

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 et al., 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 et al., 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 et al., 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., 2007). 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., 2007; 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-LinkTM NHS-biotin and ImmunoPure Avidin from PIERCE (Rockford, IL); Strearyl 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 connector (FONT Canada, Canada), a fiber coupler (Newport Corp, CA), a spectrometer (HR4000, Ocean Optics 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 hr, 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., 2007). 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™ 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 CaCl₂, 0.4 mM MgCl₂, 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 cells at room temperature. A gold-coated fiber tip was immersed into the bottom of a droplet as shown in Fig. 3a for 15 min. The sensor part was then quickly located into a flow cell and perfused with Pipes buffer with or without DNP-HSA.

3. Results

3.1 Construction and sensitivity of Optical-fiber SPR sensor

The optical fiber-SPR sensor system was constructed as shown in Fig. 1a. To confirm the performance and the sensitivity, we first analyzed spectrums of the light reflected at the sensor tip in methanol, water and ethanol. The absorption spectrums detected in these solvents reflected the inherent RI of each solvent, 1.3265, 1.3329, 1.3594, respectively. To evaluate the resolution of RI by this system, methanol and ethanol were mixed in various percentages and analyzed by peak wavelength of each solution. As shown in Fig. 1b, the optical-fiber SPR system detected a change of

peak wavelength as small as 5 % difference of the mixing rate of methanol and ethanol.

3.2 Detection of the binding of alkane thiol or proteins on a gold surface

We next investigated whether the sensor could detect molecular bindings on gold surface. As shown in Fig2.a, the peak wavelength increased, when the sensor part was immersed into 1 mM stearyl mercaptan which binds to a gold surface. When a biotinylated sensor tip was perfused with PBS in the flow cell with avidin at flow rate of 200 μ l/min, the peak wavelength increased in a dose-dependent manner to avidin. 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).

3.3 Detection of the reaction in living-cells on a gold surface

RBL-2H3 cells were fixed on an optical-fiber SPR sensor as described in the “Materials and Methods”. As shown in Fig. 3a, RBL-2H3 cells were fixed on the fiber tip confluent within 15 min. On the exposure of RBL-2H3 cells sensitized with anti-DNP IgE to 50 ng/ml DNP-HSA (flow rate 100 μ l/min), peak wavelength began to increase (Fig. 3b) until even after the end of perfusion with the antigen. The average change of peak wavelength was 1.82 ± 0.64 nm (n=5). No apparent change of peak wavelength was observed, when cells were perfused with buffer alone, or when the sensor

was exposed to DNP-HSA in the absence of RBL-2H3 cells (Fig. 3b). Cells on the sensor tip were not detached from fiber tip during measurement.

4. Discussion

We developed an optical fiber-SPR sensor system for the analysis of living cell reactions on the fiber tip. We previously reported that a conventional SPR sensor, with a relatively large flat space (1×5 mm) for cell attachment and a prism, can detect reactions of living cells (Hide et al. 2002, Yanase et al.2007, Suzuki et al., 2008, Tanaka et al., 2008).

The application of optical fiber sensors based on SPR phenomenon has been reported for the analysis of liquid or gas samples (Nelson et al. 1997, Mitsushio et al. 2004). However, it has been difficult to fix living cells on the tip of an optical fiber. In this study, we established a “droplet method” to briefly fix cells on the tip of an optical fiber. By this method, we could easily fix cells from a small amount of cell suspension onto the sensor part. If the gold surface was treated by the method to fix non-adherent cells as reported in our previous article, we could fix either adherent or non-adherent cells on the fiber tip, (Yanase et al., 2007).

For the system developed in this study, we employed an optical fiber consisting of a quartz core and plastic clad, which may be readily removed by sulfuric acid and replaced with gold film by vapor deposition. Moreover, we inserted a SC-connecter between fiber coated with gold film, and

that connected to a spectrometer and a white LED as a light source. By this configuration, the sensor part can be easily replaced for a new one as a disposable part for each measurement.

The minimal detection limit by this sensor is approximately 0.001,65 RI (5% difference of the mixing rate of methanol and ethanol). This is somewhat larger than those by conventional flat type SPR sensors, such as Biacore[®], which detects changes of RI as small as 0.000,001. Moreover, SPR signals reflected as changes of peak wavelength by this system were fluctuating probably because of the flexibility of optical fibers. However, the change of RI caused by the activation of RBL-2H3 cells on a gold film, around 0.006 (Yanase et al., 2007), is larger than the above mentioned detection limit. In fact, we successfully detected the SPR signal with RBL-2H3 cell in response to antigen beyond the noise.

