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Supporting Information

Design of target response wettability switchable core-shell-shell electrochemiluminescence nanoprobes for sensitive hyaluronidase detection

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EXPERIMENTAL SECTION

Chemicals and Reagents.

Tris (2,2′-bipyridyl) ruthenium (II) chloride hexahydrate ($Ru(bpy)_3^{2+}$), hyaluronic acid (HA), hyaluronidase (HAase), tripropylamine (TPA), phenylphosphonic acid (PPOA), and 3-aminopropylphosphonic acid (3-APPA) were purchased from Sigma-Aldrich (Shanghai China). Triton X-100, 3-aminopropyltriethoxysilane (APTES), N-hydroxy succinimide (NHS), and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) were obtained from Aladdin Bio-Chem Technology Co., Ltd (Shanghai China). Anhydrous ethanol was obtained from Sinopharm Chemical Reagent Co., Ltd. 20× phosphate buffered saline (PBS, 0.2 M) was acquired from Sangon Biotechnology Co., Ltd. (Shanghai, China). Deionized water (Milli-Q, Millipore, 18.2 MΩ·cm) was used for solution preparation throughout the experiment.

Apparatus and Instruments.

TMS-200 Thermo shaker incubator was purchased from Allsheng Instruments Co., Ltd. (Hangzhou, China). A laboratory made ECL detection system composed of a BPCL ultraweak luminescence analyzer (Institute of Biophysics, Chinese Academy of Science, Beijing, China) for ECL detection and a CHI660D electrochemical workstation (Chenhua Instruments, Shanghai, China) for electrochemical measurements. A three-electrode system composed of an ITO electrode (working electrode), a platinum wire electrode (counter electrode), and an Ag/AgCl electrode (reference electrode, saturated with KCl) were used in detection process. The ITO electrode was sonicated in deionized water for 15 min firstly, and then immersed into 1% (PPOA) solution for 2 h and dried naturally for later use. The Working area of the ITO electrode was 10 mm × 3 mm.

Preparation of Ru-SiO_2@AP NPs.

Ru-SiO_2 NPs was prepared through early reported synthesis method and dissolved in anhydrous ethanol to prepare a solution with 10 mg/mL concentration firstly.[1,2] Then 2.4 mL above-mentioned Ru-SiO_2 NPs solution and 200 μL APTES (25%) was mixed with 9.4 mL anhydrous ethanol respectively and incubate with shaking (2500 rpm) at 25 °C for 16 h. After that, the resulting solution was centrifuged at 7000 rpm for 10 min

and then washed by anhydrous ethanol and ultrapure water 3 times and dispersed in ultrapure water to obtain a solution with 5 mg/mL Ru-SiO_2@AP NPs.

Preparation of Ru-SiO_2@AP@HA NPs.

HA sodium salt solution (250 μL, 10 mg/mL) was incubated with 5 mL PBS (20 mmol/L), 3.75 mL H_2O, 250 μL 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC, 48.8 mg/mL), and 250 μL N-hydroxy succinimide (NHS, 11.5 mg/mL) for 15 min to activate carboxyl. After that, 500 μL proposed Ru-SiO_2@AP NPs solution was added into the above-mentioned mixed solution and incubated with HA for 4 h. Then, the resulting solution was centrifuged at 7000 rpm for 10 min, and centrifugal washed by ultrapure water for 3 times. Finally, the collected precipitates were dispersed in ultrapure water (4 mg/mL) to obtain purify Ru-SiO_2@AP@HA NPs.

Preparation of Interfering Samples and Real Samples.

All interfering substance solutions were prepared with PBS (10 mmol/L). The concentration of NaCl, KCl, $MgCl_2$, $CaCl_2$ and urea solution was 10 mmol/L; the concentration of glucose (Glu) and uric acid (UA) were 100 nmol/L; the concentration of GSH and human serum albumin (HAS) were 10 nmol/L; and the concentration of tyrosine (Tyr) was 100 U/mL. The urine samples obtained from the Affiliated Hospital of Putian University were centrifuged (8000 r/min) for 5 min, and the supernatant (400 μL) was mixed with 100 μL PBS (70 mmol/L) and Ru@SiO_2-NH_2@HA NPs solution (100 μL), and then incubated at 37 °C for 30 min. After that, the above-mentioned mixed solution can be obtained for detection.

Detection Procedures of HAase.

The Ru-SiO_2@AP@HA NPs solution (4 mg/mL, 100 μL) with 100 μL ultrapure water and 100 μL PBS (70 mmol/L) was dripped in different concentration of HAase solution (400 μL) and incubated for 30 min. After that, the ECL nanoprobes precipitated from the solution was collected and dissolved in PBS (2.0 mL, 10 mmol/L). Then the ECL signal of the resulting mixed solution was recorded with the scanning potential range from 0.4 to 1.6 V, the scan rate was set at 100 mV/s, and photomultiplier tube voltage was set at -1000 V. Each sample was detected and repeated three times. and the

obtained ECL intensity average was used for quantitative detection of HAase activity.

