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SUPPORTING INFORMATION

Ti_3C_2 MXene-anchored photoelectrochemical detection of exosomes by in-situ fabrication of CdS nanoparticles with enzyme-assisted hybridization chain reaction

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EXPERIMENTAL SECTION

Material and Reagents. Fetal Bovine Serum (FBS), Streptavidin-labeled alkaline phosphate (SA-ALP), N-hydroxysulfsuccinimide (Sulfo-NHS), 1-Ethyl-3-(3-dimethyl-aminopropyl) carbodiimide hydrochloride (EDC) and Carboxyl magnetic beads coupling kit were purchased from Sangon Bioengineering Technology Co. Ltd. (Shanghai, China). Sodium thiophosphate (TP), 1-thioglycerol (TG) were purchased from Aladdin Industrial Corp. (Shanghai, China). Exosomes were obtained from Zaiji Biotechnology Co., Ltd. (Fujian, China). Ti_3AlC_2 power was purchased from Forsman Scientific Co. Ltd. (Beijing, China). Cadmium chloride ($CdCl_2$), sodium chloride (NaCl), hydrofluoric acid (HF, 40wt%) and dimethyl sulfoxide (DMSO) were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). All other reagents are analytical grade. Ultrapure water was supplied by a Milli-Q purification system (S.A.S 67120 Molsheim, Millipore). After the end of the reaction, the material deposited on the FTO glass is collected. The oligonucleotides mentioned in this paper were all synthesized by Sangon bioengineering technology Co. Ltd. Their sequences are as fellow:

Capture aptamer (CD63): 5'-NH_2-TTTTTTTTTTCACCCCACCTCGCTCCCGTGAC ACTAATGCTA-3'

Hairpin 1 (H1): 5'-biotin-TTAACC**CACGCCGAATCCTAGACT**CAAAGT**AGTC TAGGATTCGGCGTG**-3'

Hairpin 2 (H2): 5'-biotin-**AGTCTAGGATTCGGCGTG**GGTTAA**CACGCCGAAT CCTAGACT**ACTTTG-3'

C1: 5'-cholesterol -TTTGCCGTCGTGCCTTATTTCTG-3'

C2: 5'-AGTCTAGGATTCGGCGTGGGTTAAGoTTT-cholesterol-3'

The underlined sequences represent the initiator that can open the hairpin structure of H1. Bold characters in H1 and H2 represent the stem regions of the hairpins.

Preparation of CD63 aptamer-modified MBs. The aminated aptamer (CD63) was immobilized to carboxyl-modified magnetic beads and applied to capture exosomes. Simply speaking, 80 μL of 50 mg·mL^{-1}·MBs was washed three times with preserving fluid and dissolved in 150 μL of activation buffer. Then, 20 μL of EDC (50 mg·mL^{-1}) and 20 μL of Sulfo-NHS (50 mg·mL^{-1}) were added to the solution to activate the carboxyl-modified magnetic beads, followed by mixing and incubating at room temperature for 15 min.[1] After the magnetic beads were fixed on the magnetic frame for 30 s, the upper waste liquid was discarded with the pipettor. 1300 μL of 1 mM amino group modified CD63 aptamer was then covalently conjugated to

resuspend the MBs. Subsequently, 200 μL coupling buffer was added and incubated in a rotator at room temperature for 90 min. Afterwards, 5 ml of the blocking solution was mixed well and incubated on the rotator at room temperature for 30 min. Finally, the supersolution was removed and redispersed in 1mL of buffer solution.

Synthesis of Ti_3C_2 MXene. According to the method reported in the literature,[2-4] the organ-like Ti_3C_2 MXene were prepared and modified. In detail, 2.0 g Ti_3AlC_2 powder was immersed in 40 mL 40% HF aqueous solution and stirred at room temperature (RT) for 48 hours to etch Al. The suspension was filtered and centrifuged with deionized water until the pH was neutral, then dried at 80 °C for 12 hours. To achieve the exfoliation, the resulting multilayer of Ti_3C_2 powder were added to 40 mL DMSO and stirred for 24 hours. The obtained mixture was centrifuged several times with deionized water to acquire the inserted powder. The collected powder was then dispersed into deionized water and layered by ultrasonic treatment. After ultrasound treatment for 1 hour, the suspension was centrifuged at 5000 rpm for 60 min to remove the superfluous unexfoliated Ti_3C_2. Finally, the supernatant containing Ti_3C_2 MXene was obtained.

