Electronic Supplementary Material (ESI) for Sensors & Diagnostics.

Supporting Information

Signal-on electrochemiluminescence immunosensor for A2780 cell detection based on the excellent catalytic performance of Co^{2+} doped TiO_2 nanodisks

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Materials

Tris (2,2'-bipyridine-n,n')-, dichloride, hexahydrate (II) ($Ru(bpy)_3^{2+}$, 98%) was obtained from Sahn Chemical Technology Co. (Shanghai, China). Bovine serum albumin (BSA, 96–99%) were bought from Biss Inc. (Beijing, China) and glutaraldehyde (GLD, 25% aqueous solution) was purchased from Shanghai Jinshan Tingxin Chemical Plant (China). 3-Mercaptopropionic acid (MPA) and p-phenylenediamine (PPD) were received from Sigma-Aldrich. Besides, N-hydroxy succinimide (NHS) and 1-ethyl-3-(3-di-methylaminopropyl) carbodiimide hydrochloride (EDC) were gotten from Shanghai Medpep Co. (China). Folic acid (FA) was acquired from Aladdin Industrial Corporation (Shanghai, China). Interleukin-6 (IL-6), carcinoembryonic antigen (CEA), prostate specific antigen (PSA) and a-fetoprotein (AFP) standard solutions were purchased from Linc-Bio Science Co. (Shanghai, China). Other reagents with analytical grade were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Phosphate buffer solution (PBS) with various pH values were prepared by mixing 0.2 M Na_2HPO_4 and 0.3 M NaH_2PO_4 under monitoring of PHS-3C exact digital pH metre (Shanghai, China). A2780 cancer cells and other cell lines were obtained from Fujian Medical University. Co-TiO_2 NDs (2.5 mg/mL) and HCNTs (5 mg/mL) were prepared in this work.

Instruments

The ECL emission was measured on a BPCL Ultra-Weak Luminescence Analyzer (Institute of Biophysics, Chinese Academy of Sciences, Beijing, China) combining with a CHI 760 electrochemical workstation and the specific operation was realized by a three-electrode system using glassy carbon electrode (GCE, 3 mm diameter) as working electrode, Pt electrode as counter electrode, and Ag/AgCl electrode (KCl-saturated) as reference electrode. Electrochemical experiments were performed on CHI 760 electrochemical workstation (Shanghai Chenhua Instrument Co., Shanghai, China). Scanning electron microscopy (SEM, S-4800 instrument) and Transmission electron microscopy (TEM, FEI F20 S-TWIN instrument) were utilized to characterize the structures and morphologies of the prepared nanomaterials. N_2 adsorption-desorption analysis was conducted on a Micro-meritics TriStar II 3020 instrument (USA). Pore size distribution plots were obtained by the Barrett-Joyner-Halenda (BJH) method. UV–vis absorption spectra were carried out on a UV 1901 spectrophotometer (Shanghai, China). X-ray diffraction (XRD) patterns were recorded on a Rigaku X-ray diffractometer using Ni filtered Cu Ka radiation (λ = 1.5406 Å) radiation. X-ray photoelectron spectroscopy (XPS) measurements were performed on a PHI Quantum 2000 XPS system with a monochromatic Al Kα source and a charge neutralizer.

Synthesis of HCNTs

HCNTs was prepared according to previous work with a minor change.[40] Typically, 10 mmol $FeCl_2 \cdot 4H_2O$ and 15 mmol citric acid monohydrate were dissolved in 50 mL ethanol and thoroughly stirred at 60 ºC for 6 h. After drying at 80 ºC, the colloid was heated at 450 ºC for 3 h in air to produce ferric oxide. Subsequently, 82 mg of ferric oxide powder was spread on a ceramic plate, which was placed in a quartz reaction tube. The above reaction tube was put in a stainless steel reaction tube equipped with temperature and gas-flow controls. After reacting for 4 h at 425 ºC in H_2, the iron oxide was reduced into Fe and the acetylene decomposition was performed at 475 ºC for 2 h at atmospheric pressure with Fe as catalyst. The final products were obtained after the system was cooled to room temperature.

