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Highly specific detection of interleukin-6 (IL-6) protein using combination tapered fiber-optic biosensor dip-probe

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ABSTRACT

We are reporting highly specific and sensitive detection of human interleukin-6 (IL-6) protein using combination tapered fiber-optic biosensor (CTFOB) dip-probe. With these probes we could successfully detect IL-6, down to a concentration of 5 pM (0.12 ng/ml) in the presence of much higher concentration of a non specific protein. Sandwich immunoassay was used to generate specific fluorescence signal. A novel strategy is developed to eliminate the false signal from non specific binding. In this new strategy it is not required to pre-treat probe surface with any kind of blocking buffer. The specificity of the sensor was established by incubating negative control probes in high concentration (1 nM) of another cytokine IL-8.

1. Introduction

In the medical field, there is a great need to investigate expression of biological markers to better understand their roles in disease diagnosis or prognosis. Biosensors, which are capable of detecting and quantifying these biomarkers, would foster better understanding of the role these proteins play in disease progression. An ideal biosensor must be adoptable to high-throughput detection while also being robust to handle, portable for point-of-care usage, cost effective and easy to use, and the sensor response time should be rapid and must use limited amounts of samples and reagents. The biomarkers that we wish to detect are in biological fluids that contain large amounts of proteins of varied nature. Therefore, the sensor should be immune to the presence of interfering agents in the biological fluid. However, no such ideal methods are currently available to the clinical community.

Evanescent wave induced fluorescence based fiber-optic biosensors have the potential to meet most of these requirements (Battaglia et al., 2005; Gentleman et al., 2004; Kapoor et al., 2004; Leung et al., 2007b; Nardone and Kapoor, 2008; Wolfbeis, 2006). Furthermore, fiber-optic sensors offer complete electrical isolation, thus are highly safe for *in vivo* use. Among fiber-optic biosensors, two highly sensitive techniques are fluorescence technique and labeled free technique. In label free techniques, signals from non specific binding (NSB) of other interfering proteins can drastically reduce the selectivity of the sensor (Battaglia et al., 2005; Gentleman et al., 2004; Leung et al., 2007a, 2008; Rijal et al., 2005). This problem further aggravates when these sensors are used in fluids containing large amounts of interfering proteins (Battaglia et al., 2005). In fluorescence based techniques, signal can be made totally blind to the presence of any auto-fluorescence from NSB of other interfering proteins.

The most popular fiber probes currently in use are tapered fiber probes (Golden et al., 1994; Kapoor et al., 2004; Ko and Grant, 2006; Leung et al., 2007a, 2008; Preejith et al., 2003; Rijal et al., 2005). Most often, small diameter fibers (<100 μm) are used in the development of fluorescence based sensors (Golden et al., 1994; Leung et al., 2007a, 2008; Preejith et al., 2003). Further tapering of such a small diameter fiber makes these probes very fragile and inhibits its use outside the lab environment. Detailed theoretical studies have shown that among the tapered fibers, combination tapered fibers have higher sensitivity than continuous tapered fibers (Nath and Anand, 1998; Sun and Kapoor, 2008).

Cytokines are hormone like polypeptides that are secreted in the course of immunologic and inflammatory responses. They function as intercellular signals and are produced by a variety of different cell types to regulate both local and systemic inflammatory responses. Cytokines are also important immunoregulators in the processes of wound healing and immunity.

This article demonstrates highly sensitive and highly specific detection of cytokine interleukin-6 (IL-6) using a combination tapered fiber-optic biosensor (CTFOB) dip-probe. A fluorescence

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based technique, by adopting the sandwich assay to CTFOB probe, was used (Wyatt et al., 1992). An important issue with such probe is the presence of false signal due to NSB of labeled detection antibody molecules on the probe surface. A novel strategy was developed to eliminate this false signal. IL-6 was detected in a media containing non specific protein levels of 1 mg/ml. This was done to simulate a serum solution conditions, as human blood samples contain many kind of different proteins. "Capture" antibodies of antigen (IL-6) were immobilized on the surface of the CTFOB probe, and fluorescence dye labeled "detection" antibodies of antigen (IL-6) were added to the bulk solution. In the absence of the antigen, the "detection" antibodies remain in solution and little fluorescence is observed. However upon addition of antigen, a "molecular sandwich" is formed on the probe surface within evanescent wave excitation volume. Evanescent wave excites and produces the characteristic fluorescence of the dye molecules attached to the detection antibodies. Intensity of the fluorescence signal from these labeled-antibodies is proportional to the number of bound antigens on the probe surface.

