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Fluoroimmunoassay System for Fiber-Optic Measurement of House Dust Mite Allergen (*Der f*1)

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A fiber-optic fluoroimmunoassay system for house dust mite allergen (*Der f*1) is proposed. We optimized or selected optical components for a system, such as optical filters, fluorescence detectors, and optical fiber probes, to detect fluorescence of a cyanine dye (Cy5). We constructed the excitation-emission system for optimized conditions and measured fluorescence intensitites of dilute solutions of Cy5. The measurement range was from 10^0 to 10^5 nmol/l. Next we applied the optical system to fluoroimmunoassays for *Der f*1. Anti-*Der f*1 capture antibodies were immobilized onto the end surface of a probe by physical adsorption. Diluted *Der f*1 solutions and Cy5-labeled antibodies were incubated with capture antibodies to form immune compexes. After all immune reactions were finished, fluorescence from antibodies was measured using the system. *Der f*1 was quantified in the range of 31.25 to 500 ng/ml. This study suggests that a fluoroimmunoassay system is a base technology to construct a monitoring device for enviromental allergens.

1. Introduction

The prevalence of allergic diseases such as asthma, atopic eczema, or rhinitis has markedly increased in developed countries since the 1960s and has become a major public health concern.[1,2] Allergic diseases develop on the basis of complex gene-environment interactions.[3] The genetic background is called atopic diathesis.[4] Many triggers can cause allergic diseases in living environments, and environmental triggers are called allergens. Examples of inhaled allergens include house dust mite (HDM),

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pollen, mold, and pet dander.[5–7] The HDM allergen is particularly common and can be found in all homes (in mattresses, upholstered furniture, and carpets) in the world.[8–10] The HDM allergen is a digestive enzyme from mite's gastrointestinal tract and is found in feces of HDM.[11,12] The allergen can be a tiny particle found in indoor air.[13] For people with the allergy, it is important to know where the allergen is in a house and to avoid exposure to it.

For the confirmation of the presence of HDM in houses, a microscopic examination of house dust samples is a direct method.[14] Simple dipstick-type test kits for HDM detection are commercially available for the public.[15,16] These tests can detect guanine, which is the major component of the nitrogenous excreta of mites. Both microscopic observation and dipstick-type tests are semi-quantitative and indirect estimation methods for the allergen of HDM. For the direct detection of HDM allergen, enzyme-linked immunosorbant assay (ELISA) has been developed and is often used.[17,18] Since ELISA makes use of monoclonal antibodies directed against the HDM allergen, an accurate determination of allergen exposure is possible using results of ELISA. This method is used mainly by research laboratories because it needs specialized apparatus and techniques. Therefore, it is desirable to develop a simple, on-site measurement technique for the direct detection of HDM allergen to control an allergic patient's environment.

For aerosol-to-hydrosol sampling, several techniques are already available.[19–21] It is considered that the on-site immunoassay for airborne allergen is possible by an appropriate biosensing system using these airborne particle samplers. On the other hand, we have already reported a fiber-optic biosensing system for HDM allergen.[22,23] The measurement principle is based on a fluoroimmunoassay. The detection antibody labeled by fluorescence molecules of cyanine 5 (Cy5) was detected using an evanescent light field generated by a laser diode light source. Since the system was originally developed as general-purpose excitation-emission equipment, it is difficult to integrate with other elements to meet the need of allergic patients or scientific researchers who want to know the concentration of the aero-allergen of HDM in a domestic environment.

In this study, we constructed a newly-designed system based on a fiber-optic fluoroimmunoassay for HDM allergen detection. The allergen detected by our system is *Der f1*, derived from *Dermatophagoides farinae*, which is a species of HDM. The aim of this study was to optimize optical components in the system and to evaluate the applicability of the system to on-site measurement of *Der f*1.