Preparation of Co-TiO_2 NDs

The previously reported method was adopted to synthesise Co-TiO_2 NDs.[41] Initially, 6 g of terephthalic acid was dissolved into a mixed solution containing dimethylformamide (DMF) (108 mL) and MeOH (12 mL) under stirring for 10 min. Then 3 mL tetra-n-butyl titanate $Ti(OC_4H_9)_4$ was added into the above solution and kept stirring for 15 min. Afterwards, 32 mg of $CoCl_2 \cdot 6H_2O$ was poured and continually stirred for 10 min. Subsequently, the above solution was transferred to a 200 mL Teflon liner. After reacted at 150 °C for 48 hours, the resultant suspension was filtered, washed with DMF and methanol. And the precipitate was collected by centrifugation, washing with ultrapure water and ethanol and dried at 60 ºC overnight. By calcining the above products at 400 ºC for 5 h under air condition, the final products of Co-TiO_2 NDs were obtained.

Fig. S1

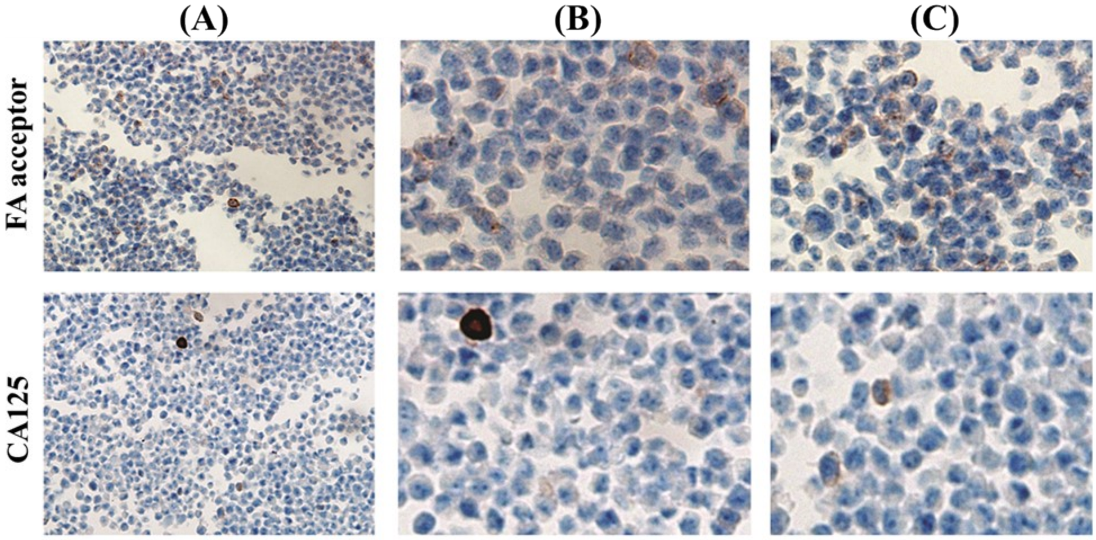

Fig. S1 The expressions of FA acceptor and CA125 on the A2780 cells.

To investigate the expressions of FA acceptor and CA125 on the surface of A2780 cells, the strept avidin-biotincomplex (SABC) experiment was performed and the results were exhibited in Figure 1. It can be observed that the FA acceptor and CA125 were significantly high-expressed in A2780 cells (Fig. S1A). Additionally, the FA acceptor and CA125 expressions in A2780 cells were more distinct in the locally enlarged images (Fig. S1B and C). The above results demonstrated the expressions of FA acceptor and CA125 on the surface of A2780 cells, further indicating the feasibility of the sensing strategy.

