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SUPPORTING INFORMATION

3D printing-based portable photoelectrochemical sensing device using digital multimeter

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EXPERIMENTAL SECTION

Material and reagent. Ammonium fluoride (NH_4F), ethylene glycol, bismuth nitrate pentahydrate [$Bi(NO_3)_3 \cdot 5H_2O$], sodium chloride (NaCl), cholesterol and glucose were acquired from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). L-α-Phosphatidylcholine (PC), 2-(morpholino) ethanesulfonic acid (MES), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), and N-hydroxysuccinimide (NHS) were purchased from Sigma-Aldrich (USA). 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[biotinyl(polyethylene glycol)-2000] (DSPE-PEG2000-biotin) was obtained from Avanti Polar Lipids Inc. (Alabama, USA). Bovine serum albumin (BSA) and carboxylated magnetic bead were purchased from Aladdin (Shanghai, China). All other chemicals used in this work were of analytical grade. Water was purified with Millipore water purification system (18.25 MΩ cm^{-1}, Milli-Q, Millipore) and used throughout the work. All oligonucleotides were synthesized by Sangon Biotech. Inc. (Shanghai, China), and the sequences are listed below:

Aptamer 1 (apt1): 5'- NH_2-AAAAAGGGGGTGAAGGGATACCC-3'.

Aptamer 2 (apt2): 5'- ATACCAGCTTATTCAATTTGAGCATAAT-biotin-3'.

Note: The underlined sequences were two aptamers of target CEA (AGGGGGTGAAGGGATACCC and ATACCAGCTTATTCAATT), respectively.

Apparatus. Scanning electron microscopy (SEM; SU8020, Hitachi Instruments, Japan), transmission electron microscopy (TEM; HT-7700, Hitachi Instruments, Japan), and X-ray diffraction (XRD; PANalytical X'Pert spectrometer, Netherlands) were used to characterize the as-prepared materials in this work. The dynamic light scattering (DLS; ZEN5600, Malvern, UK) was used to estimate the size of liposome. Six photosensitive resin printing parts were printed by a 3D printer (Formlabs, USA). The current of analysis detection was using a digital multimeter (DMM; VICTOR 86E, China).

Synthesis of TiO_2 nanotubes and BiOCl nanosheets-modified TiO_2 nanotubes. TiO_2 nanotubes (designated as TiO_2 NTs) were prepared by using a two-step anodization method according to the literature with minor modification.[1] Prior to anodization, a titanium sheet was first subjected to successive sonication in acetone, ethanol, and ultrapure water for 15 min. Subsequently, the cleaned Ti sheet was treated with HF-HNO_3-H_2O mixed solution (1:4:5 in volume) for 30 s, thoroughly washed by ultrapure water and dried with N_2. Electrochemical

anodization was carried out in an ethylene glycol solution containing 0.3% (w/v) NH_4F and 2% (v/v) ultrapure water at 50 V for 2 h, with a two-electrode system by using the titanium sheet as the working electrode and a graphite sheet as the counter electrode. After that, the resulting titanium sheet was thoroughly washed with ultrapure water and vigorously sonicated in ultrapure water to remove the surface layer. The same Ti sheet then underwent the second anodization at 50 V for 30 min. After the two-step anodization, the prepared TiO_2 NTs were cleaned with ultrapure water and dried with N_2. The as-anodized TiO_2 NTs were annealed in air at 450 ºC for 1 h with a heating rate of 5 ºC min^{-1} to crystallize the amorphous TiO_2.

Next, BiOCl nanosheets-modified TiO_2 nanotubes were obtained by a sequential chemical bath deposition method.[2] Briefly, the above-prepared TiO_2 NTs were initially immersed into 20 mM $Bi(NO_3)_3$ solution, 20 mM NaCl solution, and ultrapure water, in turn, and then incubated for 2 min (each solution) at room temperature (note: such an immersion cycle was repeated five times to achieve more deposition amount). Finally, BiOCl nanosheets-functionalized TiO_2 NTs (designated as TiO_2 NTs/BiOCl) were obtained by washing and drying under nitrogen.

