



# **Quantitative electrophysiological monitoring of anti-histamine drug effects on live cells via reusable sensor platforms**

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1 Quantitative Electrophysiological Monitoring of Anti-histamine Drug

2 Effects on Live Cells via Reusable Sensor Platforms

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**ABSTRACT:** We demonstrated the quantitative electrophysiological monitoring of histamine and anti-histamine drug effects on live cells *via* reusable sensor platforms based on carbon nanotube transistors. This method enabled us to monitor the real-time electrophysiological responses of a single HeLa cell to histamine with different concentrations. The measured electrophysiological responses were attributed to the activity of histamine type 1 receptors on a HeLa cell membrane by histamine. Furthermore, the effects of anti-histamine drugs such as cetirizine or chlorphenamine on the electrophysiological activities of HeLa cells were also evaluated quantitatively. Significantly, we utilized only a single device to monitor the responses of multiple HeLa cells to each drug, which allowed us to quantitatively analyze the antihistamine drug effects on live cells without errors from the device-to-device variation in device characteristics. Such quantitative evaluation capability of our method would promise versatile applications such as drug screening and nanoscale bio sensor researches.

**Keywords:** Anti-histamine; HeLa cell; Carbon nanotube transistor; Reusable sensor

## 1. Introduction

Histamine, which acts as a neurotransmitter in central nervous systems, can influence many biological processes such as neuronal excitation and immune regulation *via* the activation of histamine receptors on cell plasma membranes (Akdis and Blaser, 2003; Benarroch, 2010; MacGlashan, 2003; Schneider et al., 2002). One type of the histamine receptors is histamine type 1 receptor (H1R) which belongs to the family of G-protein-coupled receptors. The activation of H1Rs by histamine triggers many intracellular electrophysiological signaling pathways including the increase of intracellular calcium ions ( $\text{Ca}^{2+}$ ) by the opening of  $\text{Ca}^{2+}$  channels in a cell plasma membrane (Benarroch, 2010; Jutel et al., 2009; Molina-Hernandez et al., 2012). In non-excitabile cells such as HeLa cells, the stimulation of H1Rs embedded in the cell membrane generates the rise of cytosolic free  $\text{Ca}^{2+}$  concentrations (Barajas et al., 2008). By measuring the changes of  $\text{Ca}^{2+}$  concentrations as well as a  $\text{Ca}^{2+}$  influx into cells, the electrophysiological effects of histamine and anti-histamine drugs could be evaluated. In order to quantitatively monitor the  $\text{Ca}^{2+}$  influx into the cells, sensitive and reliable measurement techniques are required.

The effects of histamine and anti-histamine drugs have been studied extensively using various methods such as fluorescence assays and electrophysiological techniques. Conventional fluorescence assays are simple and have been utilized extensively to evaluate the histamine and antihistamine drug effects (Bootman et al., 1992; Sauve et al., 1991). However, the fluorescence signals of dyes would be degraded over time, which made it very difficult to achieve the quantitative evaluation of drug effects. In addition, the fluorescence assays need time-consuming processes such as the pretreatment of samples with fluorescent dyes. Meanwhile, electrophysiological tools such as patch clamp techniques and nanoscale devices have exhibited outstanding performances in the detection of electrophysiological signals of live

1 cells stimulated by histamine (Negulyaev Yu et al., 1993; Sauve et al., 1991). The patch clamp  
2 methods exhibit high temporal and spatial resolution for the monitoring of electrophysiological  
3 activities in a cell. However, the operation of these methods requires skilled operators.  
4 Furthermore, the patch clamp methods could measure an ion flux only at a specific location of a  
5 cell membrane rather than the electrical potential of the whole cell membrane, and, thus, it could  
6 only monitor the partial intracellular electrical signal pathways.

7 On the other hand, nanoscale devices have been developed to explore the  
8 electrophysiological activities of adherent cells with rather easy procedures (Cheng et al., 2014;  
9 Li et al., 2013; Spanu et al., 2015). Among the nanoscale devices, many researchers have utilized  
10 carbon nanotube-based transistors to monitor the activities of various cells (Sudibya et al., 2009;  
11 Tsai et al., 2008; Wang et al., 2007). However, nanoscale devices usually exhibited different  
12 characteristics from one device to another, which made it difficult to quantitatively analyze the  
13 data measured by nano-devices. Furthermore, the direct growth of cells on devices might affect  
14 the characteristics of the nano-devices, resulting in a difficulty in comparing the data measured  
15 by different nano-devices.

16 Herein, we report a method for the quantitative electrophysiological monitoring of the  
17 antihistamine drug effects on live cells using reusable sensor platforms (RSPs) based on carbon  
18 nanotube (CNT) transistors. In this work, HeLa cells were first floated in a solution, and one of  
19 the cells was placed on the junction area of a reusable sensor based on a CNT transistor *via* a  
20 microcapillary manipulation for the monitoring of its electrophysiological responses to histamine  
21 and anti-histamine drugs. Significantly, for repeated measurements, the measured cell was  
22 replaced with a new cell on the same RSP, enabling quantitative evaluation without being  
23 suffered from the device-to-device variation of device characteristics. The method was utilized to

quantitatively monitor the electrophysiological responses of HeLa cells to histamine by evaluating the conductance changes of a RSP. These conductance changes were attributed to a  $\text{Ca}^{2+}$  influx through ion channels on a HeLa cell membrane due to the activation of H1Rs by histamine. The pretreatments of HeLa cells with anti-histamine drugs such as cetirizine or chlorphenamine reduced a  $\text{Ca}^{2+}$  influx into the cells, which indicates that the activation of H1Rs was inhibited by anti-histamine drugs (Mizuguchi et al., 2013). Importantly, for each experimental set, we utilized only a single device to evaluate the activity of H1Rs in multiple HeLa cells, which allowed us to obtain statistically meaningful and quantitative results about the antihistamine drug effects without errors from device-to-device variations. Our work should provide a simple but powerful method for various biomedical applications such as drug screening and pharmaceutical studies at a single-cell-level.

