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Non-optical detection of allergic response with a cell-coupled gate field-effect transistor

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ABSTRACT: In this study, we report the label-free and reliable detection of allergic response using a cell-coupled gate field-effect transistor (cell-based FET). Rat basophilic leukemia (RBL-2H3) cells were cultured as a signal transduction interface to induce allergic reaction on the gate oxide surface of the FET, because IgE antibodies, which bind to Fcε receptors at the RBL-2H3 cell membrane, are specifically cross-linked by allergens, resulting in the allergic response of RBL-2H3 cells. In fact, the surface potential at the FET gate decreased owing to secretions such as histamine from the IgE-bound RBL-2H3 cells, which reacted with the allergen. This is because histamine, as one of the candidate secretions, shows basicity, resulting in a change in pH around the cell/gate interface. That is, the RBL-2H3-cell-based FET used in this study was originally from an ion-sensitive FET (ISFET), whose oxide surface (Ta₂O₅) with hydroxyl groups is fully responsive to pH on the basis of the equilibrium reaction. The allergic response of RBL-2H3 cells on the gate was also confirmed by estimating the amount of β-hexosaminidase released together with histamine and was analyzed using the electrical properties based on an inflammatory response of secreted histamine with the vascular endothelial cell-based FET. Thus, the allergic responses were monitored in a non-optical and real-time manner using the cell-based FETs with the cellular layers on the gate, which reproduced the *in vivo* system and were useful for the reliable

1 detection of allergic reaction.

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3 KEYWORDS: Biosensor; Field-effect transistor; Allergy; Histamine release; pH; RBL-2H3 cell

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6 BRIEFS: In this study, we developed a non-optical detection of allergic response with a cell-coupled
7 gate field-effect transistor.
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15 Allergy is one of the most common diseases at present, and the number of patients is increasing
16 worldwide.¹ In particular, allergy in infants often causes death; therefore, allergy tests are required for
17 them at an early stage. Basophils and mast cells play important roles in type I allergy or IgE-associated
18 diseases, such as food allergy, hay fever, and atopic dermatitis (AD). The binding of antigens, which are
19 called allergens in the field of allergy, to IgE bound to the high-affinity IgE receptor (FcεRI) on the
20 surface of basophils and mast cells cross-links FcεRI, resulting in the release of preformed and newly
21 synthesized inflammatory mediators, such as histamine, arachidonic acid metabolites and cytokines, and
22 causes allergic symptoms.²⁻⁴ Therefore, the identification of antigens that provoke mast-cell- and
23 basophil-induced allergic reactions is crucial to avoid anaphylactic shock and aggravations of allergic
24 diseases. The specific binding of antigens to IgE may be detected by various immunological methods,
25 such as enzyme-linked immuno sorbent assay (ELISA). However, these tests cannot be used to evaluate
26 whether the antigen-binding IgE activates mast cells and basophils in patients. Therefore, evaluating the
27 potential of antigen-specific IgE to activate mast cells and basophils from a patient is more important
28 than the analysis of simple IgE–antigen binding in the diagnosis of type I allergy.⁵⁻⁷ In particular, it is
29 very important to detect secretions such as histamine released during allergic reactions.
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51 To solve this problem, biosensors can be utilized to detect allergic responses in a cost-effective and
52 easy way. In fact, a surface plasmon resonance (SPR)-based biosensor was proposed to detect allergic
53 responses on rat basophilic leukemia (RBL-2H3) cells with IgE in previous works.^{4,8,9} In these studies,
54 RBL-2H3 cells with dinitrophenyl (DNP)-specific rat monoclonal IgE were stimulated by DNP-
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conjugated human serum albumin (DNP-HSA) as an antigen. This method enabled the imaging of cellular behaviors caused by the antigen-antibody reaction at the cell membrane in a label-free and real-time manner, but the inflammatory mediators such as histamine have not been directly detected. On the other hand, a field-effect transistor (FET)-based biosensor has received considerable interest as a non-optical biosensing device. The FET biosensor has the ability to directly detect ionic or biomolecular charges, which are induced by biological reactions such as DNA recognition events,¹⁰⁻¹³ immunological reactions,¹⁴ and cellular activities¹⁵⁻¹⁹ on the gate of a FET device. In particular, a cell-coupled gate FET realized the noninvasive analysis of cellular activities such as cellular respiration by detecting ionic behaviors around the cell/gate interface.¹⁷ Thus, a platform based on a cell-based FET is suitable for a detection device to recognize ionic secretions resulting from the allergic responses of RBL-2H3 cells.

In this paper, we propose a non-optical detection method to detect allergic responses using a cell-coupled gate FET. In particular, IgE-bound RBL-2H3 cells were utilized on the gate and allowed to react with antigens to detect secretions such as histamine around the cell/gate interface based on the principle of the field effect.