RESULTS AND DISCUSSION

Characterization of the Prepared Nanoparticles.

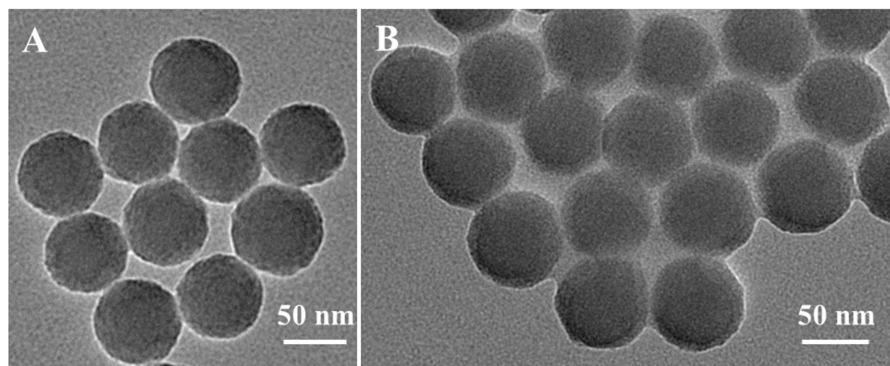

Fig. S1 TEM imagines of **(A)** Ru-SiO_2@AP NPs and **(B)** Ru-SiO_2@AP@HA NPs.

As shown in **Fig. S1A**, the size of Ru-SiO_2@AP NPs were about 60 nm, and the morphology and size of Ru-SiO_2@AP NPs were homogeneous. **Fig. S1B** showed the size of Ru-SiO_2@AP@HA NPs were about 65 nm, and the membrane structure coated on the nanoparticles could be observed, indicating the success of HA modification.

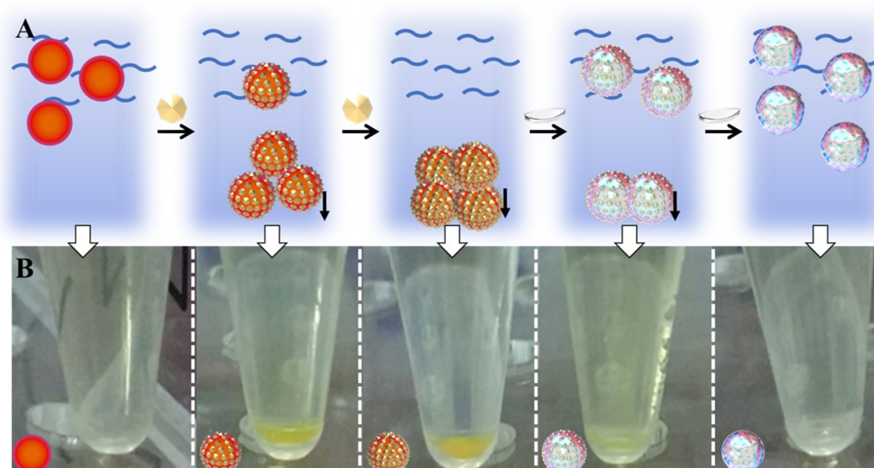

Fig. S2 (A) Controlling the precipitation capacity of nanoprobes through wettability regulation via different dossages of APTES and HA modification; (B) Solubility of (a) Ru-SiO_2 NPs, Ru-SiO_2@AP NPs modified by (b) 25%, and (c) 35% APTES, and Ru-SiO_2@AP@HA NPs modified by (d) 8 mg/mL, and (e) 10 mg/mL HA.

Performance and Application of the Proposed System.

