Electronic Supplementary Information

3D origami multiple electrochemiluminescence immunodevice based on porous silver-paper electrode and multi-labeled nanoporous gold/carbon sphere

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Reagents

Human carcinoembryonic antigen (CEA), carcinoma antigen 125 (CA125), and r-fetoprotein (AFP) standard solutions, mouse monoclonal capture antibodies ($McAb_1$) and signal antibodies were purchased from Linc-Bio Science Co. Ltd. (Shanghai, China). The clinical serum samples and surrogate animal serums were from Shandong Tumor Hospital. Thioglycolic acid (TGA), 1-ethyl-3-(3 dimethylaminopropyl) carbodiimide (EDC), luminol and N-hydroxysuccinimide (NHS) were from Alfa Aesar China LTd. $Ru(bpy)_3^{2+}$-NHS ester were obtained from IGEN (Gaithersburg, MD). Poly(diallyldimethylammonium chloride) (PDDA), bovine serum albumin (BSA), and glucose oxidase (GOx) were obtained from Sigma-Aldrich Chemical Co. (USA). Glucose was obtained from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). $AgNO_3$ (as the precursor for the formation of Ag nanoparticles (AgNPs) seeds), $NaBH_4$, and trisodium citrate were obtained from Shanghai Reagent Company (Shanghai, China). Ultrapure water obtained from a Millipore water purification system (>18.2 MΩ, Milli-Q, Millipore) was used in all assays and solutions. Phosphate buffered solutions (PBS) (pH 7.4, 10.0 mM) was prepared with NaH_2PO_4 and Na_2HPO_4. The washing buffer was PBS (10.0 mM) containing 0.05% (w/v) Tween-20. PBS (10.0 mM) containing 0.5% (w/v) BSA and 0.5% (w/v) casein was used as blocking solution. The mixture solution of 0.50 mM $AgNO_3$ and 0.25 mM ascorbic acid (AA) was prepared daily for silver-growth enhancement. All other reagents were of analytical grade and used as received. Whatman chromatography paper #1 (58.0 cm × 68.0 cm) (pure cellulose paper) was obtained from GE Healthcare Worldwide (Pudong, Shanghai, China) and used with further adjustment of size (A4 size).

Abbreviations

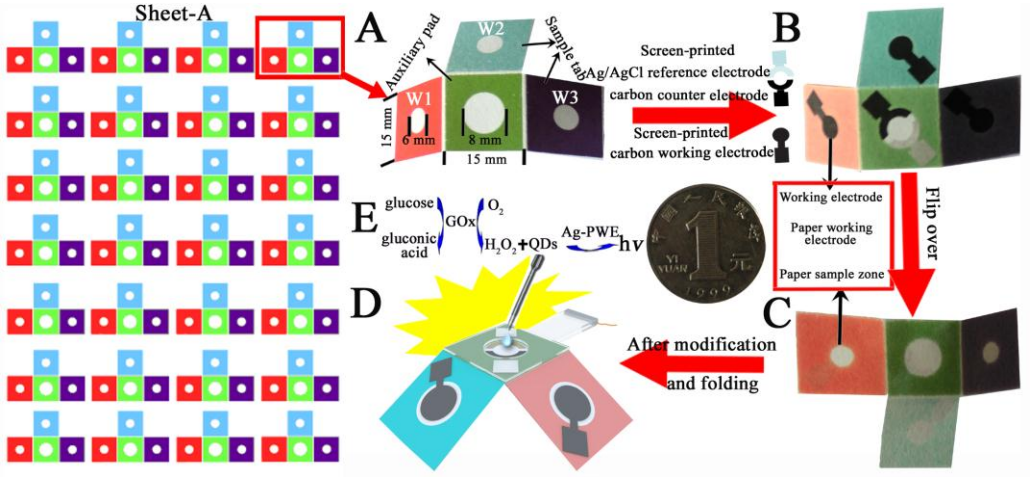
ECL	electrochemiluminescence
μ-PADs	microfluidic paper-based analytical devices
PWEs	paper working electrodes
AgNPs	silver nanoparticles
TGA	thioglycolic acid
EDC	1-ethyl-3-(3 dimethylaminopropyl) carbodiimide
NHS	N-hydroxysuccinimide
PDDA	poly(diallyldimethylammonium chloride)
GOx	glucose oxidase
AA	ascorbic acid
BSA	bovine serum albumin
DMSO	dimethylsulfoxide
PMT	photomultipier tube
CNSs	carbon nanospheres
NPG	nanoporous gold
QDs	CdTe quantum dots
PBS	phosphate buffered solutions
POC	point-of-care
SPCEs	screen-printed carbon electrodes
RSD	relative standard deviation
EIS	electrochemical impedance spectroscopy
CV	cyclic voltammetry
SEM	scanning electron microscopy
TEM	transmission electron microscopy
XPS	X-ray photoelectron spectroscopy
UV-vis	ultraviolet visible
CEA	carcinoembryonic antigen

