DETECTION OF FILARIAL ANTIBODY USING AN FIBER OPTICS IMMUNOSENSOR (FOI)

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INTRODUCTION

Recent developments in the area of Fiber Optic Sensors have opened up a new area of analytical sensing in the field of clinical, environmental, food and process analysis. Fiber Optic Sensors offer several advantages over conventional sensing techniques such as (i) Optical signals do not have electrical interference; small size, high transmission efficiency, chemical stability and flexibility of optical fibers allows for miniaturization of sensor and remote sensing in hazardous environments. (ii) Unlike potentiometric sensors, they do not require reference signal. (iii) Multiwavelength monitoring allows one to obtain sequential information as a number of different analysis. (iv) Good biocompatibility and sterility of optical fibres makes them suitable for *in vivo* measurements.

Evanescent waves generated at the surface of its optical waveguide due to the Total Internal Reflection (TIR) of light. These waves are exponentially decaying field in the rarer medium surrounding the waveguides and continued to the distance less than the wavelength of the light used from the waveguide surface. Surface property of these waves make them ideal to study the chemical reactions in the real time, at the interface which results in absorbance of Fluorescence excitation.

A special class of optical fibers used in the field of biological diagnosis are known as Fiber Optic Biosensors (FOB). The use of FOB for the detection of Immuno chemicals such as Antibody, Antigen are known as Fiber Optic Immunosensors (FOI). FOI provide a powerful analytical tool since they combine high sensitivity and specificity provided by biological component with highly sensi-

tive optical detection via optical waveguide.

Early diagnosis of various Tropical Diseases such as Filariasis, Leishmaniasis, Malaria, Tuberculosis, Leprosy is a keystone in the monitoring and subsequent eradication of these diseases. Filariasis is one of the major public health problems all over the world particularly for India. There is an urgent need for a sensitive specific detection technique to diagnose this disease at early stages to prevent the problems in advance stages. Mass Screening of the disease in the endemic areas with a simple and cost effective method is an added feature for monitoring and control of this disease. We report here preliminary results with an assay for *Satara digitata* antibody using an FOI through which the result can be read in less than 15 minutes.

Design of Fiber as Transducer

Plastic clad fused silica fibers of 600 μm core diameter and 0.40 numerical aperture (Ceramoptic GmbH, Germany) were used as a transducer for the immobilization of *S. digitata* Antigen. As reported by Anderson et. al. (1) combination of tapered fibers have significantly higher sensitivity as compared with untapered fibers and therefore combination tapered fibers were prepared by etching optical part of fiber using hydrofluoric acid (HF). Fibers were cut into 15 cms length and both the ends of the fibers were polished with the help of polishing machine as well as emery papers. Suitable fiber polishing tool has been designed and used for Fiber polishing. Nylon jacket and silicon cladding from the distal 10 cm length of fiber were removed and the fused silica core was cleaned. Combination of tapering was possible by immersing the fiber in HF and running of two different speeds using a Microprocessor controlled stepper motor. After tapering the final shape of the fiber (transducer) was as shown in Fig. 1 (a)

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Fig. 1. Diagram of (a) the combination tapered fiber, and (b) the test fiber

Fig. 2. Schematic diagram of the Fiber optics Immunosensor-Instrumental Set up. See text for details

Tapered fibers were cleaned ultrasonically in benzene, isopropanol and distilled water and then dried. Immobilisation of *S. digitata* antigen on the surface of the fiber transducer was carried out by the method of Bhatia et al (2). The immobilization was also tried out by means of physical adsorption which also gives similar magnitude of the signal. Since the fiber is a disposable type after one test, physical adsorption shall be ideal to avoid use of costly chemicals for cross linking.

Fiber Optic Immunosensor-Instrumental Set Up

An evanescent wave fluorimeter was constructed using the fiber optic accessory supplied with the Aminco Bowman Series 2 Luminescence Spectrometer (SLM AMINCO, USA), as shown in Fig.2.