2. Materials and Methods

2.1 *Reagents*

*Der f*1 derived from *Dermatophagoides farinae* (*Der f*1 standard, Lot 2761, 2500 ng/ml), Anti-*Der f*1 monoclonal antibodies as capture antibodies (Anti-*Der f*1 mAb 6A8, Lot 2828, 2 mg/ml) that can bind to *Der f*1, and Anti-*Der f*1 monoclonal antibodies as detection antibodies (Anti-*Der f*1 mAb 4Cl, Lot 2469, 2 mg/ml) were purchased from INDOOR Biotechnologies, Inc. (Charlottesville, Virginia). Cy5-labeled detection antibodies were produced using a commercial labeling kit (Cy5-Ab labeling kit, PA35000, GE Healthcare UK Ltd. Buckinghamshire, England). Bovine serum albumin

(BSA, Lot 401-041, 10 g) was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). All other chemicals used were reagent grade. The buffer used for washing optical fiber probes consisted of 10 mM phosphate buffer at pH 7.4 (PB) containing 0.05% (v/v) Tween 20 (PBT). The dilution buffer used to capture antibodies consisted of 80 mM carbonate-bicarbonate buffer; for BSA, PB; for *Der f*1, PBT; and for Cy5-labeled antibodies, it consisted of PBT containing 0.1% (w/v) BSA.

2.2 *Experimental setup for fiber-optic fluoroimmunoassay*

The principle of our measurement is based on a fluoroimmunoassay. The fluorochrome we used to label detection antibodies was Cy5. Cy5 can be excited by light of 649 nm wavelength, and it emits red fluorescence light at 670 nm. On the basis of the excitation/emission properties of Cy5, we constructed our measurement system. The system is shown in Fig. 1(a). A red light emitting diode (LED; λ_p 631 nm, OSHR511A-TU, OptoSupply Ltd., China) on a custom-fabricated LED power source (KLV Co., Ltd., Japan) was used as an excitation light source. A photomultiplier tube (PMT; H7421, Hamamatsu Photonics K.K., Japan) or a spectrometer (USB4000, OceanOptics Inc., USA) was used as a fluorescence detector. The LED and PMT were connected to an optical fiber probe using an optical fiber assembly (BIF600-VIS/NIR, Ocean Optics Inc., USA). We used three types of probes as follows: a silica-core fiber probe (F1000-900, core diameter: 1.0 mm, Ocean Optics Inc., USA), a polystyrene (PS)-core fiber probe (core diameter: 1.0 mm, Shenzhen Corpereal Photoelectric Co., Ltd., China), and a polymethyl methacrylate (PMMA)-core fiber probe (CK40, diameter: 1.0 mm, Mitsubishi Rayon Co., Ltd., Japan). The plastic optical fiber probes (PS-core and PMMA-core) were cut

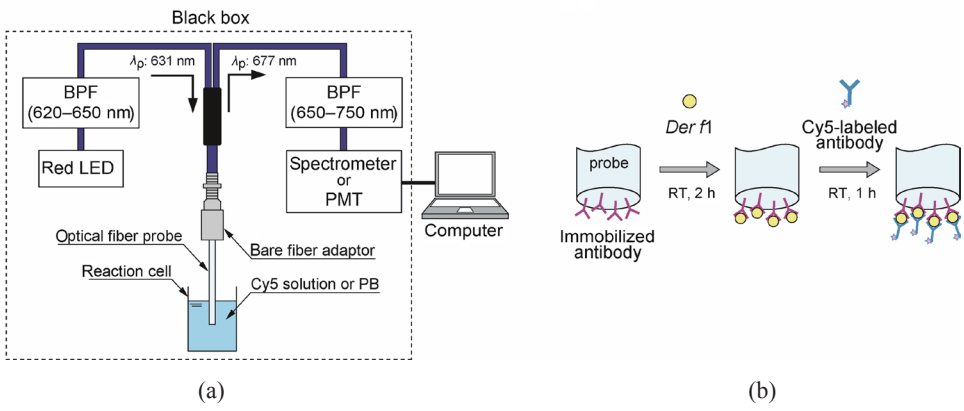

Fig. 1. (Color online) (a) Experimental setup of the fiber-optic fluoroimmunoassay system. The excitation light source is coupled to the optical fiber probe, and the fluorescence of Cy5 is detected coaxially by the photomultiplier. (b) Process of *Der f*1 measurement by fluoroimmunoassay using the optical fiber probe.

into 12 cm lengths, and both incisal end surfaces were horizontally polished using a micropolisher (Rev™, Krell Technologies Inc., USA), which is used for polishing films to a roughness of 30 μm and 9 μm for silica films and 0.3 μm for alumina films. The excitation light and the fluorescent light were filtered using two band-pass filters (BPF) which transmitted light with a wavelength of 620–650 nm (QMAX_EX 620-650, Omega Optical Inc., USA) and 670–750 nm (QMAX_EM 670-750, Omega Optical Inc., USA). As a reaction cell for fluoroimmunoassay, we used a well in a commercial microtiter plate (655061, Greiner Bio-One Co. Ltd., Japan). To evaluate the properties of our optical system, serial concentrations of Cy5 (10^{-1} to 10^{5} nmol/l) were prepared, and 100 μl of each solution was put into the reaction cell to detect Cy5 fluorescence. The Cy5 dye used in preparation of the solutions was one of the components of the Cy5 labeling kit described.

