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Sensitive Cytokine Assay Based on Optical Fiber Allowing Localized and Spatially Resolved Detection of Interleukin-6

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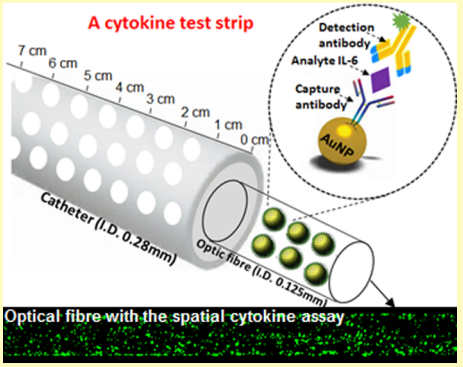
Supporting Information

ABSTRACT: We demonstrated a cytokine detection device based on gold nanoparticle modified silica optical fiber for the monitoring of locally variable cytokine interleukin-6 (IL-6) concentrations using a sandwich immunoassay scheme. The fiber is designed to be introduced into an intrathecal catheter with micrometer-sized holes drilled along its length to enable fluid exchange between the outside and inside of the catheter. An exposed optical fiber (diameter 125 μm) modified with a layer of gold nanoparticles was functionalized with the IL-6 capture antibody to form the sensing interface. The immunocapture device was incubated with a cytokine solution to capture the analyte. The device was then exposed to the IL-6 detection antibody which was loaded on the fluorescently labeled magnetic nanoparticles, making it possible to quantify the cytokine concentration based on the intensity of fluorescence. A reliable method for quantifying the fluorescent signal on a 3D structure was developed. This device was applied to the detection of cytokine IL-6 with the low limit of detection of 1 pg mL^{-1} in a sample volume of 1 μL. The device has the linear detection range of 1−400 pg mL^{-1} and spatial resolution on the order of 200−450 μm, and it is capable of detecting localized IL-6 secreted by live BV2 cells following their liposaccharide stimulation. This biological detection system is suitable for monitoring multiple health conditions.



KEYWORDS: *optical fiber, cytokines, localized and spatially resolved detection, IL-6, immunosensors, cytokine test strip*

Cytokines are small proteins (~6−70 kDa) secreted by cells, with broad biological importance, in particular for cellular signaling.[1] Certain pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α in the spinal cord, dorsal root ganglions (DRG), injured nerves, or skin are known to be involved with abnormal spontaneous activity in injured nerve fibers or compressed/inflamed DRG neurons[2] and in the process of pathological pain.[3] Current cytokine detection methods are applicable to body fluids such as blood plasma. However, the cytokines are locally released and measurements of their average content in body fluids provide only a limited insight into the underpinning processes. Thus, in order to understand the role of the immune system in pain or multiple other health conditions which lead to immunoreactivity and the expression of cytokines, it would be useful to be able to monitor spatially localized concentration of cytokine in specific locations of the body, such as the spinal cord, reproductive tract, cancer stroma, and so forth. This is challenging because the cytokine concentration in body fluids is low, normally in the pM range,[4] and cytokine assays may suffer interference from heterophilic antibodies, rheumatoid factors, and specific or nonspecific cytokine binding proteins.[5]

Benefiting from cost-effective and simple parallel array-type operation, and relatively high sensitivity, conventional enzyme-linked immunosorbent assay (ELISA) has become the most common cytokine quantification tool, used, for example, for clinical diagnosis of the "cytokine storm" in patients.[6−8] However, ELISA typically requires long incubation time (several hours) and suffers from the complicated sample labeling process. Moreover, a large amount of sample must be available to achieve a sufficient signal-to-background ratio for detection. Thus, broad interest exists in developing simple, sensitive, and rapid cytokine analysis platforms for comprehensive characterization and quantitative analysis of cytokines secreted from immune cells.[9−22] Recently, a microfluidic microsphere-based biosensor for quasi real-time detection of TNF-α was reported by Konry and co-workers.[23] A label-free localized surface plasmon resonance (LSPR) biosensing technique to detect cell-secreted tumor necrosis factor (TNF)-α cytokines in clinical blood samples was also reported.[24] This technology can detect cytokines with a blood sample volume of 3 μL and a total assay time 3 times shorter than that of ELISA (5−6 h). Revzin and co-workers