CA125	carcinoma antigen 125
AFP	r-fetoprotein
$McAb_1$	capture antibodies
$McAb_2$	$Ru(bpy)_3^{2+}$ labeled signal CEA antibodies; luminol labeled signal CA125 antibodies; QDs labeled signal AFP antibodies

Preparation of this 3D origami electrochemiluminescence device

The preparation of the 3D origami electrochemiluminescence (ECL) device was similar to our previous work[1] with major modifications and a detailed procedure was described below. Wax printing is rapid, inexpensive, and particularly well-suited for producing large lots (hundreds to thousands) of prototype microfluidic paper-based analytical devices (μ-PADs)[2]. The shape for hydrophobic barrier on origami device was designed using Adobe illustrator CS4. The entire origami device could be produced in bulk on an A4 paper sheet by a commercially available solid-wax printer (Xerox Phaser 8560N color printer). Then, the wax-printed paper sheet was placed on a digital hot plate set at 150 °C for 130 s. This fabrication process can be finished within 2 min. As shown in Scheme S1A, this origami ECL device was comprised of an auxiliary pad surrounded by three sample tabs with the same size (15.0 mm × 15.0 mm). The shape of hydrophobic barrier on origami ECL device, which contained a paper auxiliary zone (8 mm in diameter) on auxiliary pad and three paper sample zones (6 mm in diameter) on three sample tabs respectively. Then, the wax-penetrated paper sheet was ready for screen-printing of electrodes on their corresponding paper zones. The electrode array consisted of a screen-printed Ag/AgCl reference electrode and carbon counter electrode on the paper auxiliary zone and three screen-printed carbon working electrodes on the three paper sample zones respectively. Finally, the paper sheet was cut into individual origami device for further modifications (Scheme S1B,C).

After folding, the three screen-printed electrodes (working electrode, reference electrode, and counter electrode) would be connected once the paper electrochemical cell was filled with solution.



Scheme S1 Schematic representation the fabrication and assay procedure of this 3D origami ECL device. Paper sheets were firstly patterned in bulk using a wax printer (sheet-A). (A) One 3D origami ECL device without the screen-printed electrodes. Then electrodes were screen-printed on sheet-A in bulk. Finally, sheet-A was cut into individual 3D origami ECL device (B,C). After modification, the immunodevice was integrated with a transparent device-holder (D). The mechanism of the signal amplification strategy (E).

Fabrication of Ag-PWE

Prior to the $McAb_1$ immobilization, growth of AgNP layer on the surfaces of cellulose fibers in the paper sample zone of paper working electrode (PWE) was implemented to fabricate porous Ag-PWE with enhanced conductivity, enlarged effective surface area, and favorable catalysis activity for sensitive immunosensing. First, the suspension of AgNP seeds was prepared by using $NaBH_4$ as reductant and stabilized with sodium citrate according to the literature[3]. Then, as-prepared AgNP seeds solution (45.0 μL) was divided into three portions and dropped into the bare PWEs (named W1, W2, and W3 respectively below) (Scheme S1A). Then the origami device

was equilibrated at room temperature for 1 h to optimize the surface immobilization of AgNP seeds on cellulose fibers. After rinsing with water thoroughly according to the method in our previous work[4] to remove loosely bound AgNP seeds, freshly prepared silver-growth solution (15.0 μL) containing 0.50 mM $AgNO_3$ and 0.25 mM AA was applied into the AgNP seeded PWEs, respectively, and incubated at room temperature for 4 min. During the growth process, the AgNP seeds acted as catalysts for the reduction of $AgNO_3$ by AA, resulting in the enhancement of the AgNP seeds[5]. Subsequently, the resulting Ag-PWEs were washed with water thoroughly. Thus a layer of interconnected AgNP on cellulose fibers with good conductivity was obtained (Scheme 1B), which was dried at room temperature for 20 min.

Characterization of the 3D origami ECL device

The hydrophilic electrochemical cells were fabricated by printing wax on the paper surface in a high-resolution printing mode (Fig. S1A). Owing to the 3D porous structure of paper, the melted wax could penetrate into the paper to decrease the hydrophilicity of paper remarkably (Fig. S1B). For the wax-patterned paper, the printed area sustained an apparent contact angle for water of 118° after baking, and the unprinted area maintained good flexibility, hydrophilicity and 3D porous structure would not affect the further screen-printing of electrodes and modifications.