2. Materials and methods

All reagents and chemicals were either of analytical grade or chemically pure. β-Mercaptoethylamine HCl (MEA), hydrofluoric acid (50%), egg albumin (EA) powder, affinity pure goat anti-bovine serum albumin (BSA), sodium bicarbonate, NaOH, acetone and phosphate buffered saline (PBS) were obtained from Fisher Scientific (Pittsburgh, PA, USA). Recombinant human IL-6, recombinant human IL-8, purified anti-human IL-6 capture (Clone MQ2-13A5) antibodies and anti-human IL-6 detection (Clone MQ2-39C3) antibodies were obtained from Biolegend (San Diego, CA, USA). EDTA, immobilization reagent Sulfo-SMCC (Sulfosuccinimidyl 4-[*N*-maleimidomethyl]cyclohexane-1-carboxylate), and 3-aminopropyltriethoxysilane (APTS) were obtained from Pierce (Rockford, IL, USA). Amine reactive Alexa Fluor 488 fluorophore was obtained from Molecular Probes (Eugene, OR, USA) and CS-800 spin columns were from Princeton Separations (Adelphia, NJ, USA). Tween-20 was obtained from Sigma–Aldrich (St. Louis, MO, USA). Optical fiber was obtained from Polymicro Technologies (Phoenix, AZ, USA).

2.1. Antibody–dye conjugation

A 0.1 M sodium bicarbonate buffer solution (pH 8.3) was prepared in PBS. Anti-human IL-6 detection antibodies (0.5 mg/ml) were mixed with optimum concentration (with dye to protein molar ratio of 30) of amine reactive Alexa Flour 488 in sodium bicarbonate buffer solution. The reaction mixture was incubated for 2 h at room temperature with constant shaking. The labeled-antibody conjugates were separated from residual dye using a gel filtration spin column (CS-800).

Finally labeled "detection" anti-IL-6 antibody solution (1 μg/ml) was prepared with dilution buffer containing PBS and 1 mg/ml of EA.

2.2. CTFOB dip-probe preparation

Each probe was an 8 cm long multimode optical silica/silica fiber (Polymicro Technologies, Phoenix, AZ, USA) with a 600 μm core diameter. Approximately 1.5 cm of protective polyimide buffer surrounding the fiber was removed from one end by burning it off with a Bunsen burner. The fiber was then decontaminated by sonicating it in a soap solution. This was followed by sonicating the fiber in a solution of de-ionized water to get rid of any carbon soot on the surface of the fiber. The cladding of the probe part was removed by immersing the 1.5 cm uncoated part into 10% hydrofluoric acid solution. Some acid capillarily ascends into the space between fiber probe and polyimide buffer. Capillary action tapered the section between the etched probe and cladded fiber. Combination tapered probes of 300 μm core diameters were obtained by adjusting the time duration of immersion. After being taken out of the hydrofluoric acid, the probes were first sonicated for 5 min each in de-ionized water, then for 10 min in 1 M NaOH solution, then again for 5 min in de-ionized water, and then in acetone for 2 min. The taper angle for each probe was measured using a microscope. The average tapered angle for all the probes was found to be 0.058 ± 0.002 rad. Finally, appropriate antibodies were immobilized on the probe surface.

2.3. Antibody immobilization

First, the cleaned probes were kept for 1 min in 2% APTS solution in dry acetone. APTS was used to derivatize the fiber surface with primary amines ($-NH_2$). These amines subsequently reacted with heterobifunctional cross-linker Sulfo-SMCC, resulting in a maleimide-activated probe surface able to react with sulfhydryl groups on antibodies (Hermanson, 1996). First, a 4.5 mM cross-linker Sulfo-SMCC solution was prepared in PBS–EDTA (10 mM EDTA). The probes were placed into the cross-linker solution for 60 min and then cleaned using PBS–EDTA solution. To expose the sulfhydryl group of the anti-human IL-6, the capture antibodies were reduced by mixing 0.1 mg/ml of antibody solution in PBS–EDTA with 50 mM concentration of MEA. The mixture was incubated for 90 min at 37 °C. After incubation, the reduced antibody molecules were adequately desalted from excess MEA using a gel filtration spin column (CS-800). Reduced capture antibody extract was diluted in PBS–EDTA to a concentration of 10 μg/ml. Maleimide-activated probe were incubated in reduced antibody solution for 4 h at room temperature. Once removed from capture antibody solution, the probes were washed with PBS–EDTA.

