Detection of Streptavidin Using Liquid Crystal Based Whispering Gallery Mode Microbubble

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ABSTRACT

Protein is a complex chemical substance essential for human survival. Traditional protein detection methods, such as colorimetry, electrochemical analysis, and enzyme-linked immunosorbent assays, have shown good specificity and accuracy for the protein detection. However, all these methods require specialized instruments, and the detection procedures are laborious and time-consuming. As a result, a rapid, sensitive, label-free protein detection method is urgently needed. Herein, we have developed an ultra-sensitive biosensor for the detection of low-concentration protein molecules, employing liquid crystal (LC)-amplified optofluidic resonator. Since the orientations of LCs highly depend on the surface biomolecular binding processes, LCs can be employed to realize the extremely sensitive detection of biomolecules. Immobilized protein molecules interfere with the orientation of LCs by reducing the vertical anchoring force from the alignment layer in which the spectral wavelength shift was monitored as a sensing parameter. A biosensing platform based on an LC-amplified optofluidic whispering gallery mode (WGM) resonator was designed and studied accordingly. Due to the simultaneous interaction of the WGM and the LCs in the optofluidic resonator, the changes caused by the injection of protein molecules will be amplified, resulting in a shift in the resonance wavelength. Total wavelength shifts scale proportionally to the molecular concentrations of the protein within a certain range. The detection limit for streptavidin (SA) can reach as low as the femtometer level, which is significantly higher than the detection limit in the classic polarized optical microscope (POM) method visible with the naked eye. In addition to SA, the LC-based optofluidic resonator can also be applied to detect a variety of protein molecules. Our study demonstrates that LC-amplified optofluidic resonator can provide a novel solution for ultrasensitive real-time characterization of biosensing and biomolecular interactions.

Keywords: Whispering-gallery mode, Liquid crystal, Optofluidic, Streptavidin, Biosensor

1. INTRODUCTION

Protein plays a critical role in biological activities and is intimately related to human health. Numerous chemical reactions within the human body rely on proteins to function properly. Moreover, as the primary source of energy, protein also plays an essential function in promoting the body's metabolism. Since proteins are involved in almost every aspect of the human body's activities, an accurate method for detecting is urgently required.

Liquid crystals (LCs) have been extensively investigated in numerous sensing applications as a rapid-response, high-sensitivity, and easy-to-manufacture material. The development of LCs encompasses a vast array of scientific fields and applications that satisfy the technological and socioeconomic needs.[1–3] LCs readily respond to external stimuli such as electromagnetic fields, pressure, surface effects, optical properties, temperature, and chemical analytes,[4–9] and these changes can be monitored by various characterization techniques. LCs amplify and convert molecular events occurring in the vicinity into detectable macroscopic signals, and their distinctive

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optical birefringence qualities form the basis of LC-based sensing platforms and are visible to the naked eye under a polarized optical microscope (POM).[10–14] However, POM method is difficult to observe tiny changes and has low sensitivity. Recently reported LC sensor based on the whispering gallery mode (WGM) due to the simple structure, high quality factor, and small mode volume of WGM optical microcavities have been widely used in a variety of fields.[15–18] On the basis of naked eye observation utilising POM, the optofluidic sensor is implemented by integrating the POM with the WGM laser spectroscopy, resulting in a more sensitive and precise characterisation.[19–23]

In this work, we presented a biosensing platform based on the LC-amplified optofluidic WGM resonator, as shown in Fig.1. The microbubble cavity was coated with the silanized alignment reagent, N,N-Dimethyl-N-octadecyl-3-aminopropyl trimethoxysilyl chloride (DMOAP). LC molecules can experience an orientation transition from homeotropic to planar by biomolecules attached to the internal surface of microbubble, resulting in a shift in the resonance wavelength. The dual effect between the WGM and the LC amplifies the changes caused by the introduction of streptavidin (SA) as protein molecules into the optofluidic resonator. The detection limit for SA is 1.25 fM (4.7×10^{-13} g/ml). In conclusion, our platform illustrates a flexible method for label-free, ultrasensitive, and real-time protein identification.

