Real-time Cell Barrier Monitoring by Spatial Trans-epithelial Electrical Resistance Measurement on Microelectrode Arrays Integrated Transwell

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Supplementary information

Fabrication of MEA

Metal electrodes were patterned by wet etching. Using DZS500 (Shenyang Keyi, China), 10 nm chromium was evaporated on the surface of glass as an adhesive layer, followed by evaporation of 200 nm gold layer. S1813 (AZ, USA) photoresist was spin-coated at 3000 rpm with soft bake and post-exposure bake at 110 ℃ for 300 s each. Photolithography was performed by MA6 DUV Mask Aligner **(**SUSS, Germany) and development for 60 s using a bath of RZX3038 developer (Ruihong Electronic Chemical, China).

The gold corrosion solution was prepared using potassium iodide (KI): iodine (I_2): water (DI) = 4 g: 1 g: 40 g, and the chromium corrosion solution was prepared using ceric ammonium nitrate: acetic acid: water (DI) = 20 g: 10 g: 70 g. The chip was immersed in the gold corrosion solution for 45 s and chromium corrosion solution for 30 s. After removal of residual photoresist by acetone and cleaning, the electrode patterns have been successfully fabricated.

Cell culture

A549 cells (Cell Resource Center, Peking Union Medical College) were stored in Dulbecco's modified Eagle medium (DMEM, 11995065, Thermo), supplemented with 10 % heat inactivated fetal bovine serum (FBS, A5669401, Thermo), 1 % glutamine, and 1 % penicillin-streptomycin-amphotericin (PSA, 15140122, Thermo) solutions, in a constant temperature and humidity incubator at 37 ℃ and 5 % CO_2. For the long-term cell growth detection experiments, we directly added cell suspensions, which have been passaged for 10-20 generations, into the apical culture medium in our device. For drug experiments, cells were first cultured in 12-well plates for 5 days. Then, along with the transwell inserts, they were transferred to the PMMA chamber to continue with the subsequent experiments.

When conducting cell culture on chip, it was pretreated with O_2 plasma for 1 minute to enhance surface hydrophilicity. Then, the MEA device was rinsed with ethanol and phosphate buffered saline (PBS, 10010023, Thermo), and the membrane was rinsed with culture medium. A549 cells harvested with trypsin/EDTA solution (25200072, Thermo) were seeded with a density of 1.6×10^5 cells/cm^2 in the apical medium. Static culture was performed with the culture medium changed twice daily.

Drug experiments

Prepare EGTA (ethylene glycol-bis(β-aminoethylether)-N,N,N′,N′-tetraacetic acid, E1219, Thermo) with deionized water at 0.1 M, pH was adjusted to 9 by dropwise addition of NaOH, and then diluted to 1 mM and 5 mM using culture medium. Prepare cisplatin (232120, Merck) using PBS containing 140 mM NaCl at 0.01 M, then dilute the concentration to 1 mM and 5 mM using culture medium. By measuring TEER, the response of cells to different drugs can be obtained, thereby analyzing the effect of drugs on cells. EGTA is typically known to modify ion transport and reduce cell barrier function without inducing cell death. In contrast, low concentrations of cisplatin can hinder cellular growth, whereas high concentrations may trigger apoptosis in cancer cells, which is irreversible.

For the EGTA experiment, we cultured A549 for 5 days until a complete monolayer of cells formed. Then we added culture media containing 1 mM and 5 mM EGTA respectively to disrupt the cell barrier. Immediately after joining, we conducted continuous testing for 4 hours. After the electrical test, the culture medium was replaced with EGTA-free medium. The cells were further cultured for 24 hours with continued monitoring. Meanwhile, the control group underwent the same procedure using culture medium without EGTA. As for cisplatin experiment, the basic procedure was same, since it took a long time to affect cells, after adding 5 μM and 50 μM cisplatin medium and live/dead staining, we changed the medium at 24 hours for cell recovery. Similarly, cells were continuously detected for 24 hours.