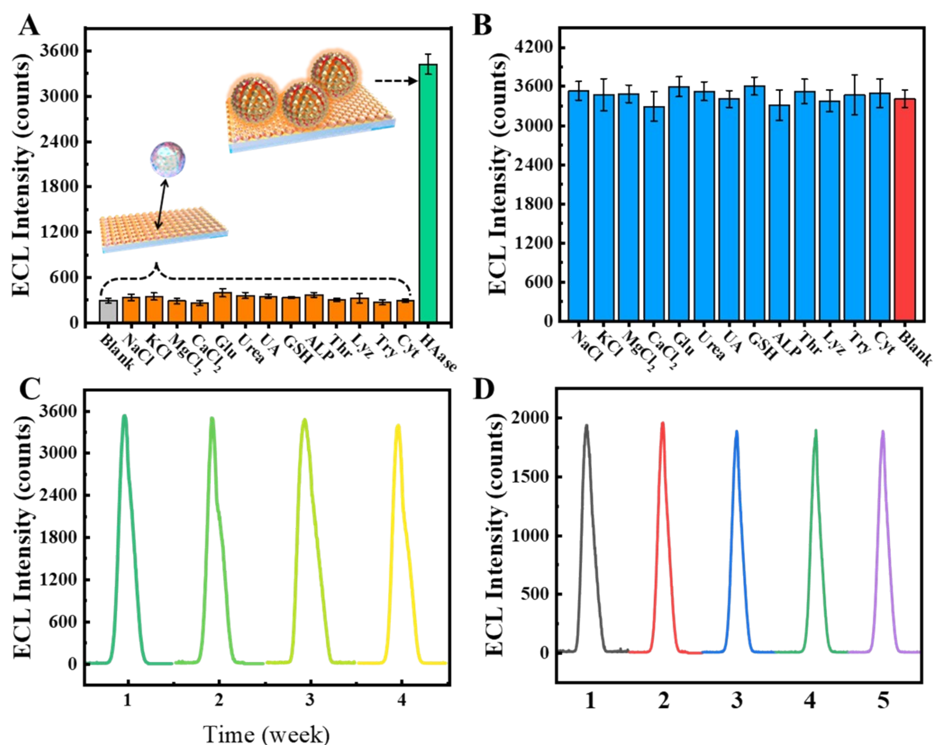

Fig. S3 (A) Selectivity of the proposed ECL biosensor, valued through the detection of 20 U/mL HAase and interfering substances (containing 10 mM NaCl, KCl, $MgCl_2$, $CaCl_2$ and urea; 100 nM glucose (Glu), uric acid (UA) and glutathione (GSH); 10 nM alkaline phosphatase (ALP), cytochrome (Cyt); and 100 U/mL thrombin (Thr), Lysozyme (Lyz), trypsin (Try)); **(B)** Anti-interference ability of the ECL biosensor during the detection of 20 U/mL HAase mixed with different interfering substances (the concentration of each interfering substance is same as above). **(C)** The stability of the proposed ECL biosensor stored at 4 °C for different weeks. HAase was 20 U/mL. **(D)** The reproducibility of the ECL biosensor. HAase was 10 U/mL.

Table S1 Comparison of different biosensors for HAase detection.

Method	Detection time (min)	Linear Range (U/mL)	LOD (U/mL)	References
Fluorescence	180	1.25 ~ 50	0.625	***Talanta, 2014, 130, 408-414.***
Fluorescence	90	0.025 ~ 0.2	0.007	***Biosens. Bioelectron.,2016, 79, 776-783.***
Fluorescence	96	0.1 ~ 8	0.05	***Anal. Chem., 2017, 89 (16), 8384-8390.***
Fluorescence	100	0.05 ~ 2 and 2.75 ~ 5	0.02	***Sens. Actuators, B 2018, 276, 95-100.***
Fluorescence	65	0.5 ~ 37.5	0.30	***Sens. Actuators, B 2019, 282, 45-51.***
ECL	150	2.0 ~ 40	0.33	***Sens. Actuators, B 2018, 275, 409-414.***
ECL	120	2.0 ~ 60	2.0	***ACS Appl. Bio Mater., 2020, 3 (2), 1158-1164.***
SERS	92	5 ~ 70	1.7	***Talanta, 2020, 215, 120915.***
ECL	30	2.0 ~ 50	0.22	This Work

Table S2 Quantification of HAase activity in urine samples with proposed ECL biosensor. (n = 3)

Urine Samples		Spiked HAase (U/mL)	Detected HAase (U/mL)	Recover Rate (%)	RSD (%)
Healthy People	**(1)**	0	2.71		8.67
		5.0	7.87	103.2	6.32
		10	12.45	97.40	4.63
		15	18.62	106.1	6.29
	(2)	0	2.39		7.90
		5.0	7.41	100.4	6.95
		10	13.04	106.5	5.71
		15	17.45	100.4	2.19
	(3)	0	3.50		7.66
		5.0	8.44	98.80	7.82
		10	13.60	101.0	8.45
		15	19.21	104.7	4.19
Patients	**(1) ***	0	37.02		3.78
		10	47.22	102.0	6.93
		20	56.98	99.80	4.13
		30	66.52	98.33	5.04
	(2) *	0	39.58		1.68
		10	49.70	101.2	3.70
		20	59.12	97.70	3.16
		30	68.26	95.60	7.65
	(3) *	0	63.16		5.24
		10	73.50	103.4	2.46
		20	83.40	101.2	2.92
		30	95.04	106.3	7.02

***** The sample was measured after twice diluted to avoid the concentration exceeding the range after standard addition.

REFERENCES

1. L. Zhang and S. Dong, *Anal. Chem.*, 2006, **78**, 5119-5123.
2. J. Qian, Z. Zhou, X. Cao and S. Liu, *Anal. Chim. Acta*, 2010, **665**, 32-38.