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Quantitative Estimation of IL-6 in Serum/Plasma Samples Using a Rapid and Cost-Effective Fiber-Optic dip-probe

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Abstract

A rapid and cost-effective combination tapered fiber-optic biosensor (CTFOB) dip-probe was used for quantitative estimation of interleukin (IL)-6 in serum/plasma samples. Sandwich immunoassay was used as the detection technique. Probes could successfully detect presence of IL-6 in two serum samples, non-neoplastic autoimmune patient (lupus) sample and lymphoma patient sample. The estimated amount of IL-6 in lupus patient sample was 4.8 ± 0.9 pM and in lymphoma patient sample was 2 ± 1 pM. It is demonstrated that the developed CTFOB dip-probe is capable of quantitative estimation of proteins in serum/plasma samples with high specificity.

Keywords

Fiber-optic; Fluorescence; IL-6; Cytokine; Serum; lupus; lymphoma

1. Introduction

Although evanescent wave based fiber-optic biosensor (FOB) technology is available for more than one and a half decade but still its application to detect proteins in body fluids is almost negligible(1). Although several works have demonstrated detection of a specific biological analytes in lab prepared solutions but FOBs are still not used for quantitative estimation or relative estimation of bio-analytes in body fluid samples(1–3). For quantitative estimation, first and most important factor is reproducibility of FOB probes. We have developed highly reproducible combination tapered fiber optic biosensor (CTFOB) probes. The most popular FOBs currently in use are tapered fiber-optic biosensors (TFOB)(1–4). Most often small diameter fibers ($< 100 \mu\text{m}$) are used in the development of fluorescence based TFOB. Further tapering of such a small diameter fiber makes these probes very fragile and inhibits its use outside the lab environment. Among the TFOBs, combination tapered fiber-optic biosensors (CTFOB) not only have highest sensitivity but probe part has a constant diameter(5–7). We have developed rugged CTFOB probes with $300 \mu\text{m}$ probe diameter as cost-effective dip-probe sensors. In this work we have demonstrated quantitative estimation of interleukin (IL)-6 in serum/plasma samples using these CTFOB probes. We could successfully detect and quantify IL-6 in two serum samples, non-neoplastic

autoimmune patient (lupus) sample and lymphoma patient sample, using these CTFOP dip-probe.

2. Materials and Methods

2-1 Materials

All solvents and chemicals were either of analytical grade or chemically pure. Egg albumin (EA) powder, b-Mercaptoethylamine HCl (MEA), Dry Acetone and Phosphate Buffered Saline (PBS) were obtained from Fisher Scientific (Pittsburgh, PA, USA). Recombinant Human IL-6, 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, 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). The SILICA/SILICA Optical Fibers were obtained from Polymicro Technologies (Phoenix, AZ, USA).

2-2 Antibody-dye conjugation

A mixture of anti-human IL-6 detection (Clone MQ2-39C3) antibodies (0.5 mg/ml) and Alexa fluor 488 fluorophore (64 μ g/ml) were prepared in PBS with 0.02M sodium bicarbonate (pH8.5). The molar ratio of dye and antibodies is 30. After 1 hour incubation in room temperature, the free dye molecule was filtered out twice by using two spin columns (CS-800).

2-2 Antibody immobilization

First, tip part of the CTFOP probes surface was derivatized by primary amines by immersing into a 2% APTS solution in dry acetone for 1 min. These amines group then react with hetrobifunctional cross-linker Sulfo-SMCC by incubating the probe into 4.5mM Sulfo-SMCC in PBS-EDTA (10mM) solution for 1 hour. Sulfo-SMCC groups could bind to amine groups in one side and create a maleimide-activated probe surface with the other side. To expose the sulfhydryl group of the anti-human IL-6 capture antibody (Clone MQ2-13A5), the antibodies were reduced by mixing 0.5mg/ml of antibody solution in 10mM PBS-EDTA and 50mM MEA and incubating at 37 °C for 90 minutes. The MEA was filtered out from antibody stock solution with the help of spin column. Finally to immobilize anti bodies on probe surface, the probes were incubated in 10 μ g/ml reduced anti-human IL-6 capture antibody for 4 hours at room temperature.