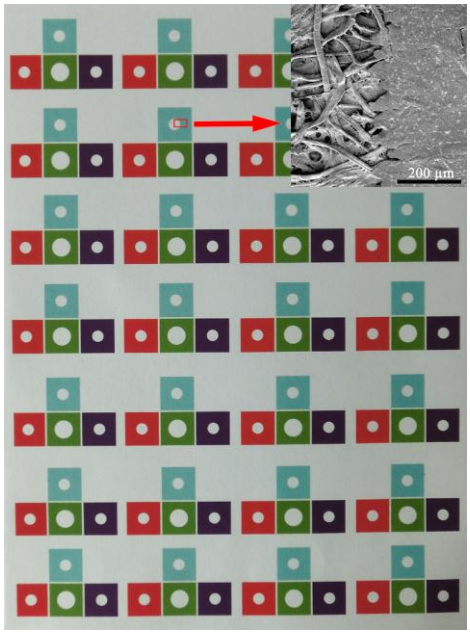


Fig. S1A Wax-printed 3D origami ECL device on a paper sheet (A4) before baking. Inset: scanning electron microscopy (SEM) image of the boundary of the wax-pattern: pure cellulose paper (left) and wax-printed paper (right).

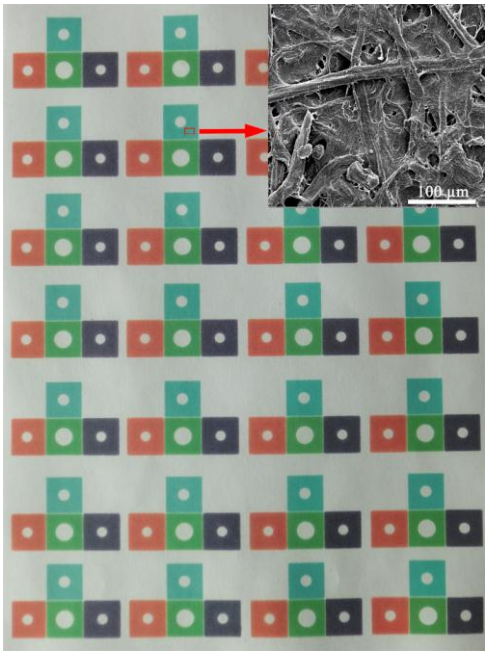


Fig. S1B Wax-printed 3D origami ECL device on a paper sheet (A4) after baking. Inset: SEM image of the wax-penetrated cellulose paper.

Characterizations of Ag-PWE

The PWE was modified through the growth of an AgNP layer on the surfaces of cellulose fibers in paper sample zone from AgNP seeds to fabricate the porous Ag-PWE. Fig. S2A showed the transmission electron microscopy (TEM) image of the as-prepared AgNP seeds solution; the average diameter was about 4 nm. As shown in Fig. S2B, the porous bare paper sample zone, possessed high ratio of surface area to weight (9.5 m^2/g)[6] with rough cellulose fibers, could offer an excellent adsorption microenvironment for the AgNP seeds. It could be seen that there was no apparent structural and surface difference between the bare (Fig. S2B) and the AgNP seeds (Fig. S2C) cellulose fibers in the paper sample zone of PWE due to the small particle size of AgNP seeds (Fig. S2D). In addition, the successful immobilization of AgNP seeds on cellulose fibers was confirmed by X-ray photoelectron spectroscopy (XPS) (Fig. S2E) and the peaks observed at 368 and 374 eV were ascribed to metallic silver (inset, Fig. S2E).

The growth process of the AgNP layer on the surfaces of cellulose fibers was tracked by scanning electron microscopy (SEM) characterization (Fig. S3). As the growth time increased (from 0 to 2 min), the AgNP seeds were rapidly enlarged by incubating in the growth solution under the self-catalytic reduction mechanism of AgNP growth[5] (Fig. S3A,C,E). Finally, as shown in Fig. S3B,D,F, a continuous and dense AgNP conducting layer with interconnected AgNPs on the cellulose fiber surface in paper sample zone was observed, indicated that a good coverage of AgNPs on the surfaces of the cellulose fibers was obtained after 4 min of growth. In addition, as shown in Fig. S3B, the AgNP modified paper sample zone still maintained good porous structure after the growth process, which would benefit the further antibodies modification and analytical application.