Fig. S2

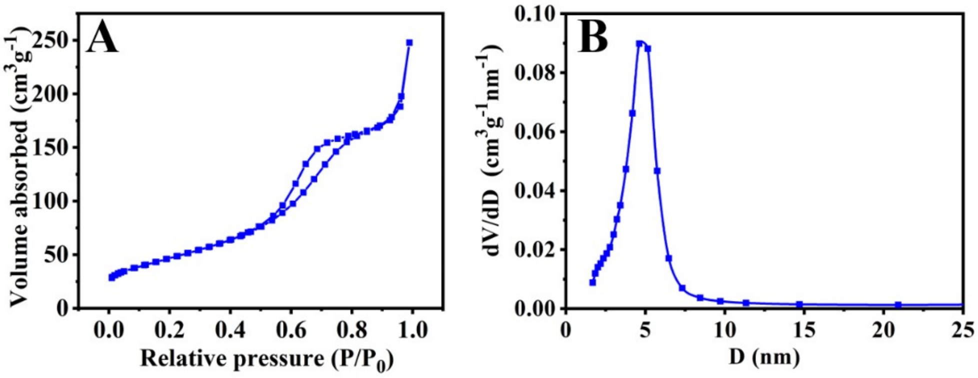

Fig. S2. (A) N_2 adsorption-desorption isotherms and (B) the corresponding BJH pore size distribution of as-prepared Co-TiO_2 NDs.

For exploring the surface area and pore nature of Co-TiO_2 NDs, the Nitrogen adsorption-desorption isotherms was conducted and the corresponding analysis were showed in Fig. S2. A type IV curve can be observed in Fig. S2A. The large surface area with 178 m^2 g^{-1} and pore volume with 0.41 cm^3 g^{-1} around 6 nm can be obtained in the as-prepared in Fig. S2B, which are propitious to exposure active sites and promote the transfer and convey of electrons and ions, resulting in the high ORR activity.

Fig. S3

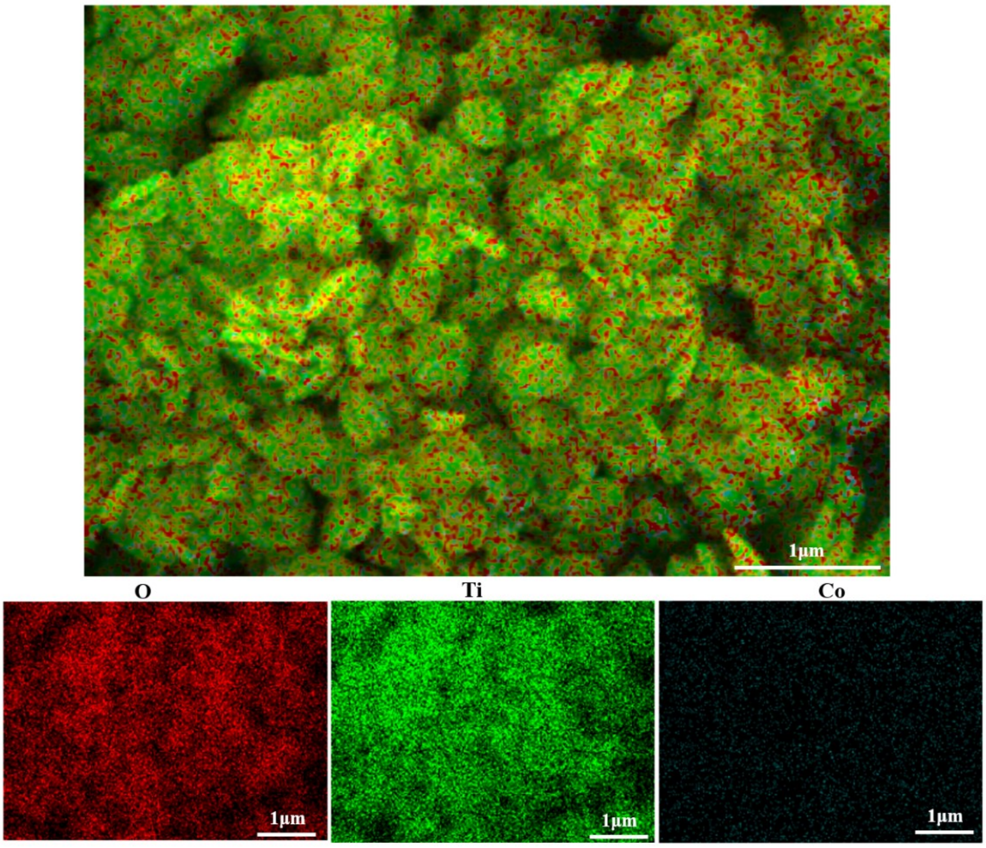

Fig. S3. Energy dispersive X-ray spectrometry (EDS) mapping of Co-TiO_2 NDs.

