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Short communication

Development of an optical fiber SPR sensor for living cell activation

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ABSTRACT

Surface plasmon resonance (SPR) sensors provide a useful means to study the interactions of biological molecules and the reaction of living cells on a sensor chip. However, conventional SPR sensors are bulky, expensive and complicated to use as common diagnostic equipment. In this study, we developed a relatively small and simple SPR system, using optical fibers of 250 μm diameter to detect the activation of living cells attached to the fiber tip. For this system, the core of 200 μm diameter with 1 cm length of an optical fiber was coated by gold film with 50 nm thickness to cause plasmon resonance. The light provided by a white LED and attenuated due to a SPR phenomenon in the sensor part was detected and analyzed using a spectrum detector. The difference in solvents with various refractive indexes and protein-bindings to the sensor tip was detected with sufficient sensitivity. Moreover, it detected a sustained increase of AR in a real-time manner, when RBL-2H3 mast cells were fixed onto the fiber tip and stimulated by an antigen. This small fiber SPR system might serve as a useful tool for various clinical examinations either within or outside the body.

1. Introduction

There is a continuously increasing demand for biosensors that are able to detect living cell activation (Ziegler and Gopel, 1998). Surface plasmon resonance (SPR) sensors are capable of characterizing the binding of detectants in the field of resonance on a sensor chip in a real-time manner without any labeling (Karlsson, 2004; Cooper, 2003; Szabo et al., 1995). They provide a useful means to study the interactions of a variety of molecules, including proteins, oligonucleotides, lipids, and even small structures, such as phages, viral particles and cells (Rich and Myszka, 2000). We previously reported that SPR sensors can detect large changes of AR, when RBL-2H3 mast cells were activated by an antigen on a sensor chip (Hide et al., 2002). We also reported that SPR sensors can detect changes other than the area of adhesion and the overall constructions in living cells observed using an ordinary light microscope (Yanase et al., 2007a,b). Moreover, we clarified that the activation of Syk, Lat, Gads and PKC is indispensable for the antigen-induced increase of AR of mast cells detected by SPR biosensors (Tanaka et al., 2008). Furthermore, we detected the change of AR with human basophils and B cells which were fixed and stimulated on a sensor chip, suggesting the potential of SPR as a new diagnostic method for allergy and immunology research/treatment (Yanase et al., 2007a,b; Suzuki et al., 2008). However, conventional SPR sensors are bulky, expensive and complicated to use as common diagnostic equipment, especially for clinical purposes. In this study, we constructed a relatively small, simple and portable system, using an optical fiber to detect the activation of living cells.

2. Materials and methods

2.1. Reagents

The chemicals used were obtained from the following sources: bovine serum albumin (BSA), dinitro-phenol-conjugated human serum albumin (DNP-HSA) and DNP-specific rat monoclonal IgE from Sigma–Aldrich Japan (Tokyo, Japan); 8-aminooctanethiol from DOJINDO (Tokyo, Japan), EZ-Link™ NHS-biotin and ImmunoPure Avidin from PIERCE (Rockford, IL); Stearyl mercaptan from TOKYO KASEI (Tokyo, Japan).

2.2. Instrument of fiber-optical SPR sensor

This optical fiber SPR sensor was composed of a light source (white LED, Ocean Optics Inc, FL), a plastic cladding multimode optical fiber with quartz core (200/230, THORLABS JAPAN, Tokyo, Japan), a SC type fiber connecter (FONT Canada, Canada), a fiber coupler (Newport Corp, CA), a spectrometer (HR4000, Ocean Optics

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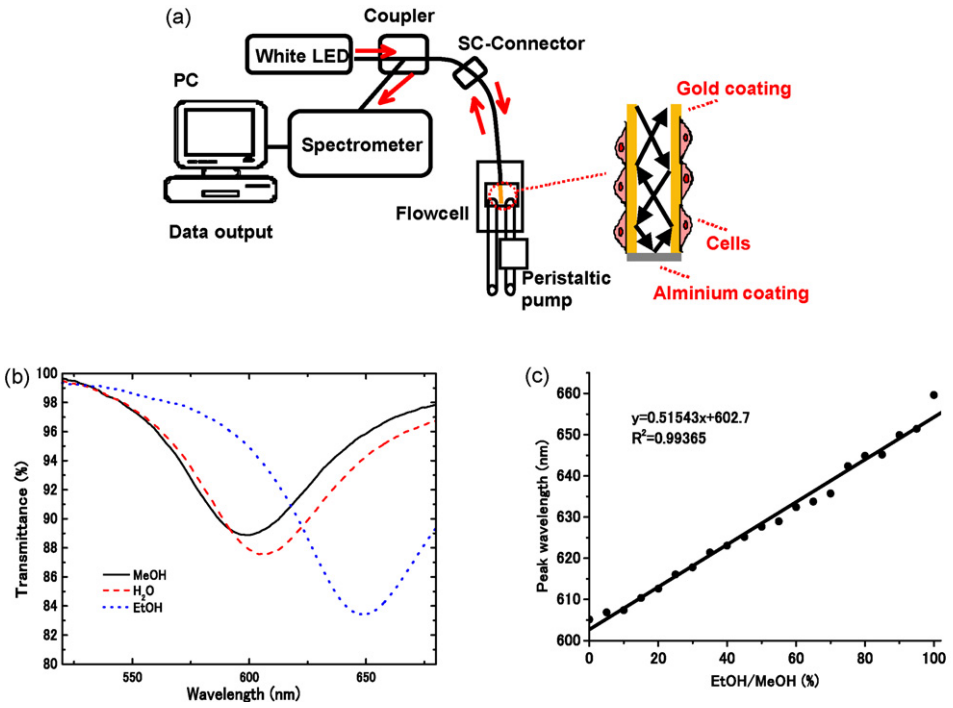