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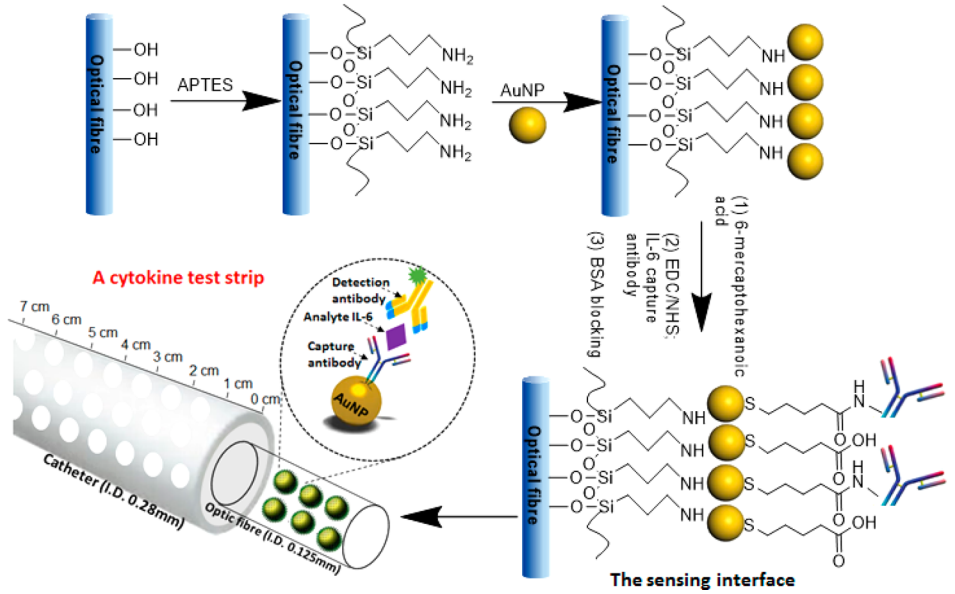
Scheme 1. Scheme of the Preparation of the Cytokine Test Strip Based on the Optical Fiber for Detection of the Localized Analyte IL-6



have reported a microscale device for detecting local release of interferon gamma (IFN-γ) from primary human leukocytes in real time.[15] However, this device is not suitable for measuring localized cytokines in vivo.

Optical fibers are chemically passive, have small physical dimensions (diameters in the range of tens to few hundred μm), and are able to access challenging environments.[25,26] Configured as fiber-optics sensors, they offer an advantage of long interaction length which, in some situations, can yield enhanced signals. Moreover, optical fiber biosensors can be used in combination with different types of spectroscopic techniques, e.g., absorption, fluorescence, phosphorescence, Raman, and surface plasmon resonance (SPR).[27] Optical fibers have also been explored as a promising platform for cytokine detection.[11,28−30] A recent report describes a fiber-optics SPR sensor for the detection of IL-1, IL-6, and TNF-α in a buffered saline solution and a spiked cell culture medium.[31] In this study, the detection limit of IL-6 of 0.44 ng mL^{-1} was achieved. The optical fiber-based sensors have also been applied to the monitoring of biologically relevant molecules in real time. For example, a label-free fiber-optic localized SPR sensor for the detection of IFN-γ was fabricated using spherical gold nanoparticles (AuNPs) on a polished end-face of the optical fiber.[12] This label-free immunoassay sensor was characterized by a short detection time (5 min), high resolution, and sensitivity (2 pg mL^{-1} for IFN-γ) due to the signal amplification from AuNPs. A recently reported photonic lab-on-a-chip sensor allowed rapid determination of IL-2 levels secreted by lymphocytes based on the measurement of optical absorbance.[32] However, none of these devices is capable of spatially localized cytokine detection along the fiber length based on the quantification of the fluorescent signal on the sensing interface.

In this study we designed an immunosening device (similar to a cytokine test strip) for monitoring of localized cytokine IL-6 concentration (Scheme 1). This cytokine capture device comprises a de-cladded optical fiber modified with a layer of AuNPs which are functionalized with the cytokine IL-6 capture antibody to form the sensing interface. This cytokine capture device can be inserted into a catheter with microscale holes drilled along its length to enable fluid exchange between the outside and inside of the catheter. It can be removed from a catheter at any stage and a new capture device may be then reintroduced for a second or subsequent measurement. The difference between the diameter of the catheter and the fiber is 155 μm resulting in very low friction between the fiber and the catheter. The removed capture device carries the analytes (cytokine IL-6) which are then detected. To achieve that, the fiber device is exposed to the IL-6 detection antibody which is loaded on the fluorescent nanoparticles. After a period of incubation and a washing step, the level of cytokines can be determined by quantifying the intensity of fluorescence from nanoparticle-labeled detection antibodies on the 3D fiber surface. This approach is different from the evanescent wave-based signal quantification in the traditional optical fiber sensors.[11] In this work, this variant of spatially resolved ELISA was successfully used for the detection of spatially localized cytokine IL-6 with the low limit of detection of 1 pg mL^{-1}, a sample volume of 1 μL, and the linear range of 1−400 pg mL^{-1}. The detection system also demonstrated high spatial resolution on the order of 200−450 μm for detecting localized IL-6 secreted by liposaccharide stimulated BV2 cells. The ability to reintroduce a new capture device into the catheter makes our design attractive for clinical applications, and this approach has the potential for the development of the point-of-care cytokine detection devices for neuroscience and other biomedical research.