EXPERIMENTAL SECTION

Reagents. The chemicals used were obtained from the following sources: bovine serum albumin (BSA), dinitrophenyl-conjugated human serum albumin (DNP-HSA), DNP-specific rat monoclonal IgE, antihistamine levocetirizine dihydrochloride,²⁰ fetal bovine serum (FBS), and *p*-nitrophenyl-*N*-acetyl- β -glucosaminide (pNPG) from Sigma–Aldrich Japan (Tokyo, Japan); endothelial cell growth medium (EGM-2; CC-3162) containing 2% FBS and vascular endothelial growth factor (VEGF) from Lonza; standard buffer solutions (pH 4.01, 6.86 and 9.18), and gelatin from Wako. Monoclonal anti-DNP (mouse IgE isotype) is derived from the hybridoma SPE-7 produced by the fusion of mouse myeloma cells and splenocytes from a mouse immunized with DNP-KLH (keyhole limpet hemocyanin).²¹

Cell culture. Rat basophilic leukemia (RBL-2H3) cells were cultured in Roswell Park Memorial Institute (RPMI) medium supplemented with 10% FBS, 100 U/ml penicillin, and 100 μ g/ml

streptomycin at 37 °C in 5% CO₂ in an incubator system. One day before the experiments, RBL-2H3 cells were harvested using trypsin. They were then cultured in the presence or absence of 50 ng/ml anti-DNP IgE on the gate of ion-sensitive FET (ISFET) devices for 1 day; one was the IgE-bound RBL-2H3 cell-based FET, while the other was the unmodified RBL-2H3-cell-based FET, as shown in **Fig. 1(a)**. RBL-2H3 cells were cultured on the gate of the FET sensor and incubated first with anti-DNP IgE (IgE(+)) and then with DNP-HAS, resulting in the activation of cells by crosslinking of the antigen with IgE antibodies, while RBL-2H3 cells without IgE (IgE(-)) on the gate were utilized as a control sensor for the stimulation of the antigen. Moreover, human umbilical vein endothelial cells (HUVEC) were cultured in EGM-2 containing 2% FBS and VEGF on a collagen-coated dish, and harvested using trypsin after the pre-culture for 1 week. Then, they were cultured on the gate surface of an ISFET, which was coated with 0.1% gelatin in advance, as shown in **Fig. 1(b)**. The number of cells seeded on the gate of the FET was controlled to 5×10^5 cells/mL for RBL-2H3 and HUVEC, respectively.

Evaluation of release of β -hexosaminidase. Different amounts of DNP-HSA antigen (1, 10, 50 and 100 ng/mL) were added to the IgE-bound RBL-2H3 cells or the unmodified RBL-2H3 cells. After allergic reactions for 30 min at 37 °C, the supernatants were dispensed to 64-well plates, then the degranulation of RBL-2H3 cells was estimated by measuring the release of β -hexosaminidase, a granule marker that hydrolyzes pNPG to the chromophore, *p*-nitrophenol. Absorbance of each well was analyzed using a microplate reader (Corona Electric Co., Ltd.; SH-9000).

Detection of histamine using ISFET. The ISFETs used in this study were composed of a silicon-based n-channel depletion-mode FET with a Ta₂O₅/SiO₂ (100 nm/50 nm) layer as a gate insulator with a width (W) and length (L) of 340 and 10 μ m, respectively. The Ta₂O₅ thin film was used as a passivation layer to prevent leakage currents in the buffer solution. The gate voltage (V_G)–drain current (I_D) electrical characteristics were measured using a semiconductor parameter analyzer (B1500A, Agilent). The change in V_G in the V_G – I_D electrical characteristics was estimated as the threshold voltage (V_T) shift, which was evaluated at a constant I_D of 1 mA and a constant drain voltage (V_D) of 2.5 V [**Fig. 1(a)**]. A Ag/AgCl reference electrode with KCl solution was connected to the measurement solution including

the gate of the FET through a salt bridge. The V_G – I_D electrical characteristics were measured in the range of pH 4.01– 9.18. Histamine was prepared with concentrations in the range of 10 nM–1 mM in the cell culture medium. The V_G – I_D electrical characteristic was measured at each histamine concentration using the ISFET device without cells.

Electrical measurement using RBL-2H3 cell-based FET. The DNP-HAS antigen was added on the IgE-bound RBL-2H3-cell-based FET and the unmodified RBL-2H3-cell-based FET in the concentration range of 1–100 ng/mL. The change in surface potential (ΔV_{out}) at the gate was measured at a constant I_D of 1 mA and a constant V_D of 2.5 V using the source follower circuit [Fig. S1 in Supporting Information]; thus, the detected ΔV_{out} was regarded as the change in the source-gate voltage (ΔV_S), which was equal to $-\Delta V_T$. Using this system, the surface potential of the FET can be monitored in real time.

Co-culture measurement system. RBL cells were cultured in the cell culture inserts with porous polyethylene terephthalate (PET) membranes, the diameter of which was 0.4 μ m, for paracrine cell–cell interactions, while the HUVEC-based FET was immersed in the common culture medium with the culture insert, where RBL-2H3 cells were cultured, as shown in Fig. 1(b). In co-culture system I, ΔV_{out} for the HUVEC-based FET was measured to evaluate the release of histamine from the IgE-bound RBL-2H3 cells upon adding antigen, while levocetirizine dihydrochloride, an antihistamine, was added at 500 ng/mL to prevent the interaction of the released histamine with the endothelial cells on the gate in co-culture system II. In co-culture system III, ΔV_{out} was monitored in the culture medium including anti-histamine and antigen using the HUVEC-based FET, but no RBL-2H3 cells were cultured on the cell culture insert.