## **2. Material and methods**

### *2.1. Fabrication of Reusable Sensor Platforms*

A RSP was fabricated by a method reported in our previous works (Kim et al., 2012; Ta et al., 2014). In brief, glass substrates were soaked into a piranha solution (the 3:1 mixture of a concentrated sulfuric acid solution and a 30% wt. hydrogen peroxide solution) to remove organic residues on the substrates (Fig. 1a–i). To disperse CNTs, the powder form of CNTs (purchased from Sigma–Aldrich) was sonicated in 1,2–dichlorobenzene (0.05 mg/mL) for 1 hour. Then, the CNTs suspension was spin-coated on the glass substrates at 5000 rpm. The source, drain, and floating electrodes (Au/Pd, 30 nm/10 nm) were fabricated by conventional photolithography processes including a thermal evaporation deposition and a lift-off process. After that, the RSPs were passivated by an aluminum oxide layer ( $\text{Al}_2\text{O}_3$ , 100 nm) using an atomic layer deposition

(ALD) system to eliminate leakage currents and possible effects from other cells during sensing experiments in a liquid environment. A further photolithography step was performed to open junction areas. Finally, the  $\text{Al}_2\text{O}_3$  layer on the junction areas was etched by an  $\text{H}_3\text{PO}_4$  solution (85% by weight in water) at 90 °C for 3 minutes (Fig. 1a-ii).

## 2.2. Repeated Measurement of Cell Responses Using Reusable Sensor Platforms

For the measurement of electrophysiological responses of HeLa cells, a RSP was assembled in a live-cell imaging chamber. HeLa cells (purchased from the Korean Cell Line Bank) were cultured on a plate in Dubecco's Modified Eagle Medium (DMEM, Gibco) supplemented with fetal bovine serum (10% v/v), L-glutamine (2 mM), penicillin (100 U/mL), and streptomycin (100  $\mu\text{g/mL}$ ) at 37 °C in an atmosphere of 5%  $\text{CO}_2$  for 4 days (Figure 1a-iii). Right before the measurements of the effect of histamine and anti-histamine drugs, HeLa cells were incubated in a fresh  $\text{Ca}^{2+}$ -free bath solution for 60 minutes. To transfer these cells onto our RSP, the cells were first washed three times with a  $\text{Ca}^{2+}$ -free bath solution, then, detached from the plate using a trypsin solution (Figure 1a-iv). A HeLa cell suspension was introduced in the imaging chamber, and the aggregate of HeLa cells was picked up using a microcapillary (Figure 1a-v). The aggregate was adjusted to locate a cell on the junction area for the screening of drugs using the RSP (Figure 1a-vi). The source-drain bias ( $V_{\text{sd}}$ ) of 0.1 V was applied to RSPs during the conductance measurements, and the source-drain currents were monitored by a data acquisition system (National Instruments, NI-9215(A)). To estimate the concentration-dependent effects of histamine on HeLa cells, 200  $\mu\text{L}$  of a histamine solution containing 15  $\mu\text{M}$   $\text{Ca}^{2+}$  with the range of histamine concentrations from 300 nM to 300 mM was injected into the chamber. For the study of the effects of anti-histamine drugs, cells were

1 incubated with the various concentrations of anti-histamine drugs (from 10 to 200  $\mu\text{M}$ ) for  
2 further 15 minutes before the loading of floating cells on a RSP was performed. And then, 200  
3  $\mu\text{L}$  of a histamine solution (300  $\mu\text{M}$ , containing 15  $\mu\text{M}$   $\text{Ca}^{2+}$ ) was injected to stimulate the cells.  
4

### 5 **3. Results and discussion**

#### 6 *3.1. Advantage of a RSP*

7 In a typical experiment, only the junction area including floating electrodes of a RSP was  
8 exposed to a single HeLa cell during drug response experiments (Fig. 1a–ii). It allows us to  
9 eliminate leakage currents and possible non-specific effects from other cells during sensing  
10 experiments in a liquid environment. Fig. 1b shows the optical image of a single HeLa cell  
11 placed on a CNT junction area using a microcapillary. Significantly, since HeLa cells were not  
12 directly cultured on device surfaces, they did not alter the characteristics of RSPs. Furthermore,  
13 the measured cell could be easily removed from the RSP surface, and the used RSP device could  
14 be reused for additional measurements with different cells, which allowed us to obtain  
15 statistically-meaningful results without suffering from possible errors due to device-to-device  
16 variations. Note that, unlike a patch clamp method, we do not need to form a hole or a high  
17 resistance seal on a cell membrane using a micropipette, and, thus, our method could be a  
18 convenient and non-invasive method. Furthermore, our method could quantitatively monitor  
19 electrophysiological signals at a single-cell-level, which would be a significant advantage for  
20 biomedical researches and applications.  
21

