A simple method to assay tumor cells based on target-initiated steric hindrance

Electronic Supplementary Information

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Experimental Section

Materials

Phosphate buffer saline (PBS, containing NaCl 136.89 mM; KCl 2.67 mM; Na_2HPO_4 8.1 mM; KH_2PO_4 1.76 mM, pH 7.4), Dulbecco's modified Eagle's medium (DMEM) and 10 mM dNTP mixture were purchased from Sangon Biotech Co., Ltd (Shanghai, China). The synthesis and HPLC purification of nucleolin aptamer (5'-3': SH-TTT TTT GGT GGT GGT GGT TGT GGT GGT GGT GG) and random DNA (5'-3': SH-TTT TTT CTA GGA TCG GTC TGT TGA AGT AAT TAT CCT AG) were also completed by Sangon. Fetal bovine serum (FBS), cell counting chamber and 0.4 % trypan blue solution were obtained from Invitrogen Trading Co., Ltd (Shanghai, China). Terminal deoxynucleotidyl transferase (TdT, 5 U/μL) and trypsin-EDTA solution were bought from Beyotime Biotechnology (Shanghai, China). Tris(2-carboxyethyl) phosphine hydrochloride (TCEP) and $Ru(NH_3)_6Cl_3$ were supplied by Macklin Biochemical Co., Ltd (Shanghai, China) and Solarbio Life Sciences Co., Ltd (Beijing, China), respectively. Mercaptohexanol (MCH) and Tris were bought from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China).

All the reagents were analytically pure without further purification. The solutions in this work were prepared with deionized water purified by Milli-Q system.

Cell passage

After cell resuscitation, the cell growth status and density were observed every 24 hours. Cell passage can be carried out when the cells covered 80 % base area of the culture dish with uniform shape and size. First, we discarded the cell culture medium, gently injected some sterilized PBS and shook slightly to wash the culture dish. Then the PBS was discarded and repeated washing dish for 2-3 times. Next, 1mL

trypsin-EDTA solution was added and the digestion was observed carefully under the microscope. When the cells began to deform and there were gaps between the cells, removed the digestion solution. Afterwards, 2 mL of fresh culture medium was added and repeatedly blew the bottom of the culture dish until most of the cells were blown down. Half of the cells were transferred to a new dish for further culture and the other half was used for subsequent experiments.

Cell counting

The obtained cells were centrifuged at 1000 rpm for 5 min. After collection, the cells were diluted to an appropriate concentration with Tris-HCl buffer solution (pH 7.4) and repeatedly blew to form cell suspension. Then, 9 μL cell suspension was mixed with 1 μL 0.4 % trypan blue staining solution, and injected into the cell counting plate after fully blowing. Taking the average of three repeated readings in different areas of the cell counter plate as the final cell concentration.

Electrochemical experiment

This experiment used a typical three-electrode system, with gold electrode as working electrode, saturated calomel electrode as reference electrode and platinum electrode as counter electrode. The gold electrode was ground with 1 μm and 0.3 μm aluminum powder successively, and then sonicated with alcohol and water for 5 min respectively. After that, electrode was put into piranha solution prepared with H_2SO_4 and 30 % H_2O_2 at a ratio of 3:1 (volume ratio) and soaked for 30 min. After rinsing, the electrode was sequentially scanned by cyclic voltammetry in 0.5 M H_2SO_4 until the electrochemical characteristics were stable. After all the pretreatments were completed, the surface of the gold electrode was dried with nitrogen, 10 μL premixed solution of 2 μM aptamer and 10 mM TCEP was dropped on the surface of each electrode and incubated at 37 ℃ for 1 hour. Afterwards, the unconnected aptamers were washed by water, 10 μL 1 mM MCH was added and incubated at 37 ℃ for 30 min. Then, the cell suspension of different concentrations was incubated on the electrode surface at 37 ℃ for 1.5 h. The surface of the electrode was rinsed with Tris-HCl to remove residual cells. Enzyme reaction mixture (1 μL TdT enzyme with activity of 5 U/μL, 1 μL 10 mM dNTP, 2 μL enzyme buffer and 6 μL Tris-HCl) was added to the electrode surface for polymerization of 30 min. Finally, the electrode was immersed in the solution containing 5 μM Ru^{3+} for 15 min, and then scanned by differential pulse voltammetry (DPV) to obtain the electrochemical signal.

Extraction of HeLa exosomes

HeLa cell derived exosomes were extracted from the cell culture medium. First, the medium was centrifuged at 5000 g and 10000 g for 10 min and 30 min, respectively. Therefore, the cells, cell fragments and other vesicles in the medium were removed by the gradient centrifugation. Then, the supernatant was collected for filtering through the filter of 0.22 μm to further remove residual biomacromolecules. Next, the filtrate was centrifuged at 120000 g for 2 h. Finally, the supernatant was discarded, and the bottom of the centrifuge tube was repeatedly blown with 200 μL buffer to resuspend exosomes. The obtained exosomes were stored at -80 ℃.