2.3 *Fiber-optic fluoroimmunoassay protocol*

The procedure of the fluoroimmunoassay is shown in Fig. 1(b). Prior to the assay, anti-*Der f*1 capture antibodies were immobilized on the distal end of the probe. The capture antibodies were diluted with carbonate-bicarbonate buffer to a concentration of 10 μg/ml. The optical probe was incubated overnight at 4 °C with the capture antibody solution. The capture antibody-coated probe was then rinsed with PBT and incubated for 1 h at room temperature (RT) in 1% BSA-PB (100 μl) to block the unoccupied parts on the surface of the probe to reduce the amount of nonspecific binding of proteins. After washing and rinsing the probe with PBT, the probe was dipped in 100 μl of *Der f1* solution (31.25–500 ng/ml) diluted with PBT for 2 h at RT. After rinsing off unbound *Der f1* with PBT, the probe was dipped in 100 μl of Cy5-labeled detection antibodies for 1 h at RT. Unbound detection antibodies were rinsed from the reaction cell with PBT, and the probe was dipped in 100 μl of PBT. Finally, the fluorescence signal from Cy5 at the end surface of the probe was recorded using a PC connected to the spectrometer or PMT. The exposure time of the PMT was fixed at 1.0 s in all experiments.

2.4 *ELISA protocol*

As a comparative experiment, we performed ELISA to quantity *Der f*1. The ELISA kit for *Der f*1 was purchased from INDOOR Biotechnologies, Inc., USA which included the same antibodies we used in our measurement. We performed the *Der f*1 ELISA in accordance with our previous reports.[19,20]

3. Results and Discussion

3.1 *Construction and evaluation of the fiber-optic fluoroimmunoassay system*

To detect fluorescence of Cy5 as distinct from the excitation light, we investigated the effects of optical filters in the system. We obtained spectra using the spectrometer when the silica-core probe was dipped in a Cy5 solution (120.6 μmol/l). Figure 2 shows the comparison of spectra obtained with/without optical filters. In the case of the system constructed without optical filters, the fluorescence emission spectrum overlapped with the excitation spectrum, and it was difficult to determine the Cy5 fluorescence from this

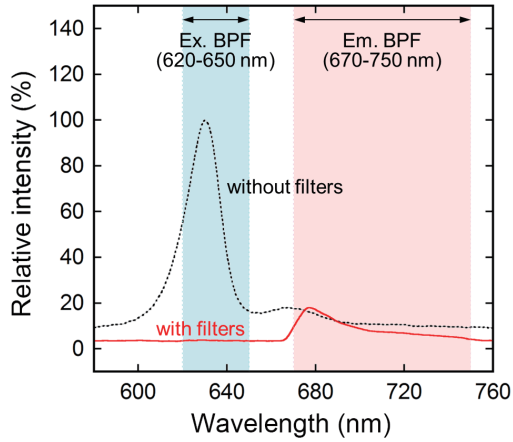

Fig. 2. (Color online) Optical spectra of Cy5 obtained from a spectrometer in the system with/without optical filters. The solid line shows the results using optical filters; the dashed line shows the results without these filters.

spectrum. On the other hand, in the case of the system constructed with optical filters, the effect of the excitation light was eliminated, and we could obtain a spectrum from Cy5 molecules which has a peak wavelength of 677 nm. The results show that the combination of the optical filters is effective in the measurement system to detect Cy5 fluorescence.

Next, we evaluated the effects of a fluorescence detector for our measurement system. We investigated the quantitative characteristics of the measurement system for *Der f*1 using both the spectrometer and the PMT. The values of the peak heights in the spectrum are plotted in red according to Cy5 concentration in Fig. 3. The circles in Fig. 3 show the mean values of each intensity of Cy5 concentration obtained using the PMT, and the rhombic symbols show the values obtained using the spectrometer. The calibration range by PMT was 10^0–10^5 nmol/l and the limit of quantitation (LOQ) was two orders of magnitude lower than that of the spectrometer. Therefore, we used the PMT as a fluorescence detector to setup the fiber-optic fluoroimmunoassay system in the following experiments.

