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Electronic Supplementary Information

Rechargeable Battery-triggered Electrochemiluminescence Detection on Microfluidic Origami Immunodevice Based on Two Electrodes

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Reagents

Human chorionic gonadotropin (HCG) antigen, capture anti-HCG antibody ($McAb_1$) and signal anti-HCG antibody ($McAb_2$) were purchased from Shanghai Linc-Bio Science Co. Ltd. (Shanghai, China). L-cysteine (L-Cys) and bovine serum albumin (BSA, 96–99%) were purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). 100 nm-thick white gold foils (Au/Ag alloy, 50 : 50 wt%) were obtained from Monarch. Dimethylsulfoxide, Nitric acid, 1-ethyl-3-(3 dimethylaminopropyl) carbodiimide (EDC) and N-hydroxysuccinimide (NHS) were obtained from Alfa Aesar China LTd (Tianjin, China). Whatman chromatography paper #1 (200.0 mm×200.0 mm) (pure cellulose paper) was obtained from GE Healthcare Worldwide (Pudong Shanghai, China) and used with further adjustment of size. Carbon ink (ED423ss) and silver/silver choride (Ag/AgCl) ink (CNC-01) were purchased from Acheson. Blocking buffer for blocking the residual reactive sites on the antibody immobilized paper was pH 7.4 phosphate buffer solution (PBS) containing 0.5 % BSA and 0.5 % casein. All other reagents were of analytical grade, and all solutions were prepared using Millipore (model Milli-Q) purified water. The clinical urine samples were from Cancer Research Center of Shandong Tumor Hospital.

Characterization of the self-assembly power supply

To verify the applicability and validity of the present power supply, a multifunction digital voltage meter (PF66G) was used to measure the output voltage. The result (Table S1) showed that the agreement between the theoretic voltage (+1.20 V) and the practical output voltage was acceptable. It can be seen that this proposed rechargeable battery-based power supply may be

suitable for fabricating two-electrode-based ECL constant-potential (+1.20 V) instead of expensive electrochemical workstations for point-of-care application. After a full charge is reached, this power supply can be used more than 1000 times with a relative error (between the theoretic voltage and the practical output voltage) within 3 %. In addition, when the output voltage was measured at internals of three days, no obvious change (relative standard deviation< 4 %) was observed after 30 weeks. More importantly, all electronic components used were purchased from a local electronics store, and the total cost of this power supply was less than ~2 £.

Table S1. The comparison between the practical output voltage and the theoretic voltage

Item \ No.	1#	2#	3#	4#	5#	6#
Theoretic voltage (V)	1.20					
Practical output voltage (V)	1.18	1.20	1.19	1.22	1.21	1.20
Relative error (%)	-1.67	0	-0.83	1.67	0.83	0
Relative standard deviation (%)	1.41					

Fabrication and characterization of microfluidic origami electronic device

The fabrication procedure of this microfluidic origami device was similarly to our previous work,[1] which (Scheme 1) consisted of wax-printing, baking the wax-patterned sheet, screen-printing electrodes, air drying the screen-printed electrodes, followed by cutting. For wax-printing hydrophobic barriers on rectangular paper sheet (20.0 cm×20.0 cm) by a wax printer (FUJIXEROX Phaser 8560DN, Japan) in bulk (Procedure (1)), the configuration of hydrophobic