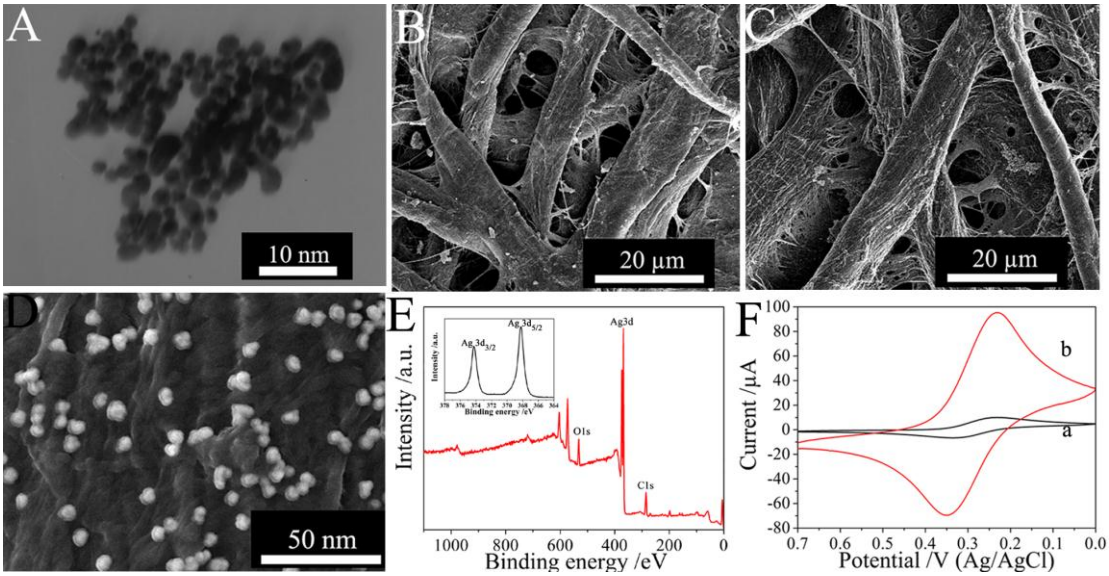


Fig. S2 A) TEM image of the as-prepared AgNP seeds solution; SEM images of B) bare paper sample zone of PWE; C) AgNP seeded paper sample zone of PWE; D) AgNP seeds on the surface of the cellulose fiber; E) Survey XPS spectrum of Ag-PWE (inset, high-resolution Ag3d XPS spectra of AgNP). F) CVs of (a) bare PWE and (b) porous Ag-PWE under 20 successive scans. Scan rate: 50 $mV \cdot s^{-1}$.

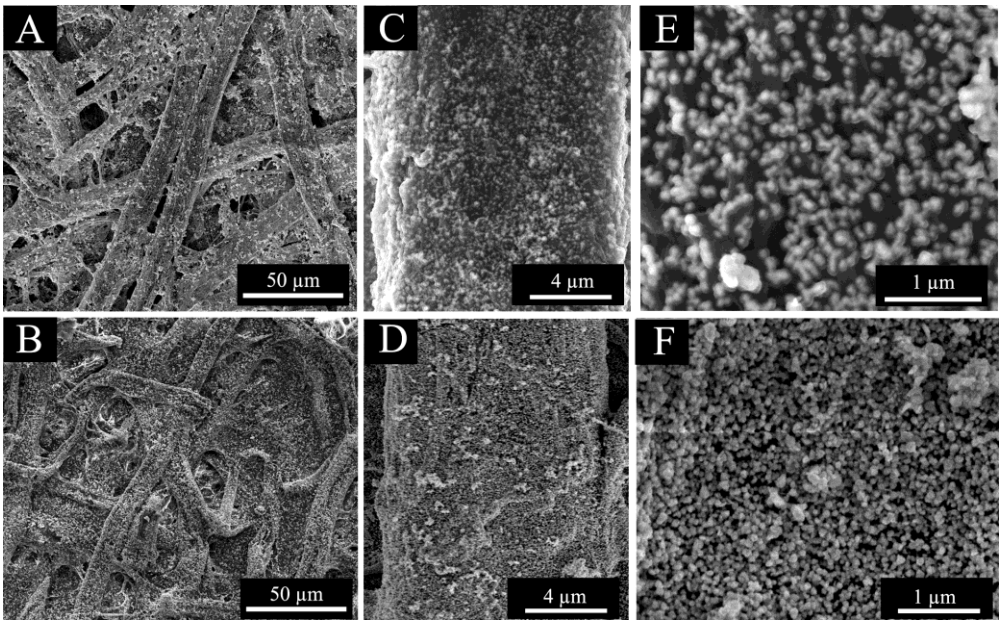


Fig. S3 Enlarged AgNPs on the surfaces of cellulose fibers in paper sample zone of PWE at different growth time under different magnification: A,C,E) After 2 min of growth; B,D,F) after 4 min of growth.

Electrical resistivity of AgNPs modified paper sample zone

To measure the resistivity of AgNPs modified paper sample zone exactly, rectangular paper

zones (1.0 mm×20.0 mm) were fabricated by wax-printing and then were modified through the growth of continuous and dense AgNPs conducting layer with interconnected AgNPs on the surfaces of cellulose fibers in these rectangular paper zones using the same method described in experimental section under the same experimental conditions in each case, the average electrical resistivity of which revealed the electrical resistivity of AgNPs modified paper sample zone in this work. Prior to the measurements, both the bare and AgNPs modified rectangular paper zones were dried in a drying oven for 3 h. Then the measurements were performed with a four-point measurement setup using a digital multi-meter (Agilent, U1251B).