RESULTS AND DISCUSSION

Monitoring of allergic response with RBL-2H3-cell-based FET. Figure 2 shows ΔV_{out} at the gate of RBL-2H3-cell-based FETs upon adding allergen at each concentration. RBL-2H3 cells were cultured on

both FET sensors, one of which was used as the sample sensor with IgE, while the other was used as the control sensor without IgE. The number of cultured cells on the gate sensing area was about 10 cells. ΔV_{out} for the IgE-bound RBL-2H3 cell-based FETs temporarily decreased twice within 10 min after the addition of antigen, while no signal was found for the control sensor even after its addition. The negative shift in the surface potential for the sample sensors indicates an increase in negative charges or a decrease in positive charges around the cell/gate interface, since the measurements are performed using the source follower circuit [Fig. S1 in Supporting Information]. Some secretions from RBL-2H3 cells based on allergic responses are assumed, as shown in Table 1. From the proposed secretions, histamine is considered to be mainly released by allergic reactions and shows basicity (pKa 9.75) in particular. On the other hand, the effect of cellular respiration on the change in pH must be considered because carbon dioxide or lactic acid generated by cellular respiration may have caused the change in pH. However, the electrical signals resulting from cellular respiration can be found after a few hours or a few tens of hours of cell culture according to our previous work.¹⁷ In this study, we observed an allergic response within 10 min upon adding the antigen to cells; therefore, we assume that the basic histamine had the greatest impact on the electrical signals, which was secreted from the cells in a short time. This is because secreted histamines are considered to have been closed around the cell/gate interface, resulting in an increase in pH around it. In particular, the cell-based FET used in this study was based on an ISFET, the gate of which was composed of an oxide membrane (Ta_2O_5) with hydroxyl groups in a solution. The ISFETs show pH responsivity on the basis of the equilibrium reaction between hydrogen ions and hydroxyl groups at the gate oxide surface.^{17,22} The ISFETs used in this study showed a response of 55.8 mV/pH [Figs. S2 and S3 in Supporting Information], which was near the Nernstian response. Actually, ΔV_{out} shifted in the negative direction by about 11 mV upon adding antigen with a concentration of 100 ng/mL [Fig. 2]. The pH in the prepared cell culture medium was 7.4. Considering the pH responsivity of this ISFET, the allergic response induced the change in pH from 7.4 to 7.6 around the cell/gate interface. That is, the change in the hydrogen ion concentration was calculated to be about 15 nM ($\Delta\text{pH}=0.2$), which roughly indicated that histamine was temporarily released around the

cell/gate interface. However, the cell culture medium has a pH-buffering action; therefore, basic histamine had to be added to a concentration of 10–100 μM in the culture medium to induce the change in pH from 7.4 to 7.6, as shown in **Fig. 3**, although the buffering effect of the culture medium may have been markedly reduced at the higher concentrations of histamine (10 and 100 mM), corresponding to a pH of more than about 8. In a previous work, the amount of histamine in a cell was reported to be approximately 1 pg/cell,²³ 10 to 80% of which would be released by a stimulation. In this case, the concentration of released histamine was calculated to be approximately 30–210 μM considering the gate size ($W/L = 340 \mu\text{m}/10 \mu\text{m}$), the gap (100 nm at most) at the cell/substrate interface,^{24,25} and the number of adhered cells (about 10 cells) on the gate. This calculation was in relatively good agreement with the expected concentration of histamine (10–100 μM) for the electrical signal shown in **Fig. 3**. Therefore, the secretions such as histamine would have been temporarily trapped in the closed space around the cell/gate interface without diffusion to the bulk solution, which could have been measured by an *in situ* measuring method, namely, the cell-based FET. Thus, the allergic response was clearly found in a label-free manner using the IgE-bound RBL-2H3-cell-based FET. Moreover, the temporal ΔV_{out} returned to the baseline after approximately 10 minutes, showing two peaks at about 2–3 and 7–8 minutes, as shown in **Fig. 2**. In the previous works, the allergic response was found within 10 min after the stimulation by allergen.^{23,26} Additionally, the exocytosis of secretions might have occurred in stages considering two peaks in the electrical signals; that is, secretory cells employed different exocytosis modes (kiss-and-run, full-collapse fusion and compound exocytosis) to control the rate and amount of vesicular content release.^{27,28} Eventually, the pH around the cell/gate interface is considered to have returned to that of the culture medium by the diffusion of secretions to the bulk solution after the allergic response, meaning that ΔV_{out} returned to the baseline. Thus, it is very important to consider ionic behaviors around a cell/gate interface, where secretions are temporarily confined, for the real-time measurement of cellular activities.

Moreover, the dependence of allergic responses on the change in the allergen concentration was also

found using the IgE-bound RBL-2H3-cell-based FETs, as shown in **Fig. 2**. A few peaks of ΔV_{out} were found at every concentration after the addition of antigen. In addition, $|\Delta V_{\text{out}}|$ slightly increased with increasing antigen concentration, but the difference between each $|\Delta V_{\text{out}}|$ was relatively small. This means that the allergic response in RBL-2H3 cells might be generated even at a low antigen concentration regardless of the number of antigen–antibody binding at the cell membrane. Once an antigen is cross-linked by IgE at the cell membrane even at a low antigen concentration, the RBL-2H3 cells would be activated to a certain extent by this stimulation, resulting in the release of secretions such as histamine.