EXPERIMENTAL SECTION

Chemicals. Aminopropyltriethoxysilane (APTES), concentrated sulfuric acid, hydrogen peroxidase (30%), toluene, ethanol, 6-mercaptohexanoic acid, bovine serum albumin (BSA), 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide hydrochloride (EDC), *N*-hydroxysuccinimide (NHS), 2-(*N*-morpholino) ethanesulfonic acid (MES), and lipopolysaccharide were purchased from Sigma-Aldrich. Mouse interleukin-6 (IL-6), anti-mouse IL-6 polyclonal antibody (capture antibody), anti-mouse IL-6 monoclonal antibody (detection antibody), and donkey anti-Goat IgG NorthernLights NL493-conjugated antibody were purchased from R&D Systems. Carboxy-

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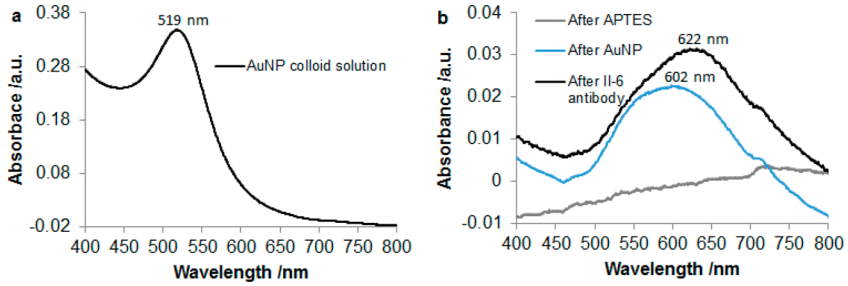

Figure 1. UV−vis absorbance of (a) gold colloid solution and (b) glass surface after stepwise modification with APTES, AuNP, and IL-6 capture antibody.

lated superparamagnetic iron oxide particles (SPIO, 1% solid, 10 mg mL^{-1}, ~0.9 μm, labeled with Dragon Green fluorophores (excitation 480 nm, emission 520 nm)) were obtained from Bangs Laboratories, USA. The optical fiber is a standard telecommunication silica fiber 62.5-μm-diameter core/125 μm acrylate cladding (LNF(TM) product from Pirelli, now Prysmian). The circular glass coverslips (diameter of 12 mm, thickness of 0.13 mm) were purchased from Fisher Scientific, Australia. Aqueous solutions were prepared using Milli-Q water. The phosphate buffer solution used in this work contained 0.1 M sodium phosphate, 0.15 M sodium chloride, adjusted to pH 7.2 with NaOH or HCl solution.

Protocol for Drilling Holes in Catheter by a Laser. The protocol for drilling holes by laser has been detailed in earlier literature.[33] The laser machining system is a Microstruct-C from 3D MicroMac, Germany with the 266 nm picosecond laser (Lumera/Coherent SuperRapid HE). A scanner with an F = 56 mm optics to drill an array of 50 of 100 μm holes was used and holes were drilled with 1 μJ pulses at 20 kHz pulse repetition frequency (80 holes along 1 cm length of fiber with 2 holes at each circumference).

Fabrication of the Sensing Interface. As shown in Scheme 1, the sensing interface was fabricated in several steps. First, the de-cladded optical fiber (or the glass coverslip used in preliminary characterizations) was immersed in a piranha solution (H_2SO_4 95% and H_2O_2 30% mixed at a volume ratio of 4:1) for 12 h in order to clean the glass and to form hydroxyl groups on its surface. After rinsing with deionized H_2O and ethanol and drying under N_2 stream, the cleaned optical fiber/coverslip was immersed in aminopropyltriethoxysilane (5% v/v) in toluene for 6 h to form an amine-terminated self-assembled monolayer. The fiber/coverslip was then rinsed with toluene and ethanol to remove the unbound monomers from the surface. The aminopropyltriethoxysilane-modified optical fiber was subsequently immersed into a 1 mL of AuNP (10 nm) solution for 5 h. Upon removal, the fiber/coverslip was copiously rinsed with deionized H_2O and finally blown dry with N_2. The AuNP modified optical fiber/coverslip thus prepared was then incubated in 0.1 mM 6-mercaptohexanoic acid solution overnight, and finally washed three times, in ethanol and deionized H_2O, respectively. To activate the carboxylic acid on the optical fiber/coverslip, the prepared fiber was incubated in the 25 mM MES buffer (pH 6.0) solution containing EDC (10 mg mL^{-1})/NHS (10 mg mL^{-1}) for 30 min. Then the fiber/coverslip was washed in the MES buffer twice. Finally, the fabricated optical fiber/coverslip was immersed in the anti-IL-6 capture antibody solution (50 μg mL^{-1}) in the MES buffer (pH = 6.0) at room temperature for 3 h and washed twice in deionized H_2O. Subsequently, the fiber/coverslip surface was blocked in a 0.25% BSA solution for 3 h to complete the preparation of the sensing interface. In order to confirm the presence of the capture antibody, the sensing interface was incubated with the anti-IL-6 secondary antibody (labeled with green fluorophores) solution (1:100) in PBS for 2 h. Then the interface was washed three times by PBS and deionized H_2O, respectively, and finally dried and imaged by confocal microscopy.