Preparation of NPG/CNSs hybrid architecture

Nanoporous gold/carbon nanospheres (NPG/CNSs) hybrid architecture was fabricated via a simple self-assembly, and a detailed procedure was described below. Firstly, CNSs were prepared by a "green" method under hydrothermal conditions[7]. NPG was made by selective dissolution (dealloying) of silver from Ag/Au alloy according to the reported method[8]. Then as-prepared CNSs were sonicated with 3:1 (v/v) H_2SO_4/HNO_3 for 2 h to obtain carboxylic group-functionalized CNSs, followed by repeatedly washing with water until pH 7.0. Subsequently, the oxidized CNSs were further functionalized with PDDA aqueous solution (0.5%) containing NaCl (0.5 M) to obtain PDDA/CNSs. After another washing with water, as-prepared NPG solution (1.0 mL) was mixed with PDDA/CNSs (200 μL, 5 mg·mL^{-1}). The mixture was then allowed to stand overnight, followed by centrifugation (three times) and redispersion in 1.0 mL water solution.

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

The $Ru(bpy)_3^{2+}$-labeled signal CEA antibodies was obtained by adding 75 μg

$Ru(bpy)_3^{2+}$-NHS ester predissolved in 1.5 μL dimethylsulfoxide (DMSO) to 50 μL of signal CEA antibodies at 1 $mg \cdot mL^{-1}$. 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 signal CEA antibodies was further diluted with PBS (pH 7.4) to 1.0 mL and kept at 4 °C until use.

Preparation of luminol labeled signal CA125 antibodies ($McAb_2$)

Luminol with amino functional groups[9] was easier to facilitate biomolecules binding via EDC chemistry. Briefly, the luminol was mixed with 200 mM EDC and 50 mM NHS in 1.0 mL of pH 7.4 PBS buffer and activated for 40 min in the dark. The mixture was centrifuged at 13 000 rpm for 15 min. Subsequently, the resulting soft sediment was dispersed in 1.0 mL of pH 7.4 PBS. Then, 50 μL of signal CA125 antibodies at 1 $mg \cdot mL^{-1}$ was added to the dispersion, and the mixture was stirred overnight. The conjugate was washed with PBS and centrifuged at 13 000 rpm for 15 min three times. The resulting soft sediment was redispersed in 1.0 mL of pH 7.4 PBS. The obtained luminol labeled signal CA125 antibodies could be used directly or stored at 4 °C for months.

Preparation of QDs labeled signal AFP antibodies ($McAb_2$)

The water-soluble CdTe quantum dots (QDs) were prepared using TGA as stabilizing agent according to a method similar to that reported previously[10]. Briefly, 86 μL of TGA was added to 20 mL of 20 mM $CdCl_2$ solution. After adjusting the pH to 10, 20 mL of 20 mM NaHTe solution (obtained by reaction of $NaBH_4$ and Te powder) was added with extensive stirring for 30 min. The red QDs solution was obtained by simply controlling the refluxing time at 100 °C overnight. The product was condensed by ultrafiltration at 10 000 rpm for 10 min, and the upper phase was

decanted and kept at 4 °C.

Similarly, the as-prepared QDs, with high-density carboxyl functional groups[11], were much easier to facilitate biomolecules binding via EDC chemistry. Briefly, the as-prepared QDs were mixed with 200 mM EDC and 50 mM NHS in 1.0 mL of pH 7.4 PBS buffer and activated for 40 min in the dark. The mixture was centrifuged at 13 000 rpm for 15 min. Subsequently, the resulting soft sediment was dispersed in 1.0 mL of pH 7.4 PBS. Then, 50 μL of signal AFP antibodies at 1 mg·mL^{-1} was added to the dispersion, and the mixture was stirred overnight. The conjugate was washed with PBS and centrifuged at 13 000 rpm for 15 min three times. The resulting soft sediment was redispersed in 1.0 mL of pH 7.4 PBS. The obtained QDs labeled signal AFP antibodies could be used directly or stored at 4 °C for months.

Preparation of NPG/CNSs-GOx-McAb$_2$ tracer

McAb$_2$ (1.0 mg·mL^{-1}) and GOx (2.0 mg·mL^{-1}) were added into the obtained NPG/CNSs solution (300 μL) adjusted to pH 9.0 with K_2CO_3 (0.1 M), followed by gently shaking at 4 °C for 1 h. The reaction mixture was then centrifuged at 15 000 rpm for 5 min, and the supernatant was discarded. The NPG/CNSs-GOx-McAb$_2$ tracer was repeatedly washed for 3-4 times with PBS buffer and was blocked with a 0.1% (w/v) BSA solution for 1 h. Finally, the tracer was collected by centrifuging and redispersed in 200 μL PBS as the assay solution.