Evaluation of β -hexosaminidase released during allergic response. To confirm the allergic response in RBL-2H3 cells on the gate, one of the secretions, β -hexosaminidase, was evaluated on the basis of the hydrolysis reaction of the pNPG substrate. IgE-bound RBL-2H3 cells or unmodified RBL-2H3 cells were cultured on the gate followed by the addition of antigen; then, the enzymatic reaction between pNPG and released β -hexosaminidase was estimated using a microplate reader. **Figure 4** shows the Michealis–Menten plots for the change in the pNPG concentration, which induced the change in the absorbance following the enzymatic reaction with β -hexosaminidase. Additionally, the concentration of released β -hexosaminidase per minute was plotted against the antigen concentration for the IgE-bound or unmodified RBL-2H3 cells. As a result, the enzymatic reaction was clearly found upon adding antigen to the IgE-bound RBL-2H3 cells, while the unmodified RBL-2H3 cells hardly showed an allergic response. Moreover, the amount of β -hexosaminidase increased regardless of the antigen concentration, indicating that the difference in the reaction rates was not very large for each antigen concentration. This result was in good agreement with that based on the electrical response using the IgE-bound RBL-2H3-cell-based FET shown in **Fig. 2**. Thus, the electrical responses for the IgE-bound RBL-2H3-cell-based FETs were caused by the allergic responses in RBL-2H3 cells, which resulted from the cross-linking of the antigen with IgE at the cell membrane.

Verification of histamine released from IGE-bound RBL cells using HUVEC-based FET. IgE-bound RBL-2H3 cells were cultured in cell culture inserts with porous PET membranes, the pore diameter of which was 0.4 μm , for paracrine cell–cell interactions, while HUVEC were cultured on the gate of ISFETs, as shown in **Fig. 1(b)**. That is, histamine released from RBL-2H3 cells should diffuse through the pores to the HUVEC-cultured gate. Then, the effect of histamine on the electrical signals of the endothelial-cell-based FET can be evaluated, meaning that histamine was secreted from the IgE-bound RBL-2H3 cells by adding allergen. This is also because histamine released from mast cells actually induces allergic inflammatory responses by the interaction with endothelial cells inside a blood vessel.²³ **Figure 5** shows ΔV_{out} for the co-culture measurement system. ΔV_{out} for the sample sensor (in co-culture system I) gradually decreased after the addition of antigen (100 ng/mL), while ΔV_{out} for two different control sensors (in co-culture systems II and III) temporarily increased for several minutes but returned to the baseline at about 20 minutes. Thus, the histamine released from the IgE-bound RBL-2H3 cells with cross-linked antigen acted on the HUVEC cultured on the gate, because the control sensor with anti-histamine (co-culture system II) showed a completely different signal from that of the sample sensor. Vascular endothelial cells contract by interacting with histamine generated by allergic reactions,²⁹ resulting in vascular permeability sthenia, that is, gaps between endothelial cells on the gate. This is why the culture medium flowed onto the gate surface through these gaps, resulting in the change in pH. Before that, the pH around the cell/gate interface changed to acidic owing to cellular respiration activities. Therefore, the pH returned to neutral or changed to alkaline, because the culture medium had invaded the cell/gate interface through the gaps. According to the electrical circuit [**Fig. S1** in Supporting Information], the decrease in ΔV_{out} for the sample sensor shown in **Fig. 5** indicates an increase in pH, which was expected. Therefore, the IgE-bound RBL-2H3-cell-based FET detected the release of secretions such as histamine from RBL-2H3 cells, which were induced by the cross-linking of antigen with IgE at the cell membrane, around the cell/gate interface. In particular, the cells themselves

such as RBL-2H3 cells or HUVEC on the cell-based FETs play an important role as a signal transduction interface between targeted biomolecules (allergen or histamine) and detection devices (FETs).

CONCLUSIONS

In this study, we realized the label-free and reliable detection of allergic response using a cell-coupled gate field-effect transistor (cell-based FET). Rat basophilic leukemia (RBL-2H3) cells were cultured as a signal transduction interface to induce allergic reaction on the oxidized gate surface of the FET, because IgE antibodies, which are specifically cross-linked by allergen, bind to Fcε receptors at the RBL-2H3 cell membrane. As a result, the surface potential at the gate of the FET decreased owing to the secretions such as histamine from RBL-2H3 cells around the cell/gate interface, which was based on the cross-linking reaction at the RBL-2H3 cell membrane caused by adding allergen to the IgE-bound RBL-2H3 cells on the gate. This is because the secreted histamine shows basicity, resulting in a change in pH around the cell/gate interface. Moreover, the RBL-2H3 cell-based FET used in this study was originally from an ion-sensitive FET (ISFET), the gate of which is composed of an oxidized film (Ta_2O_5) whose surface with hydroxyl groups is sensitive to hydrogen ions based on the equilibrium reaction. The allergic response of RBL-2H3 cells on the gate was also confirmed by the change in the concentration of β -hexosaminidase secreted together with histamine during the allergic reaction, and was analyzed using the electrical properties based on an inflammatory response of secreted histamine with endothelial cells using the human umbilical vein endothelial cells (HUVEC)-based FET. Thus, the allergic response was monitored in a real-time and label-free manner using the cell-based FET with RBL-2H3 cells or HUVEC on the gate, which was useful for the reliable detection of allergic reaction reproduced *in vivo*.

