PARALLEL SPR-IMAGING OF CELL RESPONSES:
PROOF-OF-CONCEPT OF CELL-BASED SPR ASSAY
FOR TYPE I ALLERGY
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ABSTRACT
We report a parallel surface plasmon resonance imaging (SPRI) system partially automated for functional analysis of living cells. To parallelize the assay, an array on 10 microfluidic chambers was placed in the view field of SPRI. We have shown the possibility of automated assay by demonstrating analysis of an experimental model of type I allergy. We stimulated sensitized rat basophilic leukemia cell line with the corresponding antigen in order to let them respond, and visualize the response of cells with the parallel SPRI system.

KEYWORDS: Type I allergy, Surface plasmon resonance imaging, SPRI, Cell-based assay

INTRODUCTION
Type I allergy causes a variety of allergic disorders including food allergy, hay fever, atopic dermatitis and asthma. It is quite important to identify causative allergens for medical care of the disease. However, existing serological tests show substantial deviation from clinical symptoms, and \textit{in vivo} tests have a risk for anaphylaxis \cite{1}\cite{2}. Therefore, more reliable blood testing is desired. SPRI-based assay for the activation of basophils in response to antigens is a possible solution.

SPRI maps the difference of the refractive indices of materials on sensor chip \cite{3}. It was shown that SPR can detect the activation of human immune cells in response to antigens at single-cell level and the results show concordance with clinical diagnosis \cite{4}\cite{5}. However, key factors for the technique to be a practical testing method, parallelization and automation, was yet to be developed. We targeted SPRI of 10 parallel samples because the number of candidate antigens for a single patient often numbers around ten. To approach the potential automation of the assay, we tried to execute several steps for the assay within a microfluidic chamber.

EXPERIMENTAL
SPRI instrument (optical system) was constructed as described in a previous literature \cite{6}. PDMS flow cell with 10 channels was fabricated by soft lithography technique (Figure 2). The width and height
of the channel of the flow cell is 500 μm and 300 μm respectively. Figure 3 shows the workflow of the assay. An allergic model was prepared as described before [5]. We employed a commonly used model system of type I allergy which uses RBL-2H3, a cell line originating from rat basophilic leukemia. RBL-2H3 cells were cultured in RPMI-1640 medium, which is supplied with CaCl₂ to make the final concentration of Ca²⁺ 1 mM. To sensitize the cells, a half of the cells were incubated in medium containing 50 ng/ml anti-dinitrophenol (DNP) immunoglobulin E antibody. While the original RBL-2H3 cells do not respond to stimulus by DNP antigen (‘non-sensitized’ cells), the cells incubated with the IgE become sensitive to DNP antigen (‘sensitized’ cells). ‘Sensitized’ and ‘non-sensitized’ cells were assigned to the chambers alternately.

The cell suspensions were delivered from reservoirs into the chambers by a PC-controlled pump. The cells were left still for 45 min to be immobilized by spontaneous cell adhesion to the gold film surface. Then the pump started again to deliver an antigen solution, 50 ng/ml DNP-conjugated bovine serum albumin (DNP-BSA), into the chambers. SPR-images were continuously taken while the cells were treated with DNP-BSA.

![Figure 2: A photographic image of the PDMS flow cell with the sensor chip, which is a gold-coated glass substrate. The dimension of the PDMS flow cell is 40x40x2 mm.](image)

![Figure 3: Schematic drawing of workflow and mechanism of the assay for type I allergy.](image)

**RESULTS AND DISCUSSION**

Figure 4a shows an SPR-image of 10-channel-flow cell with cells in its channels. A region of interest (ROI) on each channel was indicated as a box. Zoomed-in images of ROIs are shown in Figure 4b. ‘Sensitized’ cells turned brighter after stimulus while ‘non-sensitized’ cells kept its intensity. Figure 4c shows the time courses of cell responses. The traces of the averaged intensities in ROIs for ‘sensitized’ cells rose and those for ‘non-sensitized’ cells stayed around the initial levels. The difference of signals between those two group was already significant 7 minutes after the beginning of the stimulus, and all five signals for ‘sensitized’ cells was above the level of mean + 5x standard deviation of the ‘non-sensitized’ signals. This result indicates that the automated cell-based assay to detect causative antigens for type I allergy can be carried out within 10 minutes on the system.
CONCLUSION

We have constructed a parallel SPRI system for 10 samples of living cells. Our results revealed that causative allergens can be detected out of multiple candidates using this system. This system may be applied not only for the evaluation of basophil activations stimulated by many antigens, but also for various cell-based assays for clinical diagnosis.

REFERENCES


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