barriers was designed using Adobe illustrator CS4. The microfluidic origami electronic device was 60.0 mm×30.0 mm, which consisted of an unprinted line and two circular paper working zones (circle-A, 6.0 mm in diameter; circle-B, 8.0 mm in diameter). The unprinted line was defined as folding line, which could ensure that the electrochemical cells on the microfluidic origami electronic device are properly and exactly aligned after folding. The two circle paper working zones were, respectively, designed for screen-printing carbon working electrode (circle-A) and Ag/AgCl auxiliary electrode (circle-B). After baking the wax-patterned paper sheet in an oven at 130 □ for 150 s, the as-prepared sheet (Fig. S1) was ready for printing electrode after cooling to room temperature (within 1 min). Due to the small size of this device, the silver wires and contact pads were unnecessary and can be directly replaced by carbon ink and Ag/AgCl ink, respectively. The disk-like carbon working electrodes (5.0 mm in diameter) were screen-printed onto the circle-A paper working zones, and the half-ring like Ag/AgCl auxiliary electrodes were screen-printed onto the circle-B paper working zones (Procedure (2). Then, the screen-printed electrodes on wax-patterned paper were allowed to air dry for 5 min. Followed by cutting (Procedure (3)), a 3D electrochemical cell with a volume of ~8 μL was activated by folding the rectangular microfluidic origami electronics in half. Then, the prepared screen-printed electrodes were polished to and fro along the direction of printing the conductive ink using an agate lapping hammer until the surface turned smooth and shiny, and surface of the screen-printed electrodes was rinsed several times by Millipore purified water and dried. Ultimately, the polished screen-printed electrodes were activated in PBS (pH=7.4) solution containing potassium ferricyanide under cyclic voltammogram for 3 min.

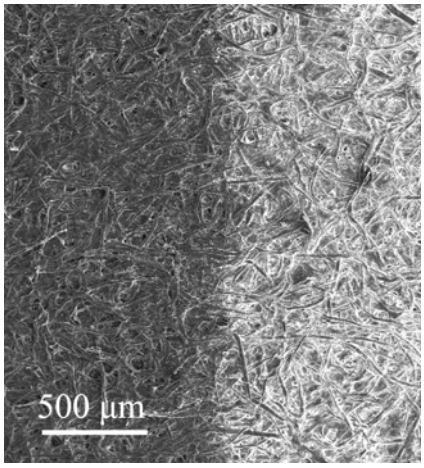


Fig. S1 Scanning electron microscope (SEM) images of the boundary of wax pattern: left is wax-patterned paper after baking, right is pure paper.

The detailed washing procedure was as follows: Because the front and back surfaces of the wax-patterned electrochemical cells on cirle-A were open to the atmosphere, the working electrode could be washed according to a reported method proposed by us:[2] The wax-patterned electrochemical cell was washed by adding a washing buffer onto the top of the wax-patterned electrochemical cell whilst pressing the bottom of the wax-patterned electrochemical cells against a piece of blotting paper. The washing buffer permeates the wax-patterned electrochemical cell into the blotting paper, thereby carrying any unbound reagents with it. The printed working electrode was firmly attached to the paper surface, owing to the penetration of binding reagents in the inks into the paper matrix, and thus did not break or peel off from the device upon washing and folding. The washing procedure was repeated twice to make sure the washing was performed completely. These washing procedures could be finished within 2 min. The washing process was important for preventing nonspecific binding and for achieving the best possible signal-to-background-noise ratio. Another purpose for this washing procedure was to stop the incubation reaction.

In this microfluidic origami electronic device, the two electrodes were, respectively, fabricated in two paper working zones, rather than one paper working zone. The hydrophobic region between the two paper working zones prevented the diffusion of reagents from the working electrode to the Ag/AgCl auxiliary electrode. As a result, influence and contaminate from the bulk operations of the immunoreactions and washing process on working electrode to Ag/AgCl auxiliary electrode can be easily eliminated.

Fabrication of the immunodevice and ECL assay procedure

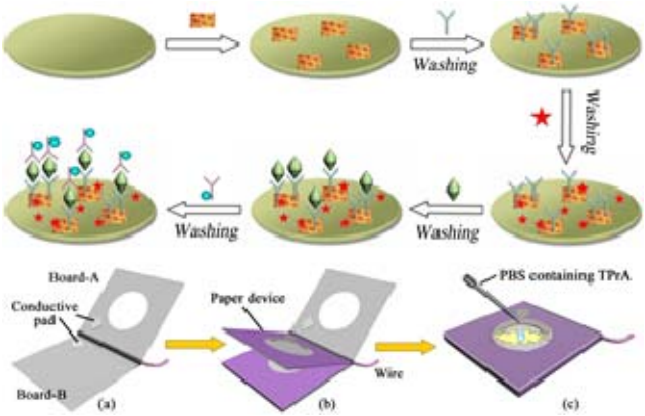
Preparation of $Ru(bpy)_3^{2+}$ labeled signal antibody ($McAb_2$)