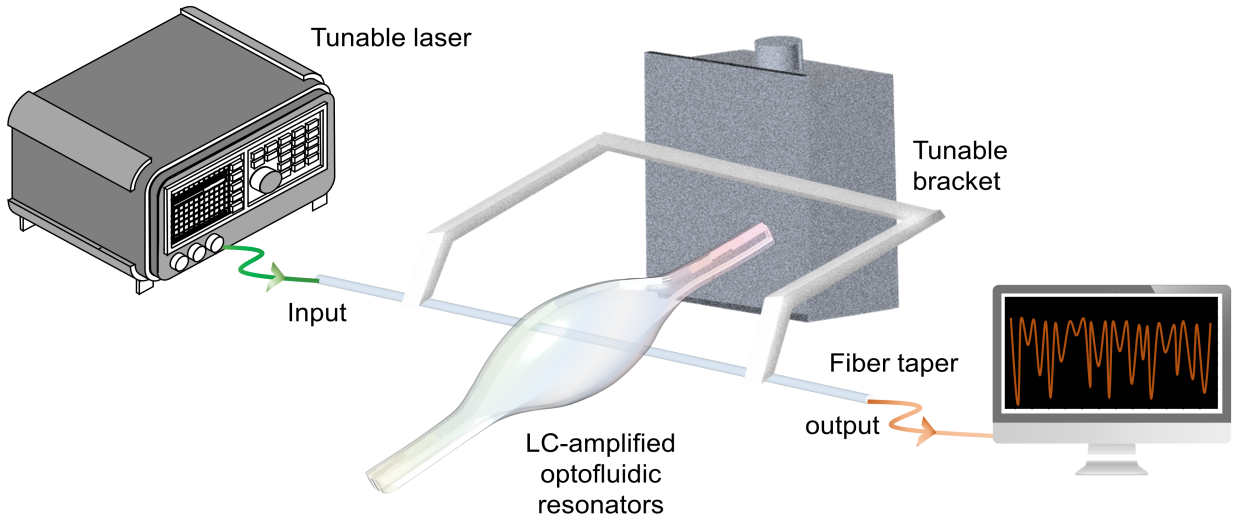

Figure 1. Schematic of the experimental platform. The light from the tunable laser was coupled into the LC-based optofluidic resonator through the fiber taper to obtain the WGM spectrum.

2. MATERIALS AND METHODS

2.1 Preparation of microbubble and fiber taper

The hollow microbubble cavities was employed as optofluidic sensors. Microbubbles can be created by heating and pressing the silica material to take advantage of its surface tension. A hydrogen-oxygen flame with a centimeter-diameter fire flame was used to heat and stretch the silica capillary. It was then switched to a small flame with a millimeter-diameter and a constant pressure was applied, and the expansion process was observed using a digital microscope. The width and wall thickness of the microbubble chamber can be changed during the expansion process by adjusting the stretching length, flame size, and extra pressure. Microbubbles with a diameter of 220 μm and a wall thickness of 3 μm were used in this article. After obtaining the microbubbles, both ends of the microbubbles are bonded to the copper plate with UV adhesive to prevent them from being destroyed. The microcavity is coupled via the tapered fibre coupling. A light taper machine can also be used to build the optical fibre cones with a diameter of 1-2 μm.

2.2 Functionalization of DMOAP and biomolecules

DMOAP at a concentration of 1 % (v/v) was injected into the microbubbles at a flow rate of 0.7 μL/min for 30 min using a syringe pump. The excess DMOAP solution was then rinsed with deionized water. The configured streptavidin with different concentrations was filled into the microbubble for 1 hour. Then the deionized water was used to remove excess protein molecules. Finally, LCs were filled into the micro-resonator at a flow rate of 0.7 μL/min for the protein sensing.