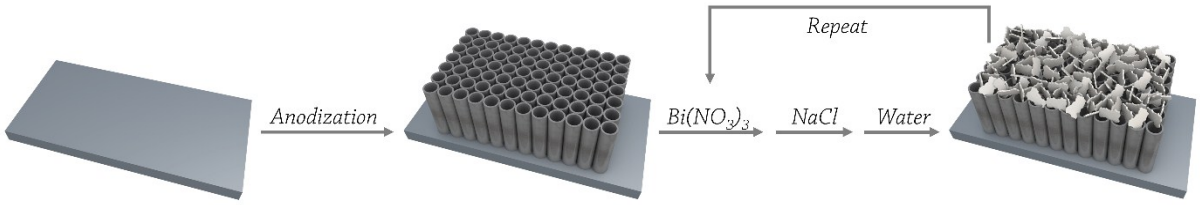

Fig. S1 Schematic illustration for fabricating the TiO_2 NTs/BiOCl electrode.

Preparation of glucose-encapsulated liposome. Glucose-encapsulated liposome (GEL) was prepared by the reverse-phase evaporation strategy according to the literatures with minor modifications.[3,4] In a typical procedure, PC, cholesterol, and DSPE-PEG2000-biotin (molar ratio: 7: 1: 2) were co-dissolved in a chloroform solution. And then, the organic solvent was then removed by rotary evaporation under reduced pressure at 45 °C to form a thin lipid film. Following that, glucose aqueous solution (10 mL, 0.1 M) was injected into the lipid film using a syringe. After incubation at 30 °C for 30 min, the resulting mixture was sonicated for 5 min. To produce the homogeneous suspension with the uniform size, the mixture was filtered several times by using 0.22 μm polycarbonate filter. The homogeneous suspension of the prepared liposome was stored in a refrigerator before use.

Conjugation of carboxylated magnetic bead with aptamer 1 (MB-apt1). Aminated DNA

strands were conjugated with carboxylated magnetic beads via a typical carbodiimide couple. Prior to use, magnetic beads were washed using MES buffer (100 mM, pH 6.0) three times, and finally resuspended in MES buffer to a final concentration of 1 mg mL^{-1}. The as-prepared MB (200 μL) was mixed with NHS (200 μL, 10 mM) and EDC (200 μL, 40 mM) in centrifuge tube, and the mixture was incubated for 15 min at room temperature, which was to active the -COOH groups on the MB. Following that, apt1 (100 μL, 20 μM) was added into above suspension, and was allowed to incubated overnight night at RT for the assembly of the MB-apt1 conjugates. And then the resulting suspension was washed with several times with PBS (10 mM, pH 7.4) and finally obtained a homogeneous suspension by adding PBS (500 μL) containing BSA (1%), which was incubated for 2 h at RT. The main aim of the process is to remove the possibility of nonspecific adsorption on MB. Finally, the product was dispersed into in PBS and stored at 4°C when not in use.

Biosensing for CEA. Initially, the analytes with different concentrations were initially added in PBS (100 μL, 10 mM, pH 7.4) containing MB-apt1 (25 μL, 5 mg mL^{-1}) and apt2 (25 μL, 1.0 μM), and then the mixture was incubated for 60 min at 37 °C under slight shaking on a shaker (note: MB-apt1 and apt2 sandwiched the target CEA during this process, generating a complex). Following that, the mixture was magnetically separated and washed with pH 7.4 PBS. After that, 50 μL of streptavidin solution (1.25 μg mL^{-1}) was added and incubated for 30 min at 37 °C. Subsequently, separation and wash were performed followed by 50 μL of the GEL injected. After the mixture further incubated for another 30 min, the unreacted GEL was removed with magnetic separation followed by washing the resulting mixture three times. Next, 50 μL of 1% Triton X-100 was added into the resulting mixture and maintained for 15 min to lyse the captured liposomes for liberating the encapsulated glucose. Following that, the supernatant was transferred into the homemade detection cell for measurement.

