Diagnosis of immediate-type allergy using SPR imaging sensor

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Abstract
Surface plasmon resonance imaging (SPRI) sensors can sensitively visualize refractive index (RI) changes in living cells on the surface of a sensor chip, without labeling, in real time. In this study, we developed a technique for the clinical diagnosis of immediate-type allergy using basophil separation chip for SPRI.

1. Introduction
A technique to visualize individual living cell activation in a real time manner without any labeling is required in the fields of life sciences and medicine. Surface plasmon resonance (SPR) sensors detect the refractive index (RI) changes on the surface of sensor chips in label-free and on real time basis. We previously reported that SPR sensors could detect real-time large changes of RI in response to activation of living cells, such as mast cells, keratinocytes, basophils and B lymphocytes on a sensor chip without labeling, suggesting the potential of SPR as a new method for clinical diagnosis and drug screening. Thus, SPR sensor possesses great potential to reveal nano-scale living cell actions in evanescent field. However, conventional SPR sensors detect only an average RI changes in the presence of thousands of cells in an area of the sensor chip, and could offer only small number of sensing channels. Therefore, we developed SPR imaging (SPRI) sensor with a CMOS camera and an objective lens in order to visualize RI distribution of individual living cells and their changes upon stimuli [1-4].

2. Results
SPRI sensors can visualize the activation of human peripheral blood derived-basophils in response to specific antigens, at the single cell level (Fig.1). Establishing a technique to rapidly isolate basophils from small blood samples, transport them to multiple SPRI-sensing regions, and stimulate them with various antigens will enable SPRI to rapidly and reliably diagnose type I allergy. It is also less invasive than conventional techniques for diagnosing type I allergy. To isolate human basophils from a small amount of peripheral blood, a microfluidic chip containing magnetic particles has been developed. Fig. 2 shows the basophil isolation chip for SPRI. The combination of a microfluidic device and the magnetic separation of living cells has been previously reported. To enhance cell capture, the basophil separation chip involves magnetic particles implanted in the microfluidic device. The chip is composed of three parts: inlet (sample gate), cell separation area with magnetic particles (magnetically active area), and SPRI analysis area containing a gold film (SPR sensor surface).

Fig. 1 RI changes in individual basophils with and without stimulation, as visualized by SPRI. Red and blue indicate high and low RI areas, respectively. The white scale bar indicates 20 μm. [From Yanase et al., Opt Mater Ezpress. 6, 1339-1348 (2016).]

Fig. 2 Structure of the basophil separation chip for SPRI analysis. [From Yanase et al., Opt Mater Ezpress. 6, 1339-1348 (2016).]

The separation chip consists of a high RI glass slide (RI = 1.72) and a patterned polydimethylsiloxane (PDMS) film formed by SU-8 lithography containing magnetic particles (100–500 μm). The chip can capture magnetic bead-labeled non-basophils in the flow channel. It then transports non-labeled basophils to the SPRI-sensing region (gold film). Neodymium magnets are placed on the chip to enhance the magnetic field. When a mixture of non-basophils labeled with magnetic beads and basophils without magnetic beads are injected into the chip inlet, cells flow into each channel by capillary force. The magnetic bead-labeled non-basophils are then captured at the magnetically active area. Finally, basophils reach the SPRI area, and RI changes of individual
basophils in response to allergens are monitored by SPRI. Fig. 3 shows a microfluidic chip with (Fig. 3(b)) and without (Fig. 3(a)) magnetic particles. A blue-stained cell suspension flows through fluidic channels by capillary force, without pumping. Magnetic bead-labeled cells are captured by the magnetic particles (Fig. 3(c), green). The activation of basophils on the SPR sensor is visualized by SPRI (Fig. 3(d)). The basophil separation chip with multiple SPR analysis areas enables us to isolate basophils from a small amount of blood, and detect their activation in response to allergens by SPRI in a single procedure [5,6].

Fig. 3. Evaluation of basophil separation chip for SPR analysis. [From Yanase et al., Opt Mater Express. 6, 1339-1348 (2016).]

3. Conclusions
SPRI sensors can visualize the activation of human basophils in response to specific antigens (allergens), at the single cell level in real time. These techniques can be performed with a small number of basophils isolated from peripheral blood. The SPR detection of cell activation is a useful tool. It is reliable for the clinical diagnosis of type I allergy, and is less invasive than conventional techniques. SPRI detection is applicable to freshly prepared basophils.

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References