Characterization of NPG/CNSs-GOx-McAb$_2$ tracer

The SEM image of CNSs displayed a uniform size of approximately 300 nm (Fig. S4A). As shown in the SEM image (Fig. S4B), the carboxylated CNSs obtained by chemical oxidation were wrapped in a thin layer of cotton-shaped material. NPG displayed a type of sponge-like morphology with a 15-20 nm sized pore structure (Fig. S4C). Subsequently, by using positively

charged PDDA as a bridge, negatively charged NPG could electrostatically attach onto the surface of the carboxylated CNSs and further attach $McAb_2$ and GOx for the biofunctionalization of the CNSs.

The NPG/CNSs-GOx-$McAb_2$ tracer was further characterized using ultraviolet visible (UV-vis) spectroscopy (Fig. S4D). According to the UV-vis spectral experiments, no absorption peak was observed for the NPG/CNSs hybrid architecture (curve a). Three distinct adsorption peaks from pure GOx were observed (curve b). One adsorption peak at about 276 nm was corresponding to the amino acid residues in proteins (the pure GOx and $McAb_2$ (curve c)), and two characteristic absorption peaks of the oxidized form of the pure GOx were located at about 382 and 452 nm respectively. After the immobilization of the $McAb_2$ and GOx, the adsorption peaks at 276, 382 and 452 nm were also observed in the tracer (curve d), which could further demonstrated that the NPG/CNSs had good biocompatibility for the $McAb_2$ and GOx.

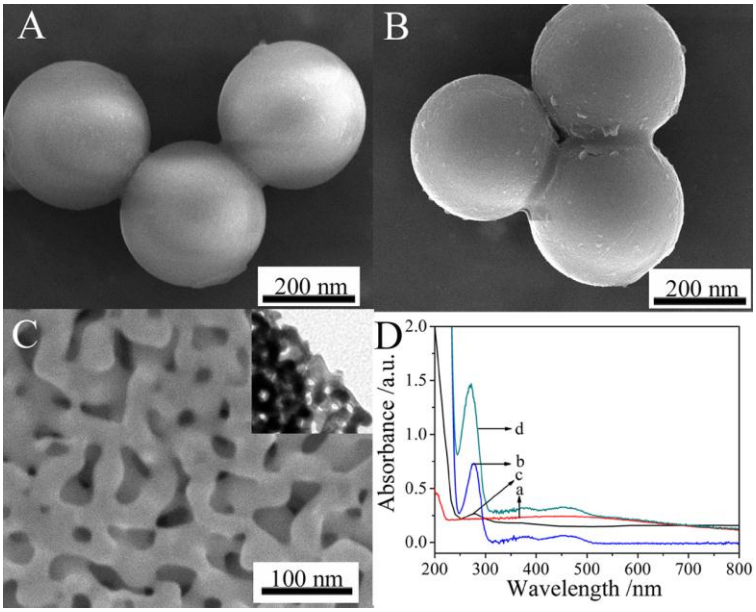


Fig. S4 SEM images of A) CNSs, B) carboxylated CNSs, C) NPG (inset, TEM image of NPG); D) UV-vis absorption spectra: NPG/CNSs (curve a), pure GOx (curve b), $McAb_2$ (curve c), NPG/CNSs-GOx-$McAb_2$ (curve d).

Preparation of this 3D origami ECL immunodevice

As shown in Scheme 1, the origami ECL immunodevice was constructed by immobilizing $McAb_1$ in the paper sample zone of Ag-PWE on sample tabs through chemical absorption between AgNP and $-NH_2$ of antibodies. In brief, 4.0 μL of 20 $\mu g \cdot mL^{-1}$ $McAb_1$ (anti-CEA, anti-CA125, and anti-AFP, respectively) were dropped onto the Ag-PWEs (W1, W2, and W3 respectively), and incubated for 40 min. Subsequently, physically absorbed excess antibodies were washed out with washing buffer. A drop of 4.0 μL of blocking solution was then applied to each Ag-PWE and incubated for 40 min at room temperature to block possible remaining active sites against nonspecific adsorption. After another washing with washing buffer, the resulting 3D origami ECL immunodevice was obtained and stored at 4 °C in a dry environment prior to use.

ECL assay procedure of this 3D origami ECL immunodevice

The ECL assay procedures of this 3D origami ECL immunodevice were shown in Scheme 1, and a detailed procedure was described below. To carry out the immunoreaction and ECL detections, the origami immunodevice was firstly incubated with a 2.0 μL of sample solution containing different concentrations of CEA, CA125, and AFP in PBS for 180 s at room temperature. After washing with washing buffer to prevent nonspecific binding and achieve the best possible signal-to-background ratio, the origami immunodevice was then incubated with 4.0 μL mixture of the designed tracers for 180 s at room temperature, followed by washing with washing buffer. Thereafter, three sample tabs were folded down below the auxiliary pad successively and clamped into the device-holder, which was comprised of two circuit boards (named Board-A and Board-B respectively below) with conductive pads on them, to fix and connect this origami immunodevice to the electrochemical workstation (Shanghai CH Instruments