To investigate the elemental distribution of Co, Ti and O in the Co-TiO_2 NDs, SEM-EDS mapping was carried out and the results were demonstrated in **Fig. S3**. Obviously, the three elements Co, Ti and O were uniformly distributed in the nanodisk and the contents of Co is about 0.32 at. %, which verified the successful doping of Co.

Fig. S4

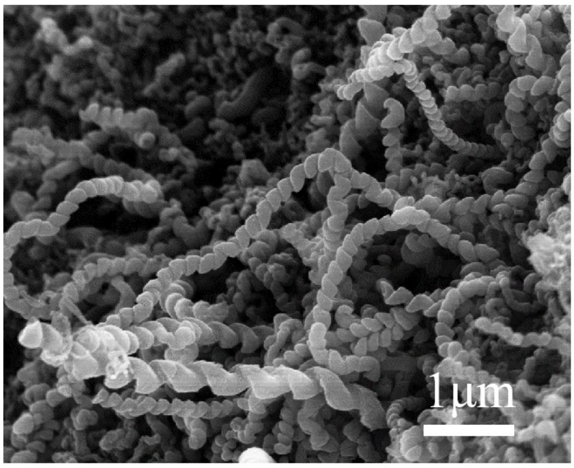

Fig. S4 SEM image of HCNTs.

Fig. S5

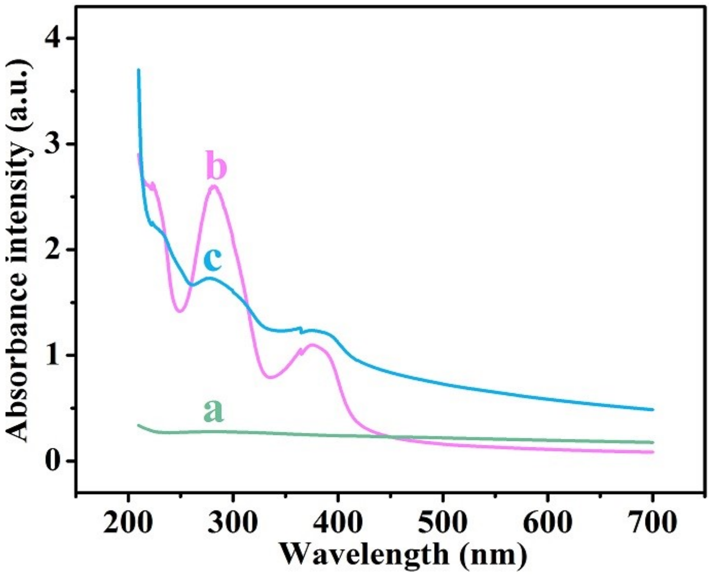

Fig. S5 The UV-vis absorption spectra of (a) Co-TiO_2 NDs, (b) FA and (c) Co-TiO_2 NDs@FA hybrids.

To prove the successful preparation of Co-TiO_2 NDs@FA hybrids, the UV-vis spectrum was performed in Fig. S5. Apparently, no obvious absorption peaks were observed at Co-TiO_2 NDs (curve a), while three adsorption peaks at 223 nm, 282 nm and 365 nm were gained from FA (curve b). These absorption peaks are attributed to π-π* transitions of FA and the absorption peak at 282 nm is associated to the pterin ring.[S1] After FA was embedded onto Co-TiO_2 NDs, the characteristic absorbance peaks of FA were remained (curve c), indicating the successful formation of Co-TiO_2 NDs@FA hybrids.