Photoelectrochemical (PEC) Measurement. The PEC measurements were carried out in 0.1 M pH 6.0 PBS and an UV LED (365 nm, 1 W) was utilized as the light source. The TiO_2 NTs/BiOCl electrode and Pt wire electrode were used as the working electrode and counter electrode, respectively. The released glucose scavenged the hole and accelerated the PEC reaction of TiO_2 NTs/BiOCl photoelectrode under light irradiation. The produced charges between two electrodes were collected by capacitor with the switch turned to TiO_2 NTs/BiOCl

electrode (for 30 s). Turning switch from the photoelectrode to DMM, the capacitor discharged the collected charges to produce an instantaneous current. The variation of instantaneous current value recorded by a nimble DMM registered as the sensor signal to intuitively monitor the CEA concentration.

ADDITIONAL RESULTS AND DISCUSSION

Fig. S2 shows 3D model of the sensing device printed by the photosensitive resin: the black arrows indicate the name of the corresponding model part, and the red arrows reveal the function of the pointed position. A capacitor was fixed in the capacitor compartment. Three square hollows in the pedestal (Fig. S3) and a switch compartment (Fig. S4, d and e) were used for the placement and fixation of a toggle switch (type: MTS-102).

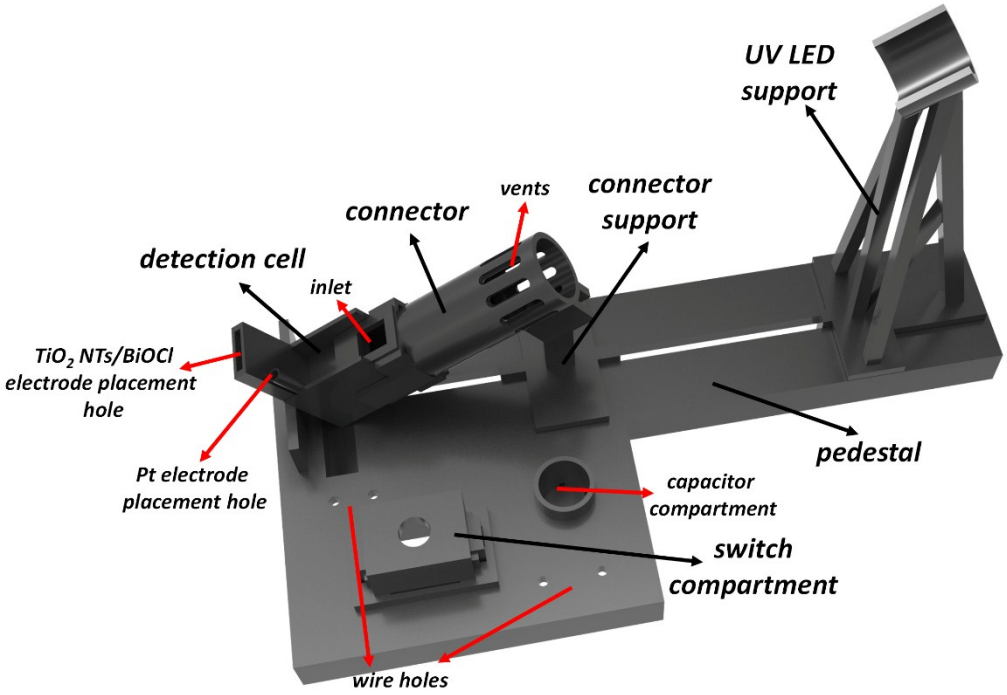

Fig. S2 3D model of the sensing device printed by the photosensitive resin.

