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SPR imaging sensor for visualization of individual cell activation and clinical diagnosis of allergy

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Abstract

In this study, we developed a technique based on SPR to evaluate the activation of living cells and investigated its potential as a tool for clinical diagnosis based on individual cell-reactions.

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 a 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. Methods

The sensor we developed is composed of a light source (red laser), P-polarizer, prism (RI=1.72), objective lens (×4) and CMOS camera (Fig.1). The SPRI sensor chips (RI=1.72, 20 mm × 20 mm × 1 mm) coated with gold thin film (1 nm Cr layer and 49 nm gold layer) by means of vapor deposition. Human basophils from peripheral blood were fixed to the surface of SPRI sensor chip via anti-basophilic antibody. Living cells on the surface of SPRI sensor chip were visualized by the difference of RI between buffer and living cells. Obtained images and changes of light intensity in living cells were analyzed with image-Pro™.

3. Results

The sensor chip was 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, resonance angle shifted to higher degree, resulting in the increase of reflected light intensity at the angle of 56 °(Δintensity). When cells were stimulated, the resonance angle shifts further and intensity of reflected light at the cell area increased (Fig.2). 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 (Fig.3). Furthermore, we also succeeded in distinguishing reactions of basophils activated by antigens from those of non-activated basophils spotted on an area.

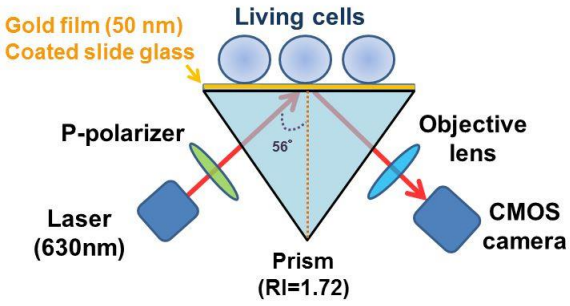

Fig. 1 Scheme of SPRI sensor for detection of individual living cells reactions.

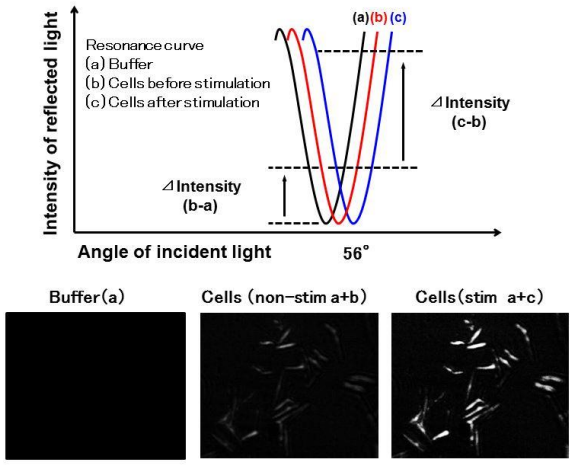

Fig. 2 Principle of detecting individual living cell reactions by SPRI

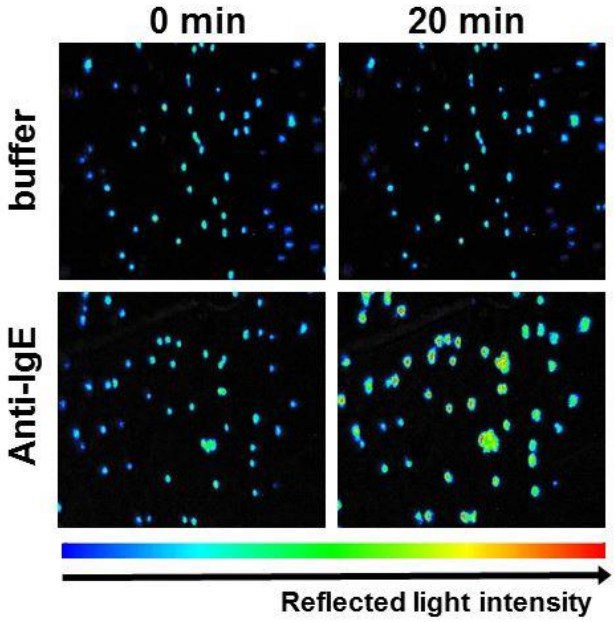

Fig. 3 RI distribution changes in individual human basophils observed by SPRI sensor.

4. Conclusions

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 allergy and drug hypersensitivity, and as a tool to reveal novel phenomena in evanescent fields around plasma membrane.

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