#### 22 *3.2. Characteristics of a RSP*



A topography image of a RSP taken by an atomic force microscopy (AFM) system (MFP-3D, Asylum Research) in a tapping mode shows the junction area of a RSP device with three floating electrodes (Fig. 2a). The dimensions of each floating electrode are 2  $\mu\text{m}$  wide and 10  $\mu\text{m}$  long. Moreover, the width of an exposed junction area is 15  $\mu\text{m}$ , which fits the size of a single HeLa cell, which allowed us to monitor electrophysiological responses at a single-cell-level. Another advantage of our reusable sensor is a high sensitivity, which was enabled by aligned CNTs networks and floating electrodes. Previous works reported that the alignment of CNTs could increase the density of CNTs in a junction area and improve the connectivity between electrodes while reducing lateral connections, which resulted in the significant enhancement of a device transconductance and a sensor sensitivity (Lee et al., 2010; Lee et al., 2009). Besides, the formation of floating electrodes could also enhance the sensitivity of our RSP due to the increased number of Schottky barriers in the devices as reported previously (Kim et al., 2012).

Fig. 2b shows the gating effect curve of a RSP obtained by using an Ag/AgCl liquid gate electrode as a liquid gate in a  $\text{Ca}^{2+}$ -free bath solution. A bias voltage between source and drain electrodes was maintained at 0.1 V, while a gate bias voltage ( $V_{lg}$ ) was swept from -0.5 to 0.5 V. The source-drain current ( $I_{ds}$ ) of RSPs was measured by a semiconductor characterization system (Keithley, 4200, USA). The curve shows a decreasing source-drain current with an increasing gate bias. It implies that our device exhibited *p*-type characteristics. In addition, the current decreased drastically by a small gate bias change, indicating the high sensitivity of our device.

### 3.3. $\text{Ca}^{2+}$ influx into HeLa cells

To confirm a histamine-induced  $\text{Ca}^{2+}$  influx into HeLa cells assembled onto our RSP devices, we performed conventional fluorescence  $\text{Ca}^{2+}$  assays. HeLa cells were loaded with 2  $\mu\text{M}$  fluo-4-AM as a  $\text{Ca}^{2+}$  indicator and intracellular calcium changes were measured upon stimulation by histamine (details in Supplementary Information). Throughout the incubation process, the indicator molecules permeated into the cells, which made it possible for us to detect intracellular  $\text{Ca}^{2+}$  ions. Fig. 2c and 2d show the histamine responses of *HeLa cells cultured on a glass substrate* and *floating HeLa cells assembled on our RSP device*, respectively. Note that, in both of cases, the fluorescence intensity of HeLa cells significantly increased when a 100  $\mu\text{M}$  histamine solution was injected. These results confirmed that histamine induced  $\text{Ca}^{2+}$  concentration changes even inside the floating HeLa cells assembled onto our RSP devices (Bristow and Zamani, 1993; Sauve et al., 1991).

### 3.4. Effect Monitoring of histamine by using a RSP

The changes of  $\text{Ca}^{2+}$  concentrations in cells could be mediated by two well-known mechanisms, namely a  $\text{Ca}^{2+}$  release from intracellular stores and a  $\text{Ca}^{2+}$  influx through specialized channels on a plasma membrane (Berridge, 1997; Streb et al., 1983). Fig. 3a depicts the mechanism of a  $\text{Ca}^{2+}$  influx *via* store-operated calcium channels (SOCCs) and voltage-dependent calcium channels (VDCCs) opened by the histamine stimulation on H1Rs. HeLa cells are known to possess histamine receptors including H1Rs (Mizuguchi et al., 2013; Sauve et al., 1991). In a resting state, ion channels such as SOCCs and VDCCs on a cell membrane are closed. When histamine molecules interact with H1Rs,  $\text{IP}_3$  molecules are generated as a second messenger in an intracellular signaling pathway. Subsequently, the activities of  $\text{IP}_3$  induces  $\text{Ca}^{2+}$  release from intracellular stores which mediates a  $\text{Ca}^{2+}$  influx

1 through SOCCs referred to as capacitive  $\text{Ca}^{2+}$  entry. In turn, the further entry of  $\text{Ca}^{2+}$  is promoted  
2 through VDCCs which are opened by membrane depolarization (Barajas et al., 2008; Dai and  
3 Zhang, 2002; Nemeth and Kurucz, 2005). Since  $\text{Ca}^{2+}$  flows into a cell through the opening of ion  
4 channels, a negative potential on the extracellular side of a plasma membrane increases (Fig. 3b).  
5 Note that the electric currents of our RSPs increase with a negative gate bias (Fig. 2b). Thus, the  
6 stimulation of HeLa cells by histamine can cause the conductance increase of RSPs, which could  
7 be used to evaluate the interaction between ligands and receptors on a cell membrane.

8 Fig. 3c shows real-time relative conductance changes measured by a floating  
9 electrode-based RSP *with* or *without* a HeLa cell during the injection of histamine. The relative  
10 conductance level of the RSP with a HeLa cell increased by ~10% when a histamine solution  
11 was injected, while that of the RSP without a cell exhibited no response. The conductance  
12 increase by the histamine injection could be attributed to the activity initiated by H1Rs in the  
13 HeLa cell. Once histamine molecules bound to H1Rs,  $\text{Ca}^{2+}$  flowed into the cell, which generated  
14 a temporary negative potential in a gap between the cell and the CNT junction area of the RSP  
15 (Fig. 3b). Note that since our RSP exhibited *p*-type characteristics, the increase of a negative  
16 potential would cause the increase of its conductance (Ta et al., 2014). The increased  
17 conductance was recovered back to the original value after 50 seconds. This conductance  
18 recovery was probably due to the repolarization process of the cell and the charge balance of a  
19 bath solution. As reported previously, the influx of  $\text{Ca}^{2+}$ , which increased positive charges within  
20 a cell, was followed by the outflux of potassium ions ( $\text{K}^+$ ) from the cell (Benarroch, 2010).  
21 Moreover, cations in a bath solution could diffuse into the gap to neutralize excess negative  
22 charges. Both of these factors probably resulted in the conductance recovery of the RSP after the  
23 response of the histamine injection. This data clearly indicates that our RSPs could detect the

1 electrophysiological effects of histamine on HeLa cells. Significantly, since our method just  
2 measure the surrounding potential changes without damaging cells, it could be applied for the  
3 study of the electrophysiological responses of various cell lines in a non-invasive manner.