The $Ru(bpy)_3^{2+}$ labeled $McAb_2$ was obtained by adding 2.0 μL DMSO containing 100 μg $Ru(bpy)_3^{2+}$ to 50.0 μL of phosphate buffer saline (PBS, pH 7.4) solution containing $McAb_2$ (1.0 $mg \cdot mL^{-1}$). After 40 min incubation at room temperature in the dark, the mixture was filtered through a Sephadex G-25 PD-10 desalting column and eluted with PBS (pH 7.4). The purified $Ru(bpy)_3^{2+}$ labeled $McAb_2$ was further diluted with PBS to 1.0 mL and kept at 4 □ until use.

Preparation nanoporous gold (NPG) modified working electrode

NPG was prepared by selective dissolution (dealloying) of silver from Au/Ag alloy according to a reported method.[3] The alloy (1.0 cm×1.0 cm) was dispersed in the concentrated nitric acid (16.0 M) at 25 ℃. Upon silver dissolution, gold atoms left behind will self-organize into an interconnected network of pores and ligaments. The NPG was repeatedly washed with Milli-Q water until the pH was neutral. Neutral NPG solution was added onto the working electrode and dried. Finally, the NPG modified platform was washed by PBS.

Fabrication of the immunodevice



Scheme S1 Schematic diagram of the fabrication of the immunosensor (A), and ECL assay procedure after inserting the as-prepared μ-PADs into the device-holder (B): (a) the folder-type device-holder, (b) the as-preparedμ-PADs in the device-holder, (c) after folding the device-holder, PBS solution (pH 7.4) containing TPrA substrate was added through the hole on the board-A by a commercially available micropipettor in this work (or a home-made, low-cost, and one-off dropper with a total volume of 2.0 μL for the field application in our future work).

The immunodevice fabrication procedures were shown in Scheme 2A, and a detailed procedure was described below. 2.0 μL of L-Cys solution (0.10 M) was added onto the NPG modified working electrode and dried. After washed with Milli-Q water for three times, 2.0 μL of PBS (0.10 M, pH 7.4) containing 1.0 $mg{\cdot}mL^{-1}$ $McAb_1$, 10 $mg{\cdot}mL^{-1}$ NHS and 20 $mg{\cdot}mL^{-1}$ EDC were added and dried at room temperature. Excess $McAb_1$, NHS and EDC were washed with PBS. Then, the working electrode was blocked by adding 2.0 μL of PBS blocking buffer to block possible remaining active sites against nonspecific adsorption, and allowing the working electrodes to dry for 15 min. After another washing, the resulting immunodevice and all reagents were stored under dry conditions at 4℃ prior to use.

ECL assay procedure

The ECL assay procedure was shown in Scheme 2A, and a detailed procedure was described below. Briefly, HCG antigen solution (2.0 μL) with a varying concentration was added and allowed to incubate for 10 min at room temperature, followed by washing with PBS. The prepared $Ru(bpy)_3^{2+}$ labeled $McAb_2$ (2.0 μL) was dropped onto the working electrode and allowed to

incubate for 10 min at room temperature. After washing with PBS, the as-prepared 3D microfluidic origami immunodevice was put into the device-holder (Scheme 2B). Then, 2.0 μL of PBS solution containing 1 mM TPrA (pH 7.4) was added through the hole on board-A, and the prepared ECL immunodevice was placed in front of the PMT ready for HCG antigen detection. Finally, a +1.20 V constant-potential (vs. Ag/AgCl auxiliary electrode) provided by the rechargeable battery-based power supply was applied on the two electrodes of the microfluidic origami electronics to trigger the ECL at room temperature. By analyzing the ECL emissions related to the HCG antigen concentrations, HCG antigen was detected.