Finally, an SPR or impedance-based biosensor enables the analysis of the morphological changes of RBL-2H3 cells activated by the allergic response, but not the detection of chemical secretions such as histamine, while a FET biosensor permits ionic charges based on secretions such as histamine to be

detected. Furthermore, the gate surface of the FET can be chemically modified to specifically detect histamine by coating functional polymer membranes such as a molecularly imprinted polymer (MIP) for histamine.³⁰ Therefore, a combined system with a common electrode employing a few detection principles will contribute to more accurate analysis of the allergic response because some information of the allergic response is simultaneously observed. In fact, a quartz crystal microbalance (QCM)-based biosensor was integrated with a FET biosensor to simultaneously detect biomolecular recognition events on a common electrode, which led to a change in molecular charges as well as the mass and viscoelasticity.³¹

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FIGURE CAPTIONS:

Figure 1 Schematic illustration of cell-coupled gate field effect transistor (cell-based FET). (a) RBL-2H3-cell-based FET. RBL-2H3 cells were cultured on the gate oxide surface. Dinitrophenyl-conjugated human serum albumin (DNP-HSA) was added in the culture chamber with the IgE-bound or unmodified RBL-2H3-cell-based FET. RBL-2H3 cells with anti-DNP IgE (IgE(+)) were activated by crosslinking of the antigen with IgE antibodies. (b) HUVEC-based FET. IgE-bound RBL-2H3 cells were cultured in the cell culture inserts with porous PET membranes, the diameter of which was 0.4 μm , while HUVEC were cultured on the gate oxide surface of ISFET.

Figure 2 Change in surface voltage (ΔV_{out}) with incubation time using IgE-bound RBL-2H3-cell-based FET. Cell culture medium with or without antigen was added in the chamber including the IgE-bound RBL-2H3-cell-based FET. The antigen concentration was controlled to 1, 10 or 100 ng/mL in the measurement solution (medium).

Figure 3 Change in surface potential of ISFET for change in histamine concentration in cell culture

medium (pH 7.4). The measurements were performed using the electrical circuit [Fig. S1 in Supporting Information]. (b) shows a part of (a). The initial pH was 7.4. The ISFETs used in this study showed a response of 55.8 mV/pH [Figs. S2 and S3 in Supporting Information]. Therefore, the change in pH was calculated on the basis of the change in surface potential shown in (a) and (b).

Figure 4 Michealis–Menten plot for change in the pNPG concentration obtained by enzymatic reaction with β -hexosaminidase. IgE-bound (IgE(+)) or unmodified (IgE(-)) RBL-2H3 cells were used for the reaction with antigen. The antigen concentration was controlled to 1, 10, 50 or 100 ng/mL in the measurement solution (medium).

Figure 5 Change in surface voltage (ΔV_{out}) with incubation time using co-culture cell system shown in Fig. 1(b). Cell culture medium with antigen was added to a concentration of 100 ng/mL to the chamber including the IgE-bound RBL-2H3 cells cultured on the porous PET membrane. ΔV_{out} for the HUVEC-based FET was measured. In co-culture system I, the IgE-bound RBL-2H3 cells were cultured in the cell culture insert, where antigen was added. In co-culture system II, levocetirizine dihydrochloride, an anti-histamine, was added to a concentration of 500 ng/mL in co-culture system I. In co-culture system III, ΔV_{out} was monitored in the culture medium including anti-histamine and antigen, but no RBL-2H3 cells were cultured on the cell culture insert.

Table 1 Secretions from RBL-2H3 cells based on allergic responses.