2.3 Optical Setup

The pump source, a tunable laser, was connected by the fiber taper to the micro-resonator. The high refractive index of LC molecules and the micro-thin wall of resonator enable the evanescent field to be coupled into the optofluidic resonator and used to monitor the changes of LCs.

3. RESULTS AND DISCUSSION

By detecting variations in the laser emission spectrum, the impact of the interaction between biomolecules and LC molecules was quantitatively studied. The shift in the laser spectrum is attributed to the change in the alignment of LCs due to the interaction between the biomolecules and the LC molecules. As a result, the information contained in biomolecules in the surrounding environment can be intuitively reflected in the change of the spectrum that is directly caused by the alteration of the LC orientation. Spectroscopy is a more reliable, sensitive, and accurate monitoring technique.

The DMOAP was used to functionalize the surfaces of microbubbles and induced the homeotropic alignment of LCs at the interface. With the attendance of biotargets, the orientation transition of LC molecules from homeotropic to planar and the wavelength shift of lasing spectra were induced, as shown in Fig. 2. In response to various concentrations of SA, spectral responses exhibited red-shift at first, then blue-shift characteristics, which resulted from the biomolecular random absorption and resonance frequency changes due to the LC reorientation.

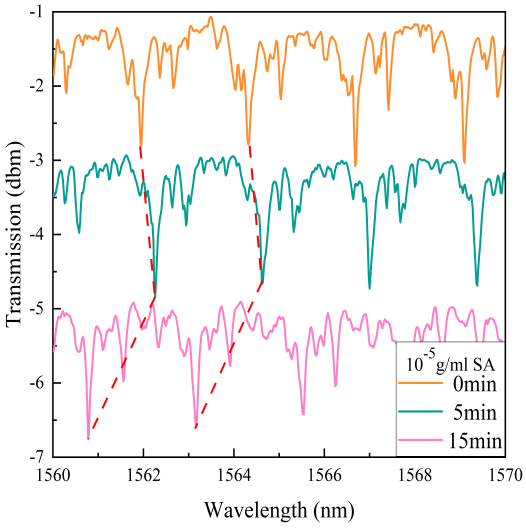

Figure 2. The WGM spectrum of the LC-based optofluidic sensor under 10^{-5} g/ml streptavidin.

In order to characterize SA concentrations, total wavelength shifts of WGM spectra (absolute value of red shifts plus absolute value of blue shifts) were used. As shown in Fig.3, signal responses at various SA concentrations (ranging from 10^{-12} g/ml to 10^{-3} g/ml) were observed for 15 minutes, respectively. The total spectral wavelength shifts and SA concentrations had a linear relationship within 10^{-8} g/ml to 10^{-3} g/ml, and the limit of detection for SA reached as high as 1.25 fM (4.7×10^{-13} g/ml). This suggests that LC orientation transitions and WGM spectra can effectively capture the slight variation in SA.