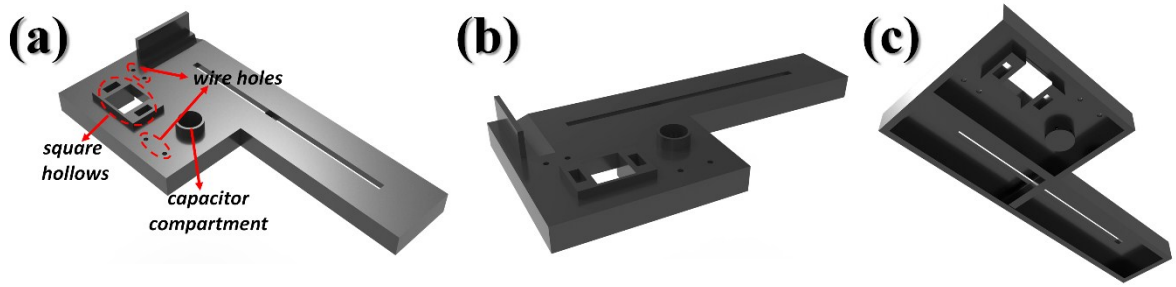

Fig. S3 3D model of the pedestal: (a, b) side view and (c) upward view.

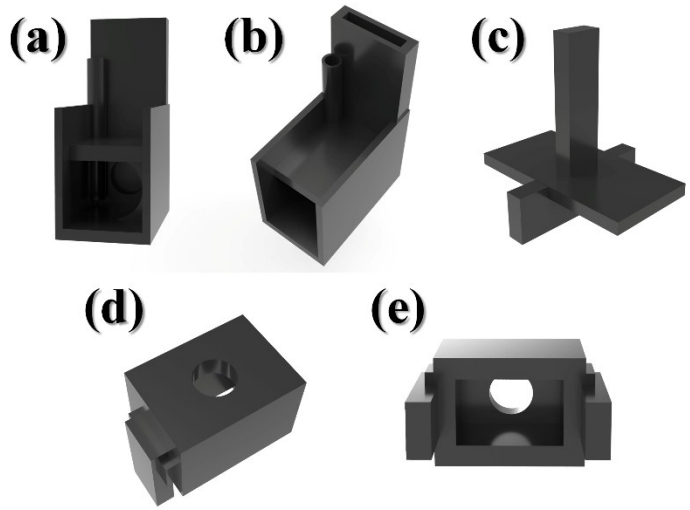

Fig. S4 3D models of (a, b) detection cell, (c) connector support, and (d, e) switch compartment.

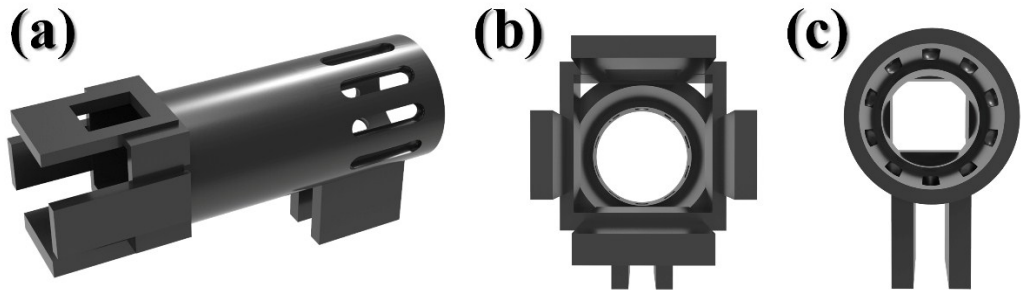

Fig. S5 3D model of the connector: (a) side view, (b) front view, and (c) rear view.