4 The responses of a HeLa cell to histamine could be quantified by the relative conductance  
5 changes of our RSP. Fig. 3d shows the conductance responses of a RSP by the stimulation of  
6 different concentration histamine solutions in the range from 100 nM to 100 mM. To confirm the  
7 repeatability of our RSP, we carried out measurements three times for each concentration of  
8 histamine using the same RSP (Fig. S1). The dose–response curve was fitted by a Hill equation  
9 written as (Bristow and Zamani, 1993)

$$\Delta G/G_0 = \Delta G/G_{0\max} \frac{C^n}{(EC_{50})^n + C^n}$$

11 Here,  $\Delta G/G_0$  and  $\Delta G/G_{0\max}$  are *a relative conductance change* and the *maximum value of*  
12 *relative conductance changes*, respectively.  $C$  and  $EC_{50}$  are *the concentration of histamine in a*  
13 *solution* and *the concentration of histamine giving a half–maximal relative conductance change*,  
14 respectively.  $n$  is a Hill coefficient. In case of drug screening experiments, the potency of  
15 specific drugs can be represented by the values of  $pEC_{50}$  which is defined as  $-\log_{10}(EC_{50})$ . The  
16 fitting analysis on our data shows that the estimated value of  $pEC_{50}$  by histamine stimulation was  
17  $\sim 5.78 \pm 0.23$ , which was similar to a previously-reported value measured by a fluorescent  
18 method (Bootman et al., 1992). Also, note that when the concentrations of histamine solutions  
19 was larger than 10  $\mu\text{M}$ , the cell responses saturated and have similar values for different  
20 concentration histamine solutions, which is consistent with previous works (Bootman et al.,  
21 1992; Bristow and Zamani, 1993; Volpi and Berlin, 1988). It should be noted that such a  
22 statistically-meaningful quantitative measurement was made possible because our method allow

us to perform repeat measurements for multiple cells using a *single* RSP device and thus to minimize possible errors from device-to-device variations of its characteristics.

### 3.5. Effect Monitoring of anti-histamine drugs by using RSPs

Fig. 4a describes the mechanism of anti-histamine drug effect on a  $\text{Ca}^{2+}$  influx into a HeLa cell. In this work, we evaluated the effects of cetirizine and chlorphenamine as anti-histamine drugs which were known as inverse agonists. Once inverse agonists bind with H1Rs, the H1Rs are converted to inactive forms, and the receptor activity of cells is down-regulated (Mizuguchi et al., 2013). Thus, the activation of H1Rs by histamine is degraded, resulting in the decrease of a  $\text{Ca}^{2+}$  influx through SOCCs into the cells. In turn, a  $\text{Ca}^{2+}$  influx *via* the opening of VDCCs should be decreased due to the reduced membrane depolarization. Thus, the quantitative effects of anti-histamine drugs to HeLa cells can be evaluated by monitoring the membrane potential change caused by the  $\text{Ca}^{2+}$  influx into the cells.

Fig. 4b shows the real-time electrophysiological responses of HeLa cells pretreated *with* or *without* cetirizine, one of the anti-histamine drugs. To study the effect of cetirizine, HeLa cells were incubated with a  $\text{Ca}^{2+}$ -free bath solution including 50  $\mu\text{M}$  cetirizine for 15 minutes before the cells were transferred and assembled on a RSP. To trigger a  $\text{Ca}^{2+}$  influx into cells, a bath solution with histamine was injected so that the final concentration of histamine was 100  $\mu\text{M}$ . The relative conductance change of the RSP with the HeLa cell pretreated with cetirizine was ~5%, which was significantly smaller than that of the RSP with a non-pretreated HeLa cell (~13%). This result indicates that cetirizine worked as an anti-histamine drug on the HeLa cells assembled on the RSP, and our method can be utilized to measure the effect of anti-histamine drugs in real time.

We could also quantitatively monitor the electrophysiological effects of different anti-histamine drugs with various concentrations (Fig. 4c). Here, HeLa cells were incubated with bath solutions including cetirizine or chlorphenamine with various concentrations ranging from 10 to 200  $\mu$ M. Then, the response of individual HeLa cells by the injection of 100  $\mu$ M histamine was measured using a single RSP. For a reliable statistical analysis, we repeated measurements three times for each anti-histamine drug concentration using the same RSP. Dose-response curves showed the drastic decrease of the relative conductance changes of a RSP for HeLa cells pretreated with chlorphenamine. Importantly, the relative conductance changes of a RSP for HeLa cells pretreated with cetirizine were smaller than those with chlorphenamine. The result is also consistent with previous reports showing that chlorphenamine was a stronger inverse agonist than cetirizine (Christophe et al., 2003; Mizuguchi et al., 2013).

