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Application of SPR Imaging Sensor for Detection of Individual Living Cell Reactions and Clinical Diagnosis of Type I Allergy

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ABSTRACT

A technique to visualize living cell activation in a real time manner without any labeling is required in the fields of life sciences and medicine. We have reported that surface plasmon resonance (SPR) sensors detect large changes of refractive index (RI) with living cells, such as mast cells, human basophils and lymphocytes. However conventional SPR sensors detect only an average change of RI with thousands of cells at detectable area on a sensor chip. Therefore, we developed an SPR imaging (SPRI) sensor with a CMOS camera and an objective lens in order to visualize RI of individual living cells and their changes upon stimuli. The SPRI sensor we developed could detect reactions of individual rat basophilic leukemia (RBL-2H3) cells and mouse keratinocyte cells in response to specific or nonspecific stimuli. Moreover, the sensor could detect the reactions of individual human basophils isolated from patients in response to antigens (allergens). Thus the technique can visualize the effect of various stimuli, inhibitors and/or conditions on cell reactions as change of intracellular RI distribution at single cell levels. Establishment of the technique to rapidly isolate cells from patient blood should enable us to utilize SPRI system as a high throughput screening system in clinical diagnosis, such as type I hypersensitivity and drug hypersensitivity, and as a tool to reveal novel phenomena in evanescent fields around plasma membranes.

KEY WORDS

basophils, biosensor, diagnosis of type I allergy, single cell analysis, surface plasmon resonance imaging

ABBREVIATIONS

SPR, Surface plasmon resonance; SPRI, SPR imaging; RI, Refractive index; RA, Resonance angle; CD, Cluster of differentiation; DNP-HSA, Dinitrophenol-human serum albumin; RBL, Rat basophilic leukemia; EGF, Epidermal growth factor; IgE, Immunoglobulin E; PKC, Protein kinase C; CMOS, Complementary metal oxide semiconductor; PMA, Phorbol 12-myristate 13-acetate.

INTRODUCTION

The identification of antigens that provoke mast cell activation is crucial to avoid anaphylactic shock and the aggravation of atopic diseases, such as atopic der-

matitis, allergic rhinitis, food allergy and asthma. The detection of antigen specific IgE in serum implies hypersensitivity against the antigen. Thus, a variety of immunological methods, such as ImmunoCAPTM, Ala-STATTM and AD-VIA CentaurTM, to detect anti-

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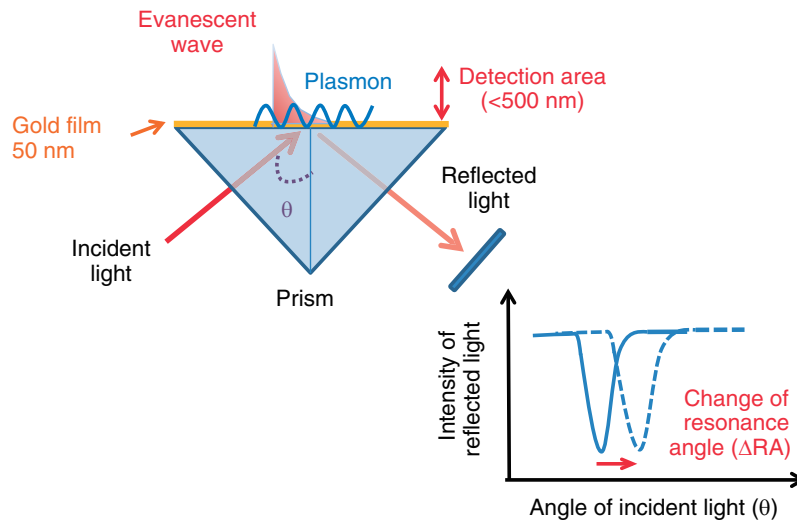


Fig. 1 Principle of SPR sensor. The SPR sensor can detect changes in the refractive index within a detection area (<500 nm) on the surface of gold film. Reflected light is attenuated at the resonance angle by surface plasmon resonance.

