Supporting Information

Functionalized nanopipettes for pH-gated single cell DNA delivery

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Table of Contents

Experimental section

Materials

4T1 cells, HeLa cells and L929 cells were obtained from Shanghai Moxi Boil Co., Ltd. 1640 medium, dulbecco's modified eagle medium (DMEM), fetal bovine serum (FBS), penicillin/streptomycin, and pancreatic ferment used to prepare the cell culture medium were obtained from Sangon Biotech, Shanghai. All DNA oligonucleotides were synthesized by Sangon Biological Engineering Technology Co., Ltd. (Shanghai, China) with high-performance liquid chromatography (HPLC) purification. Calcein-AM/PI probes were obtained from ThermoFisher Scientific Inc. (Waltham, MA, USA). Agarose gel was purchased from Thermo Scientific. $K_3[Fe(CN)_6]$ and $K_4[Fe(CN)_6]$ were purchased from Macklin (Shanghai, China). 1×PBS buffer (pH 7.4) contained 136.7 mM NaCl, 2.7 mM KCl, 8.72 mM Na_2HPO_4, and 1.41 mM KH_2PO_4. The silver wire (0.25 mm) used as the electrode was purchased from Alfa Aesar. All electrolyte solutions were prepared using ultrapure water obtained through a Millipore water purification system with an electric resistance of >18.2 MΩ (EMD Millipore, TONDINO, Shanghai).

Instrumental methods

Nanopipettes were fabricated using a P-2000 laser puller (Sutter Instrument Co., Novato, CA). An Au layer was modified on the inner surface of the nanopipette tip by using a Leica coating machine (EM ACE200). The morphologies of the nanopipettes were characterized using a field-emission scanning electron microscope (SEM, Ultra 55, Carl Zeiss Ltd., Germany). All reagents were centrifuged in a centrifuge (Eppendorf Centrifuge 5430). An inverted microscope (Eclipse TiU, Nikon, Japan) equipped with a 100x objective lens and a white light source was used to obtain images of nanopipettes and cells. Gel imaging was obtained using a Bio-Rad molecular imager. The nanopipette was fixed under the microscope for electrochemical measurements by a holder (Axon Instruments, Union City, CA) connected to an Axopatch 200B low-noise amplifier and an Axon Digidata 1550B low-noise data acquisition system (Molecular Devices, Sunnyvale, CA). Electrochemical impedance spectroscopy (EIS) was done on a CHI660E electrochemical workstation (CHI660E, Shanghai Chenhua Co., LTD., China). All materials were weighed with an analytical balance (ME 104, METTLER TOLEDO).

Cell culture

4T1 cells were cultured in 1640 medium with 10% FBS, 100 μg/mL penicillin, and 100 μg/mL streptomycin. HeLa cells and L929 cells were cultured in DMEM with 10% FBS, 100 μg/mL penicillin, and 100 μg/mL streptomycin. The cells were cultured in a humid atmosphere at 37 °C with 5% CO_2 and 95% air.

Gel electrophoresis analysis

First, 2.1 g of agarose was weighed and dissolved in 70 mL of 1×Tris-borate-ethylenediaminetetraacetic acid (TBE) buffer. The mixture was heated to effect dissolution, followed by the addition of ethidium bromide (a DNA staining agent), and then allowed to cool to form a gel. Subsequently, the agarose gel containing a 0.5 μM DNA solution was placed in 1×TBE buffer and subjected to electrophoresis at 70 V for 45 min. Finally, the gel was imaged under blue light using a Bio-Rad molecular imager.

Fabrication of laser-pulled nanopipettes

The nanopipettes used in this work were fabricated using a Sutter P-2000 laser puller, and the parameters of the nanopipettes as shown in Table S1. The variation of nanopipette pulling times was controlled within 0.2 s to ensure the reproducibility of the aperture geometry.

Preparation of Au-coated nanopipettes

Glass nanopipettes were fixed on the sample stage using conductive adhesive with their tips facing upward. The sample stage was then placed at the center of the vacuum chamber of the sputter coater, ensuring that the sample surface was aligned with the Au target. After setting appropriate parameters, a 10 nm-thick Au film was deposited on the inner surface of the nanopipette tips via ion sputtering. Subsequently, the Au-coated nanopipettes were removed for further modification.