A 150 W Xe arc lamp was used as the excitation source. Excitation light of 488 and 16 nm bandwidth was selected using a monochromator (MC1) and was carried using a rectangular to circular fiber bundle (FB1) to the fiber optic module. After proper collimation the optical beam was further filtered using a low bandpass filter (Omega Optical, Vermont, USA). Excitation beam was then reflected by a dichroic beam splitter (90% reflection at 488 nm, Omega Optical) and focused by a microscope objective of numerical aperture NA= 0.30 through a mechanical chopper on to the fiber. Excitation beam was coupled to the test fiber by using a V-groove fiber coupler. A fraction of fluorescence signal at 525 nm generated on the tapered fiber surface was coupled back into the fiber. Coupled fluorescence was transmitted by

the dichroic mirror (90% transmission at 525 nm) and focussed on to the optical fiber bundle (FB2) after passing through a low bandpass filter (Omega Optical). The optical fiber bundle sends the fluorescence chromator (MC2). Output from the PMT was connected to a lock-in amplifier (Stanford Research Systems SR850) which was referenced to the chopper frequency. The signal was scanned at a rate of 1 Hz and the result was displayed in graphical form.

Sample holding Chamber

Glass capillary as shown in Fig 1b of 1 cm internal diameter and 10 cm length with a silicon bulb on the top was used to make the sample chamber. The chamber has been designed with a view to use the set up even by an unskilled person to handle the sample solutions. The tapered region of the fiber is sealed into the glass capillary with the jacketed region of the fiber protruding out of the silicon bulb. The distal end of the fiber kept just outside the capillary to prevent the excitation of bulk fluorescence. 150 μ l of sample was drawn into the capillary by suction using a silicon bulb. The test fiber coupled with the main fiber using a V-groove fiber coupler.

Test with FOI

In order to determine the optimum conditions (FOI) initial tests were carried out using immobilised human IgG and Anti-human IgG labelled with Fluorescent Isothiocyanate (FITC). The assay for the detection of filarial antibody (*S.*

digitata) is similar to sandwich type. Antigen immobilised fibers were incubated with different dilutions of the *S. digitata* antibody for 10 minutes. After this the antibody solution was removed from the capillary and the fibers were incubated with anti-human IgG*FITC. Then through the FOI the signal changes with respect of time was monitored.

Results

Human IgG with anti-human IgG*FITC :

Fig. 3 shows the output of the lock in amplifier for immunoassay with different concentration of FITC labelled anti-human IgG. It can be seen that the background fluorescent signal at the time of introduction of FITC labelled antihuman IgG in the sample chamber is much higher for the higher concentration of the antibody as compared to the lower concentration of anti-human IgG. This is due to the presence of higher concentration of the free FITC labelled anti-human IgG within the range of evanescent wave. To determine the contribution of non-specific adsorption of proteins from the bulk sample, fibers were incubated with 1 μ g/ml of FITC labelled anti-rabbit IgG and FITC labelled anti-human IgG. Signal obtained after 10 minutes of incubation was in the ratio of 1:13 showing the specificity of the sensor. Another parameter of importance from the point of view of sensitivity of this sensor is the system noise level. System noise level is due to the presence of characteristic noise from the light source, optics, detection and lock in amplifier system which in this case was 45 v.

Fig. 3 Signal output from the lock-in-amplifier form IgG+different concentration of IgG*FITC binding assay.

Evanescent wave FOI eliminates totally the repeated washing steps to separate the bound and unbound fluorescent tagged antibody of limited spatial reach of evanescent wave. Thus fluorescent signal generated as a result of binding of tagged antigen to the fiber immobilised antibody can be directly monitored in realtime hence allowing for the kinetic study of the binding reaction.

***S. digitata* Antigen-Antibody-anti human IgG*FITC**

Fig. 4 shows the change of signal output with time for the different dilutions of *S. digitata* antibody. Individual graphs for each dilution of antibody has been retraced on the same plot for signal comparison. Signal represents the change in fluorescence signal over the background. Rate of change just after the addition of the FITC labelled anti human IgG as well as the signal change decreases with the increase in the dilution. Time taken for the rate of change of signal to stabilize was about 5 minutes.

Summary

Optical waveguides based immunoassay has

been reported in the literature for the detection of pathogens like *C. botulinum* and F1 antigen of *Y. pestis* (3) and also for the antibodies to pathogens like the Rubella virus (4) in the serum or the whole blood. In this line we have demonstrated the FOI for the detection of *S. digitata* antibody. Experiments are in progress in our laboratory to standardise the sensor for detection of Bancroftian filariasis caused by *W. bancrofti*. Few modifications are also in the process so as to improve the signal amplification at evanescent region as well as to reduce the two step method into single step method. The FOI has an advantage over other conventional methods because no extensive washing steps are required and the whole procedure takes just 15 minutes to get the result. The FOI designed for this experiment can be made portable for use in the field level for epidemiological studies.

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