2.4. Design of portable signal detection setup

A schematic of our portable signal detection setup is shown in Fig. 1. A laser diode (Nichia, Japan) of wavelength 476 nm was used as an excitation source. Output from the laser diode was collimated by mounting it in a collimation tube (Thorlabs Inc., NJ, USA). To block any red tail emission from the diode laser, a dichroic band pass filter (Edmund Optics, NJ, USA) with peak transmission at 476 nm and

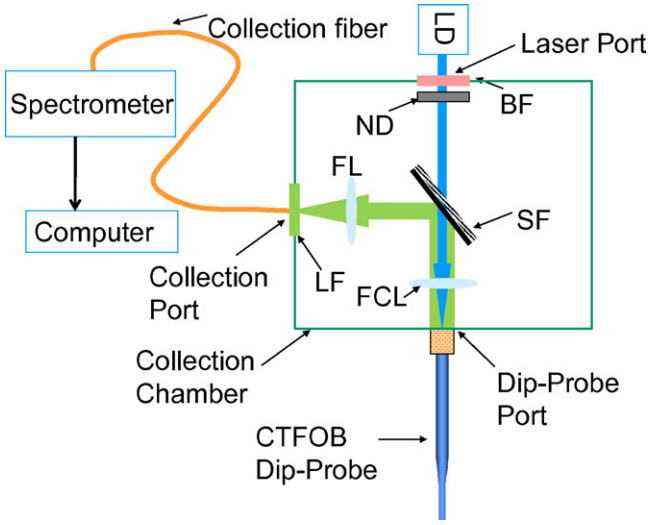

Fig. 1. A block diagram of the experimental setup: LD, laser diode; BF, band pass filter; ND, neutral density; SF, short pass filter; FL, focusing lens; LF, long pass filter; FCL, focusing/collection lens.

band width of 10 nm was placed in front of the diode laser. The laser tube was mounted at one port of the collection chamber and the CTFOB dip-probe was mounted on a diagonally opposite port with the help of a SMA adoptable bare fiber terminator (Thorlabs Inc., NJ, USA). This arrangement helped in convenient changing of the dip-probes for different experiments. One end of the fluorescence collection fiber is mounted on a collection chamber port at right angle to the laser port and dip-probe port, other end is mounted on to the collection port of a miniature charged coupled device (CCD) based fiber-optic spectrometer (model HR2000, Ocean Optics Inc., FL, USA). A short focal length focusing/collection lens (FCL) was used to focus the laser beam onto a 600 μm core dip-probe. The evanescent wave induced fluorescence from the probe surface couples back into the probe fiber and the same FCL lens now acts as fluorescence collection and collimation lens. A dichroic short pass filter (Maier Photonics Inc., VT, USA) was placed in the laser beam path, making 45° angle with the laser beam. The peak emission wavelength for Alexa 488 dye is 530 nm. Therefore the short pass filter with high reflectivity (95%) at wavelengths longer than 488 nm and high transmission (90%) for the excitation laser wavelength at 476 nm was chosen. The short pass filter allowed the laser light to pass while reflecting the collected fluorescence towards the collection port. A focusing lens (FL) focused the fluorescence into the collection fiber. A 488 nm laser line cut off long pass filter (Edmund Optics, NJ, USA) was placed in front of the collection fiber. This arrangement further improved the signal to noise ratio by blocking unwanted scattered light from the diode laser. The laser line cut off filter has about 99% transmission for all wavelengths longer than 500 nm, but extremely small transmission (0.0001%) for wavelengths shorter than 500 nm. The spectrometer was interfaced to a notebook computer (DELL). All the spectra were collected with the help of this computer.