Fig. S6

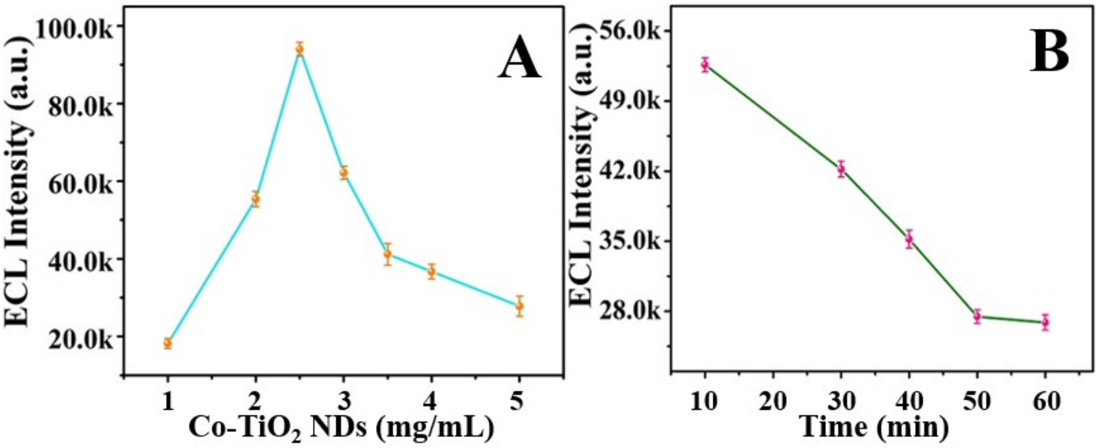

Fig. S6 Effects of (A) the concentration of Co-TiO$_2$ NDs and (B) the reaction time of FA for the ECL responses of the biosensor.

