Fiber Optic based Biosensors

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ABSTRACT
Fiber optic Biosensors (FOBS) are basically an analytical device that is derived from an optical fiber. It has the ability to measure various biological species like proteins, toxins, DNA etc. by making use of an optical field. FOBS offer an effective alternative to other traditional immunological devices because of its qualities like accuracy, rapidness and cost effectiveness. One of the types of FOBS is a tapered optical fiber that utilizes special geometries so that its evanescent field is exposed to interact with the target. In order to increase its effectiveness it makes use of different transduction mechanisms like absorbance, fluorescence, refractive index changes and Surface Plasmon Resonance which increases the selectively and sensitivity. In this paper the basic principles of FOBS, its types and some of its applications are discussed.

Keywords
Biosensors, Evanescent field, Immunoassay, FRET, Microarray

1. INTRODUCTION
Biosensors are analytical devices that can convert the physical and chemical properties of the target into a measurable signal with the help of a transducer. There are different types of biosensors available like electrochemical, optical, piezoelectric, calorimetric etc. In the past decade issues regarding environmental pollutants, food safety concerns, diagnosis and monitoring of diseases has increased rapidly. Hence there is a requirement of different analytical techniques that are fast, accurate and cost effective which can produce results with greater frequency and accuracy. These biosensors should not just detect the presence of a target but also describe its chemical composition, bioavailability and toxicity. FOBS fulfill the above mentioned

2. FIBER OPTIC EVANESCENT SENSORS
A typical optical fiber consists of a core and a cladding surrounding the core. In order to achieve total internal reflection (TIR), the refractive index of core has to be slightly higher than that of cladding. Therefore core is doped with Germanium and the light propagates through TIR. Light that propagates through the core consist of two components: guided field in the core and the exponentially decaying evanescent field in the cladding. A fiber that has uniform diameter, this evanescent field decays to zero within the cladding. Therefore, this evanescent field cannot interact with the surroundings In order to make these fibers useful for sensing purposes, this evanescent field must interact with the surroundings. For example, cladding can be etched out so that the evanescent field can be exposed to the surroundings. The distance to which this evanescent field extends beyond core-cladding interface is described by penetration depth and is mathematically given as[2]

$$E(x) = E_0 exp\left(\frac{-x}{d_p}\right) \quad (1)$$

Where x is the distance from the fiber core, E_o is the magnitude of the field at the interface, and d_p is the penetration depth. The penetration depth is given by[2]

$$d_p = \frac{\lambda}{2\pi\sqrt{n_{co}^2 sin^2\theta - n_{cl}^2}} \quad (2)$$

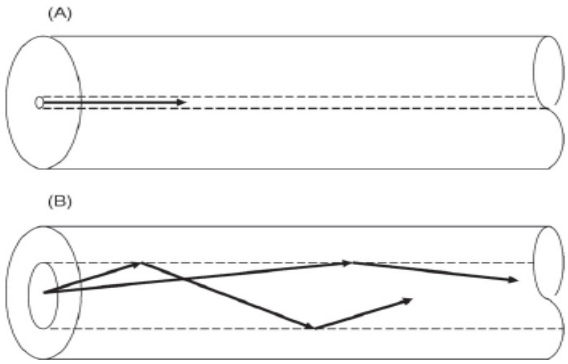

Fig 1: (A) step index sm fiber. (B) step index mm fiber[2]

Where λ is the wavelength of the light, θ is the angle of incidence of light, n_{co} and n_{cl} are refractive indices (RI) of core and cladding respectively. Fig 2 shows a fiber that is cut along the axis showing penetration depth. If the cladding is etched out then the output power is the measured in terms of changes in magnitude of evanescent field. But, the penetration depth of the evanescent field that normally occurs in optical fiber is very small for sensing. The characteristic dimensions of bacterial cells and proteins are on the order of 1µm and 10nm respectively. Depending on the size of the analyte the penetration depth has to be increased so that it can be used as an effective sensing tool. The properties of light are determined by its mode number N which has a one to one relationship between with incident angle.

$$V_{core} = \frac{2\pi}{\lambda}\rho(n_{core}^2 - n_{clad}^2)^{1/2} \quad (3)$$

Where ρ is the core radius, λ *i*s the wavelength of light, n_{core} is the refractive index of core and n_{clad} is the refractive index of cladding. The total number of modes in a multimode fiber N is directly proportional to V^2.