Thus, the system is cheap, small and can easily detect reactions of living cells on the gold coated fiber tip in a real time manner, as well as RI of various solvents and reactions of molecules/proteins-binding. Since the sensing part of this system is so small and data can be collected at the other end of an optical fiber connected to a portable device, it is feasible to embed the sensor part and fibers into an endoscope for humans to support clinical diagnosis and perhaps for treatments. The improvement of sensitivity and resolution of this system together with the accumulation of data of various kinds of cells including those of cancers and inflammations should be a subject for future/subsequent studies to develop this system for clinical use.

5. Conclusion

We have developed a small and simple optical fiber-SPR system that can detect reactions of living cells attached to the fiber tip in a real-time manner. It might be a useful tool not only for basic research, but also for clinical diagnosis based on cell functions, both *in vitro* and *in vivo*.

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Figure legends

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 connector, a fiber coupler, a spectrometer, and a personal computer with analysis software. One cm 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.

Fig. 2. *Detection of the binding of alkane-thiol and proteins on the gold surface*

(a) Binding of stearyl mercaptan (1mM) 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 μ l/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)

Fig. 3. *Detection of reactions of living-cell on the gold surface*

(a) RBL-2H3 cells were fixed on the sensor tip surface by means of the “droplet method” described

in “Materials and Methods” and observed under a phase contrast microscope. (b) RBL-2H3 cells on the surface of an optical fiber were stimulated with DNP-HSA (50 ng/ml) at room temperature, and flow rate 100 μ l/min. The average change of peak wavelength was 1.82 ± 0.64 nm (n=5). Horizontal bars show the period of DNP-HSA perfusion.