Cytokine Measurement. After modification of the optical fiber/coverslip surface with the anti-IL-6 capture antibodies, the optical fiber/coverslip was incubated in PBS solution or serum containing IL-6 in different concentrations for 2 h. Then the fiber/coverslip was washed with a wash buffer (PBS, 0.1% Tween-20) and dried by N_2 stream. Finally the optical fiber/coverslip was exposed to Dragon Green magnetic nanoparticles loaded with IL-6 detection antibody (DG_SPIO_IL-6_Ab; see the details of making DG_SPIO_IL-6_Ab in the Supporting Information) for 1 h by washing with wash buffer (PBS, 0.1% Tween-20) and deionized H_2O before fluorescence measurements described below.

Confocal Laser Scanning Microscopy Imaging and Fluorescent Signal Quantification. The fluorescence spectra for the fiber/coverslip based cytokine assay were collected at excitation wavelengths of 493 nm for green dye (NL493) with the emission range of 510−650 nm, and 480 nm for Dragon Green with the emission range of 500−650 nm using a Fluorolog Tau3 system (JY Horiba, Edison, NJ) in 10 mm quartz cuvettes at room temperature. The spectral band passes were 0.5 nm in both excitation and emission. The PMT voltage was adjusted to 950 V. The optical fiber samples were imaged using a SP2 (Leica) confocal microscope with objective HC PL FLUOTAR, magnification 10×, NA 0.3, xy-resolution 651 nm, pinhole 1× Airy disc, and field of view 1500 × 1500 μm^2. The z-stack of 10 z-planes over 125 μm height were collected, with the separation of around 12.5 μm between planes. All ten images were then processed, to calculate the maximum pixel value from these 10 planes. This maximum pixel value was then assigned as the pixel value in the combined image (Z-projection in ImageJ). For every concentration of IL-6, two different locations were imaged so that 3 mm fiber length in total was imaged for each cytokine measurement. The intensity of the green dots representing the Dragon Green fluorescent labels was quantified by integrating over a spatial window of 450 μm using ImageJ and Matlab software. In this way the spatial resolution of 450 μm was realized.

RESULTS AND DISCUSSION

Evaluation of the IL-6 Immunosensor Performance on the Glass Surface. The sensing interface in Scheme 1 was first produced on a glass coverslip instead of the optical fiber for ease of characterization. The self-assembled AuNPs deposited on a glass coverslip were characterized by UV−vis spectrophotometry (Figure 1). The spectrum of the AuNP colloid solution has a characteristic plasmon peak at 519 nm. This peak is absent for the glass surface after modification of APTES; however, a similar feature at 602 nm appears after modification of glass with AuNP, which confirms the successful attachment of AuNP. This modified spectral characteristics suggests that the Au colloids self-assembled on glass are close enough to affect the coupling of plasmons of individual particles resulting in an increased absorbance at wavelengths >600 nm when compared to that of the original AuNP in solution.[34] The plasmon peak showed a further redshift (622 nm) after the attachment of IL-6 capture antibody due to the change of the surrounding environment of AuNPs.[35] Fluorimetry was used to monitor surface modifications of the glass surface after the attachment of detection antibodies (Figure S1). The back-

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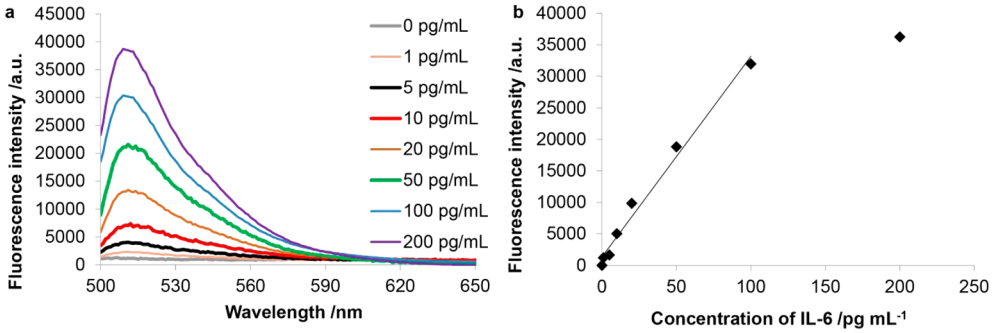

Figure 2. (a) Relationship between fluorescence intensity and the concentration of IL-6. (b) Calibration curve of IL-6 based on the sensing interface fabricated on a glass coverslip.