gen specific IgE, have been developed and utilized in clinical practice.¹ However, there are often substantial discrepancies between these serological tests and clinical symptoms.¹ *In vivo* tests, such as skin test and antigen challenge test, are more reliable in reflecting clinical conditions. However, these tests may be painful, and could potentially evoke anaphylactic shock when a patient is extremely sensitive to a particular antigen.² Moreover, the intradermal injection of an antigen may sensitize subjects who are not sensitized to the antigen. Skin reactions to antigens are induced by the activation of mast cells sensitized with IgE against the antigen in skin tissues. Since basophils in peripheral blood are sensitized with the same repertoire of IgE in individual subjects, the release of various substances, including histamine, arachidonic acid metabolites and cytokines from basophils in response to antigens well represent the sensitivity of the subjects. They also express several molecules on their surface, such as CD203c and CD69. Therefore, the *in vitro* histamine release test and cell surface analysis by flow cytometry for basophils of peripheral blood is sensitive, safe and gives reliable information regarding antigen that causes type I hypersensitivity. Griese *et al.* reported that the histamine release test showed higher sensitivity and specificity than skin test or serum analysis, such as ImmunoCAP, based on the comparison with bronchoprovocation of extrinsic asthmatic children.³ Moreover, the basophil activation test based on CD203c upregulation has been validated as a reliable tool for the diagnosis of IgE-mediated allergies.⁴ Thus, detection of basophils' reaction against antigens (allergens) may be a useful indicator of type I hypersensitivity. Although changes

of Ca²⁺ concentration in cells monitored with calcium fluorescent probe is well used as an indicator of cell activation, the introduction of non-biological fluorescent probe into cells may change behaviors of the cells. The technique to detect basophils' activation in response to antigens (allergens) with a small amount of peripheral blood without any labelling had not been developed until recently. We therefore applied Surface Plasmon Resonance (SPR) sensors to detect individual basophil activation.

SPR SENSORS

It is well known that SPR sensors can characterize the binding of detectants in the detection area on a sensor chip coated with gold film (50 nm) in real time without any labeling. They provide a useful means to study interaction of any biological molecules from proteins, oligonucleotides, and lipids to small particles, such as phages, viral particles and cells.⁵⁻⁷ As shown in Figure 1, when p-polarized light is reflected at the interface between glass and gold film at various incident angles (condition for total reflection), reflected light is strongly attenuated at a specific incident angle due to the resonance between plasmon derived from gold film and the evanescent wave derived from reflected light. The specific angle is called resonance angle (RA) which is changed by the refractive index (RI) in a detection area (<500 nm) on the surface of gold film (Fig. 1). Thus, SPR sensors can detect the RI changes in a detection area from the change of RA.

Figure 2 shows the typical shape of an RA change during the course of the experiment of antigen-antibody interaction. When a sensor chip modified

Detection of Cell Reactions by SPR

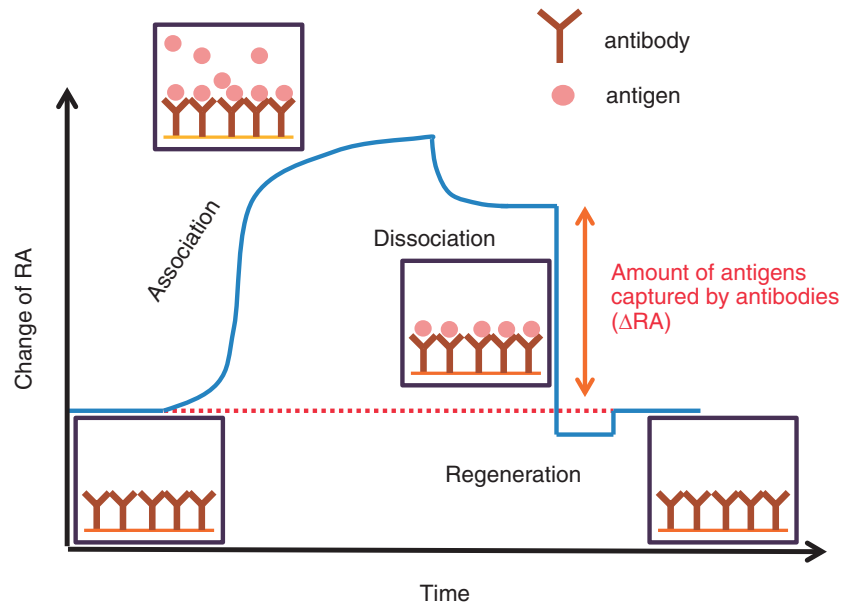


Fig. 2 Principle of detection of antigen-antibody interactions by SPR sensor.