Co., China) (Scheme S1D). Ultimately, PBS solution (pH 7.4, 40 μL) containing glucose (10.0 mM) was dropped into the paper electrochemical cell through the hole on Board-B and cyclic voltammetry (CV) measurements were performed at room temperature in a potential range from -1.5 to 0 V (W1), from 0 to 0.8V (W2), and from -0.9 to 0 V (W3), a modulation amplitude of 50 mV, a pulse width of 50 ms and a step potential of 5 mV (Scheme S1D). The ECL emission was detected using an IFFM-E chemiluminescence analyzer equipped with a photomultipier tube (PMT) biased at 800 V. The ECL signals related to CEA, CA125 and AFP concentrations could be detected successively.

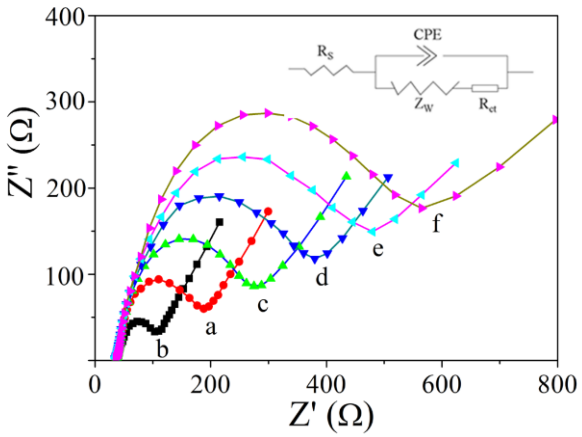


Fig. S5 EIS of bare PWE (a), Ag-PWE (b), $McAb_1$ modified Ag-PWE (c), BSA/$McAb_1$ modified Ag-PWE (d), antigen/BSA/$McAb_1$ modified Ag-PWE (e), and NPG/CNSs-GOx-$McAb_2$/antigen/BSA/$McAb_1$ modified Ag-PWE (f).

Cross-reactivity

To investigate the cross-reactivity of determination, cross-reactivity were evaluated by comparing the ECL responses of the 3D origami ECL immunodevice incubated with blank solution, 0.1 ng·mL^{-1} CEA, 0.1 U·mL^{-1} CA125, or 0.1 ng·mL^{-1} AFP. As expected, only the Ag-PWE prepared with the corresponding $McAb_1$ showed obvious ECL responses (Fig. S6). Obviously, the cross-reactivity between analytes and non-cognate antibodies was negligible.

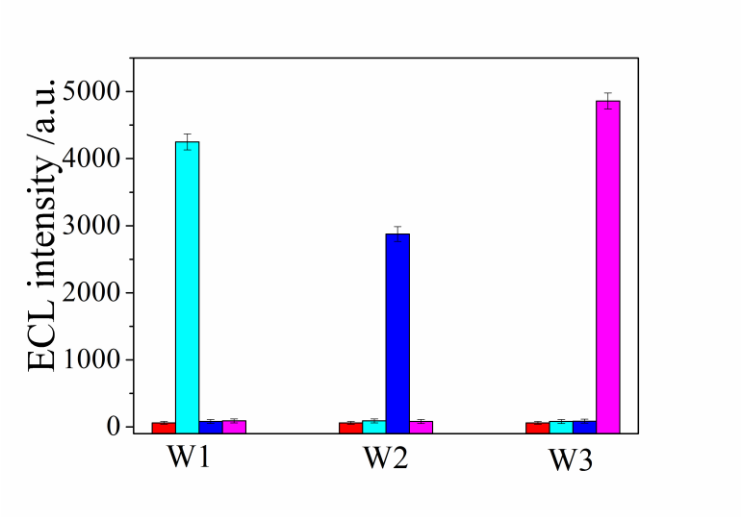


Fig. S6 ECL responses for different antigens on three Ag-PWEs: W1 (CEA antibodies modified Ag-PWE); W2 (CA125 antibodies modified Ag-PWE); W3 (AFP antibodies modified Ag-PWE).

Optimization of Detection Conditions

The immunoreaction time was a significant parameter for the capture and the specific recognition of the corresponding tracer on the Ag-PWE. With the increasing incubation time used in sandwich-type immunoassay, all the ECL responses for CEA, CA125, and AFP increased gradually and reached a plateau after 180 s (Fig. S7A), indicating a tendency to complete immunoreaction on the Ag-PWE surface. Thus, 180 s was used as the optimal immunoreaction time.

The effect of detection solution pH on the ECL signals of the immunodevice was displayed in Fig. S7B. In the examined pH range, the maximum ECL response of this immunodevice occurred when pH was 7.4. Taking into account the bioactivity of immunoreagents, a pH 7.4 PBS was selected as the detection solution.