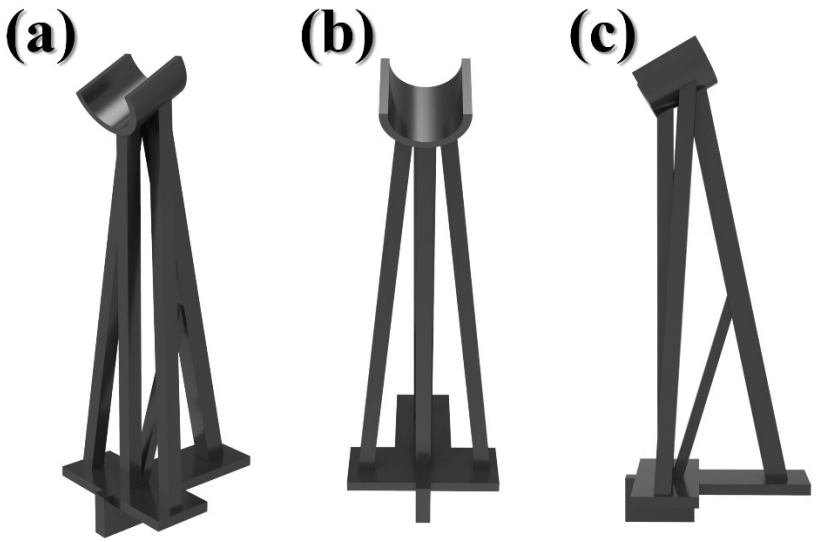

Fig. S6 3D model of the UV LED support: (a) side view, (b) vertical view, and (c) right elevation.

The six printing parts were designed by Cinema 4D (R18.057) and exported as .obj files for importing PreForm (Version 2.17.1), and finally printed by 3D printer (Form 2; Formlabs). The printing parameters were shown below (Fig. S7): Layer Thickness was chosen as 0.05 mm,

Density of Supports was 1.00, and Point Size was available in two sizes (0.60 mm for pedestal and 0.80 mm for the others). Black resin cartridge V4 (RS-F2-GPBK-04) was used in this work. The models with .stl files were also provided in the supporting information. As an example, the size of pedestal was displayed in Fig. S8.