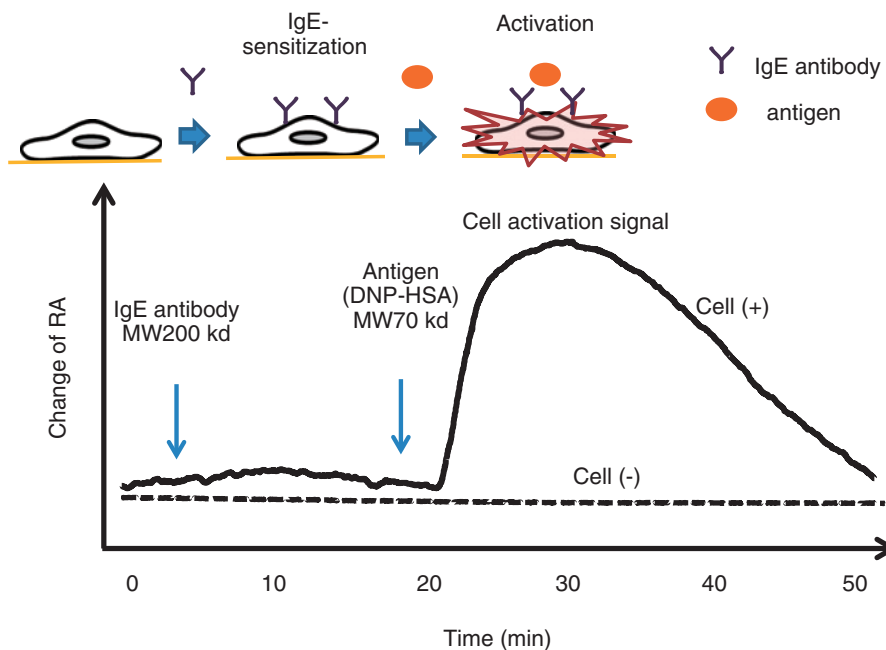


Fig. 3 RI changes of RBL-2H3 cells in response to antigen and antibodies. SRR signals obtained by the binding of anti-DNP IgE and DNP-HSA to RBL-2H3 cells were cultured on the surface of the SPR sensor and incubated first with anti-DNP IgE and then with DNP-HSA.

with antibody which can capture antigen is exposed to antigens, RA increases due to binding of antigens to antibodies (association step). Then RA decreases slightly, when antigens which did not bind to antibodies are removed by a washing process (dissociation step). The amount of antigen bound to antibody is calculated from ΔRA . The sensor chip with antigens

captured by antibodies is regenerated by removing antigens with sodium hydroxide (regeneration step). Thus, SPR sensors can detect the RI changes derived from antigen-antibody interaction on a sensor chip as the change of RA in a detection area in a real time manner.

DETECTION OF LIVING CELL REACTION BY MEANS OF SPR

In 2002, we first reported that SPR sensors do not only detect binding and dissociating of biochemical substances, but also reflect large changes of RI in re-

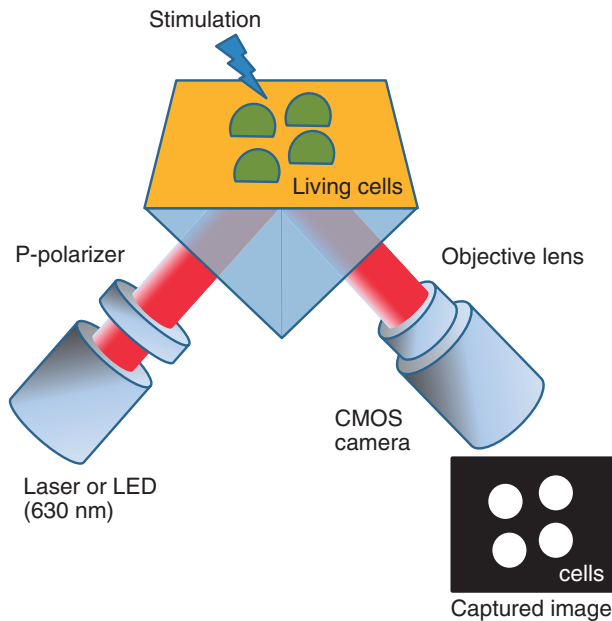


Fig. 4 Structure of SPR imaging sensor.