Fig. S7

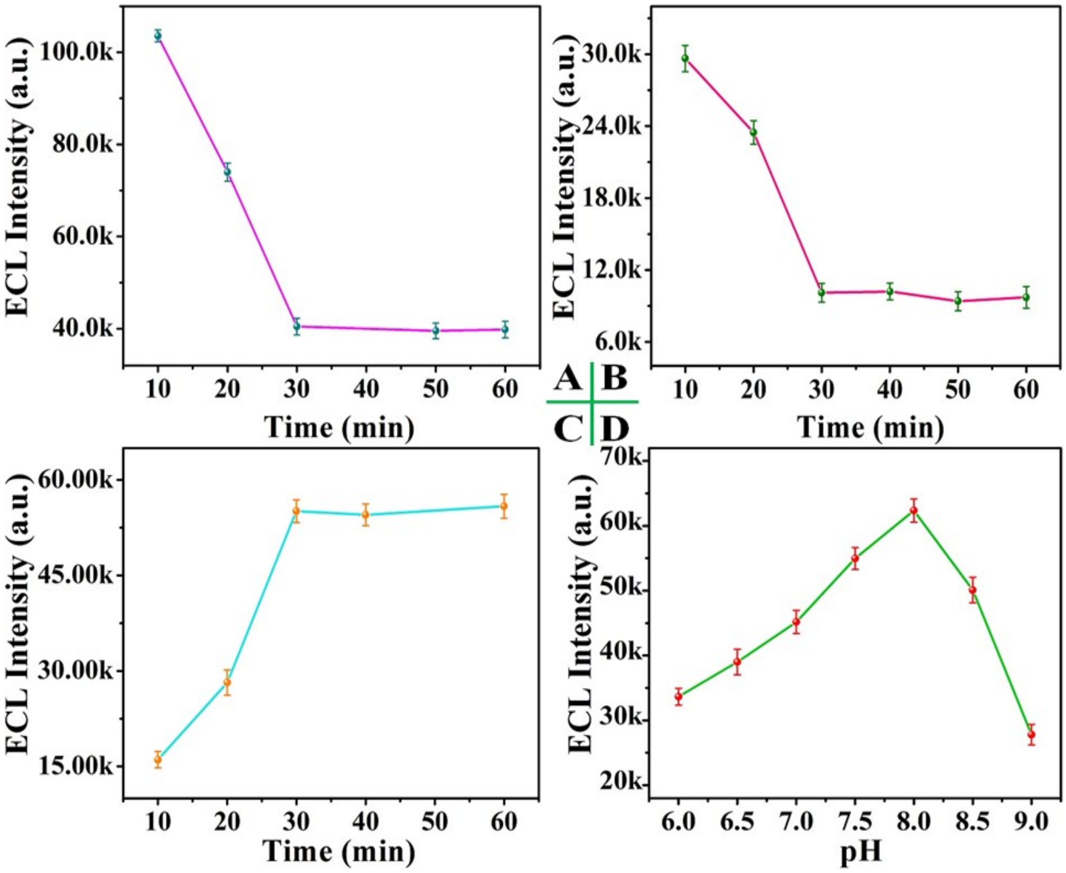

Fig. S7 Effects of the immobilization time of Ab_{CA125} (A), the incubation time of A2780 cells (B), Co-TiO_2 NDs@FA composites (C) and the pH value of the testing solution (D) for the ECL responses of proposed biosensor.

Fig. S8

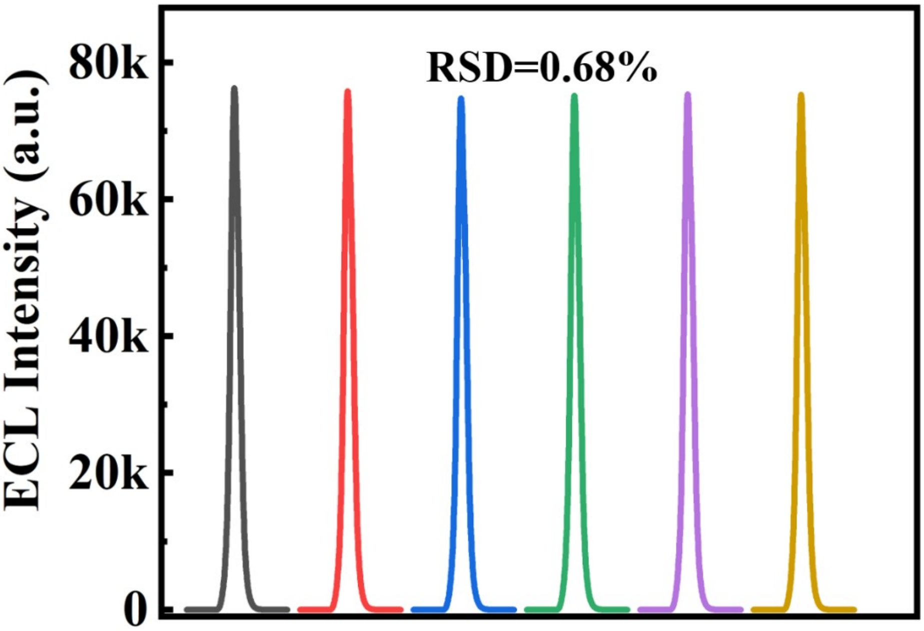

Fig. S8 Reproducibility of the six independent proposed biosensor for 2.6×10^4 cells/mL A2780 cells analysis.

Table S1

Comparison of the proposed ECL biosensor with other cell-based biosensors.

Method	Target cells	Linear range (cells/mL)	LOD (cells/mL)	Advantages	Disadvantages
CV[43]	Hela	$5\times10^2 \sim 10^5$	273	Excellent linearity, high sensitivity	Poor portability and discrimination
EIS[44]	HL60	$5\times10^2 \sim 5\times10^6$	500	High stability, biocompatibility	Low sensitivity and poor portability
CL[45]	CCRF-CEM	$5\times10^2 \sim 10^5$	270	Excellent linearity, high sensitivity	Poor selectivity and portability
ECL[This work]	A2780	$2.6\times10^2 \sim 2.6\times10^6$	100	High sensitivity, good stability and reproducibility	Poor portability

Table S2

Recoveries tests of A2780 cell in PBS (n=3) [a].

Samples	A2780 (cells/mL)	Added (cells /mL)	Found (cells /mL)	Recovery (%)
PBS	0	260	275	105.8
	0	2.6×10^3	2.744×10^3	105.5
	0	2.6×10^5	2.5×10^5	96.2

[a] n is the repetitive measurements number.

References

S1 D. D. M. Profirio and F. B. T. Pessine, *European Polymer Journal*, 2018, **108**, 311-321.