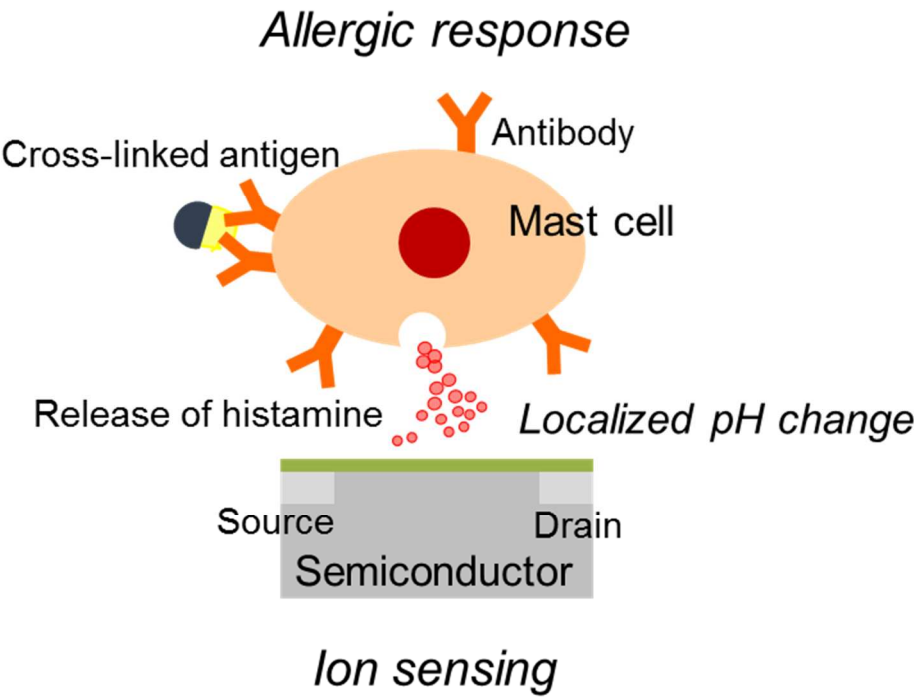
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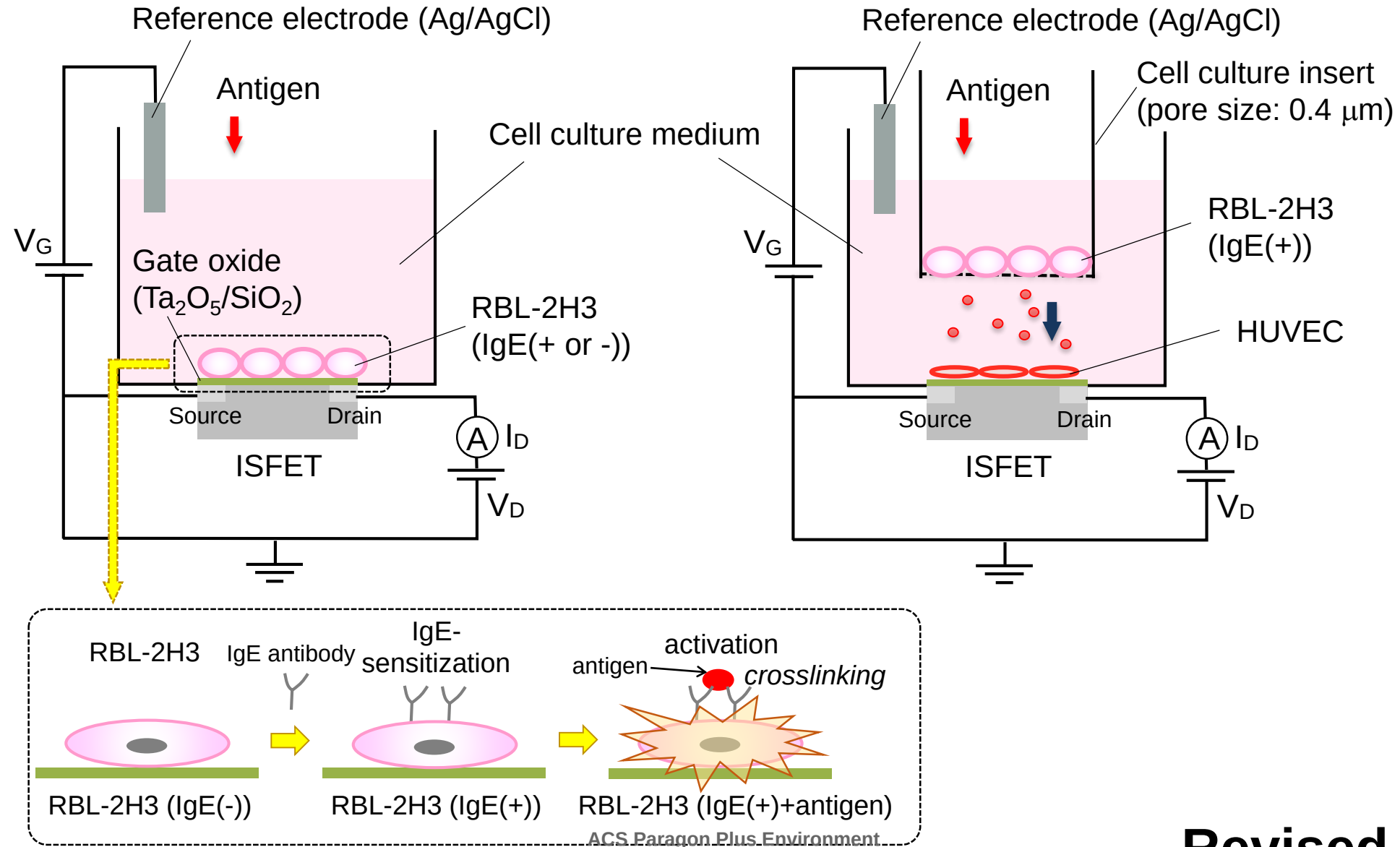
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for TOC only



(a)

(b)