Characterization of NPG modified working electrode

To investigate the effect of NPG, electrochemical impedance spectroscopy (EIS) of the working electrode was performed in a background solution of 5.0 $mmol \cdot L^{-1}$ $[Fe(CN)_6]^{3-/4-}$ containing 0.1 $mol \cdot L^{-1}$ KCl. Fig. 2A showed the Nyquist plots of EIS corresponding to the stepwise modification processes. The curve a showed the EIS of bare working electrode after polishing, including a small semicircle portion and a linear portion, and the semicircle diameter corresponds to the electron-transfer resistance. When NPG was used to modify the bare working electrode, a much lower resistance was obtained (curve b), implying that the NPG could accelerate the electron transfer probably due to the high surface area and high conductivity of NPG. Compared with the working electrode surface modified only with capture antibody ($McAb_1$) (curve d), the working electrode surface modified with NPG and $McAb_1$ (curve c) displayed a lower resistance, further demonstrated that the NPG could improve the electron transfer of the immuno-electrode effectively to enhance the sensitivity. Furthermore, the enhancement effect of NPG for sensitivity was confirmed through control experiments using unmodified working

electrodes as solid supports for $McAb_1$ (Fig. S2B). The result displayed that it showed higher ECL intensity when NPG modified working electrode (curve a) was used as the solid support than unmodified working electrode solid support (curve b). Thus, NPG indeed enhanced the ECL response of $Ru(bpy)_3^{2+}$-TPrA system. This enhancement effect could be attributed to the fact that when NPG were used to modify the working electrode, the NPG accelerated the electron transfer and then enhanced the ECL response.

In addition, as mentioned above, although NPG modified working electrodes showed excellent properties, when $McAb_1$ was immobilized on the modified electrode surface, however, the increment of resistance on NPG modified working electrodes (curve b to curve d) was similar to that on unmodified working electrode (curve a to curve c), indicated that more $McAb_1$ were successfully immobilized on NPG modified working electrode than unmodified working electrode.

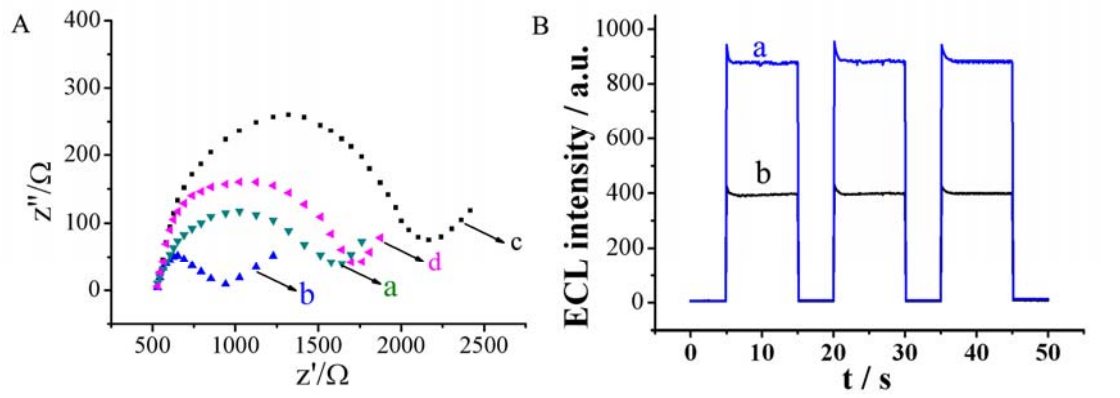


Fig. S2 (A) Electrochemical impedance spectroscopy (EIS) of the bare working electrode after polishing (a); NPG modified working electrode (b); capture antibody ($McAb_1$) modified bare working electrode (c); NPG and $McAb_1$ modified working electrode (d); (B) The ECL performance of 100 $mIU \cdot mL^{-1}$ using different working electrodes: (a) NPG modified working electrode and (b) unmodified working electrode

Optimization of experimental conditions

A series of experiments were conducted to select optimal analytical conditions. The highly

acidic or alkaline surroundings would affect the immobilization of $McAb_1$ and the immunoreaction of antibody-antigen, so the effect of pH was investigated. With the increasing of pH from 6.0 to 7.4, the ECL intensity increased. When the pH value of the buffer solution was bigger than 7.4, the ECL intensity was decreased. Accordingly, the PBS solution of pH 7.4 (Fig. S3A) was chosen for further ECL detection. The incubation time is an important parameter affecting the analytical performance and time efficiency of immunoassay. The effect of incubation time on the ECL intensity was investigated. With an increasing incubation time, the ECL intensity increased quickly and reached a plateau from 10 min to 16 min as shown in Fig. S3B. Thus, the ECL immunoassay can be performed indistinguishably in this time range. Furthermore, in the field application, a cheap and simple wrist watch is competent to control the incubation time in this time range.