2-3 Serum samples preparation

The two serum samples used were part of a larger study for biomarker discovery. Sample collection was accomplished after approval by the Institutional Review Board (IRB) of the University of Alabama at Birmingham and in accord with an assurance filed with and approved by the U.S. Department of Health and Human Services. No data allowing the identification of patients were provided for this study. Analysis was performed on small aliquots of samples collected for routine laboratory tests. For the current study, serum

samples were obtained from a lupus patient and from a lymphoma patient before they were treated with chemotherapy. Handling and processing was similar for the samples. In brief, blood samples were collected without anticoagulant into red-top Vacutainers and allowed to coagulate for 20 to 30 min at room temperature. Sera were separated by centrifugation, and the specimens were immediately divided into portions, frozen, and stored in a dedicated -80°C freezer. No more than two freeze-thaw cycles were allowed before testing of samples.

2-4 Signal generation and recording

Sandwich immunoassay was used to generate the fluorescence. The spectra profile of the fluorescence was generated by using the detection setup reported earlier(7). All the detection procedures were operated at room temperature. The probes immobilized with capture anti-IL-6 antibodies were dipped into various concentrations (5, 20, 50, 100, 200, 500, and 1000pM) of human IL-6 sample solutions for 1 hour and two serum samples (lupus and lymphoma) for 2 hour. To simulate the serum samples, all human IL-6 sample solutions were mixed with 1mg/ml egg albumin (EA). Four probes were used for each concentration. After 1 min washing by washing buffer (PBS with 0.02% Tween-20), background signal spectral profiles of each probe was recorded. After recording, all probes were then incubated in 1 $\mu\text{g}/\text{ml}$ labeled anti-IL6 detection antibody solution (still diluted with EA). Finally, the fluorescence spectral profile of each probes were recorded after washing. The signals for each probe were extracted by using least square fitting method(7).

3. Result and Discussion

3-1 Calibration curve

Quantitative estimation needs a calibration curve. The calibration curve was obtained by recording signal from various known concentrations (5 pM–1000 pM) of IL-6 using identical fiber-optic dip-probes. Commercial IL-6 (Biolegend Inc.) was used for calibration curve. The Fig. 1 shows the calibration curve with concentration from 5pM–1000pM. An exponential growth function $S(C) = A[1 - \exp(-B \times C)]$ was fitted to the curve, where S is the fluorescence intensity extracted by least square fitting method, C is the concentration of the samples. It can be seen in Fig. 1 that the function fits well to the experimental data with $R^2 = 0.994$.

3-2 IL-6 measurement in serum samples

Fig 2 and Fig 3 show one of the spectral profiles of two serum samples. Background spectral profile is also shown along with total signal profile. Specific signal was extracted from the total signal by least square fitting method. Since the incubation time for serum samples were 2 hours and for the calibration curve data it was only one hour, therefore the serum sample signal intensity was divided by two to compute IL-6 concentration. The average computed IL-6 concentration from the probes incubated in lupus sample was 4.8 ± 0.9 pM while average computed IL-6 concentration from the probes incubated in lymphoma serum sample was 2 ± 1 pM.

4. Conclusion

The estimated amount of IL-6 in sample in lupus patient serum sample was 4.8 ± 0.9 pM and in lymphoma patient serum sample was 2 ± 1 pM. Both the results indicate that lupus serum sample contains higher concentration of IL-6 than lymphoma patient sample. It demonstrates that CTFOB dip-probe is capable of quantitative estimation of proteins in serum/plasma samples with high specificity.

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Fig. 1.

Calibration 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 $R^2 = 0.994$.

Fig. 2.
Recorded spectral profile of the fluorescence collected from a probe incubated in a serum from a lupus patient.

Fig. 3.
Recorded spectral profile of the fluorescence collected from a probe incubated in serum from a lymphoma patient.