To quantitatively estimate the effect of anti-histamine drugs, the results were analyzed by a Hill equation for inhibitors like

$$\Delta G/G_0 = \Delta G/G_{0\max} + (\Delta G/G_{0\min} - \Delta G/G_{0\max}) \frac{A^n}{(IC_{50})^n + A^n}$$

Here,  $\Delta G/G_{0\min}$  is the minimum value of relative conductance changes.  $A$  and  $IC_{50}$  are the concentration of an anti-histamine drug in a solution and that of an anti-histamine drug giving a half-maximal relative conductance change, respectively. Anti-histamine drugs are ‘inhibition’ drugs whose role is blocking the binding of histamine to receptors. In this case, the potency of such drugs can be evaluated by a  $pIC_{50}$  value which is defined as  $-\log_{10}(IC_{50})$  and representing an inhibition efficiency rather than a binding efficiency. By fitting the data,  $pIC_{50}$  values for cetirizine and chlorphenamine were directly calculated from dose-response curves to be 4.70 and 5.77, respectively. These  $pIC_{50}$  values quantitatively indicate that the measured potency of

chlorphenamine was higher than that of cetirizine as reported previously (Christophe et al., 2003). Significantly, previous works show that chlorphenamine inhibited the activity of H1Rs with  $pIC_{50}$  values in the range of 3.86 – 6.28, which is consistent with our measurement results (Markwardt et al., 1996; Sakuta, 1994; Salata et al., 1995). This result supports the validity of our method. Furthermore, the measured response data in Fig. 4c can be utilized to estimate the quantity of H1Rs which were still active with anti-histamine drugs. For example, the HeLa cells pretreated with 10  $\mu$ M chlorphenamine exhibited a relative conductance change of ~40% compared with the response of non-pretreated cells. It implies that ~60% of H1Rs on a cell membrane shifted to inactive forms by chlorphenamine, which was consistent with previous results measured by conventional methods (Mizuguchi et al., 2013).

To fully appreciate our method, the Cheng-Prusoff equation was used to estimate inhibitor constants,  $K_i$ , which are indicators for the efficiency of drugs (Lazareno and Birdsall, 1993).

$$K_i = \frac{IC_{50}}{1 + \frac{C}{EC_{50}}}$$

$pK_i$  values, defined as  $-\log_{10}(K_i)$ , for cetirizine and chlorphenamine were calculated from the Cheng-Prusoff equation to be 6.49 and 7.56, respectively. These results clearly show that our method can be utilized to *quantitatively* evaluate the effect of anti-histamine drugs. Considering that the statistically-meaningful quantitative measurement of drug effects using high-performance nanoscale devices have been extremely difficult due to rather large device-to-device variations of its characteristics, our strategy can be a significant breakthrough in the various

1 applications of nanoscale devices such as drug screenings and electrophysiological study of cell  
2 activities.

#### 4 **4. Conclusions**

5 In summary, we report a strategy using a single RSP device to quantitatively monitor the  
6 electrophysiological responses of multiple individual cells to histamine and anti-histamine  
7 drugs. Here, we could repeatedly measure the real-time response of HeLa cells to histamine  
8 drugs quantitatively using the same RSP device. Results have showed that our method enables  
9 the statistically-meaningful quantitative evaluations without suffering from the errors by the  
10 device-to-device variations of sensor characteristics. Using this method, we demonstrated the  
11 quantitative evaluation of the effect of antihistamine drugs such as cetirizine and chlorphenamine  
12 and found that chlorphenamine had a better efficacy than cetirizine as reported previously  
13 (Christophe et al., 2003). Since our strategy takes an advantage of high-performance nanoscale  
14 devices while still enabling the statistically-meaningful evaluations, it can be a powerful tool for  
15 various applications such as drug screening and therapeutic monitoring.