The sensitivity of the proposed immunodevice was relied on the formation of the immunocomplex on the electrode which was dependent on the amount of GOx and $McAb_2$

conjugated on the NPG/CNSs. To obtain the best performance of the immunodevice, NPG/CNSs modified with GOx and $McAb_2$ at different mass ratios was synthesized and used for the construction of the immunocomplex (Fig. S7C). As shown, the maximal ECL signal was achieved at a mass ratio of GOx to $McAb_2$ of 40.

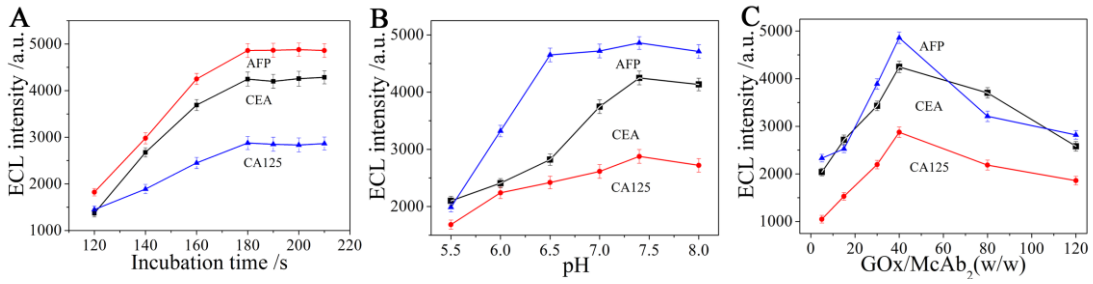


Fig. S7 Effect of incubation time (A), pH (B) and weight ratio of GOD to $McAb_2$ for preparation of tracers for 0.1 $ng \cdot mL^{-1}$ CEA and AFP, and 0.1 $U \cdot mL^{-1}$ CA125, where n = 10 for each point.

Analytical Performance

The reproducibility of this 3D origami ECL immunodevice for cancer markers was investigated with inter-assay precision. The relative standard deviation (RSD) for the parallel detection of 0.5 $ng \cdot mL^{-1}$/$U \cdot mL^{-1}$ antigens (CEA, CA125, and AFP) with five immunodevices was 2.82% (CEA), 2.41% (CA125), and 3.20% (AFP), indicating good precision and reproducibility. In addition, over 93.0% of original ECL response retained after 15 days of storage in 10.0 mM PBS at 4 °C for CEA, CA125, and AFP, indicating this immunodevice had acceptable storage stability.

Application in human serum samples

Table S1 Assay results of human serum samples by the proposed and reference method

Samples	CEA concentration ($ng \cdot mL^{-1}$)				CA125 concentration ($U \cdot mL^{-1}$)				AFP concentration ($ng \cdot mL^{-1}$)			
	1	2	3	4	1	2	3	4	1	2	3	4
Proposed method	25.2	31.7	46.2	38.4	6.7	16.5	32.4	72.1	67.2	21.5	17.6	13.4
Reference method	24.8	32.5	47.6	37.6	6.5	15.9	31.7	73.2	68.4	21.2	17.4	13.1
Relative error (%)	1.6	-2.5	-3.0	2.1	3.0	3.6	2.2	-1.5	-1.8	1.4	1.1	2.2

*Average of eleven measurements.

Table S1 Assay results of human serum samples by the proposed and reference method.

In the reference method (a commercially-used signal-analyte ECL method), the ECL measurements were carried out on Elecsys 2010 Immunoassay Analyzers (Roche Diagnostics, Basle, Switzerland)[12] in Shandong Tumor Hospital.

According to the criteria suggested by Li[13] the blood donor of sample-1 may be at the highest liver cancer and germ cell cancer risks, the blood donor of sample-2 may be placed in the class of the highest intestinal cancer and pancreatic cancer risk, the blood donor of sample-3 may be placed in the class of the highest breast cancer risk, the blood donor of sample-4 may be at the highest ovarian cancer risk.

Table S2 Recovery of CEA in human serum samples

sample	c_{CEA} (ng·mL^{-1})	added	detected	RSD (%, n=11)	recovery (%)
1	×	1.0 pg·mL^{-1}	0.96 pg·mL^{-1}	3.2	96.0
2	×	50.0 pg·mL^{-1}	50.4 pg·mL^{-1}	3.7	100.8
3	×	2.0 ng·mL^{-1}	1.95 ng·mL^{-1}	4.0	97.5
4	×	20 ng·mL^{-1}	20.6 ng·mL^{-1}	3.5	103.0
5	×	48 ng·mL^{-1}	47.3 ng·mL^{-1}	4.2	98.5

Each sample was repeated for ten times and averaged to obtain the recovery and RSD values.

Samples 1-5 were from healthy people; "×"represents not detectable.

Table S2 Recovery of CEA in human serum samples

Reference

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