Preparation of i-motif/anti-i-motif DNA double strands

The i-motif is a typical four-stranded nucleic acid structure formed by cytosine-rich sequences. In this study, three distinct sets of repeated i-motif/anti-i-motif sequences were prepared. The i-motif strands were conjugated with a thiol (-SH) group at the 5'-end, while the anti-i-motif strands were labeled with a FAM fluorescent group at the 5'-end. To prepare i-motif/anti-i-motif DNA double strands (dsDNA), hybridization

was performed using anti-i-motif strands (10 μM) complementary to i-motif strands (10 μM). The DNA sequences of the i-motif and anti-i-motif strands are listed in Table S2.

Fabrication of pH-sensitive nanopipettes

First, dsDNA (5 μM) was injected into Au-coated nanopipettes. Subsequently, the nanopipettes were centrifuged at 5000 rpm for 2 min to drive the solution into their tips. The nanopipettes were then allowed to stand for approximately 6 h.

ICR analysis

Two Ag/AgCl wires were introduced for obtaining electrochemical signals, as one of them was used as the working electrode inserted into the nanochannel filled with electrolyte solutions (0.02×PBS) of varying pH values, and the other one was used as the reference electrode bathed in 0.02×PBS corresponding to the respective pH values. Before electrochemical measurements, the liquid-filled tip of the nanochannel was centrifuged the exclude of the air bubbles. Then the nanopipette was fixed on a holder and connected to the headstage of the Axopatch 200B device. A previously designed protocol was edited to perform electrochemical experiments with pClamp 10.7 (Axon Instrument, Forest City, USA) run on a PC. The record mode was gap-free with a sampling frequency of 100 kHz and a 5 kHz low-pass Bessel filter.

DNA delivery to single cells using pH-responsive nanopipettes

One Ag/AgCl wire was inserted as the working electrode into the nanochannel filled with 0.02×PBS, and the other one was immersed as the reference electrode in the 1640 solution. The fluorescence intensity of the nanopipettes and the cells was observed by a microscope under 488 nm lasers.

EIS measurement

A conventional three-electrode system consisting of the working electrode (bare or modified planar glass plates), the reference electrode (Ag/AgCl), and the counter electrode (platinum wire) was employed. EIS analysis was conducted in 10 mM $[Fe(CN)_6]^{3-}$/$[Fe(CN)_6]^{4-}$ dissolved in 1×PBS (pH 7.4) containing 100 mM KCl at room temperature. The experimental parameters were as follows: open circuit potential, +0.25 V; potential amplitude, ±10 mV; frequency range, 0.1-100,000 Hz.

Calcein-AM/PI live/dead staining assay

First, 4T1cells cultured in a confocal dish were rinsed twice with pre-warmed 1×PBS to remove residual culture medium and serum that might interfere with dye binding. Subsequently, a serum-free medium working solution containing calcein-AM (at a final concentration of 5 μM) was added to the dish, and the cells were incubated for 20 min at 37 °C in a 5% CO_2 atmosphere under dark conditions. After this incubation period, a serum-free medium working solution of propidium iodide (PI, at a final concentration of 5 μM) was added to the dish, followed by an additional 5 min incubation. Upon completion of the incubation, the staining solution was aspirated, and the cells were rinsed 2-3 times with PBS. Finally, fluorescence images were acquired immediately using a fluorescence microscope: the excitation wavelengths were set to 488 nm for calcein-AM (to visualize viable cells) and 535 nm for PI (to visualize dead cells).

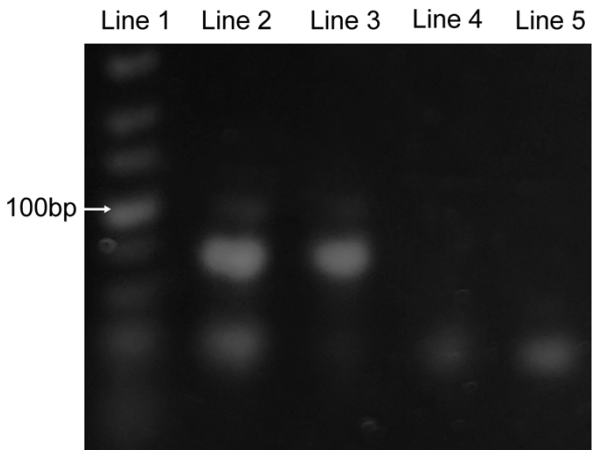

Fig. S1 Hydrogen ions induce the folding of i-motif chains to release anti-i-motif chains evaluated by agarose gel electrophoresis. From lanes 1 to 5: ladder (Line 1), A dsDNA solution with a pH of 6.0 (Line 2), A dsDNA solution with a pH of 7.5 (Line 3), an i-motif single strand (Line 4), and anti-i-motif single strand (Line 5). The agarose gel was run in 1×Tris-Borate-EDTA buffer at 70 V for 45 min and then stained with ethidium bromide. The gel was imaged on a Bio-Rad molecular imager under blue light.