2.5. Signal generation and recording

Sandwich immunoassay was used to generate fluorescence signal proportional to sample concentration. The CCD based spectrometer was used to record signal spectral profiles. Probes with immobilized "capture" antibodies were incubated in sample solution for an hour at room temperature. After removal from the sample solution, probes were washed for 2 min in a washing buffer containing PBS and 0.02% Tween-20. After drying the probes, background signal spectral profile was recorded.

Next the probes were incubated for 2 h at room temperature in labeled "detection" anti-IL-6 antibody solution (1 μg/ml). Probes were subsequently washed for 2 min then dried before recording signal spectral profile. Probes were washed for another 2 min (and dried), then signal was re-recorded. If a probe showed no signal change, no further washing was done. Otherwise, 2 min washing and subsequent signal recording continued until no change in signal was observed. It typically did not require more than two 2 min washings.

2.6. Signal extraction by least square fitting method

The signal spectrum profile was a sum of two spectral profiles: one auto-fluorescence from any kind of protein bindings (background) to the probe surface and two the characteristic spectral profile of labeled detection antibodies. If we represent the background spectral profile due to auto-fluorescence as $f_B(\lambda)$ and the spectral profile of characteristic fluorescence of labeled-antibodies as $f_{Fl}(\lambda)$, the recorded signal spectral profile $f_{Signal}(\lambda)$ will be a linear combination of these two profiles:

$$f_{signal}(\lambda) = af_B(\lambda) + bf_{Fl}(\lambda), \quad (1)$$

where λ represents wavelength, a is the coefficient of background signal and b is the coefficient of characteristic fluorescence signal.

First, a standard fluorescence spectral profile $f_{Fl}(\lambda)$ of Alexa 488 was recorded by immobilizing only the labeled-antibodies on the probe surface. The background spectral profile $f_B(\lambda)$ for each probe was recorded before incubating the probe in labeled detection antibodies. After incubating in labeled detection antibodies, the signal spectral profile $f_{Signal}(\lambda)$ for each probe was recorded.

Least square fitting to obtain coefficients a and b, was achieved using "solver method" of Microsoft excel. Sum shown in Eq. (2) was obtained from the data of the three recorded profiles, signal $f_{Signal}(\lambda)$, background $f_B(\lambda)$ and standard profile $f_{Fl}(\lambda)$. Initially guessed values of a and b were used.

$$\sum (f_{Signal}(\lambda) - [af_B(\lambda) + bf_{Fl}(\lambda)])^2 \quad (2)$$

By minimizing the sum shown in Eq. (2) using "solver method", actual values of coefficient a and b were obtained. CCD integration time for recording of background spectral profile $f_B(\lambda)$ of each probe was taken to be 1 s, while integration time for recording of signal spectral profile $f_{Signal}(\lambda)$ varied from probe to probe to keep the signal in linear range of the CCD. The ratio of coefficients b/a, for each probe was taken as the normalized fluorescence signal value of that probe.

3. Results and discussion

3.1. Degree of labeling

Degree of labeling (DOL) for the labeled "detection" antibodies was calculated using the following formula:

$$\frac{[\text{dye}]}{[\text{antibody}]} = \frac{210,000[A_{495}]}{71,000[(A_{280} - (0.11A_{495})]} \quad (3)$$

A_{495} and A_{280} represent the absorbance at wavelengths of 495 nm (λ_{max} for Alexa 488) and 280 nm, respectively. The molar excitation coefficients of typical IgG antibody and Alexa 488 are 210,000 cm^{-1} M^{-1} and 71,000 cm^{-1} M^{-1}, respectively. The correction factor to account for absorption of the dye at 280 nm is 0.11. Absorbance at 495 nm and 280 nm was measured in labeled-antibodies solution at concentration of 0.1 mg/ml in PBS using a spectrophotometer (Model UV-3101PC, Shimadzu, Japan). Degree of labeling (dye protein ratio) of our labeled-antibody was calculated to be about 14.

3.2. Immobilization strategy for evanescent based probes

During antibody immobilization, the orientation of attached antibodies needs to be controlled to avoid the associated problem of mixed avidity. This is because an antibody binding site oriented towards the probe surface may cause it not to be available to bind its intended target. To avoid the mixed avidity, the immunoglobulin (IgG antibody) should be attached to the probe surface from its fab portion.