Construction of the PEC biosensor platform. Firstly, FTO conductive glass was ultrasonically cleaned three times with mixture (1:3, v/v) of deionized water and C_2H_5OH, and the FTO electrode was immersed in 0.01 M NaOH solution for 5 hours. Then, the exposure area of FTO glass was controlled to be 0.2827 cm^2. The Ti_3C_2 MXene/Cd^{2+} solution was obtained by adding Ti_3C_2 MXenes dispersed in ethanol solution (1 mg/mL) to $CdCl_2$ (0.2 M) and ultrasonic treatment for 2 h. Soon afterwards, 20 μL Ti_3C_2 MXene/Cd^{2+} solution was uniformly dropped on the clean FTO electrode, and blow-dried with N_2. The modified Ti_3C_2 MXene/Cd^{2+}/FTO was successfully obtained.

Signal amplification via HCR. Firstly, the different concentrations of exosomes were mixed with CD63 aptamer-modified MBs and incubated at room temperature for 2 h. Shortly afterwards, the stock solutions of H1 and H2 was heated to 95 °C for 5 min before use, respectively, and then allowed to gradually anneal to 25 °C to form the hairpin structure. Subsequently, 100 μL of 1 μM H1 were mixed with 100 μL of 1 μM H2 and incubated at 37 °C for 1 h. At the same time, the stock solutions of C1 and C2 were pre-hybridized from 90 °C to 25 °C at a final concentration of 1 μM to form DNA anchor with a sticky end. After that, the MBs combined with exosomes were resuspend with 200 μL of the above mixture, and then incubated at 37 °C for 1 h. After washing with PBS buffer for three times, H1 and H2 were added and then incubated in a thermostatic mixer for 1 h. The above solution was washed for three times, then SA-ALP was added and incubated for 1 h. As above, washed with PBS buffer and re-dispersed in 2mL preservation solution. Finally, 10 μL of 0.7 M thioglycerin and 60 μL of 8 mM sodium thiophosphate were added to the solution to react at 37 °C for 90 min.

PEC determination. The above solution (20 μL) was incubated for 30 min on the PEC biosensor to obtain FTO/MXenes/CdS. Finally, FTO/Ti_3C_2 MXene/CdS was inserted into the test solution (0.1 M Na_2SO_4)

and the PEC response was recorded under xenon lamp irradiation at a potential of 0 V (vs. SCE). And then the photocurrent responses were recorded using an electrochemical workstation (CH Instruments, 760D, USA).

Polyacrylamide gel electrophoresis (PAGE) analysis. 4 μL of different DNA solutions were mixed with 2 μL of 6× glycerin loading buffer and then loaded into 1× TRIS-Borate-EDTA (TBE) buffer in 12% polyacrylamide gel, respectively. Electrophoresis tank (Dingguo, DG-24DN, China) was performed on the electrophoresis apparatus (Dingguo, DL-300C, China) at constant pressure of 200 V for 45 min. The gel was dyed 4 S red plus for about 90 min and then rinse with ultrapure water. Finally, the gel was photographed by a fully automatic gel image analysis system (Peiqing, JS-2012, China).

PARTIAL RESULTS

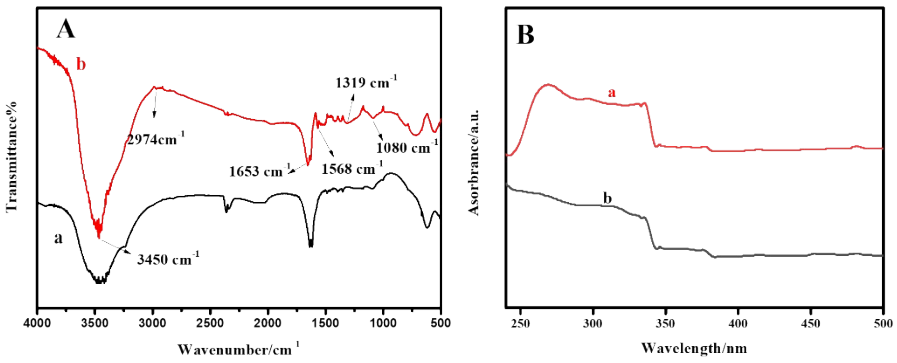

Fig S1. (A) FT-IR spectra of (a) carboxy-modified MBs and (b) CD63 modified MBs, (B) UV-vis absorption spectra of (a) CD63-MBs conjugate and (b) carboxy-modified MBs.

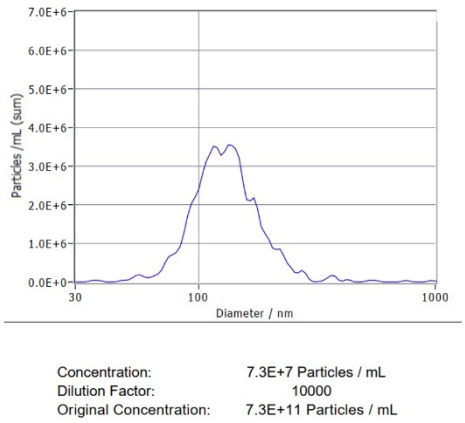

Fig S2. Size distribution of exosomes measured by NTA.

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