LOCALIZED SURFACE PLASMON RESONANCE (LSPR) OPTOFLUIDIC BIOSENSOR FOR LABEL-FREE CELLULAR IMMUNOPHENOTYPING
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ABSTRACT
We developed an optofluidic biosensor by integrating a microfluidic device into a Localized Surface Plasmon Resonance (LSPR) detection system to quantitatively analyze a cell-secreted cytokine, TNF-alpha. The device was designed for on-chip trapping and incubation of immune cells and subsequent detection of cytokines by a gold nanoparticle-based LSPR sensor. During the 2 hour incubation of 50,000 THP-1 cells in the microfluidic device, we observed a shift of 0.2877nm in the LSPR surface absorbance spectrum peak, which corresponds to a TNF-alpha concentration of 327ng/mL. This device enables LSPR technique for cell-secreted cytokine assay detection by scaling down the cell secretion volume.

KEYWORDS: Localized Surface Plasmon Resonance (LSPR), Optofluidic, Biosensor

INTRODUCTION
Comprehensive characterization of immune cells and their functions is critical to precisely monitor immune conditions of the human body upon screening infectious diseases and examining the efficacy of drug delivery [1-3]. In particular, the quantitative analysis of cytokines, which are intercellular signaling proteins secreted from immune cells, is a key to precise identification of cellular immune functions [1]. The conventional gold standard method for cytokine secretion assay is the Enzyme-linked immuno sorbent assay (ELISA). However, this technique requires a large cell sample volume, a complex labeling process, and a separate cell culture system for detecting cell-secreted cytokines [4]. To overcome these disadvantages of the current method, this research develops a single microfluidic platform for cell trapping and incubation and localized surface plasmon resonance (LSPR) detection for cytokine analysis. The approached device requires an only 3uL-volume chamber for cell incubation, which is approximately 100 fold smaller than that of a 96 well plate used in the conventional method. Furthermore this platform performs the whole assay process in a single chip, which decreases the total assay time around 4 times compared to ELISA detection.

METHOD
Localized surface plasmon resonance (LSPR) is a plasmonic phenomenon that arises on the metal nano-particle deposited surface. LSPR occurs when the incident light coupled to a sensing surface and the photon frequency of the incident light matches the natural frequency of the surface electron oscillation. LSPR-based detection measures a shift in the absorbance (or alternatively, scattering) spectrum of a nanostructured metal surface. When biomolecules captured on the LSPR detection surface, the near field of sensing surface’s refractive index (RI) will change. As a result, the absorbance spectrum of light incident to the sensing surface changes. A LSPR-based biosensing technique analyzes the absorbance spectrum change promises to quantify the cytokine secretion kinetics of immune cells in a label-free manner. However LSPR detection has a limitation for cytokine secretion analysis owing to its relatively poor sensitivity resulting in a limit of detection (LOD) of ~100ng/mL as compared to that of fluorescence-based detection (LOD ~ 10pg/mL). To compensate for the lack of sensitivity, , we propose a device incorporating both a microfluidic chamber to accumulate immune cells and a LSPR sensor surface, which concentrates the cell-secreted cytokines on a chip prior to LSPR detection. The device is composed of three parts (Figure 1 a),b)): (1) a top Polydimethylsiloxane (PDMS) supporting layer, (2) a middle PDMS layer for the microfluidic channels with micro-pillar arrays, and (3) a bottom sensor layer of gold-nanoparticles deposited for LSPR sensing. Having a 5μm center-to-center distance, the micro-pillar arrays physically trap THP-1 cells and allow only cell-secreted cytokines (TNF-alpha) to be diffused into the detection area for their small sizes.

EXPERIMENTAL
We fabricated the microfluidic channel using PDMS-based soft lithography. Our device processing started with fabricating a silicon mold by deep reactive ion etch (DRIE). The silicon mold surface was subsequently salinezed with (tridecafluoro-1,1,2,2,-tetrahydrooctyl)-1-trichlorosilane vapor (United Chemical Technologies) for 1 h under vacuum to facilitate subsequent release of PDMS structures from the mold. The soft lithography process first entailed thorough mixing of the PDMS curing agent with the PDMS base monomer (wt : wt = 1 : 10) to prepare a PDMS precursor (Sylgard-184, Dow Corning). We then poured the PDMS precursor onto the mold and cured it overnight in an oven at 60°C [5]. We used a commercially available substrate with a gold nanoparticle-deposited surface (P8 SAM, Lamdagen) as the bottom layer of the device for LSPR signal detection.