Fig. 1. Construction and sensitivity of optical fiber SPR sensor. (a) The optical fiber SPR sensor was composed of a light source (white LED), a plastic cladding multimode optical fiber with quartz core (200/230), a fiber connecter, a fiber coupler, a spectrometer, and a personal computer with analysis software. One centimetre of the fiber tip was treated with sulfuric acid to remove cladding and coated by gold thin film (50 nm) by means of vapor deposition. (b) The absorption spectrums detected in methanol (solid line RI = 1.3265), water (dotted line RI = 1.3329), ethanol (broken line RI = 1.3594). (c) Each peak wavelength was detected in solutions consisting of methanol and ethanol mixed at various ratios (5% increment in between) and plotted.

Inc.) and a personal computer with analysis software (Fig. 1a). The software to detect and plot the wavelength of maximum absorption (peak wavelength) was produced by Toyo Advanced Technologies Co., Ltd. (Hiroshima, Japan). The sensor parts (1 cm) were exposed by sulfuric acid to remove clad, followed by coating with gold thin film (50 nm) by means of vapor deposition. For the analysis of protein-binding and living cell reactions, we used a flow cell made of transparent acryl which can be perfused with various sample solutions at a proper flow rate with a peristaltic pump (PERISTA pump, ATTO, Tokyo, Japan) and observed by phase contrast microscopy (Fig. 1a).

2.3. Biotin coating of gold surface

An optical fiber tip coated with gold film was immersed in 1 mM 8-aminooctanethiol in ethanol for 1 h, in 1 mM NHS-biotin, 10% DMSO in PBS for 30 min, and finally in 1% BSA in PBS for the purpose of blocking.

2.4. Cell culture

RBL-2H3 cells were cultured in RPMI-1640 medium supplemented with 10% fetal calf serum (FCS), 100 U/ml penicillin, and 100 μg/ml streptomycin as described previously (Yanase et al., 2007a,b). The day before experiments, cells were harvested using trypsin. They were then cultured (4×10^5 cells/ml) in the presence of 0.05 μg/ml anti-DNP IgE in HydroCell^{TM} dishes (Cellseed, Tokyo, Japan) for floating culture.

2.5. Preparation of cells for SPR analysis

Recovered cells were suspended in Pipes buffer containing 119 mM NaCl, 5 mM KCl, 1.0 mM $CaCl_2$, 0.4 mM $MgCl_2$, 5.6 mM glucose, 25 mM piperazine-*N*-*N*′-bis (2-ethanesulfonic acid) (pipes) and 1 mg/ml BSA, pH 7.2 (Hide et al., 2002) at a concentration of 1×10^6 cells/ml. A furrowed plate was filed with the cell suspension and turned so as to make an elongated droplet containing living

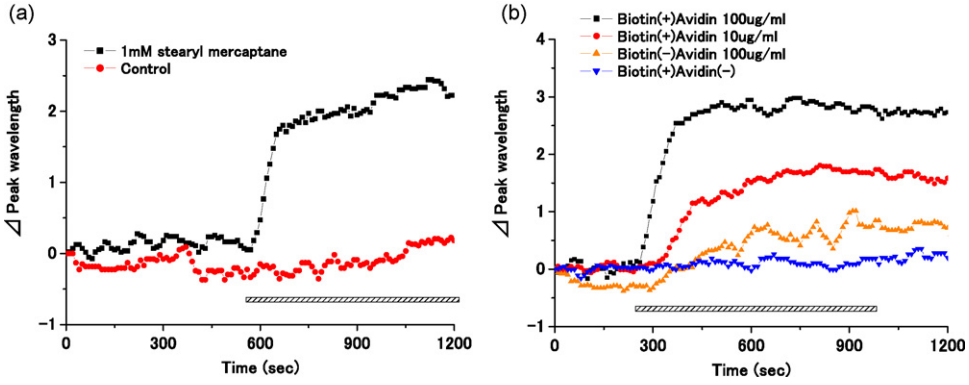

Fig. 2. Detection of the binding of alkane thiol and proteins on the gold surface. (a) Binding of stearyl mercaptan (1 mM) on the gold surface was detected in a real-time manner. (b) Binding of avidin to biotin arranged on the gold surface was detected in a real-time manner at flow rate 200 μl/min. Horizontal bars show the period of avidin perfusion. The average peak wave lengths at the beginning of each measurement obtained by vehicles for respective molecules were 649.7 ± 5.6, $n = 4$ (ethanol) and 628.6 ± 1.7, $n = 4$ (PBS).