There can be two approaches to achieve this goal: First and most efficient approach to fix the IgG on probe surface from its fab portion is the use of a cross-linker and A-protein. Second approach is to introduce sulfhydryl residues into hinge region of IgG structure for conjugation with maleimide-activated probe surface (Hermanson, 1996).

Fluorescence signal in CTFOB dip-probe is produced by evanescent field excitation. In the CTFOB dip-probes, evanescent field decays exponentially with distance from the probe surface with ~200 nm penetration depth (distance at which field decays to 1/e or 37% of its value at the probe surface). Therefore, efficient signal gen-

eration requires smallest possible distance between dye molecules and the probe surface.

In first approach the addition of an extra A-protein molecule between the cross-linker and IgG, increases the dye molecules distance from probe surface and makes the fluorescence generation inefficient. Therefore we decided upon the second approach of IgG immobilization.

3.3. Non specific signal

In fluorescence based techniques, there are two sources of unwanted fluorescence signals. One source is the "auto-fluorescence" from non specific bindings of any kind of proteins to the probe surface. Other source is "false characteristic fluorescence" contribution from NSB of labeled "detection" antibodies or residual dye molecules to the probe surface.

3.3.1. Removal of auto-fluorescence signal

To avoid NSB of any kind of protein, traditionally the probe surface is treated with different types of blocking buffers. As we have discussed, background spectral profile due to auto-fluorescence can be discriminated against the characteristic fluorescence spectral profile. By using least square fitting method as described above, the actual characteristic fluorescence contribution can be extracted by subtracting the background spectral profile from the total fluorescence signal profile. Therefore there is no need to treat the probe surface with any kind of blocking buffer to avoid NSB of these proteins.

3.3.2. Reduction of false-fluorescence signal

However, there is no way to discriminate between the false-fluorescence signal and the actual fluorescence signal. Both are produced by same dye molecules. Such a false signal can drastically reduce the detection sensitivity. A novel strategy is developed to eliminate such false signal. A dilution buffer containing PBS and 1 mg/ml egg albumin was prepared. The labeled "detection" anti-IL-6 antibodies were then diluted with this buffer to the required concentration of 1 μg/ml.

The hypothesis was, when the dip-probes are incubated into this solution, there will be a competition for nonspecific adsorption between EA molecules, labeled anti-IL-6 antibody molecules and residual dye molecules; a relatively high concentration of EA molecules (more than 1000,000:1) will inhibit the nonspecific adsorption of labeled "detection" antibodies on the probe surface.

To test our hypothesis an experiment was planned with two probes immobilized with anti-IL-6 capture antibodies. Two separate dilutions (1 μg/ml each) of labeled detection anti-IL-6 antibodies were prepared; one dilution was prepared in PBS containing no EA protein while other dilution was prepared in PBS containing 1 mg/ml of EA protein. Without incubating in any IL-6 sample solution, the probes were directly incubated in respective labeled detection anti-IL-6 antibodies solutions for 2 h at room temperature. After washing with washing buffer (PBS and 0.02% Tween-20), the signal spectra were recorded and extracted signals of both the probes are shown in Fig. 2. In the absence of any sample antigen (IL-6) both the probes were not suppose to show any signal. If at all we see any signal from these probes that should be the false signal. It can be seen in Fig. 2 that the probe incubated in labeled detection antibodies solution without EA protein gives a strong false signal, while the one incubated in solution containing EA shows no false signal. This demonstrates that our strategy can successfully be used to reduce the false signal due to NSB of labeled detection antibodies or residual dye molecules.

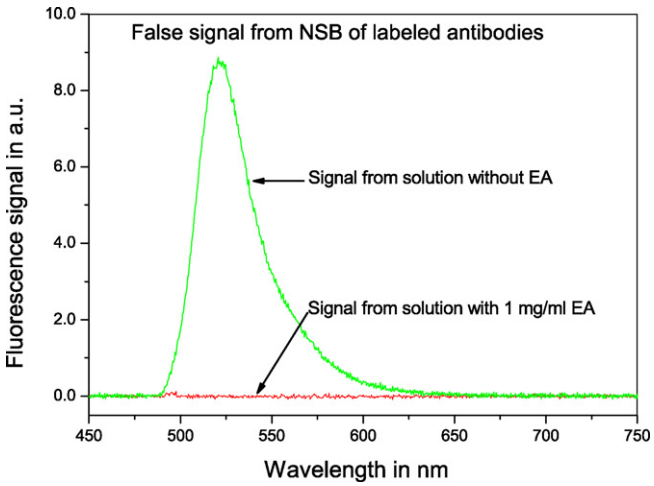

Fig. 2. Reduction of false signal, due to non specific binding of labeled detection antibodies, using high concentration (1 mg/ml) of EA in labeled anti-IL-6 antibody solution.