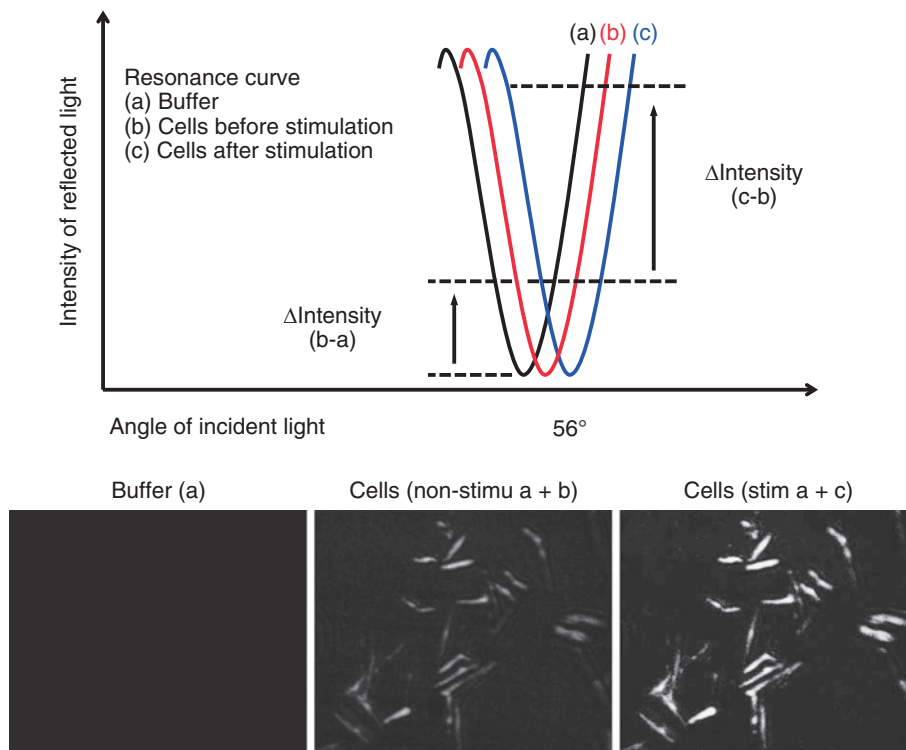


Fig. 5 Principle of detecting individual living cell reactions by SPRI.

sponse to the activation of living mast cells.⁸ Consequentially, activation of various cells, including keratinocytes, human basophils, B and T lymphocytes on a sensor chip were successfully analyzed without any labeling, suggesting the potential of SPR as a new diagnostic method for allergy and immunology.⁹⁻¹² The SPR sensor can only detect RI changes near plasma membrane of cells adhered to the sensor surface in a detection area (<500 nm) (Fig. 1). Therefore, the sensor needs to attach floating cells to the sensor surface with linker molecules, such as antibodies.¹⁰ Moreover, detection of RI changes of cells in a tissue and tissue slices is difficult because of such a narrow detection area. Figure 3 shows the SPR signal (real-time RI changes) in RBL-2H3 mast cells (adherent cells) treated with IgE antibodies followed by DNP-HSA antigens. In mast cells, the binding of antigen to IgE bound to the high-affinity IgE receptor (FcεRI) on the cell surface cross-links FcεRI, results in the release of preformed and newly synthesized mediators, such as histamine and arachidonic acid metabolites, and causes anaphylactic symptoms.^{13,14} When RBL-2H3 mast cells are sensitized with IgE antibodies, RBL-2H3 cells are not activated. Therefore, RI in RBL-2H3 cells does not increase. When RBL-2H3 cells sensitized with IgE antibodies are stimulated with antigen (DNP-HSA), RI rapidly increases and lasts for 40 min (Fig. 3). This result suggests that SPR sensors detect cell activation in response to stimuli, rather than the binding of extracellular molecules to the cell surface.