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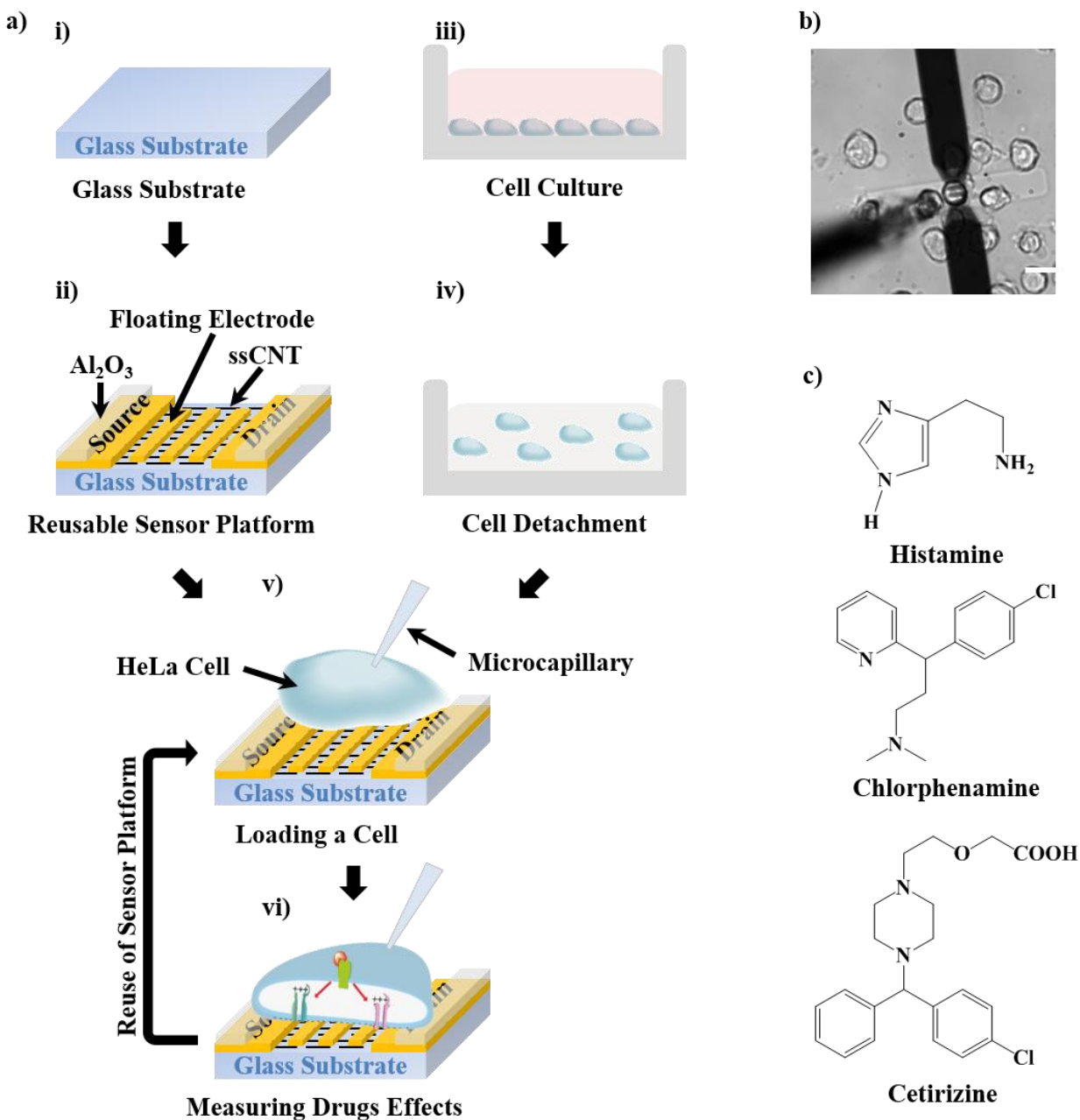
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## 1 Figures



2

3 **Fig. 1.** Schematic diagram depicting an experimental procedure and the molecular formulas of

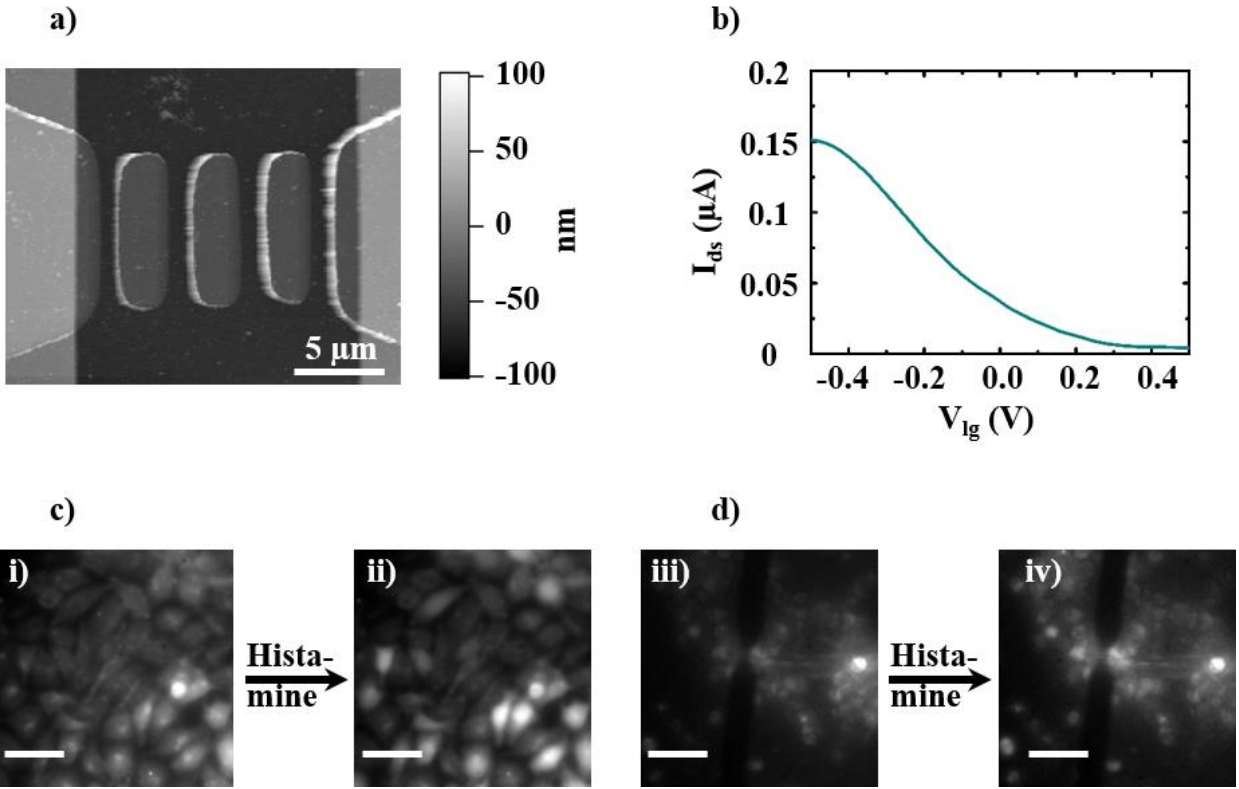
4 chemicals used in experiments. (a) Schematic diagram of an experimental procedure: (i)