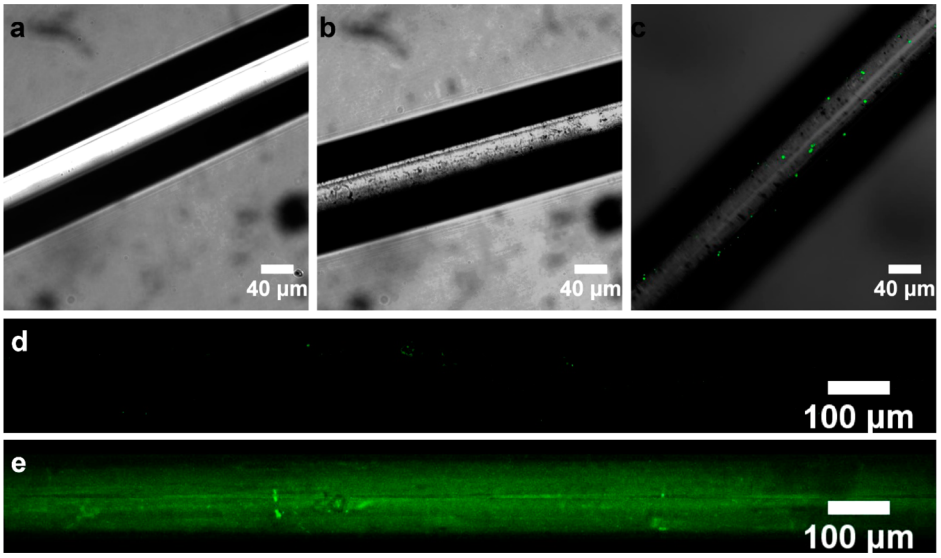

Figure 3. Confocal images for the stepwise modification of optical fiber: (a) original optical fiber, (b) AuNP modified optical fiber, and (c) IL-6 capture antibody modified optical fiber for determination of IL-6 after incubation with DG_SPIO_IL-6_Ab; (d) fiber surface without modification of IL-6 capture antibody; and (e) IL-6 capture antibody modified optical fiber after exposure to green dye (NL493) labeled secondary antibody, respectively.

ground fluorescence signal for the blank glass surface was observed around 530 and 600 nm, respectively; it disappeared after modification of the glass surface with APTES, due to the formation of a layer of amine groups on the surface. After further stepwise modification of the glass surface with 6-mercaptohexanoic acid, AuNP, incubation with the anti-IL-6 capture antibody followed by the incubation with IL-6 (100 pg mL^{-1}) and the fluorescent detection antibody (DG_SPIO_IL-6_Ab), the characteristic fluorescence peak of the Dragon Green beads appeared at about 518 nm, indicating a successful attachment of the detection antibody. Thus, this fabricated system is capable of detection of IL-6.

The sensing interface fabricated on the glass coverslip was used to detect IL-6 at different concentrations. Figure 2a shows the relationship between fluorescence intensity and the concentration of IL-6, and the fluorescence signal of the detection antibodies conjugated to Dragon Green beads increased linearly with the concentration of IL-6. The calibration curve of this sensing interface for IL-6 is plotted in Figure 2b. The lowest detectable concentration was 1 pg mL^{-1} with the linear range of 1−100 pg mL^{-1} which is within the physiological concentration range of IL-6 in the body.[4] Thus, this assay can be used to quantify the IL-6 concentration in vitro. This immunosensor scheme has been further applied to the optical fiber, as described in the following sections.

Performance of the Fabricated Sensor on Optical Fiber Surface. In order to realize the localized detection of cytokines, in the next stage the ELISA surface detailed previously was fabricated on an optical fiber, to be able to carry out a cytokine assay for the detection of Il-6. A stepwise modification of optical fiber was carried out, as previously described and the resulting fiber surface was characterized by confocal microscopy (Figure 3). The original de-cladded optical fiber showed a smooth and clean surface. After the fabrication of AuNPs on the fiber surface, some small black dots could be observed, suggesting the presence of AuNPs clusters resulting in an increased surface area for binding the IL-6 capture antibody. The SEM image of AuNP was included in Figure S2, further suggesting the presence of AuNP with the size of about 10 nm. No significant change could be seen in the confocal images after the attachment of capture antibody on the fiber surface. After the incubation of the sensing interface with the analyte IL-6 and the IL-6 detection antibodies (DG_SPIO_IL-6_Ab), very bright green dots were observed (Figure 3c), suggesting that the DG_SPIO_IL-6_Abs were attached to the sensing interface to form a sandwich structure with the IL-6 and IL-6 capture antibodies. The intensity of this green fluorescence

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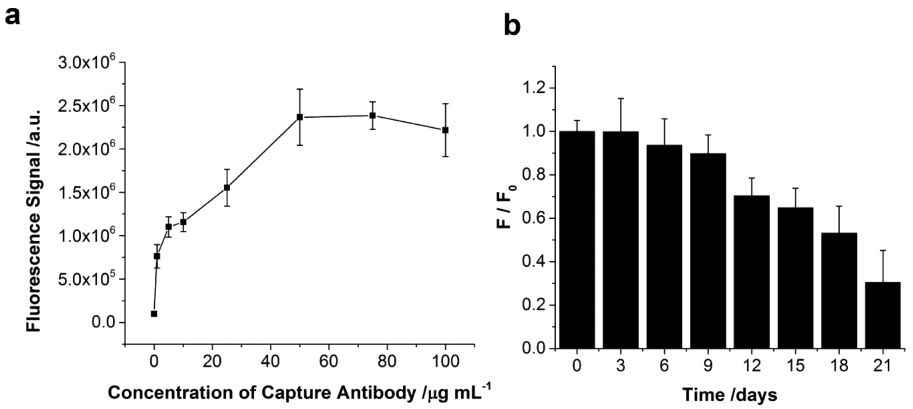

Figure 4. (a) Change in fluorescence signal of green dye with increasing concentration of IL-6 capture antibody. (b) Stability of the capture antibody modified sensing interface. F_0 and F are the fluorescence signals from the secondary antibody for the freshly prepared fiber and the same fiber after being stored in PBS for different periods of time.