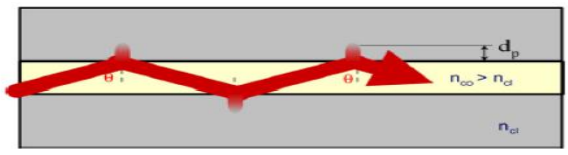

Fig 2 Evanescent field and penetration depth [2]

2.1 Tapering effects

When optical fiber is tapered, the evanescent field gets exposed to a much greater extent which in turn increases the penetration depth. Tapering can be performed in two ways: one by chemically etching out the cladding and tapering the fiber, or by keeping both core and cladding in place and tapering the entire fiber.

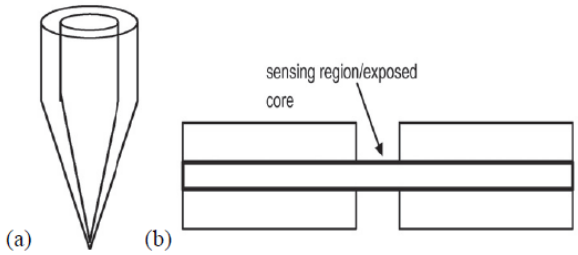

Fig 3: (a) Tapered fiber (b) uniform de-cladded fiber [2]

Tapered optical fibers obtained in both the ways were compared by measuring the evanescent absorption. The absorption characteristics in both the cases followed Lambert-Beer's law which is expressed as [2]

$$A = \alpha L \eta \quad (4)$$

Where α is absorption coefficient, L is the length of the fiber η is the fraction of evanescent domain. A is given as

$$\alpha = \varepsilon C \quad (5)$$

Where ε is molar absorptivity and C is concentration of species. Sensitivity of de-cladded uniform core fiber is given by [2]

$$S = \varepsilon L \left(\eta + C \frac{\partial \eta}{\partial C}\right) \quad (6)$$

2.2 Bending Effects

Bending in an optical fiber is performed basically to generate higher order modes. These higher order modes increase the magnitude of evanescent field and penetration depth [1, 2, 3]. A bent fiber is generally called as U probe. Absorption coefficient in case of U probe was found to be larger than in straight probe. This indicates that the evanescent field is much stronger in case of U probe. The reason why this absorption coefficient is low in straight probe is that the ray propagation angle is inversely proportional to core radius. In U probe, ray propagation angle decreases. The penetration depth increases with decrease in ray propagation angle. It was reported that there is a limit to the bending angle [4] beyond which the sensitivity decreases. This is because power transfer takes place between the two arms which results in evanescent field overlap that does not allow evanescent field pass through the bent sensing region.

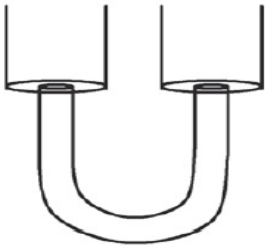

Fig.4 U probe [2]

2.3 Effects of Change in Launching Angle

In single mode fiber the launch angle of light is usually very small since it can support only one mode. Whereas in case of multimode fibers, light can be launched in a number of angles that correspond to different modes. These modes are supported simultaneously. The evanescent field was increased to 300% in certain designs of tapered fibers. [5]

3. DETECTION PRINCIPLES

Penetration depth is too low as compared to target dimensions. Hence it is imperative to use additional transduction mechanisms to amplify the signal. The mechanisms used in evanescent sensing are as follows:-

3.1 Output Power Changes due to Change in Refractive Index

This method was the earliest reported and most widely used. By measuring the changes in output power changes in the magnitude of evanescent field can be detected. The total amount of evanescent field that propagates through the fiber depends on the difference in refractive index of core and sampling. Geometric parameters like bending, radius, length and taper ratio influence the sensitivity in such cases.

For example, sensitivity can be increased by bending the fiber because the fraction of evanescent field increases.[6-8]. When it comes to radius, sensitivity can be increased by using smaller diameters as it increases the evanescent field. Decreasing waist diameter increases sensitivity[10] and longer fibers can be used to overcome small dp. Taper ratio is defined as the ratio of the waist diameter of a tapered fiber to the total diameter of uniform fiber. Sensitivity can also be varied by varying the taper ratio. For larger taper ratios the evanescent field becomes negligible whereas for smaller taper ratios the mode is very highly spread that it has a tendency to move towards free space wave [9]. Taper ratio of unity gave minimum dp, while dp increases upto 300% in certain designs of taper.