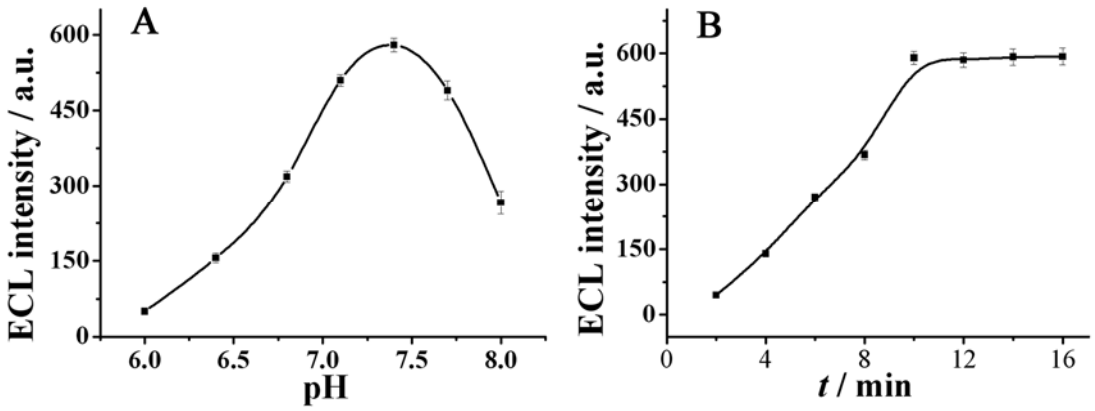


Fig S3 Optimization of experimental conditions: effect of pH value (A) and incubation time (B) on ECL intensities with 10 $mIU \cdot mL^{-1}$ HCG.

Analytical performance

The limit of detection is calculated at the concentrations which produced three times ECL_{blank} (signal-to-noise (S/N) = 3, n=11). As shown in Table. S2, the HCG antigen solution of 0.0019

mIU·mL^{-1} produced three times ECL_{blank}. Consequently, the limit of detection was calculated as 0.0019 mIU·mL^{-1}.

Table S2 The calculation of detection limit.

c_{HCG} / mIU·mL^{-1}	ECL intensity / a.u. (n=11)	Average	S/N
0	10, 12, 12, 11, 12, 10, 10, 12, 11, 12, 11	11.18	3.01
0.0019	32, 34, 32, 32, 34, 35, 33, 34, 35, 34, 35	33.64	

Considerable efforts have been directed at using various detection methods, including enzyme-linked immuno sorbent assay (ELISA),[4] lateral flow tests,[5] electrochemical immunoassay (ECIA),[6] mass spectrometric analysis (MSA)[7] and immunochemiluminometric assay (ICMA),[8] to achieve accurate detection results. Compared with these above methods (Table S3), our proposed immunosensor exhibited a wider linear range and a lower detection limit, especially much better than that using ELISA or lateral flow tests.

Table S3. Comparison of the linear range and detection limit using different methods for determination of HCG.

Method	Linear range /mIU·mL^{-1}	Detection limit /mIU·mL^{-1}	References
ELISA	6-200	Not given	[4]
Lateral flow tests	25-250	Not given	[5]
ECIA	0.05-150	0.023	[6]
MSA	0.2-100	0.1	[7]
ICMA	1-100	0.3	[8]
ECL	0.005– 4000	0.0019	This work

When this paper-based immunodevice and the ECL reagents ($Ru(bpy)_3^{2+}$ labeled signal antibody ($McAb_2$) and TPrA) was stored and measured at intervals of three days, no obvious change (4.12 % relative standard deviation) was observed after 2 weeks under ambient conditions while 3 weeks (2.93 % relative standard deviation) under dry conditions at 4□. These results indicated that the immunosensor array has acceptable stability and suitability for storage or long-distance transport to remote regions and developing countries. The interference of foreign species was studied by analyzing a standard solution of 1.0 mIU·mL^{-1} HCG antigen to which increasing amounts of interfering analyte was added. The results indicate that 200 mg·mL^{-1} L-cysteine, 100 mg·mL^{-1} ascorbic acid (Vc), 50 mg·mL^{-1} glucose, 100 mg·mL^{-1} human serum albumin (HSA), 100 mg·mL^{-1} carcinoma embryonic antigen (CEA) and 200 mg·mL^{-1} r-fetoprotein had no interference on the determination of HCG antigen (Table S4).