Optimization of experimental conditions

In order to obtain optimal detection performance, we have optimized four major factors: aptamer concentration, TdT catalysis time, Ru^{3+} concentration and incubation time. The concentration of aptamer is a key factor so we optimized it firstly. On the one hand, if the aptamer concentration is too low, the distance between aptamers is too far and the captured cells may not be able to cover the surrounding aptamers. On the other hand, if the aptamer concentration is too high, there will be excess aptamers on the electrode surface, which can also be catalyzed by the TdT enzyme to generate strong background signal. Therefore, both of the two situations are not conducive to highlight the difference between the experimental group and the blank group. In order to improve the signal-to-noise ratio (S/N), we have tested several blank and experimental groups with different aptamer concentrations. The results show that a relatively higher S/N can be obtained at 2 μM aptamer (Fig S1A). With the extension of TdT catalysis time, the aptamer can be extended continuously until stable. However, once the catalysis time is too long, some aptamers in the experimental group that are difficult to get close to the polymerase due to the steric hindrance effect will also be slowly tailed, causing interference signal and reducing the S/N of the results. Therefore, it is necessary to optimize the TdT catalytic time. As shown in the Fig S1B, when the catalytic time is controlled at 30 min, a satisfactory S/N ratio can be obtained. To ensure that the DNA strand can fully combine enough Ru^{3+} to achieve the strongest electrochemical signals, we have also optimized the concentration of Ru^{3+}. It can be found that when the concentration of Ru^{3+} reaches 5 μM, the electrochemical signal remains basically unchanged (Fig S1C). So, 5 μM Ru^{3+} ions can ensure DNA strands binding enough electrochemical signal molecules. Finally, we have optimized the incubation time of Ru^{3+} to reduce the time cost of the method. When the incubation time is extended from 5 min to 15 min, the electrochemical

signal continues to increase until it keeps stable (Fig S1D). In a conclusion, we have selected 2 μM aptamer, 30 min TdT catalytic time, 5 μM Ru^{3+} and 15 min Ru^{3+} incubation time as the optimal reaction conditions for subsequent experiments.

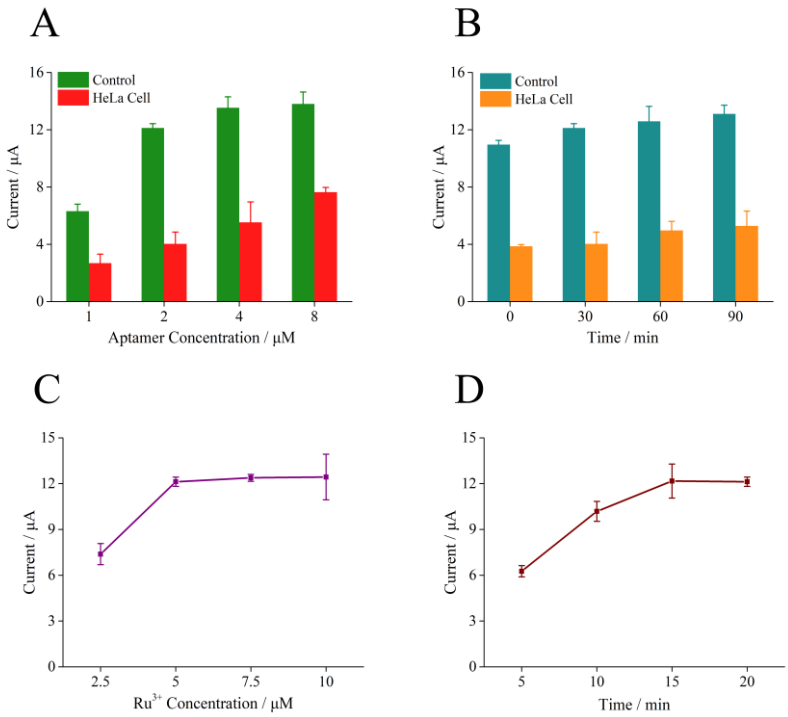

Fig. S1 The influence of aptamer concentration (A), TdT enzyme catalytic time (B), Ru^{3+} concentration (C) and Ru^{3+} incubation time (D) on the detection performance.

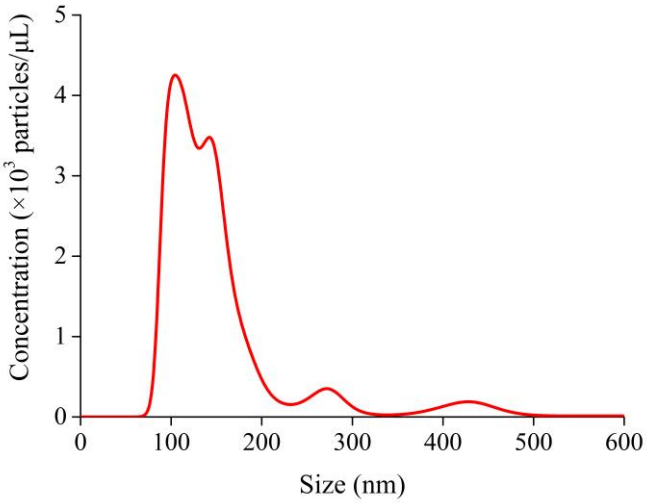

Fig. S2 The concentration and size distribution of HeLa exosomes.