3.2 Evanescent Field Absorption

When the light is transmitted through the optical fiber evanescent field interacts with the target in the tapered region and the transmission decreases as the target analyte absorbs the wavelength used for transmission. Hence for absorbance measurements, the light source used must be at a wavelength which the analyte can absorb. This is also influenced by geometric factors like radius, length, taper ratio and bending. Reducing the core radius increases absorption by increasing η. The number of ray reflections are also affected by radius[1] For straight fiber the number of ray reflections increases with decreasing radius and hence increases absorption coefficient. Lower taper ratios cause higher absorbance. Bending of fiber increases dp as the angle of guided ray decreases in bent region and hence increases absorption [1].

3.3 Evanescent Wave Fluorescence

There are two methods of using fluorescence. One is sandwich assay where a primary antibody is immobilized in fiber and analyte is introduced so that it binds with the primary antibody. This is confirmed by using a fluorescent secondary antibody that fluoresces when excited by the incident light from the evanescent wave. The second method is the direct method where a fluorescent dye is attached to surface of taper. When the analyte is near the surface, fluorescence quenching is measured.

Factors that influence the fluorescence are length, angle of incidence, bending and surface area. Longer taper can be used to overcome low dp [11]. When the angle of incidence is increased then maximum percentage of light in evanescent field is obtained and hence more light is available for fluorescence excitation. Looping and bending of fiber increases coupling and sensitivity in fluorescence sensing. Tapered fibers have small surface area which leads to lower source collection efficiency. It was found that tapers having more than 10μm diameter have 5-10 times higher coupling efficiency [12].

3.4 Surface Plasmon Resonance

SPR sensor is basically a dielectric that is metal coated in which light propagates. Instead of having evanescent field interact with the analyte, optical energy is transferred to the surface of metal layer as packets of electrons known as plasmons on satisfying the resonance condition of the light beam. This resonance condition depends on angle of incidence, wavelength and dielectric functions. The factor on which resonance occurs depends on RI of analyte. From this RI of analyte can be obtained.

Gold or silver is usually used as coating for SPR sensors. Gold increases the shift in resonance with respect to changes in refractive index whereas in case of silver, signal to noise ratio(SNR) increases as it narrows the resonance curve. Gold coatings can increase sensitivity as it increases the separation between the resonance curves whereas silver coatings reduce sensitivity as it reduces the width of resonance curve [13].

Factors that affect the performance of SPR include launch angle, waist diameter, length and metal thickness. Thickness of the metal coating must be on the order of the wavelength used [14]. If the length of the sensing region is increased then the number of reflections increases causing a broader SPR curve with lower SNR [13]. Thicker waist diameter increases sensitivity because of sharper resonance peaks [14].

4. CLASSIFICATION OF FIBER OPTIC BIOSENSORS

Classification of FOBS is based on the type of biological recognition element. There are five types which are described below [15].

Enzyme fiber optic biosensors employ enzymes as the biological recognition element that is immobilized on the fiber. Enzymes catalyze the chemical reactions and its products or intermediate products are detected either directly or using an indicator.

Immunoassay fiber optic biosensors which make use of binding between an antibody and antigen which is monitored indirectly using a fluorescent optical label.

Nucleic acid fiber optic biosensors makes use of DNAs and hence it is also called as DNA sensors or genosensors. These types of biosensors basically make use of a single stranded DNA to form a double stranded DNA with complementary sequences. This is achieved by labeling one of the single stranded DNA with an optical indicator.

Whole cell fiber optic biosensors checks for reactions of analyte on an intact microorganism. The detection process is performed by employing an indicator or by analyzing the optical characteristics of the cells.

An enzyme optical fiber biosensor is presented where glucose is detected by making use of fluorescence mechanism [16]. This is basically an enzymatic reaction in which glucose oxidase (GOD) is used as catalyst.

$$\text{D- Glucose + O2} \xrightarrow{\text{GOD}} \text{D – Gluconolactone + H2 O2}$$

There are two ways in which glucose can be detected. One by measuring hydrogen peroxide or by measuring the concentration of oxygen consumed. The amount of glucose in the solution has a quantitative relation with the amount of glucose. Therefore, glucose can be detected by simply measuring the concentration of oxygen. This also makes use of a fluorescent dye layer to show any change in colour. When glucose and oxygen gets diffused in the enzyme, oxygen gets consumed and the residue oxygen diffuses into the fluorescent dye layer. This causes a change in fluorescence. The linear ranges of detection is described using Stern – Volmer equation

$$\frac{I_0}{I} = \frac{\tau_0}{\tau} = 1 + K[\mathrm{Q}] \qquad (7)$$

Where I_0 and I are intensity of fluorescence in the absence and presence of the oxygen quencher respectively, K is Stern-Volmer constant, τ_0 and τ are fluorescence lifetime in the absence and presence of oxygen quencher in sample respectively, [Q] is oxygen concentration. The light source from LED is a sine modulated signal and hence fluorescence signal in the output is also a sine signal with a phase delay. Light which reflects from fluorescence lifetime is changed to phase change with the help of lock –in amplifier.