3.2 *Fabrication and evaluation of plastic optical fiber probes*

As an optical fiber probe in our system, three types of optical fibers made from different materials were applied and tested. We prepared a silica-core probe, a PS-core probe, and a PMMA-core probe. In the experiment described in § 3.1, we used a silica-core probe to detect Cy5 fluorescence. However, it was difficult to immobilize antibodies on the untreated-silica probe by physical adsorption. Therefore, in terms of immobilization of molecules, we chose plastic-core optical fibers (PS and PMMA) as probes in the measurement system.

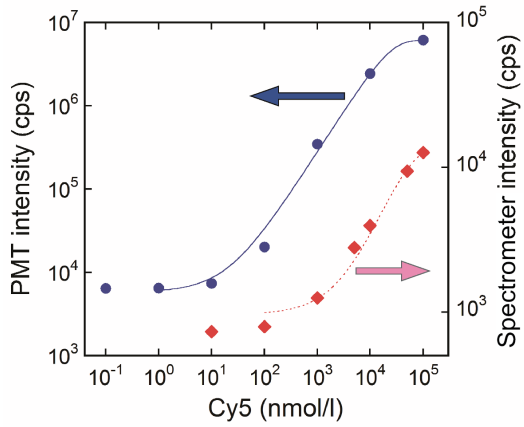

Fig. 3. (Color online) Comparison of calibration curves for Cy5 measured by a spectrometer and PMT. The PMT could detect fluorescence of Cy5 at lower concentration.

Optical fibers were pretreated by polishing their end surfaces to detect and propagate the light efficiently as a probe. Figure 4 shows photographic images of the end surface of a PS-core fiber at each stage of polishing. The end surface of the fiber became smooth as a polishing film was replaced with a finer one. We used an optical fiber as a probe after polishing with an alumina film (mineral size: 0.3 μm).

To investigate the detectability of Cy5 fluorescence by the optical probe, we prepared dilute Cy5 solutions and measured the fluorescence intensity of each concentration using PS or PMMA-core probes. The results are shown in Fig. 5. The PS-core optical probe could detect fluorescence at a lower concentration of Cy5 than the PMMA-core probe. In the case of the PMMA-core probe, Cy5 fluorescence from low concentrations could not be discriminated from optical background noise. Although the numerical apertures (NA) of the PS-core fiber and the PMMA-core fiber are 0.55 and 0.5 respectively, the light transmission rate of PMMA-core fiber is generally higher at the wavelength of Cy5 fluorescence than that of PS-core fiber.[24] This suggests that the PMMA-core fiber has a possibility to have higher background noise. In this study, we regarded the PS-core probe as suitable to detect Cy5 fluorescence using our system.

3.3 *Fiber-optic fluoroimmunoassay for Der f1*

The Cy5 detection system was applied to measure *Der f*1 based on the fluoroimmunoassay principle. First, we prepared the optical probe immobilized with capture antibodies described in § 2.3. Then the probe was dipped in *Der f*1 and Cy5-labeled antibody to form sandwich-type immune complexes on the probe surface. After immobilization of immune complexes, the probe was connected to the optical system, and fluorescence intensity was measured as the tip of the probe was immersed in PB. The fluorescent intensities obtained from each probe after immunoassay for *Der f*1 are

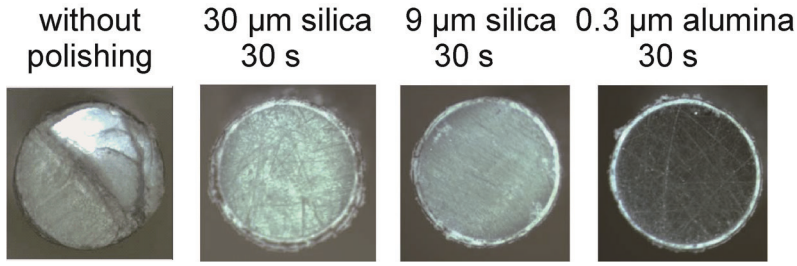

Fig. 4. (Color online) Microscopic images of the end surface of the PS-core fiber after each stage of polishing. The end surface became smooth as a polishing film was replaced with a finer one.