Fig. S2 Rectification ratios derived from *I-V* curves of bare, Au-coated, and DsDNA nanopipettes corresponding Figure 1b.

Fig. S3 Optimization of PBS electrolyte concentration for nanopipette measurements.

Fig. S4 *I-V* curves of bare nanopipettes measured in buffers at pH 6.0 and 7.5 over different time intervals.

Fig. S5 *I-V* curves of Au-coated nanopipettes measured in buffers at pH 6.0 and 7.5 over different time intervals.

Fig. S6 *I-V* curves of dsDNA-modified nanopipettes measured in buffers at pH 6.0 and 7.5 over different time intervals.

Fig. S7 Reversibility of a representative i-motif-functionalized nanopipette measured under optimized conditions. Downward arrows: On state (acidic pH), and upward arrows: Off state (neutral pH).

Fig. S8 Optimization of dsDNA-FAM concentration. (a) Microscopic images of nanopipettes modified with 1, 5, and 10 μM dsDNA-FAM. Scale bar: 10 μm. (b) Average fluorescence intensity of dsDNA-modified nanopipettes at different concentrations. (c) *I-V* curves of nanopipettes functionalized with dsDNA-FAM at different concentrations.

Fig. S9 Photos showing the electrochemical monitoring system. The nanopipette was fixed on a holder and connected to the headstage of the Axopatch 200B device. The nanopipette was controlled to move along the X, Y, and Z directions when the micromanipulation system was turned on, until the nanopipette tip was moved to approach the targeted location. The whole process was monitored under an inverted microscope.

Fig. S10 Time-resolved *I-V* curves of an i-motif functionalized nanopipette recorded at different time points during DNA delivery to a 4T1 cell.

Fig. S11 Microscopic fluorescence images of dsDNA-FAM-modified nanopipettes and cells from four experiments. BF: Bright field. Scale bar: 10 μm.

Fig. S12 Average fluorescence intensity of 4T1 cells and i-motif-functionalized nanopipettes before and after insertion from five experiments.

Fig. S13 Left: Microscopic fluorescence images of dsDNA-FAM-modified nanopipettes and HeLa cells. BF: Bright field. Scale bar: 10 μm. Right: *I-V* curves of the nanopipette before and after insertion into HeLa cells and aspiration of cytoplasmic fluid. Data were expressed as mean ± standard error of five experiments.

Fig. S14 Left: Microscopic fluorescence images of dsDNA-FAM-modified nanopipettes and L929 cells. BF: Bright field. Scale bar: 10 μm. Right: *I-V* curves of the nanopipette before and after insertion into L929 cells and aspiration of cytoplasmic fluid. Data were expressed as mean ± standard error of five experiments.

Fig. S15 Cell viability assay (calcein AM/PI staining) of 4T1 cell before and after nanopipette injection. BF: bright field, calcein: green channel, PI: red channel. The cells are stained by calcein AM for 20 min and PI for 5 min. Scale bar: 10 μm.

Table S1. Parameters for the fabrication of nanopipettes using a P-2000 laser.

	Heat	Fil	Vel	Del	Pul
Line 1	650	3	35	128	70
Line 2	700	4	30	132	125

Table S2. Oligonucleotides sequences.

Name	Sequence (5`----------3`)
i-motif 1	SH-$(CH_2)_6$- AAAAAAAAAAAACCCTAACCCTAACCCTAACCC
Anti-i-motif 1	GGGTTAGTGTTAGTGTTAG
i-motif 2	SH-$(CH_2)_6$- AAAAAAAAAAAACCCTAACCCTAACCCTAACCCAACCC TAACCCTAACCCTAACCC
Anti-i-motif 2	GGGTTAGTGTTAGTGTTAGAAAAGGGTTAGTGTTAGTG TTAG
i-motif	SH-$(CH_2)_6$- AAAAAAAAAAAACCCTAACCCTAACCCTAACCCAACCC TAACCCTAACCCTAACCCAACCCTAACCCTAACCCTAA CCC
Anti-i-motif	GGGTTAGTGTTAGTGTTAGAAAAGGGTTAGTGTTAGTG TTAGAAAAGGGTTAGTGTTAGTGTTAG
Anti-i-motif- FAM	FAM- GGGTTAGTGTTAGTGTTAGAAAAGGGTTAGTGTTAGTG TTAGAAAAGGGTTAGTGTTAGTGTTAG