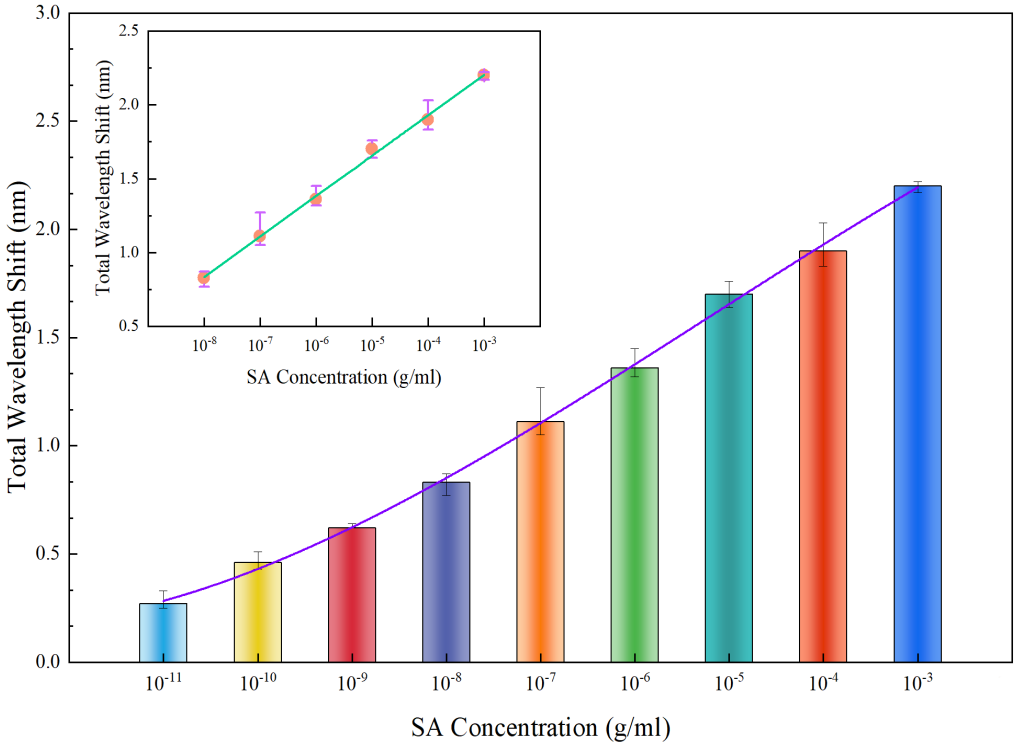

Figure 3. The overall wavelength shifts under different streptavidin concentrations (from 10^{-12} to 10^{-3} g/ml) under various concentrations of human IgG.

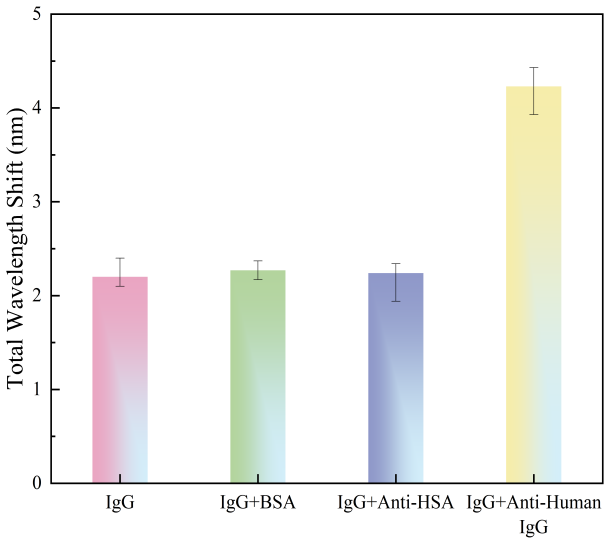

Figure 4. Relative spectral response of the specific binding of LC-based WGM biosensor against different substances.

In addition, we examined the specific ligand-receptor binding. Human immunoglobulin G (HIgG) and anti-human immunoglobulin G (AHIgG), are used to study the high sensitivity and specific biosensing capability of our sensing platform . As a substrate for modifying the inner surface of the microcavity, a certain concentration of HIgG was utilized. HIgG-modified microcavity was subsequently subjected to blank control group, anti-human albumin (anti-HSA), anti-mouse IgG, and AHIgG at the same concentration (10^{-3} g/ml), respectively.

Compared to the control group, only the AHIgG group displayed a pronounced wavelength response, as depicted in Fig. 4.

4. CONCLUSIONS

We have developed a highly sensitive optofluidic resonator that employs liquid crystal (LC) amplification for quantitative analyses of protein molecules. Proteins immobilized in modified whispering gallery mode (WGM) microcavity trigger the orientational transition of LC molecules, which can be amplified and used to assess the concentration of target molecules via wavelength changes. Streptavidin (SA) was taken as the example for the detection of protein molecules. The overall spectral shift increased linearly with the SA concentration and the detection limit can reach as low as the femtomole-level. Our LC-amplified WGM sensor is capable of detecting various types of protein molecules, which provides a high-sensitivity, fast-response and low-cost solution for the ultrasensitive label-free protein detection.

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