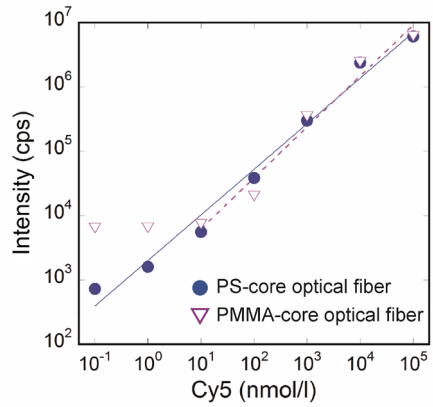

Fig. 5. (Color online) Calibration curves for Cy5 measured using the PS-core probe and PMMA-core probe.

shown in Fig. 6. The fluorescent intensity was higher as the concentration of *Der f*1 increased. The calibration curve was expressed as the following equation:

$$\text{Intensity (cps)} = 20.29 + 19.48\ [\textit{Der f}1\ \text{(ng/ml)}],\ (r = 0.989). \quad (1)$$

Concentrations of *Der f*1 solution were quantified by the fiber-optic fluoroimmunoassay system in the range of 31.25–500 ng/ml. Since LOQ of a commercial ELISA was generally 1 ng/ml, the LOQ we obtained was not low enough for the measurement of environmental *Der f*1 allergen. On the other hand, the upper limits of detection of the fluoroimmunoassay system and ELISA were 500 and 250 ng/ml, respectively. It is necessary to improve the sensitivity; however, the fluoroimmunoassay system can detect fluorescence as a function of concentration of *Der f*1 and has more advantages than existing methods of *Der f*1 detection, such as flexibility, simplicity, and user-friendliness in terms of using it in daily life. The optical setup can be compact

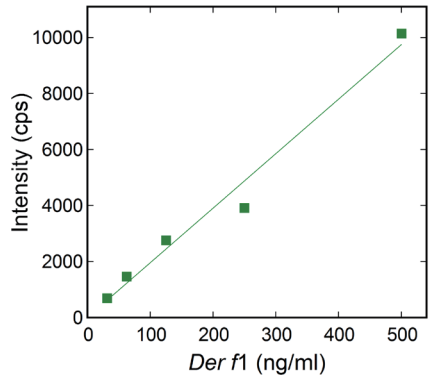

Fig. 6. (Color online) Calibration curve for *Der f*1 measured by the fiber-optic fluoroimmunoassay system. Fluoroimmunoassay for *Der f*1 was carried out using the PS-core optical fiber probe in the system.

in size because we avoided the use of large-scale optical components. Moreover, our laboratory group has already developed a flow cell to use in biochemical gas sensing (for example, refs. 25 and 26). The flow cell can be integrated with the probe in a reaction cell to improve the flexibility of the probe. Immunoassays may be performed several times in a row in the same cell of the probe by controlling the suction/discharge of reagents. Because of the simplicity of the optical system and the introduction of the flow cell, we suggest that automatization of the fluoroimmunoassay process is possible to improve user-friendliness. Generally, monitoring of an antigen level by immunoassay is difficult because an immune reaction is non-reversible. Therefore, *Der f*1 monitoring over time could be realized by repeated immunoassays as we improve our system in the future. This study suggests that the fiber-optic fluoroimmunoassay system can be basis of a new method which can monitor allergen levels in the domestic environment.

4. Conclusions

A fiber-optic fluoroimmunoassay system for the detection of HDM allergen was constructed and tested. We optimized optical components used in the system and achieved an excitation-emission system capable detecting the fluorescence of Cy5 with high sensitivity. To apply the system to measure HDM allergen, we used a polystyrene-core optical fiber as a probe and immobilized anti-*Der f*1 (HDM allergen) antibodies on the end surface of the probe to form immune-complexes with *Der f*1. The system measured *Der f*1 in the range of 31.25 to 500 ng/ml. The fluoroimmunoassay system could be useful as a basic technology for monitoring environmental allergen levels in the domestic environment to help in the prevention and treatment of allergies. It is expected that allergen monitoring devices can be realized by further study focusing on the improvement in sensitivity and on the introduction of a flow cell into the system.

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