3.4. Detection of interleukin-6 in the presence of interfering agent

Samples of various IL-6 concentrations (2 pM, 5 pM, 20 pM, 50 pM, 100 pM, 200 pM, 500 pM, 1 nM and 5 nM) were prepared. To simulate a serum solution condition, we used a dilution buffer containing PBS and 1 mg/ml of EA. Capture anti-IL-6 antibodies were immobilized on the surface of all dip-probes. Four probes were used for measurements of each concentration. Probes were incubated in respective concentrations of sample (IL-6) solution for 1 h at room temperature. After removal from the sample solution, probes were washed for 2 min in a washing buffer containing PBS and 0.02% Tween-20. After drying the probes, background signal was recorded.

Next the probes were incubated for 2 h at room temperature in labeled "detection" anti-IL-6 antibody solution (1 μg/ml). After appropriate washing and drying procedure, fluorescence spectral profile of each probe was recorded.

Least square fitting method was used to extract the true IL-6 signal from recorded profiles for all the probes with smallest value of $R^2 = 0.994$. Signal from probe incubated in 2 pM sample solution were negligibly small or un-detectable while the signals were easily detectable from probes incubated in 5 pM and higher concentration sample solutions.

Average signal value of each concentration was used to draw dose–response curve shown in Fig. 3. The signal saturates at concentrations higher than 500 pM. This saturation is due to limited number of capture antibodies available on the probe surface. An exponential growth function was fitted to dose response curve with $R^2 = 0.993$. The curve has linear response from 5 pM to about 500 pM. For higher concentration detection, saturation limits can be increased by decreasing the incubation time in sample solution.

3.5. Specificity and sensitivity check with control probes

Sensitivity and specificity of the developed CTFOB dip-probes for IL-6 detection can be checked if we compare above results to the signals from a control probe. Two control probes were prepared by immobilizing anti-IL-6 antibodies. The probes were incubated for 1 h at room temperature in 1 nM concentration of another cytokine IL-8. After washing, the probes were then incubated for 2 h at room temperature in labeled "detection" anti-IL-6 antibody solution. This solution had a concentration of 1 μg/ml in dilution buffer containing 1 mg/ml EA. Subsequently, probes were washed for 2 min and signal was recorded after drying the probes. As expected both

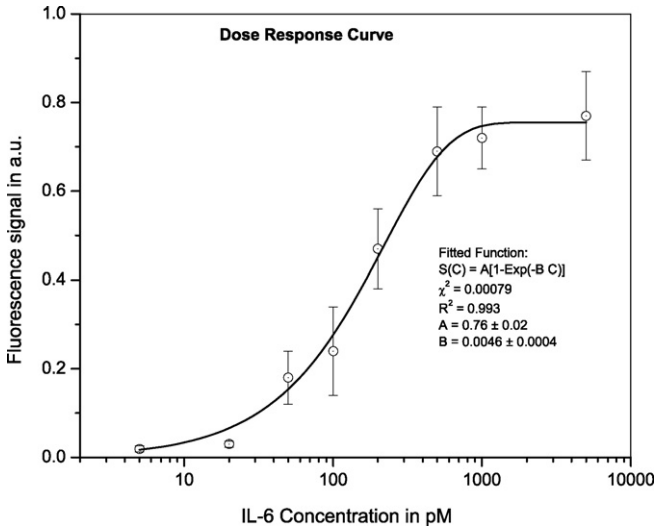

Fig. 3. Dose–response curve plotted from average fluorescence signal of various concentrations. An exponential growth curve function $S(C) = A[1 - \exp(-B \times C)]$ was fitted to the experimental curve with $R^2 = 0.993$.