5 preparation of a clean glass substrate; (ii) fabrication of a reusable sensor platform *via*

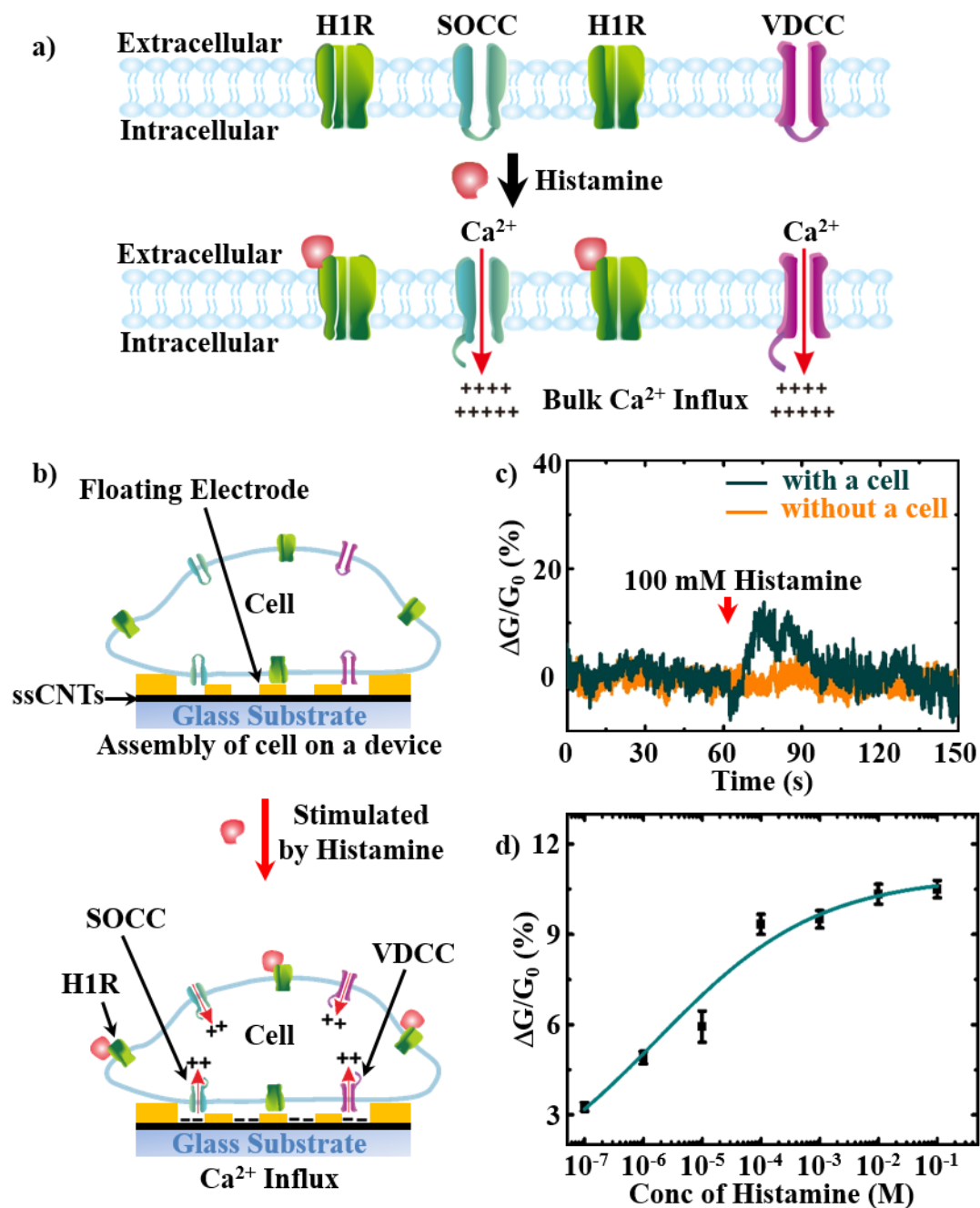
6 conventional photolithography processes; (iii) culturing of HeLa cells on a plate; (iv) detachment

1 of the HeLa cells using a trypsin solution; (v) loading of a floating HeLa cell on the RSP; (vi)  
2 monitoring of the electrophysiological responses of the cell to drugs using the RSP. Steps (v) and  
3 (vi) can be repeated for different cells using the same RSP device. (b) Microscopic image  
4 showing a Hela cell transferred to the junction area of a RSP using a microcapillary. The scale  
5 bar represents 20  $\mu\text{m}$ . (c) Chemical formulas of histamine, chlorphenamine, and cetirizine.

6



**Fig. 2.** Characterization of a reusable sensor platform (RSP) based on carbon nanotubes. (a) AFM topography image of a RSP with three floating electrodes connected by carbon nanotube networks. Individual floating electrodes have the dimensions of a 2 μm width and a 10 μm length. (b) Liquid gating effect of a RSP. Here, an Ag/AgCl electrode was used as a liquid gate electrode, and a gate bias was swept from -0.5 to 0.5 V with the source–drain bias of 0.1 V. (c) Fluorescent images of HeLa cells cultured on a glass substrate were taken before (i) and after (ii) the injection of histamine. (d) Fluorescent images of HeLa cells assembled on a RSP were taken before (iii) and after (iv) the injection of histamine. The scale bars represent 50 μm.