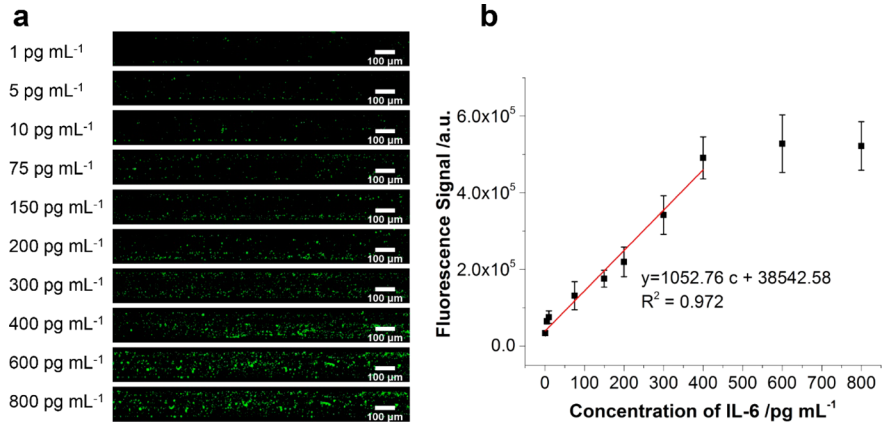

Figure 5. (a) Z-stack maximum intensity projection images of the optical fiber after its exposure to different concentration of IL-6 followed by the incubation of DG_SPIO_IL-6_Ab (27.5 μg mL^{-1}). (b) Calibration curve of IL-6 based on the fluorescence signal and IL-6 concentration obtained from (a).

could be used to quantify the analyte. To further confirm the presence of capture antibody, the green dye (NL493) labeled secondary antibody was applied on the capture antibody modified sensing interface and the fiber surface without the attachment of IL-6 capture antibody. Negligible levels of fluorescence on the fiber surface were detected after the incubation with a green dye-labeled secondary antibody (Figure 3e). However, the sensing interface demonstrated bright green fluorescence (Figure 3d), indicating the presence of the green dye-labeled secondary antibody and the capture antibody with a homogeneous distribution.

To maximize the amount of IL-6 capture antibody on the sensing interface required for high sensitivity, its concentration was optimized by quantifying the fluorescence intensity of the green dye-labeled secondary antibody (Figure 4a). The fluorescence signal on the 3D optical fiber was quantified using ImageJ and MatLab software. This signal increased with the concentration of IL-6 capture antibody used for fabrication of the sensing interface, and a maximum fluorescence signal was obtained when the concentration of capture antibody was 50 μg mL^{-1}. In addition, the stability of the capture antibody was investigated by leaving the fabricated sensing interface in PBS for extended periods of time followed by monitoring the concentration of IL-6 capture antibody using the green dye labeled secondary antibody (Figure 4b). The fluorescence signal was stable for the first 9 days, indicating that the capture antibody was still attached on the fiber surface. The fluorescence signal then continued to decrease to less 50% of the original intensity after 20 days, which might be due to limited stability of C−Au bonds between alkanethiols and AuNPs[36] resulting in a progressive release of the capture antibodies from the glass surface.

Detection of IL-6 Using the Fabricated Cytokine Immunosensing Device. After verifying the performance of the cytokine capture surface on glass slides we fabricated an identical capture surface on a glass fiber 125 μm in diameter. In order to quantify the fluorescence signal reporting on the presence of analyte molecules captured on fiber surface we developed a tailored approach. Drawing on the capability of laser scanning confocal microscope to reject out-of-focus signal and its depth of field that is much smaller than the fiber diameter, we recorded multiple images at different axial planes (Z-stack), around 12.5 μm apart, in order to image the total visible fiber area. This Z-stack was further processed to select the maximum pixel value from each image. This maximum value was then assigned to the corresponding pixel (maximum Z-projection in Image-J). This final composite image produced from a Z-stack was taken as a representation of the total fluorescence signal of a section of the fiber and it was used for further quantification of the signal. These data are shown in Figure 5a.