Simplified electric double layer effect

Due to the electric double-layer effect, there is an electric double-layer capacitor on the electrode surface, whose impedance varies with frequency and is represented as a hybrid element of resistance and capacitance. Typically, it is approximated as a constant phase element, (***CPE***), with its formula given by:

$$Z_{CPE} = \frac{1}{Q_M(j\omega)^{\alpha_M}} \#\ (1)$$

If the impedance is purely resistive, then the CPE index ($\boldsymbol{\alpha_M}$) equals 0, and for pure capacitance it equals 1. For most electrodes, the index varies between 0.5 and 0.9. The magnitude of impedance is defined by the parameter Q_M. In the frequency range of 1 kHz to 1 MHz used in these experiments, the dielectric impedance is close to the resistance.

Determination of parameters

To evaluate the differences between electrodes, EIS scans were first performed on 16 electrodes, and 2 mL of culture medium was added to PMMA chamber. the overall impedance size of Z_{CPE}, R_{up}, and R_B in the electrical model was determined by the collected impedance data. Due to the frequency variation of Z_{CPE}, all data is collected at a fixed frequency (10k Hz), and the average impedance of 16 electrodes is 275.57 ± 2.58 Ω. The conductivity of the culture medium was measured using a conductivity meter (YSI 3200) to be 1.26 S/m. Based on the geometric dimensions of the device and electrodes, as well as the conductivity values of the culture medium, it can be calculated that the longitudinal and transverse resistances of the basolateral medium are approximately 430 Ω and 4300 Ω.

Explanation of R_{down} and R_B

Both R_{down} and R_B represent longitudinal resistance, but they are expressed differently in different models. R_{down} corresponds to the solution impedance represented in the equivalent electrical model (**Fig. 2a**), while R_B represents the solution impedance in the dual-route model (**Fig. 2b**). The dual-route model was proposed to explain the different equivalent circuits at various electrode positions, simplifying them into two branches to help readers better understand the principle of spatial difference measurements and the subsequent calculation of the 16 branches. The renaming of R_B was done to align with the transverse resistance R_A, in order to explore the impact of the transverse to longitudinal impedance ratio on spatial TEER monitoring.

The calculation process of Z_X

The calculation formula of the impedance Z_{U1} measured by channel 1 is as follows, which is equivalent to 16 channels of Z_{UX} connected in parallel and then connected in series Z_F:

$$Z_{U1} = Z_F + \left(Z_1{}^{-1} + \left(Z_2 + R_{1-2}\right)^{-1} + \left(Z_3 + R_{1-3}\right)^{-1} + \left(Z_4 + R_{1-4}\right)^{-1} + \ldots + (Z$$

After transforming the formula:

$$\left(Z_{U1} - Z_F\right)^{-1} = 1 * Z_1{}^{-1} + \frac{1}{1 + {R_{1-2}}/{Z_2}} * Z_2{}^{-1} + \frac{1}{1 + {R_{1-3}}/{Z_3}} * Z_3{}^{-1} + \ldots + \frac{1}{1 + {R_{1-N}}} + \ldots + \frac{1}{1 + {R_{1-16}}/{Z_{16}}} * Z_{16}{}^{-1}$$

16 channels can obtain 16 such equations to form a system of equations：

$$\mathbf{A}=\begin{pmatrix}(\mathbf{Z_{U1}}-\mathbf{Z_F})^{-1}\\ \vdots\\ (\mathbf{Z_{UX}}-\mathbf{Z_F})^{-1}\\ \vdots\\ (\mathbf{Z_{U16}}-\mathbf{Z_F})^{-1}\end{pmatrix},\ \mathbf{B}=\begin{pmatrix}\lambda_{1-1} & \cdots & \lambda_{1-16}\\ \vdots & \ddots & \vdots\\ \lambda_{16-1} & \cdots & \lambda_{16-16}\end{pmatrix},\ \lambda_{N-M}=\frac{1}{1+R_{N-M}/Z_{OM}},\ \mathbf{C}=\begin{pmatrix}(\mathbf{Z_1})^{-1}\\ \vdots\\ (\mathbf{Z_X})^{-1}\\ \vdots\\ (\mathbf{Z_{16}})^{-1}\end{pmatrix}$$

Matrix A can be obtained through measurement, coefficient matrix B can be obtained by solving the lateral impedance R_{N-M}, and matrix C is the required accurate area spatial TEER. The calculation formulas for the three are as follows:

$$\mathbf{A}=\mathbf{B}\times\mathbf{C}$$

$$\mathbf{B}^{-1}\times\mathbf{A}=\mathbf{B}^{-1}\times\mathbf{B}\times\mathbf{C}$$

$$C=\mathbf{B}^{-1}\times\mathbf{A}$$

Matrix C can be obtained through the above calculation process.