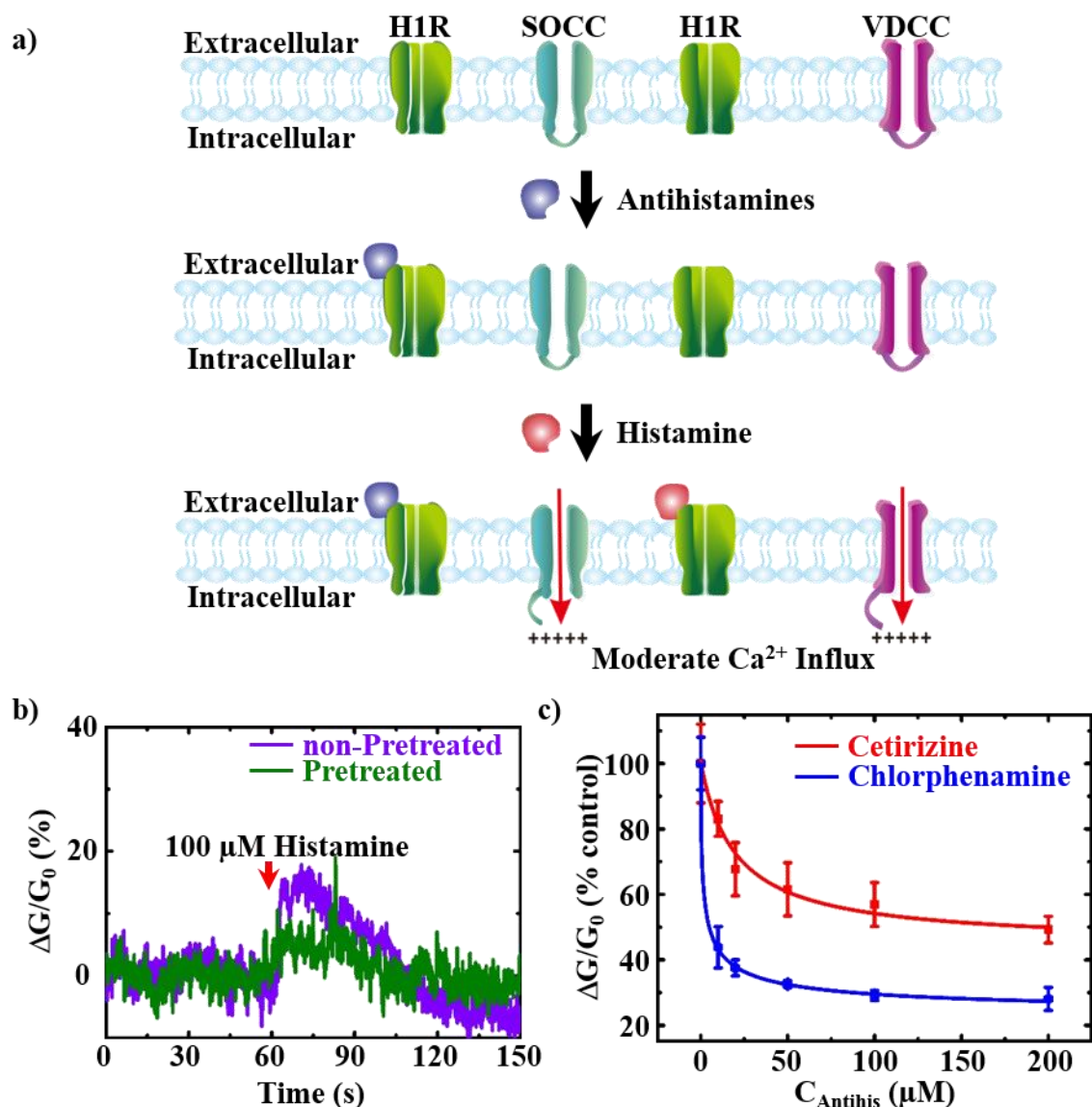


**Fig. 3.** Quantitative electrophysiological monitoring of the effect of histamine drug on HeLa cells. (a) Schematic drawing sketching the mechanism of a  $\text{Ca}^{2+}$  influx *via* the activations of SOCCs and VDCCs by histamine. (b) Schematic diagram showing the sensing mechanism of our RSP device by the histamine stimulation on a cell. (c) Real-time conductance changes measured

1 by a RSP *with* (dark cyan line) and *without* a HeLa cell (orange line) during the injection of 100  
2 mM histamine. (d) Relative conductance change of the RSP in response to the histamine with  
3 different concentrations. A dose–response curve was fitted by a Hill equation (solid line). All  
4 measurements were performed using a single RSP device. Data are expressed as means  $\pm$  SEM  
5 (standard error of the mean), ( $n = 3$ ).

6





**Fig. 4.** Quantitative electrophysiological monitoring of antihistamine drug effects using RSP devices. All electrophysiological measurements for each anti-histamine drug were performed using a single RSP device. (a) Schematic diagram of the effect of an anti-histamine drug on a  $\text{Ca}^{2+}$  influx into a HeLa cell. (b) Real-time electrophysiological responses of cetirizine pretreated (marker by “Pretreated”, green) and non-pretreated (marker by “non-Pretreated”, violet) HeLa cells to the injection of 100  $\mu\text{M}$  histamine. (c) Modulation of a  $\text{Ca}^{2+}$  influx into HeLa cells by the

1 various concentrations of anti-histamine drugs. HeLa cells were pretreated with the solutions of  
2 cetirizine or chlorphenamine with various concentrations in the range of 10 – 200  $\mu$ M before the  
3 injection of 100  $\mu$ M histamine. Data are expressed as means  $\pm$  SEM (standard error of the mean),  
4 ( $n = 3$ ).  
5