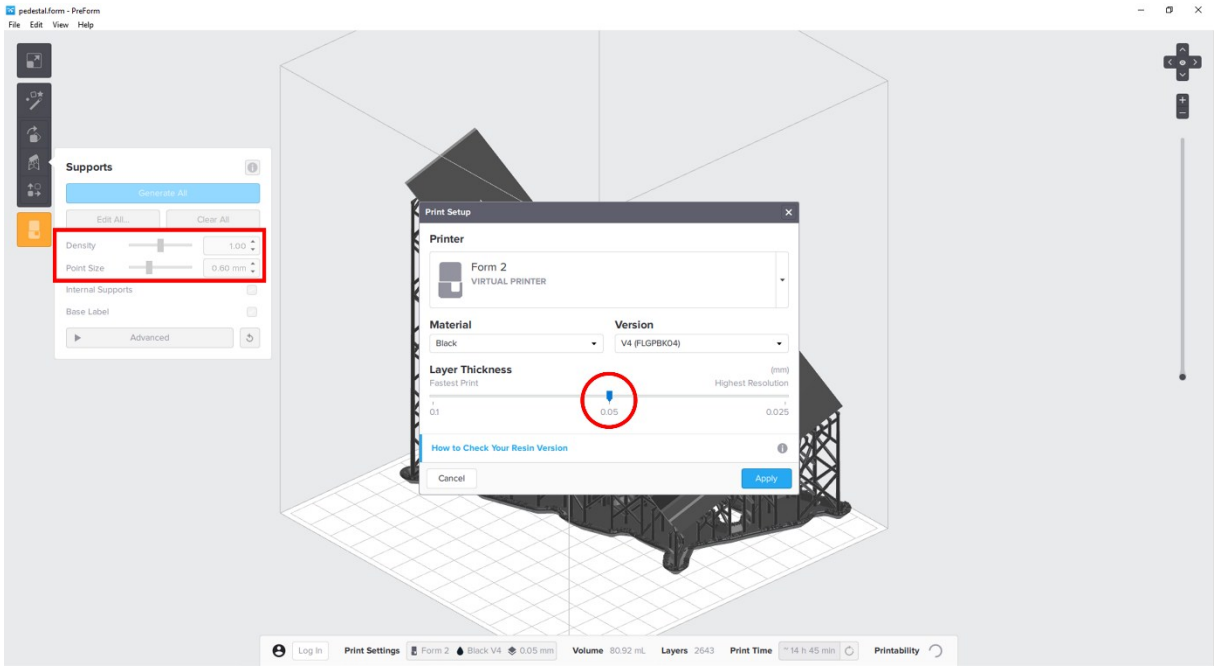

Fig. S7 The printing parameters of pedestal.

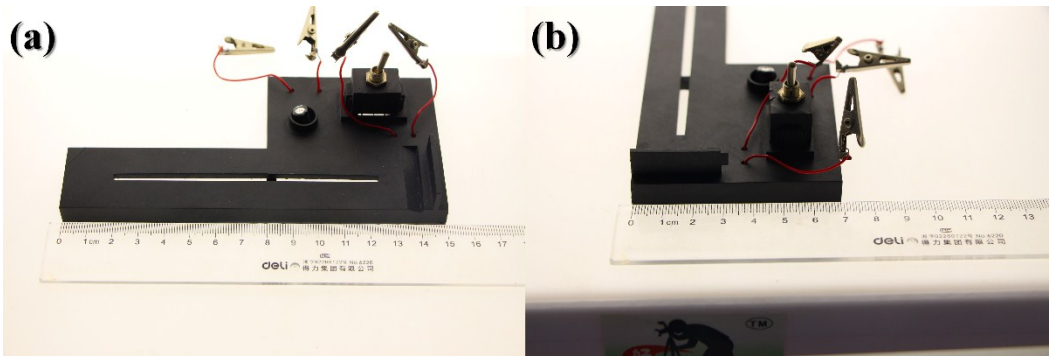

Fig. S8 The size of pedestal.

We might roughly estimate the number of liposome molecules that one magnetic bead nanoparticle could bind at most by the following algorithm:

$$n = \frac{S_{MB}}{S_{GEL}} = \frac{4\pi r_{MB}^2}{\pi r_{GEL}^2} \quad (1)$$

where n, S_{MB}, S_{GEL}, r_{MB}, and r_{GEL} stand for the number of captured GEL, spherical surface area of magnetic bead, the area of GEL's radius-based circle, the radius of magnetic bead, and the radius of GEL.[5]

The diameter of magnetic bead used in this work is 300 nm, and the diameter of the GEL could be obtained from the DLS data in the text, assuming that the hydrodynamic diameter is equal to the diameter of the GEL. The average hydrodynamic diameter of GEL measured by DLS was approximately 164 nm, thus, one magnetic bead could simultaneously capture 13 GEL with a diameter of 164 nm at most according to the above-mentioned formula. As for other sizes of GEL, the algorithm is similar.

Optimization of experimental conditions. To acquire an optimal analytical performance of the developed PEC sensing method, pH of the electrolyte solution and incubation time of CEA were investigated (1.0 ng mL^{-1} CEA used in the all cases). Fig. S9a demonstrates the correlation between the signal-to-background (S/B) ratio (*i.e.*, relative to the background signal) and the pH of electrolyte. It could be found that a maximum S/B ratio was acquired at pH 6.0 PBS. Higher or lower pH could lead to lower S/B ratios. Therefore, pH 6.0 PBS was used as the electrolyte during the photocurrent measurement process. Next, the incubation time was studied. A short incubation time was unfavorable for the formation of the aptamer-CEA-aptamer complex. As seen from Fig. S9b, the photocurrent increased with the increasing incubation reaction time and tended to level off after 60 min. A longer incubation time did not cause a significant increase in the photocurrent response. To save assay time, 60 min was used for the aptamer-CEA-aptamer reaction.