Table 1

Influence of R_A/R_B on circuit spatial difference detection.

	$\mathbf{R_A/R_B}$	$\mathbf{Z_X/Z_Y}$	$(Z_{UX}-Z_F)^{-1}/(Z_{UY}-Z_F)^{-1}$
Case.1	1	1	1
Case.2	5	1	1
Case.3	10	1	1
Case.4	1	5	0.778
Case.5	5	5	0.524
Case.6	10	5	0.444
Case.7	1	10	0.727
Case.8	5	10	0.455
Case.9	10	10	0.331

We can change R_A/R_B by adjusting the distance between the microelectrodes and PETE membranes to achieve better spatial resolution. When the TEER of the cell layer X area and Y area are the same ($Z_X/Z_Y = 1$), no difference can be detected no matter what value R_A/R_B is. When there is a significant difference in TEER between the cell layer X area and the Y area ($Z_X/Z_Y = 10$), the larger R_A/R_B is, the greater the detected difference is.

Table 2

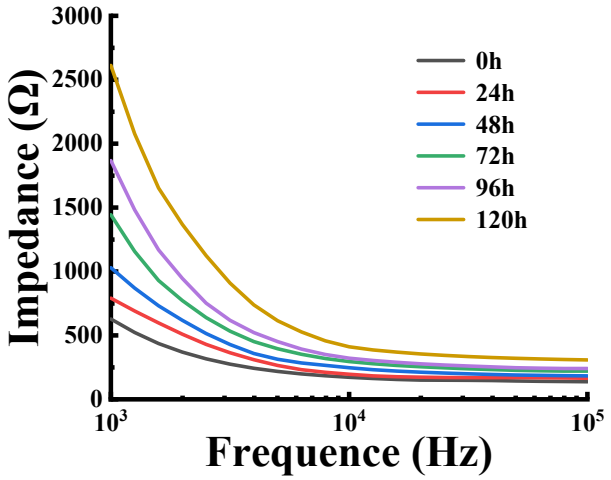
Values of factor $\boldsymbol{K_{1-M}}$, $\boldsymbol{R_{1-M}=K_{1-M}R_0}$. For example, $\boldsymbol{K_{1-9}}$ represents the transverse resistance equivalent coefficient from the first electrode to the 9th electrode, which is 0.460. So $\boldsymbol{R_{1-9}}$ equals

$K_{1-9} R_0$.

	1	2	3	4
1	0	0.588	0.460	0.382
2	0.588	0.536	0.467	0.407
3	0.460	0.467	0.436	0.391
4	0.382	0.407	0.391	0.35

The remaining lateral resistances can be obtained in the same way. $R_{N-M} = K_{N-M} R_0$

Supplementary Fig. 1



This EIS impedance spectrum shows the changes, demonstrating measured by one electrode and the upper electrode in MEA, demonstrating how we conduct impedance monitoring. At frequencies below 10 kHz, the impedance change is primarily influenced by the capacitance and resistance of the entire cell layer, while at frequencies above 10 kHz, the impedance gradually stabilizes. For this study, we selected the impedance value at a frequency of 10 kHz. We obtained EIS impedance spectrum at different time points through continuous impedance scanning, and finally generated the graph showing the relationship between impedance variation and time.

