid into the six inlet reservoirs; (2) sealing the air holes on reservoirs by 3M adhesive tape; (3) baking the microfluidic device inside an oven at 85 ºC for 30 min. No leakage of liquid into the downstream microchannels is observed before and after baking.

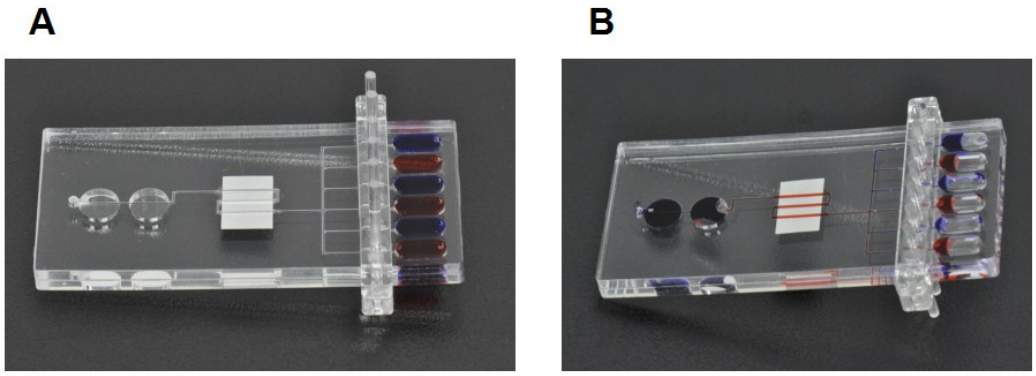

Fig. S3 The photographs of microfluidic chip before (A) and after (B) automated immunoassay. In order to improve visual effect, two different colored dyes are loaded into the inlet reservoirs.

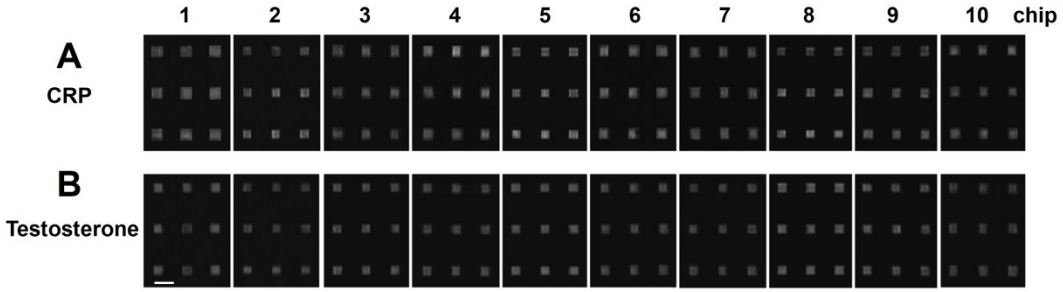

Fig. S4 Reproducibility test. (A) Images of ten chips for CRP (200 ng/mL) detection. (B) Images of ten chips for testosterone (10 ng/mL) detection. Scale bar is 1000 μm.

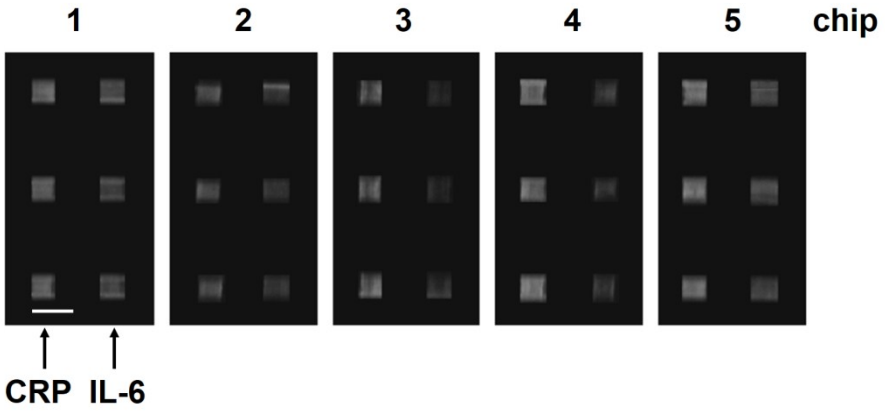

Fig. S5 Simultaneous detection of CRP and IL-6 within the single microfluidic device. The tinfoil layer has two stripes of patterned antibodies: one stripe for CRP-Ab_1 (25.0 μg/mL), and the other stripe for IL-6-Ab_1 (52 μg/mL). 60 μL of mixture sample of CRP (200 ng/mL, 30 μL) and IL-6 (3 ng/mL, 30 μL) is introduced into the device from the sample inlet (reservoir 2). Scale bar is 1000 μm.

Table S1. The intra-assay RSDs (every chip) and inter-assay RSDs (ten chips) for detection of CRP and testosterone. The concentrations of CRP and testosterone are 200 and 10 ng/mL, respectively.