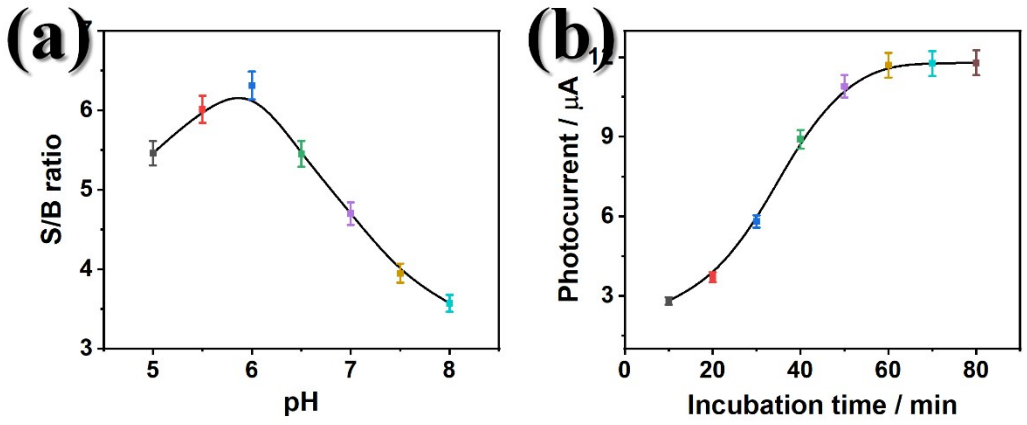

Fig. S9 Effects of (a) pH of electrolyte solution and (b) incubation time for the aptamer-CEA-aptamer reaction (1.0 ng mL^{-1} CEA used in all cases).

The stability of the as-prepared TiO_2 NTs/BiOCl photoelectrode was studied over a period of four weeks. The photocurrent response could retain 98.8%, 97.5%, 96.6% and 94.7% of the

initial signal at the 7th, 14th, 21th, and 28th, respectively. Besides, the storage stability of glucose-encapsulated liposome was also investigated over three an three-weeks. The signal could retain 98.6%, 95.2%, 89.3%, and 81.7% of the initial signal at the 3rd, 7th, 14th, and 21th, respectively. This is most likely a consequence of partial breakage of GEL during storage.[6]

Calculation method for *t*-test statistics. To investigate the method accuracy between two methods, statistical comparison based on the experimental results was first carried out with an unpaired Student's *t*-test preceded by the application of an F-test. The statistics for each sample was calculated by using independent two-sample *t*-test with equal sample sizes and equal variance as follows:

$$t = \frac{|\bar{x}_1 - \bar{x}_2|}{S_{x1x2}} \sqrt{\frac{n}{2}} \quad (2)$$

where

$$S_{x1x2} = \sqrt{\frac{S_{x1}^2 + S_{x2}^2}{2}} \quad (3)$$

The $\bar{x}$, S_x and n represent the mean, standard deviation and times of parallel detection of the sample (1 means the data obtained from the proposed method and 2 means the data obtained from referenced method), respectively.

Table S1 Comparison of the assay results by using the PEC sensing platform and the ELISA method

Sample no.	Method; Concentration (mean ± SD, n = 3, ng mL^{-1})		t_{exp}
	Found by PEC sensing platform	Found by ELISA	
1	15.37 ± 1.18	13.91 ± 1.05	1.60
2	0.96 ± 0.07	1.03 ± 0.08	1.14
3	7.52 ± 0.56	8.05 ± 0.47	1.26
4	28.69 ± 2.14	27.12 ± 2.35	0.86
5	2.61 ± 0.15	2.87 ± 0.21	1.75
6	21.13 ± 1.71	22.52 ± 1.42	1.08

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