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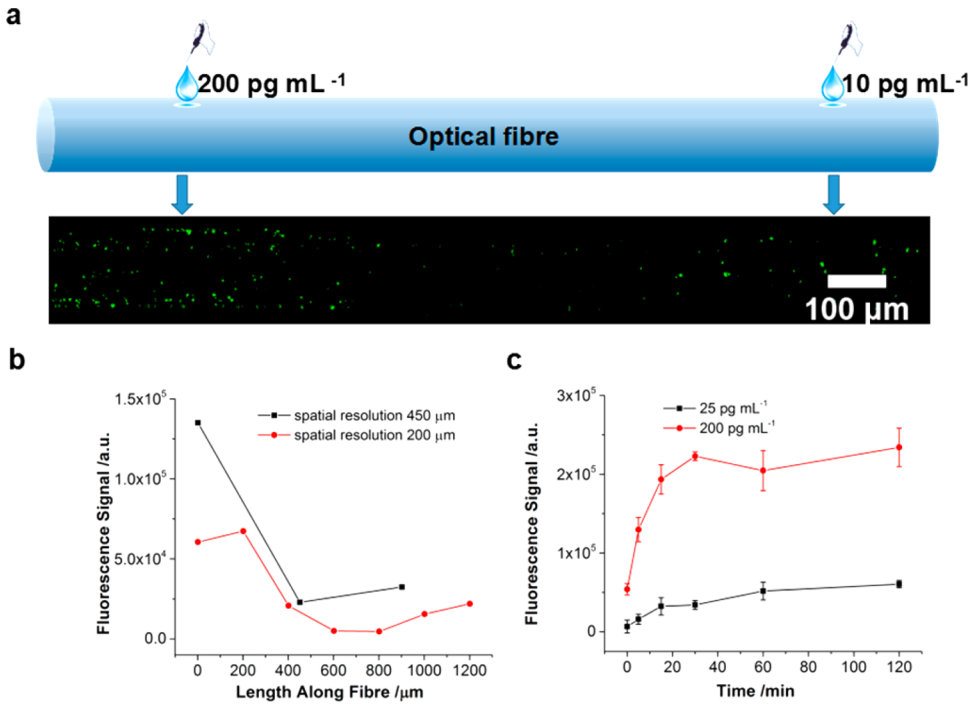

Figure 6. (a) Results of spatially localized cytokine detection experiments using our device. We simultaneously placed 1 μL of serum containing 10 and 200 pg mL^{-1} IL-6 on the fabricated sensing interface, respectively, followed by incubation with detection antibody and signal quantification. (b) Relationship between fluorescence signal in the 200 and 450 μm windows along the imaged length of the fiber. (c) Relationship between the fluorescence signal and the response time of the immunosensing device for the determination of IL-6 with the concentrations of 25 and 200 pg mL^{-1}.

The nonspecific protein adsorption of this sensing device was investigated using BSA as the blocking reagent (Figure S3). In the absence of BSA blocking, a significant nonspecific DG_SPIO_IL-6_Ab absorption was observed on the capture antibody-modified interface after the exposure to the detection antibody solution, likely due to physical adsorption of DG_SPIO_IL-6_Ab on surface defects. However, when the capture antibody-modified sensing interface was blocked with 0.25% BSA, only a few green dots were observed in the confocal image, suggesting negligible nonspecific absorption (5 orders of magnitude lower than the signal for 100 pg mL^{-1} IL-6). Such low nonspecific adsorption is required to achieve high detection specificity of IL-6. The capture antibody-modified sensing interface (with 0.25% BSA blocking) was further used for detection of IL-6 in the PBS solution. As shown in the Z-stack maximum intensity projection images (Figure 5a), the Dragon Green intensity increased with increasing IL-6 concentration, indicating that IL-6 could be quantified by integrating Dragon Green fluorescence by Image-J and Matlab software. A linear relationship between the fluorescence intensity and the concentration of IL-6 in the range 1−400 pg mL^{-1} was obtained (Figure 5b), which is within the physiologically relevant range.[4] The lowest detectable concentration of IL-6 was 1 pg mL^{-1}, which is similar to that of an electrochemical immunosensor based on ferrocene-loaded porous polyelectrolyte nanoparticles as labels (1 pg mL^{-1}).[37] The lowest detection limit is lower than the value reported in a recently developed liquid-gated field-effect transistor sensor based on horizontally aligned single-walled carbon nanotubes for detection of IL-6 (1.37 pg mL^{-1}),[38] and it is 1 order of magnitude lower than a fluorescence-based immunoassay (20 pg mL^{-1}).[39] The application of AuNPs on the sensing interface for loading large amounts of capture antibodies and the brightness of the nanoparticles labeled with detection antibody have contributed to high sensitivity achieved in this work. The reproducibility of the fabricated cytokine assay was evaluated by fabricating 10 separate pieces of optical fibers used for the detection of 60 pg mL^{-1} IL-6 (Figure S4). The relative standard deviation of these ten immunosensors was ±3.6%, indicating that the fabricated assay was closely reproducible in our test conditions.

The feasibility of the fabricated cytokine assay for the spatially localized detection of IL-6 was studied by placing single drops (~1 μL) of the serum sample containing 10 pg mL^{-1} and 200 pg mL^{-1} IL-6 onto various locations of the fabricated optical fiber surfaces (Figure 6), followed by incubation with the detection antibody. Subsequently, the optical fiber exposed to two different concentrations of IL-6 in two close locations was imaged (3 mm length in total) and signal quantification carried out. The intensity of the green dots representing the Dragon Green fluorescent labels were quantified by integrating over a spatial window of specific width (typically 100−500 μm) using ImageJ and Matlab software. This spatial window was chosen to ensure enough Dragon Green beads are imaged for the fluorescence quantification, even for the lowest cytokine concentration. The width of the spatial window is one of the factors determining the achievable spatial resolution. We found that the fluorescence in the fiber area exposed to 200 pg mL^{-1} IL-6 was significantly higher (5 times) than the fluorescence produced with 10 pg mL^{-1} IL-6, suggesting that the fabricated sensing interface was capable of differentiating IL-6 at different concentrations from the sample volume of 1 μL. Thus, the cytokine immunosensing device developed here requires minimal sample consumption and offers excellent assay performance, making it highly suitable for analyzing biomarkers