Supplementary Fig. 2

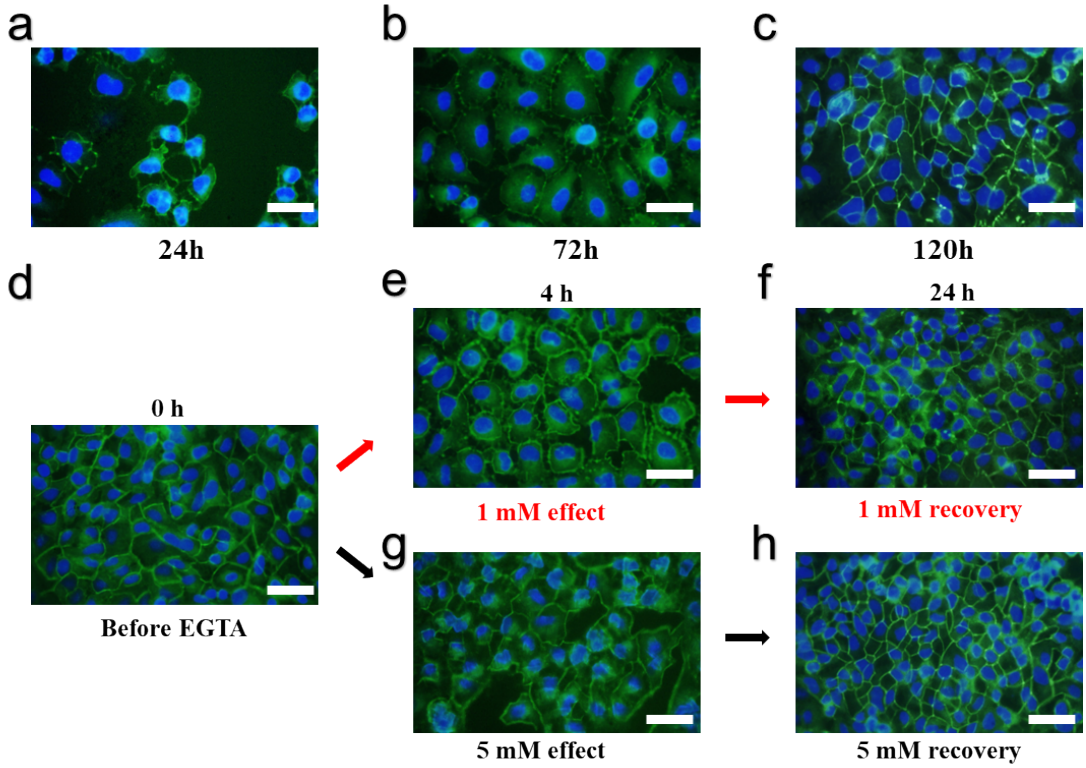


Fig. S2 ZO-1/DAPI staining image of A549 cells. a-c. The process of A549 gradually forming monolayer cells in long-term culture. d-h. Validation experiment of EGTA effect on the barrier of A549 cells. Nuclei stained in blue and ZO-1 in green. The scale is 30 μm.

ZO-1/DAPI staining

The transwell inserts were rinsed with preheated PBS buffer and fixed by 4% paraformaldehyde in PBS for 25 min. Followed by three washing steps with PBS for 30 min at room temperature. 0.2% Triton X-100 (SS0049, Biofount) were added and permeated for 3 minutes. The cells were then washed three times for 30 min with PBS containing 5% BSA (HXH504384, Biofount), and the inserts were incubated overnight at 4 ◦C with the first antibody rabbit anti-ZO-1 (33-9100, Thermo) which was diluted in PBS to a concentration of 2 μg/mL. After decanting the solution and washing three times for 15 min in PBS with 1% BSA the second antibody goat anti-rabbit with Alexa-488 (A-11008, Thermo) with a concentration of 5 μg/mL was applied to the cells for 30 min at room temperature in the dark. Lastly, after one washing step in PBS with 1 % BSA and two washing steps in PBS without BSA for 15 min, the cells were incubated with 200 μg/mL DAPI (4',6-diamidino-2-phenylindole) diluted in PBS for 10 min in the dark and at the end washed with PBS. Finally, 95% glycerol were added to cover the surface.

Authorship contribution statement

Yimin Shi: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing - Original Draft. **Sheng Sun:** Investigation. **Hui Liu:** Investigation. **Mingda Zhao:** Investigation. **Meiyan Qin:** Investigation. **Jinlong Liu:** Investigation. **Yang Zhao:** Investigation.

Jingfang Hu: Investigation. **Mingxiao Li:** Investigation. **Lingqian Zhang:** Methodology, Writing - Review & Editing, Supervision, Funding acquisition. **Chengjun Huang:** Writing - Review & Editing, Supervision, Funding acquisition.