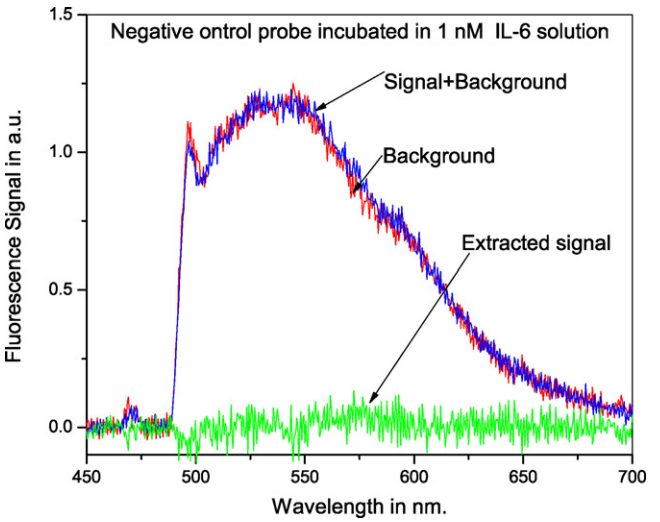

Fig. 5. Recorded spectral profile of the fluorescence collected from one of the negative control probe incubated in 1 nM concentration of cytokine IL-6. Instead of capture anti-IL-6 antibodies, goat anti-BSA antibodies were immobilized on the probe surface. Signal spectral profile was separated from the background spectral profile using least square fitting method with $R^2 = 0.995$.

probes do not show any significant signal. Extracted signal from a control probe is shown in Fig. 4. Absence of any signal even in the presence of such a high concentration of another cytokine (IL-8) demonstrates high specificity of these probes for IL-6 detection.

Computed average signal of control probes in arbitrary units (a.u.) was 0.003 ± 0.002. If we compare control probe results with those obtained from the IL-6 detection probes, we found that the average signal from probes kept in 5 pM IL-6 solution was 0.019 ± 0.007. While average signal obtained from probes kept in 2 pM IL-6 solution was 0.002 ± 0.002. This is comparable to that from the control probes. The margins of error for all the average signals are computed for 95% confidence level. Common performance metrics of the developed CTFOB probes were computed from a confusion matrix (Fawcett, 2006). The matrix was generated by using signal values obtained from the (negative) control probes and the 5 pM sample signal (positive) probes. Beside IL-8 control probes, data from control probes used in Section 3.6 was also used. It was found that at a threshold signal level of 0.01, the sensitivity was 100% with 83% specificity. It can be seen that the average signal from 5 pM probes is about 2 times more than the used threshold level and is about 6 times more than the average signal of control probes. These results indicate that developed CTFOB dip-probes can detect cytokine IL-6 with high specificity at concentration levels as low as 5 pM.

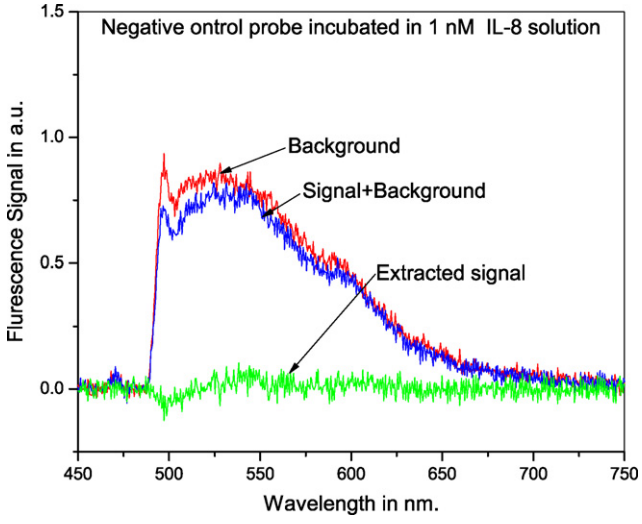

Fig. 4. Recorded spectral profile of the fluorescence collected from one of the negative control probe incubated in 1 nM concentration of cytokine IL-8 solution. Signal spectral profile was separated from the background spectral profile using least square fitting method with $R^2 = 0.993$.