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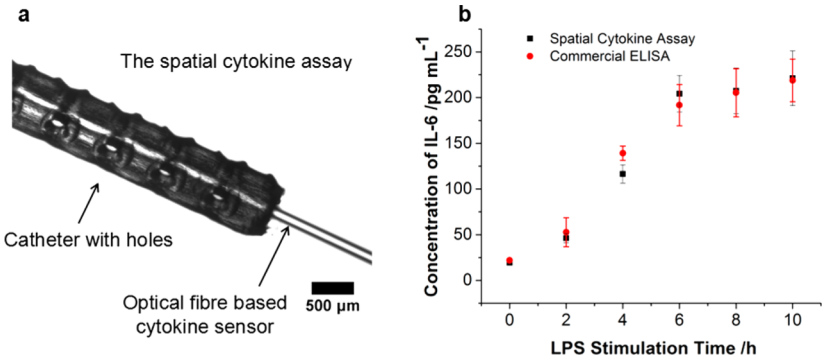

Figure 7. (a) Image of the optical fiber based cytokine assay (with catheter on) under bright field microscopy. (b) IL-6 secretion profile of BV2 cells after LPS stimulation for the commercial ELISA and the herein fabricated spatial fiber based cytokine assay.

and cytokines in precious biological samples. Moreover, our fiber has the capability of spatially resolving detection of localized IL-6 with resolution on the order of 200 to 450 μm. To our knowledge, so far only the Olink Bioscience's Proseek protein assay enables sensitive detection and quantification of proteins in a 1 μL sample volume,[40] but it does not offer spatial resolution. We also determined the response time of the immunosensing device for the detections of IL-6 at the concentrations of 25 and 200 pg mL^{-1}, respectively. The fluorescence signal of the SPIO-Ab (Figure 6c) increased dramatically with increasing incubation time for IL-6 (200 pg mL^{-1}) and it saturated around 30 min. In the case of lower concentration of IL-6 (25 pg mL^{-1}), the fluorescence signal increased about 1 order of magnitude more slowly than that for the high concentration of IL-6 (200 pg mL^{-1}). This more rapid transition toward the equilibrium is as expected by the basic laws of chemical kinetics.[41] We emphasize that for the concentration of 200 pg mL^{-1}, a measurable signal increase was observed already after 5 min incubation in the cytokine-containing medium.

Finally, the optical fiber sensor with the catheter on was applied for the detection of IL-6 secreted by live BV2 cells (Figure 7). Figure 7a shows the device which we used for the measurements in the medium. The concentration of IL-6 secreted into the medium increased with the LPS stimulation time and the maximum concentration was obtained after 6 h LPS stimulation. A similar IL-6 secretion pattern for BV2 cells was obtained by conventional ELISA but the lowest detection limit of our fiber device (1 pg mL^{-1}) was 1 order of magnitude lower than that in the conventional ELISA Kit from R&D System for IL-6 (10 pg mL^{-1}). Critically, in these experiments no media need to be removed from the culture; instead, repeated sampling can be achieved by replacing the fiber. Therefore, the cytokine assay device presented here is capable of monitoring cytokines ex vivo.

CONCLUSIONS

We fabricated and characterized a sensitive cytokine assay based on the optical fiber, which could be used for monitoring the localized cytokine concentration ex vivo. A spatially resolved ELISA sandwich assay was built on the optical fiber surface so that the fiber could be inserted into a perforated catheter. After exposure of the device to the cytokine-containing solution for a period of time, the optical fiber forming a cytokine test strip was removed from the catheter which could be inserted into the body. The fiber was then exposed to the solution of the detection antibody conjugated to fluorescent Dragon Green beads and washed, followed by quantification of cytokines based on the intensity of fluorescence by laser scanning microscopy. This variant of spatial ELISA was successfully used for the detection of cytokine IL-6 with the low limit of detection of 1 pg mL^{-1} and sample volume of 1 μL, and it showed high specificity to IL-6. The sensor interface was stable for up to 9 days in PBS solution, and it was capable of detecting localized IL-6 secreted by BV2 cells with liposaccharide stimulation. This technology provides a new strategy for monitoring spatially varying concentration of cell secreting products, and it has the potential to be developed as a point-of-care device for multiple health conditions.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acssensors.6b00619.

Supplementary figures for fluorescence spectra of the glass surface modified with different components, the SEM image of optical fiber modified with AuNPs, antifouling property of the sensing interface, reproducibility of the fabricated cytokine test strip. Experimental section describing the preparation of Dragon Green magnetic nanoparticles loaded with IL-6 detection antibodies and details for cell culture and ELISA measurement. (PDF)

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Notes

The authors declare no competing financial interest.

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