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Fig.1

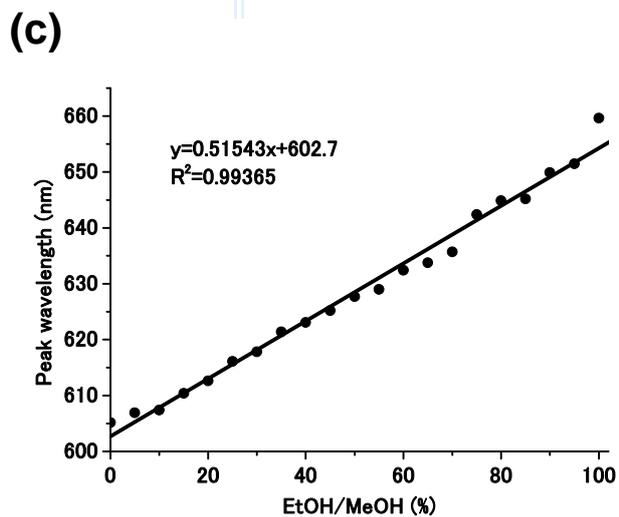
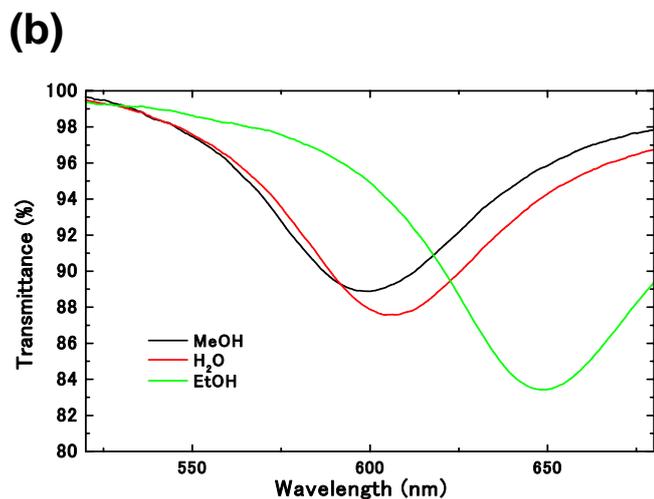
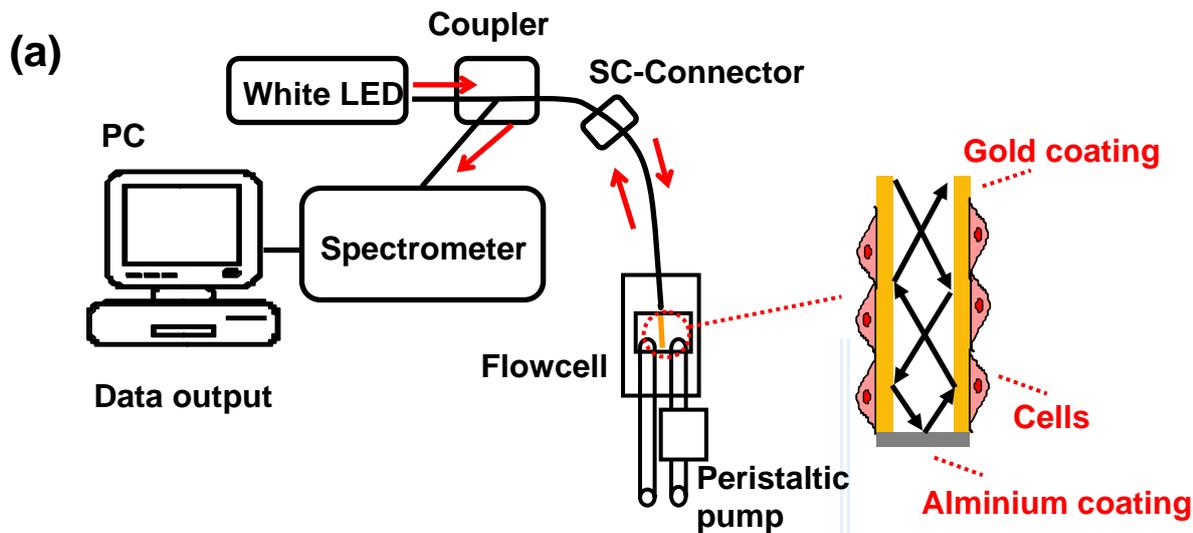
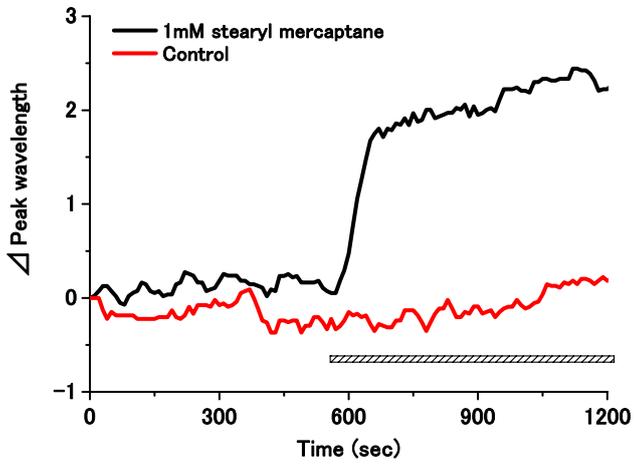


Fig.2

(a)



(b)

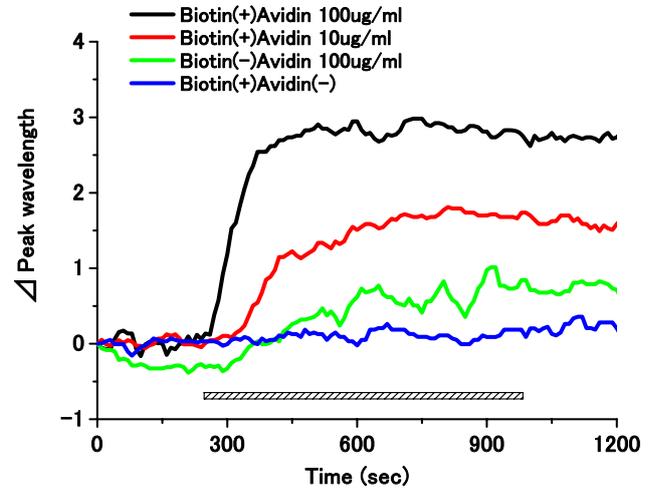
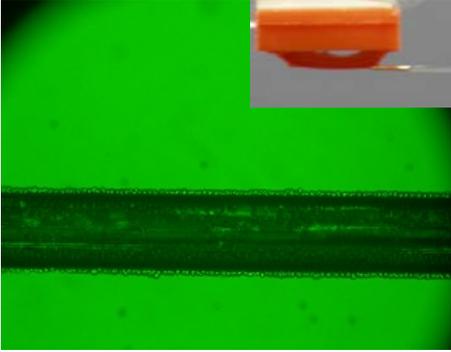


Fig.3

(a)



(b)