**Revised Figure 1**

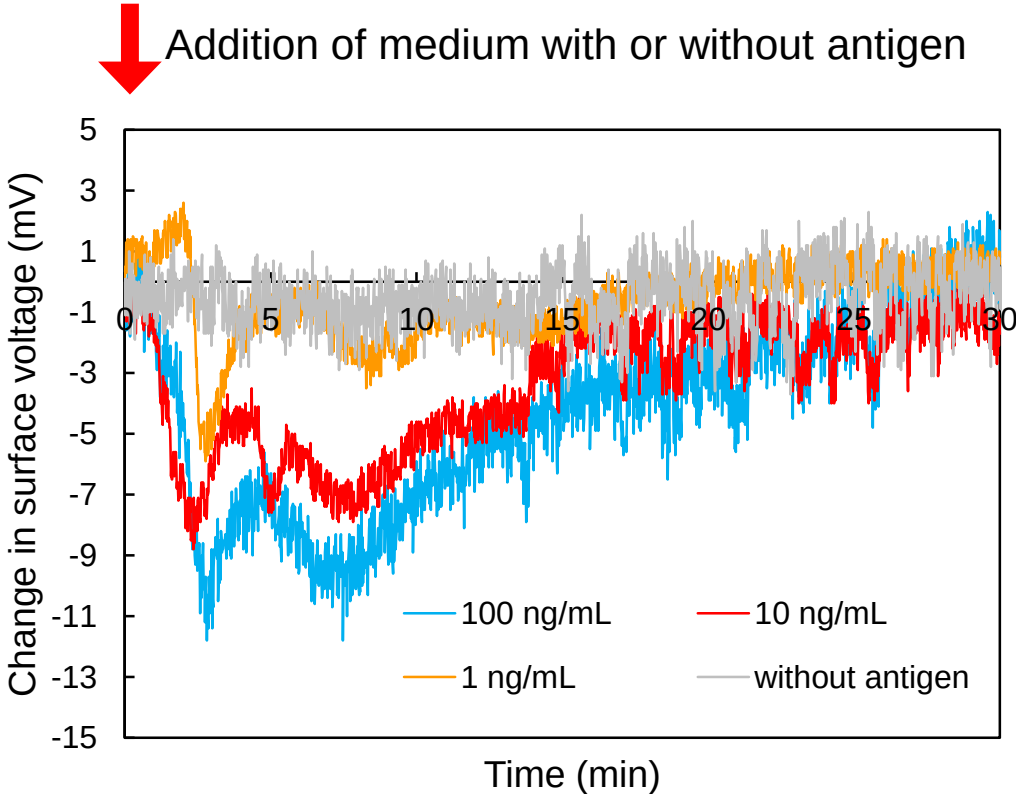
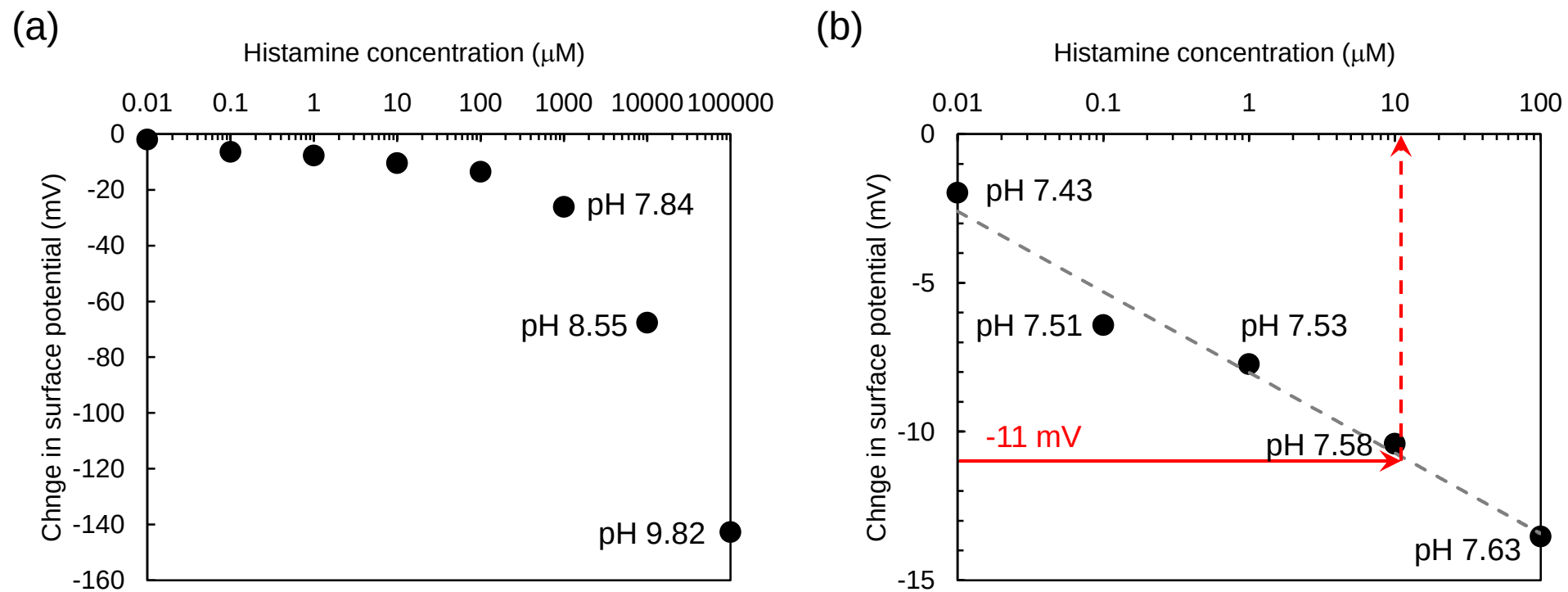