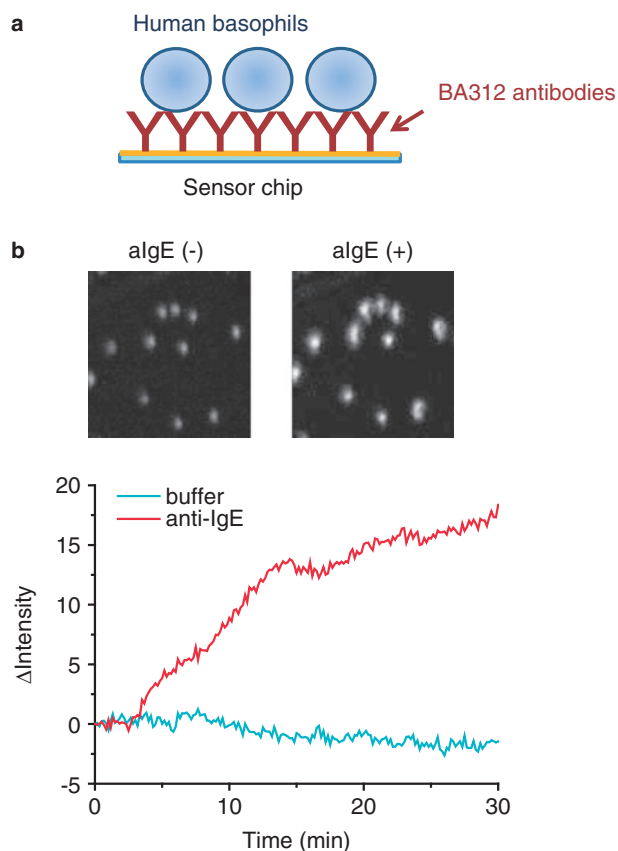


Fig. 6 RI changes in individual human basophils detected by SPRI. (a) Basophils isolated from peripheral blood were fixed on the surface of sensor chip via BA312 antibody. (b) RI changes (images and graph) in individual basophils in the absent or presence of anti-IgE.

We have also demonstrated that reactions detected by SPR sensors are not limited to changes of the area of cell adhesion and subcellular structures of living cells which may be observed using an ordinary light microscopy.⁸ We also demonstrated that RI changes is not proportional to Ca^{2+} mobilization in cells using PMA which also induced partial RI changes in RBL-2H3 cells without Ca^{2+} mobilization.¹⁵ In mast cells, the activation of protein kinases Syk, Lat, Gads and protein kinase C β (PKC β) are indispensable for the antigen-induced RI increase of mast cells detected by SPR biosensors.¹⁵ We also demonstrated that epidermal growth factor (EGF) induced RI changes via the phosphorylation of EGFR, and suggested that the SPR biosensor could be applied to the real-time detection and diagnosis of malignant tumors.¹⁶ Furthermore, we have developed a relatively small, simple and portable system, using an optical fiber to detect the activation of small numbers of living cells attached to the fiber chip in a real-time manner.¹² To date, relations between RI and cell functions have also been reported by other groups. Chabot *et al.*, re-

ported that SPR sensors may determine real time adhesion and morphological changes in cells following activations by various agents.¹⁷ An SPR sensor based on Fourier Transform infrared FTIR-SPR operating in the near infrared wavelength range could monitor changes in cell occupancy and membrane biochemical composition, such as cholesterol.^{18,19} Lee *et al.* reported that an SPR sensor combined with olfactory receptor expressing cells provides a new olfactory biosensor system for selective quantitative detection of volatile compounds.²⁰ A technique to detect reaction of cancer cells against an anti-cancer drug with SPR sensor is reported by Kosaihira *et al.*^{21,22}

DEVELOPMENT OF SPR IMAGING SENSOR TO VISUALIZE REACTIONS OF INDIVIDUAL CELLS

Thus, SPR sensor possesses great potential to reveal nano-scale living-cell actions in the field of evanescence. However, conventional SPR sensors detect only an average RI change in the presence of thousands of cells in an area of the sensor chip, and could offer only a small number of sensing channels (<10). Therefore, it was difficult to construct an array system for cell activation, and reactions of target cells may be readily overlooked when they were in a mixture of different cell types. Moreover, they could not reveal the intracellular distribution of RI, which is important to understand the mechanism of RI changes upon cell activation. A recently developed SPR imaging (SPRI) system determines a spatial RI distribution at the SPR-active surface by measuring the distribution of light intensity which proportionally reflects RI on the surface of sensor chip in an image captured by camera.²³⁻²⁵ We therefore developed an SPRI sensor for living cell analysis in order to detect RI changes in individual cells and to analyze subcellular distribution of RI changes. The sensor we developed consists of a light source (diode laser 630 nm), CMOS detector, optical prism (RI = 1.72) and a sensor chip with thin gold film (50 nm) matched to the prism via reflected index matching fluid (Fig. 4). Figure 5 shows the principal of visualization of individual cells activation. Expected resonance curve of buffer, cells before stimulation and cells after stimulation are (a), (b) and (c) respectively (Fig. 5). The sensor chip is exposed to the laser beam at the angle of 56° , achieving the maximum resonance of surface plasmon with buffer solution in the absence of cells. In the presence of cells whose RI is higher than buffer, the resonance angle shifts to a higher degree, resulting in the increase of reflected light intensity at the angle of 56° (Δ intensity) and the illuminance of the area when cells are attached. When cells are stimulated, the resonance angle shifts further to the level that varies according to the stimulus (Fig. 5) and intensity of reflected light at the cell area increases. Using this system, we could detect reactions of individ-