Table S4. The interference of foreign species studied using a 1.0 mIU·mL^{-1} HCG antigen standard solution

HCG	Foreign species		Relative error (%)
(ECL /a.u.)	Tolerable concentration	ECL /a.u.	
424	200 mg·mL^{-1} (L-cysteine)	440	3.77
426	100 mg·mL^{-1} (Vc)	406	-4.69
422	50 mg·mL^{-1} (glucose)	439	4.03
423	100 mg·mL^{-1} (HSA)	406	-4.02
427	100 mg·mL^{-1} (CEA)	408	-4.45
428	200 mg·mL^{-1} (r-fetoprotein)	443	3.50

Analysis of HCG in real human urine samples

To verify the applicability and reliability of the proposed ECL immunodevice for real biological samples, the detection of HCG antigen in clinical urine samples was conducted by this method and compared with reference values obtained by commercially used electrochemiluminescence method in Cancer Research Center of Shandong Tumor Hospital. A total of 4 urine samples were analyzed and the results are shown in Table S5. HCG antigen contents detected by the two methods agreed well, indicating acceptable reliability and accuracy. The total assay process of this proposed ECL immunosensor included incubation, washing, and detection. The incubation time has been optimized as 10 min. Since a washing procedure can be finished within 2 min, the required time to run an assay from start to finish is less than 25 min, which was much shorter than those of more than 1 h to overnight with other methods.[9]

Table S5 Detection of results of real samples by the proposed method and reference method

Method \ Sample		1 #	2 #	3 #	4 #
Proposed Method	Detection result* ($mIU \cdot mL^{-1}$)	8.73	26.95	49.58	108.55
	Standard deviation (SD)	0.34	0.81	2.06	4.91
	Relative standard deviation (RSD, %)	3.89	3.01	4.15	4.52
Reference method* ($mIU \cdot mL^{-1}$)		8.41	28.06	51.98	104.23
Relative error (%)		3.69	-3.96	-4.62	4.14

References

1 P. P. Wang, L. Ge, M. Yan, X. R. Song, S. G. Ge and J. H. Yu, *Biosens. Bioelectron.*, 2012, **32**, 238.

2. J. X. Yan, L. Ge, X. R. Song, M. Yan, S. G. Ge, J. H. Yu, *Chem. Eur. J.* 2012, **18**, 4938.

3. M. N. Kashid and L. K. Minsker, *Ind. Eng. Chem. Res.*, 2009, **48**, 6465.

4. P. Podrouzek, Z. Krabec, P. Mancal, J. Presl, *J. Hyg. Epidemiol. Microbiol. Immunol.,* 1988, **32**, 467.

5.http://www.berktree.com/sa-scientific-lateral-flow-cassette-tests-hcg-serum-urine-tests-model-87730-pack-of-30.html.

6. H. C. Yang, R. Yuan, Y. Q. Chai, H. L. Su, Y. Zhuo, W. Jiang, Z. J. Song, *Electrochim Acta.*, 2011. **56**, 1973.

7. L.H. Gam, S. Y. Tham, A. Latiff, *J. Chromatogr. B.,* 2003, **792**, 187.

8. R. Pandian, J. L Lu, J. O. Plewnia, *Clin.Chem.*, 2003, **49**, 808.

9. E. R. Goldman, A. R. Clapp, G. P. Anderson, H. T. Uyeda, J. M. Mauro, I. L. Medintz and H. Mattoussi, *Anal. Chem.,* 2003, **76**, 684; G. Liu, J. Wang, J. Kim, M. R. Jan and G. E. Collins, *Anal. Chem.,* 2004, **76**, 7126.