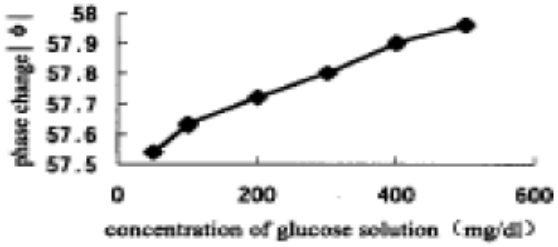

Fig 5. Detection of curve of glucose [16]

Fig. 5 shows a linear relationship between 50mg/dl~ 500mg/dl. It has a response time of 30 seconds. The normal amount of glucose found in blood is about 80mg/dl and in pathological cases it can go upto 3 to 4 times. Therefore, this biosensor can be used for diagnosis. The thickness of fluorescent dye layer can also be increased. But this increases the response time from 30 seconds to 7 minutes. This shows that the response is controlled by the amount of oxygen dispersed in the dye layer.

Another biosensor that belongs to the type immunoassay based FOBS for early detection of myocardial infarction [17]. The detection method used for this biosensor is called as fluorescence resonance energy transfer method (FRET).

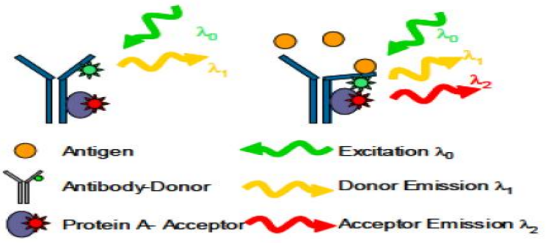

Fig 6. FRET immunosensor [17]

This particular mechanism employs two fluorophores. One is called as donor and the other one is called as acceptor. When both these fluorophores are kept in close proximity then donor absorbs the energy provided by the excitation source and nonradiatively transfers this energy to acceptor. This process causes the acceptor to emit fluorescent energy. FRET exhibits distance-dependent property which is utilized to detect conformational changes when antibodies combine to the respective antigens.

Both the fluorophores in this biosensor are conjugated to a specific antibody-Protein A or Protein G which ensures successful antibody orientation and proper separation between the fluorophores. When donor and acceptor fluorophores are within the Försters distance, distance at which the power transfer between donor and acceptor is 50%, the donor absorbs energy from excitation source λ0 and transfers the energy to acceptor which in turn emits fluorescence at λ2. If the fluorophores are not within the Försters distance, then donor will accept energy in λ0 and will emit fluorescence in λ1 with little emission from λ2. Since only proper agents will cause conformational changes, FRET technique has the potential to reduce the number of false results.

Here generic goat anti-human IgG (GaHuIgG) antibodies were utilized with Alexa Fluor 546 (AF546) and Alexa Fluor 594 (AF594) as donor and acceptor respectively. Human IgG (HuIgG) was the antigen to be detected. FRET induced fluorescence was measured by considering the ratio of maximum areas covered under donor and acceptor emission spectrums P1 and P2 respectively.

A decrease in the value of P1/P2 indicates power transfer from donor to acceptor. The nonradiative transfer of energy will increase the value of P2 which will mathematically decrease the value of the ratio which indicates that conformational change has occurred in the antibody. After the addition of the HuIgG antigen the ratio P1/P2 is reduced when the immobilized fiber is exposed to 70.0µg/ml concentration. The percentage difference of P1/P2 before and after addition of antigen was found to be in the range of 3.3% - 18.7%.

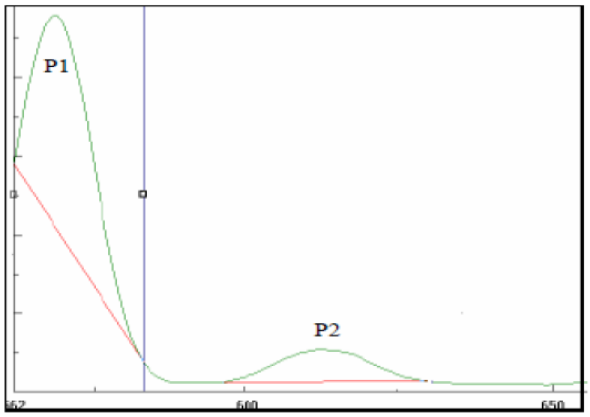

Fig 7. Donor (P1) and acceptor (P2) emission spectrums [17].