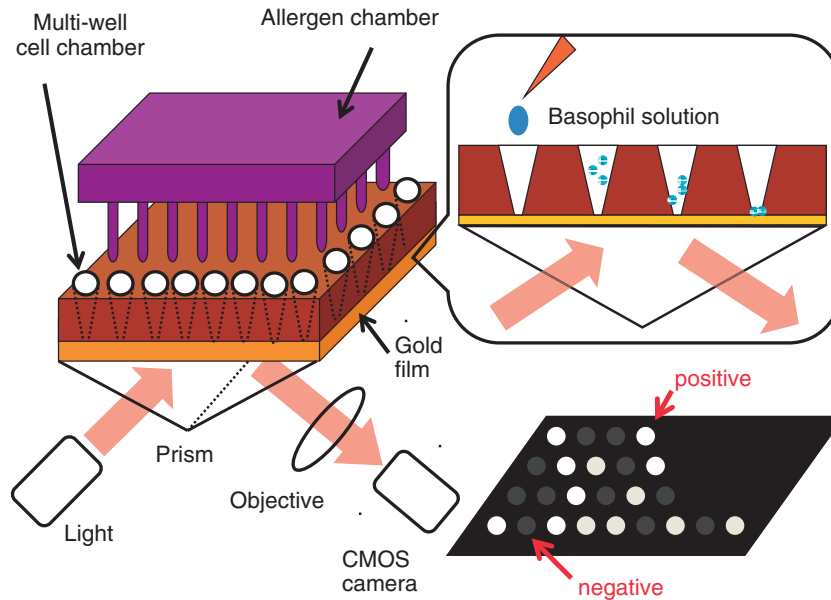


Fig. 7 Concept of high throughput screening SPRI system for clinical diagnosis of type I allergy.

ual rat mast cells (RBL-2H3 cells), mouse keratinocytes (PAM212 cells), and human epidermal carcinoma (A431) cells in response to either specific or non-specific stimuli, such as antigen, phorbol ester or EGF, with or without their inhibitor, resembling signals obtained by conventional SPR sensor. Moreover, we could distinguish reactions of different type cells, co-cultured on a sensor chip, and revealed that increase of RI around nuclei is rapid and potent as compared to that in peripheries in the reaction of RBL-2H3 cells against antigen. Thus, the SPRI sensor we developed could visualize the effects of various stimuli, inhibitors and/or conditions on cell reactions as changes of intracellular RI distribution at single cell levels.²⁶ Similar results with SPRI sensors were reported by another group.²⁷

APPLICATION OF SPRI FOR CLINICAL DIAGNOSIS OF TYPE I ALLERGY

We further developed a technique based on SPRI to detect reactions of individual human basophils, isolated from peripheral blood, and investigated the potential of this sensor as a tool for diagnostics of type I allergy. As shown in Figure 6, human basophils isolated from peripheral blood were fixed to the surface of gold film via BA312 antibody (anti-basophilic antibody) and changes of RI distribution in response to antigens were monitored by SPRI sensor. This system successfully detects the reactions in a basophil in response to anti-IgE, as well as histamine release, at the single cell level (Fig. 6). Moreover, the sensor could detect the difference of reaction in a basophil in response to several kinds of antigen: mite antigen, cedar pollen antigen or sweat antigen with a very small

drop of sample (<0.7 μ l). Furthermore, we could also distinguish reactions of basophils activated by antigen from those of non-activated basophils in the same area.²⁸ These results suggested that we could readily construct arrays of basophils for the analysis of various antigens in SPRI. Thus, the technique we developed can visualize the effect of various stimuli or inhibitors on basophils as change of intracellular RI distribution at the single cell level. Moreover, the sensor can also detect lymphocyte reactions.¹⁰ In combination with a device to rapidly isolate basophils or lymphocytes from peripheral blood and a multi-well chamber, the technique may be a useful tool as a high throughput screening system in clinical diagnosis for type I allergy and probably other hypersensitivity situations, such as drug hypersensitivity, with a drop of patient blood (Fig. 7).

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