Figure 2



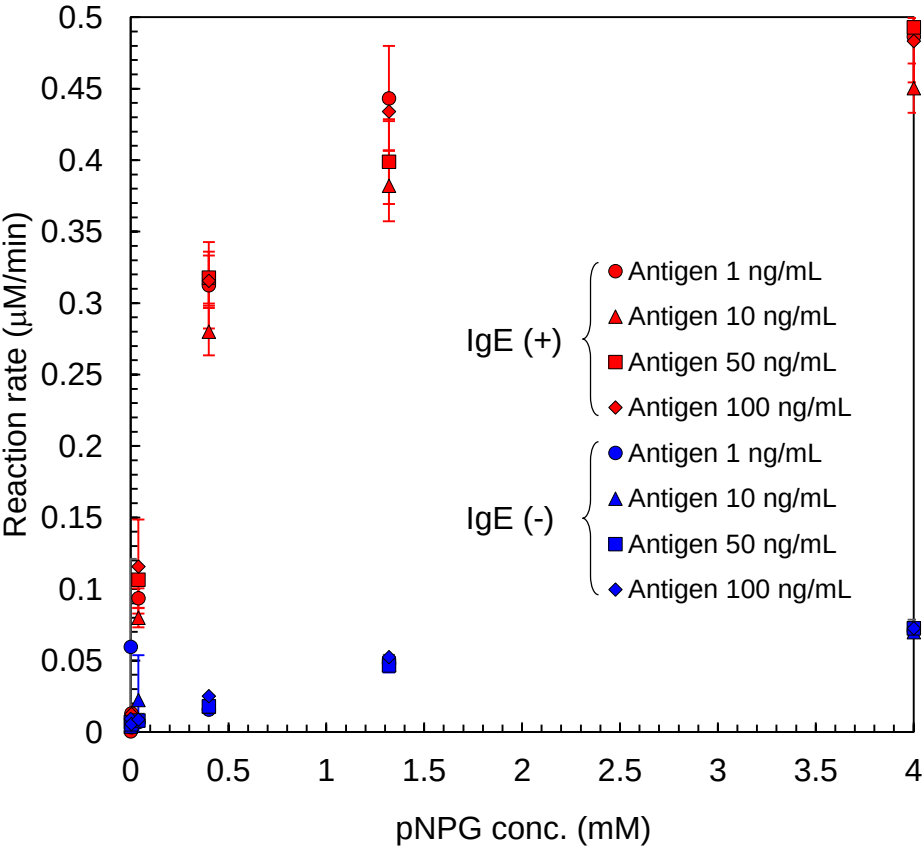
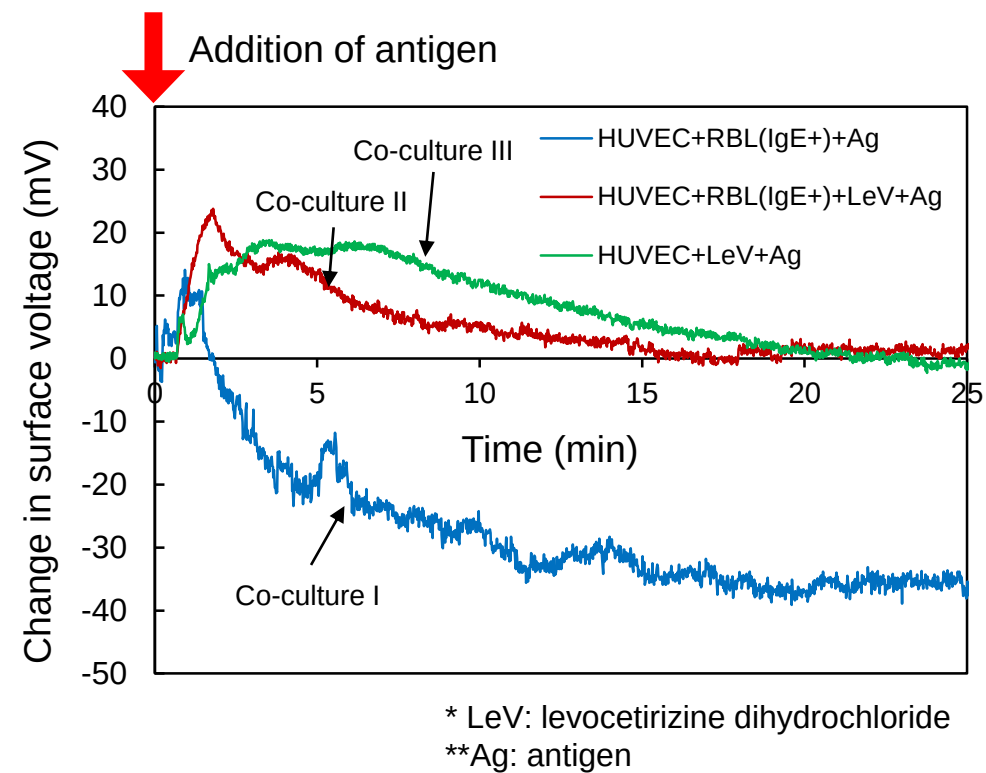
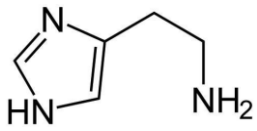
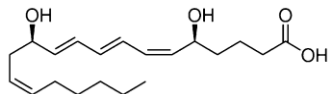


Figure 4



Influential factors	Respiration activity	Histamine	Leukotriene	Others
Chemical structures	$\text{CO}_2 + \text{H}_2\text{O} \leftrightarrow \text{H}^+ + \text{HCO}_3^-$	 pKa=9.75		Degranulation
Effect of charges on ΔV_{out}	↑ Increase	↓ Decrease (Basicity)	↑ Increase	Small impact (small amount of secretion)