A living bacterial cell array was also used to make an effective fiber optic based biosensor for the detection of genotoxin [18]. A microwell array was formed at the end of an imaging fiber bundle. The bacterial cell array was fabricated by inserting live bacteria into this microwell. Each fiber in the imaging fiber bundle has its own light pathway. This allows for simultaneous monitoring of thousands of individual cell responses with spatial and temporal resolution as well. E.coli cells having a recA:gfp fusion were used as sensing elements. GFPmut2 protein was used as the reporter protein to provide maximal sensitivity. Different concentrations of following genotoxins were added in different microwells: mitomycin C (MMC), N-methyl-N-nitro-N-nitrosoguanidine (MNNG), hydrogen peroxide, nalidixic acid (NA), formaldehyde (FA). Imaging fiber from the one end transmits the fluorescent signal emitted from the individual cells which and captured by the camera present at the other end. Fluorescence intensity was recorded every 5 min over a 90 min period.

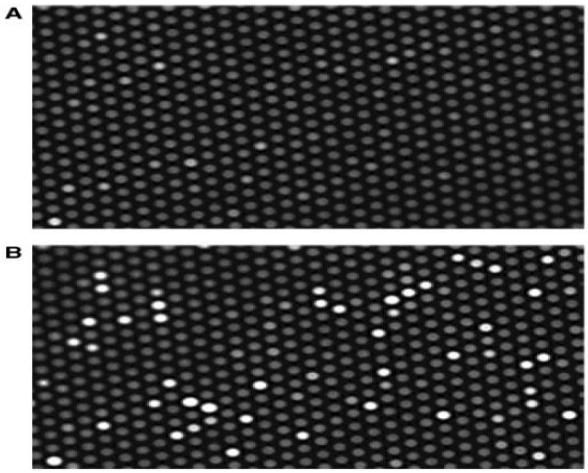

Fig 8. Fluorescent images of single cell array biosensor [18]

Figure 8 shows the fluorescent images taken at t = 0min (A) and at t = 90 min (B) after the cells were incubated with medium containing 5µg/ml MMC. The bright spots in the figure indicate increase in fluorescence. Figure 9 shows the response time of this biosensor after it was exposed to three different concentrations (0, 1, 5µg/ml). These graphs indicate the cell responses to MMC are both dose and time dependent. The GFP expression increased with incubation time after an initial lag phase. This lag phase is inversely proportional to the concentration of genotoxin. When the concentration of MMC was increased the time required to obtain significant response became shorter.

For 5µg/ml concentration of MMC took 30 min and for 1µg/ml of MMC took 45min. this biosensor showed sensitivity of 1ng/mL after 90 min response.

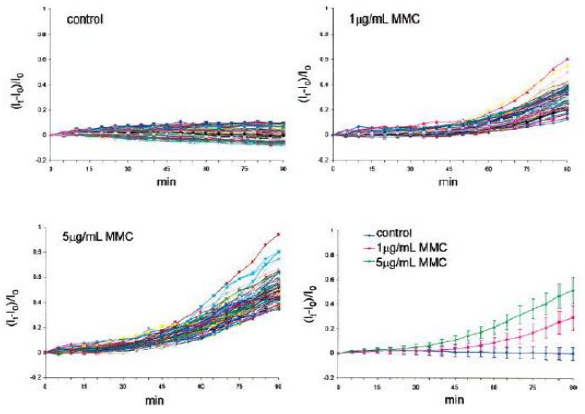

Fig 9. Kinetics of GFP expression in 0, 1, 5µg/ml concentration and the average intensity of all the three concentrations [18].

5. CONCLUSION

The design of FOBS has evolved from simple de-cladded fibers to tapered geometries along with surface modifications in the last 20 years. FOBS has a wide range of applications ranging from detection of pathogens, medical diagnosis based on protein, glucose, cell concentration and real time DNA detection. It has been observed that the use of fluorescence based FOBS are more widely used as compared to intensity or absorbance based biosensors for the detection of low levels of biomolecules. Intensity and absorbance based biosensors are not sensitive enough because of the small size of biomolecules. SPR FOBS have much better sensitivity as compared to the fluorescence based biosensors. Both these methods have reached a plateau in terms of detection limit. Combination of SPR and fluorescence can enhance the SPR signal. When the surface conditions and stability of recognition molecule is improved, then it increases the sensitivity and robustness of FOBS. This proves advantageous for intensity based FOBS because it is most sensitive when the molecules are bound on the surface. Considering its promising advantages, FOBS will remain a popular choice among researchers and practitioners for detection of biological agents.

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