	CRP			Testosterone		
	Spiked (ng/mL)	**Detected (ng/mL)**	**Intra-assay RSDs (%)**	**Spiked (ng/mL)**	**Detected (ng/mL)**	**Intra-assay RSDs (%)**
Chip 1	200	230.53	13.40	10	13.61	10.47
Chip 2	200	229.99	11.10	10	5.10	11.40
Chip 3	200	222.01	1.19	10	5.34	8.69
Chip 4	200	196.18	6.35	10	5.14	8.59
Chip 5	200	230.84	5.64	10	6.44	8.48
Chip 6	200	227.59	8.83	10	9.23	9.56
Chip 7	200	200.16	3.73	10	0.31	11.18
Chip 8	200	189.45	12.65	10	4.01	8.60
Chip 9	200	172.00	0.85	10	1.43	8.70
Chip 10	200	206.57	0.85	10	8.43	11.40
Inter-assay RSDs(%)		9.35			13.07	

Table S2. The LoD of CRP and testosterone. .

	CRP			Testosterone		
	Spiked (ng/mL)	**Gray value**	**Detected (ng/mL)**	**Spiked (ng/mL)**	**Gray value**	**Detected (ng/mL)**
Chip 1	0	169.84	2.12	0	12049.71	-1.65
Chip 2	0	220.72	3.20	0	12294.47	-2.31
Chip 3	0	126.62	1.20	0	12060.26	-1.67
Chip 4	0	142.5	1.54	0	12741.95	-3.52
Chip 5	0	151.49	1.73	0	11838.13	-1.07
Average		162.23			12196.90	
STD		36.23			308.43	
Average±3STD		270.92	4.27		11271.61	0.45

STD = standard deviation

Table S3. Comparison of detection of CRP and testosterone in serum samples by the automated microfluidic chemiluminescent immunoassay and conventional CL-ELISA. Relative values = values from chips/values from 96-well plates.

Samples	CRP			Testosterone		
	Chip (μg/mL)	96-well plates (μg/mL)	Relative values (%)	Chip (ng/mL)	96-well plates (ng/mL)	Relative values (%)
1	34.8	35.0	0.99	3.70	3.81	0.97
2	48.4	51.0	0.95	3.72	3.98	0.94
3	61.3	56.8	1.08	3.08	3.17	0.97
4	17.2	17.7	0.97	5.26	5.28	1.00
5	52.7	46.1	1.14	4.13	3.96	1.04
6	37.8	30.5	1.24	5.06	4.68	1.08
7	53.8	53.4	1.01	4.00	3.49	1.15
8	22.0	20.6	1.07	5.46	5.94	0.92
9	97.3	95.6	1.02	5.26	5.39	0.98
10	58.3	56.7	1.03	3.91	3.57	1.10
11	40.2	40.2	1.00	6.76	6.54	1.03
12	107.8	97.4	1.11	4.57	4.70	0.97
13	98.1	89.7	1.09	3.03	3.44	0.88
14	75.3	73.6	1.02	5.54	4.92	1.13
15	77.8	67.5	1.15	2.96	2.97	1.00
16	71.7	55.7	1.29	4.15	3.89	1.07

Table S4. Storage stability of patterned CRP-Ab_1 (25.0 μg/mL) on tinfoil layer, which is assessed by detecting standard CRP samples (100 ng/mL) inside microfluidic devices for a period of six consecutive days.

CRP	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**
Spiked (ng/mL)	100	100	100	100	100	100
Detected (ng/mL)	112.68	97.19	103.78	95.06	92.55	101.24
Intra-assay RSD (%)	7.02	5.14	0.09	11.29	8.47	6.13

Table S5. Simultaneous detection of CRP and IL-6 within the single microfluidic device.

	CRP			IL-6		
	Spiked (ng/mL)	**Detected (ng/mL)**	**Intra-assay RSDs (%)**	**Spiked (ng/mL)**	**Detected (ng/mL)**	**Intra-assay RSDs (%)**
Chip 1	200	196.64	0.47	3	3.52	5.04
Chip 2	200	195.40	3.65	3	3.15	15.5
Chip 3	200	209.18	10.56	3	2.56	14.11
Chip 4	200	196.34	0.25	3	2.62	16.2
Chip 5	200	228.91	1.86	3	3.22	2.87
Inter-assay RSDs (%)		7.00			13.63	

Table S6. Comparison between microfluidic device and conventional ELISA.

	Conventional ELISA[1]	Microfluidic Device
Function integration	Low	High
Sample volume (μL)	~ 100	≤ 60
Time (Min)	≥ 240	~ 70
Reagent volume (μL)	~ 100	≤ 60
Sensitivity	μg/mL	ng/mL
Manipulation	Complicated	Simple

References

1. T. Wu, K. Tsao, C. P.-Y. Chang, C. Li, C. Sun and J. T. Wu, *Clin. Chim. Acta*, 2002, **322**, 163-168.