For LSPR signal detection, we illuminated the sensing surface with a white light source (HL-2000 tungsten halogen light, Ocean optics) via an optical probe light (R400-7-UV-VIS, Ocean optics) and collected reflected light by the
same light probe. The light collected by the probe was delivered to a spectrometer (HR-4000, Ocean optics), and the light signal was converted into electrical signal to analyze it by a computer. (Figure 1 c))

![Image](image.png)

Figure 1: a) Schematic of developed microfluidic device with LSPR detection surface. b) The microfluidic device has micro-pillar arrays around the detection area and cells are captured in the specific area, and secreted cytokine will be diffused into the detection area. The detection area is deposited with gold nanoparticles. c) Schematic of LSPR optical detection setup.

To prepare the sensing surface of the device, we activated the gold nanoparticle deposited surface with a 1:1 ratio mixture of 0.4M EDC (1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride, Thermo Scientific) and 0.1M NHS (N-hydroxysuccinimide, Thermo Scientific) solutions for 20mins. After the surface activation, we immobilized primary capture antibody (DY210, R&D Systems) molecules at a concentration of 103 μg/mL on the detection surface with an incubation time of 60mins. To eliminate the non-specific binding on the detection surface, we loaded a 1% BSA (Albumin, from bovine serum, SIGMA) solution and the 1x casein (5x Casein block solution, Surmodics BioFX) blocking buffer onto the sensing surface and incubated the surface for 20mins. After preparing the sensing surface, we loaded purified TNF-alpha (DY210, R&D Systems) of known concentration in the range of 100–500ng/mL to the device and obtained the standard curve. In our TNF-alpha cell secretion assay, a total number of 50,000 THP-1 cells (THP-1, ATCC), which was estimated through hemocytometer cell counting, were stimulated with LPS (Lipopolysaccharides, SIGMA) of 250ng/mL and incubated in the device for 2 hours. Between every step, we washed the sensing surface with 1x PBS to remove surplus biomolecules unbound on the sensing surface.

For quantification of TNF-alpha, we collected the absorbance spectrum at each process and determined the absorbance peak wavelength of the sensing surface as a function of the TNF-alpha concentration. A MATLAB code was used to find the peak wavelength from the original data collected by Spectra Suits (Ocean Optics).

RESULTS AND DISCUSSION

![Image](image.png)

Figure 2: Fluorescent image of Calcein AM stained THP-1 cells captured by device. It shows the good viability of all the loaded cells.

We first characterized the cell trapping performance of the device by taking a fluorescence image of captured Calcein AM-stained cells. We captured the cells on the device with micro-pillar arrays fabricated in the microfluidic chamber.
We measured the intensity of the cell-emitting fluorescence to quantify the number of the captured cells. Up to 90% of the total cells loaded into the device was captured by the micro-pillar arrays and subsequently incubated in the device. Prior to loading cells to the device, we immobilized antibodies onto the sensor surface (Figure 3 a) and the Figure 3 b) shows the real-time LSPR signal shift during the sensor preparation process and subsequent analyte detection. As shown in the Figure 3 b), the detection preparation processing time (step 1~3) took 2.5 hr which is faster than conventional method ELISA which typically takes over 18hr requiring a large number of reagent loading and washing steps.

We loaded 50,000 THP-1 cells onto the device with the biosensor surface prepared above, stimulated them by lipopolysaccharide (LPS) of 250ng/mL, and incubated them on the chip for 2 hours (Figure 4a). After the cell stimulation assay on the device, we washed the inside of the device chamber and obtained an LSPR signal shift of 0.2877 nm (Figure 4c), which corresponds to the concentration of 327ng/mL TNF-alpha was in the chamber according to the standard curve (Figure 4 b) blue dot).

CONCLUSIONS

We have established a label-free optofluidic technology integrating a multifunctional microfluidic device that enables cell separation, cell incubation, and LSPR optical detection of cell-secreted cytokines on a single chip. Our device has several practical advantages; by trapping and incubating the cells on the chip, this device eliminates the sample collection process that extracts cytokines secreted by the cells. Also, by confining the cells in the small volume chamber, cell secreted cytokines can be concentrated and able to amplify their concentration level, which is high enough to be reliably detected by the LSPR technique. The result shown in this paper represents our first-step progress toward achieving label-free time-course monitoring of cytokine secretion by immune cells under a well-controlled microenvironment. We anticipate that observing the dynamic cytokine secretion profile may permit rapid assessment of the healthy or diseased condition of immune cells without a long incubation time. Biologically in-depth information obtained by this method may enable clinicians to make an accurate prognosis of infectious diseases.

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REFERENCE


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