3.6. Efficacy of immobilization

In adopted immobilization method, MEA reduction is not consistently efficient in cleaving certain antibodies. It may impact stability of capture antibodies and ultimately antigen–antibody interaction (Glockshuber et al., 1992). Efficacy of immobilized "capture" antibodies was verified by comparing our IL-6 detection results with another control probe results. Two control probes were prepared without immobilization of "capture" anti-IL-6 antibodies. To make these control probes chemically comparable to IL-6 detection probes, "capture" goat anti-BSA were immobilized on the probe surface. The probes were incubated for 2 h at room temperature in high concentration (1 nM) of IL-6 solution. After washing, the probes were incubated for 2 h in labeled "detection" anti-IL-6 antibody solution. Subsequently, probes were washed and signal was recorded. No signal was observed on any of the probes. Extracted signal profile along with recorded profiles from one of the probe is shown in Fig. 5. If we compare these control probe results with those obtained from the IL-6 detection probes, we can conclude that the signal in the IL-6 detection probes is specifically due to interaction of IL-6 with immobilized capture anti-IL-6 antibodies. The result also demonstrates that the protocol followed for reduction of antibodies with MEA retains the efficacy of immobilized capture anti-IL-6 antibodies.

3.7. Effect of temperature on probe signal

We also tried to find out the effect of temperature on the response of these CTFOB dip-probes. Four probes were prepared with immobilization of anti-IL-6 capture antibodies. Two probes were incubated for 1 h in 100 pM IL-6 solution at room temperature of 24 °C. Other two probes were incubated for 1 h in 100 pM IL-6 solution at 37 °C. Next the probes were incubated for 2 h at room temperature in labeled "detection" anti-IL-6 antibody solu-

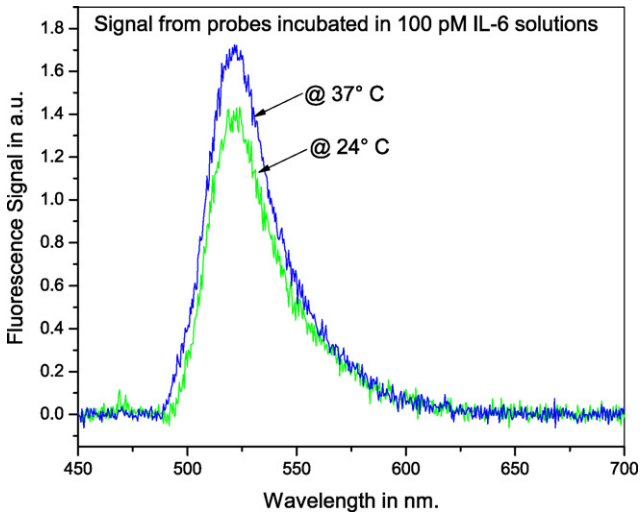

Fig. 6. Comparison of signal from the probes incubated in 100 pM of cytokine IL-6 solutions at 24 °C and 37 °C. Signal spectral profiles were separated from the background spectral profiles using least square fitting method with $R^2 \geq 0.997$.

tion (1 μg/ml). After appropriate washing and drying procedure, fluorescence spectral profile of each probe was recorded. Extracted signals of the probes are shown in Fig. 6. It was found that average signal at 37 °C was about 1.2 times higher than the average signal at 24 °C. The higher signal indicates higher antibody antigen interaction at higher temperature.

4. Conclusion

We have demonstrated that the developed CTFOB dip-probe can successfully detect cytokine IL-6 with high sensitivity and specificity even in the presence of much higher concentration of other non-specific proteins and cytokine like IL-8. The reported CTFOB probes can detect cytokine IL-6 concentration levels as low as 5 pM (0.12 ng/ml) with high specificity. IL-6 is one of the lower concentration cytokines that may be present in a healing wound. Its concentration in a healing wound is 0.2 ng/ml; however, in non healing wounds its levels could be as high as 3 ng/ml (Battaglia et al., 2005). The lower concentration of IL-6 in wounds makes it a much more challenging sensing target. We have also demonstrated that the CTFOB dip-probes can easily be used for IL-6 detection even at body temperature of 37 °C. From these observations we can conclude that the probes can be used to detect biologically relevant levels of the cytokine IL-6 in human blood/serum samples. We have also demonstrated that the proposed novel method effectively reduces any false signal due to NSB of labeled detection antibodies. The method presented here also eliminates the need of probe pre-treatment with any kind of blocking buffer. The dose–response curve indicates that the developed CTFOB dip-probes can also be used for quantitative estimation of IL-6 in blood/serum samples.

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