Detection of DNA Hybridization Using Liquid Crystal Based Whispering Gallery Mode Microbubble

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ABSTRACT

DNA detection based on DNA hybridization has wide applications in various fields, such as clinical diagnostics, food safety, and environmental monitoring, etc. At present, common DNA detection approaches include fluorescence-based microarray technique, electrochemical method and surface plasmon resonance (SPR), etc. However, these methods require additional precise equipments and relatively complicated detection process. In this work, we developed a biosensor platform based on a liquid crystal (LC)-amplified optofluidic whispering gallery mode (WGM) resonator to achieve ultra-sensitive, label-free, and quick-response DNA hybridization detection. Liquid crystal is a material with high sensitivity, rapid response, and low cost. It exhibits significant directional and positional ordering, and it is sensitive and responsive to external stimuli. LC molecules exhibit a uniform orientation on the surface of the resonator, when the surface is covered with an appropriate amount of ssDNA. Once complementary DNA and ssDNA hybridize on the surface, the homotropic orientation of the LCs will be destroyed. Due to the simultaneous interaction of the WGM and the LCs in the optofluidic resonator, changes caused by the DNA hybridization can be amplified, resulting in a shift in the resonance wavelength. In this experiment, we used the spectral wavelength shift as a sensing parameter to achieve the detection of target DNA, and a lower detection limit compared to traditional DNA detection methods was obtained. At the same time, this biosensor platform also shows good selectivity. Our research results suggest that the LC based WGM optical microcavity sensing platform can provide an ultra-sensitive, label free solution for DNA detection.

Keywords: Liquid crystal, Whispering-gallery mode, Biosensor, DNA hybridization, Optofluidic

1. INTRODUCTION

DNA-based biosensors are useful tools for molecular biomarker detection. As a kind of biological macromolecule, DNA has good stability, high specificity and excellent biocompatibility. Therefore, the detection of DNA targets has a wide range of applications in clinical detection,[1,2] food safety,[3] gene expression[4] and environmental monitoring.[5,6] At present, the most common DNA detection methods include fluorescence-based methods,[7–9] electrochemical methods[10] and surface plasmon resonance (SPR),[11,12] etc. However, these methods have the problems of high cost and complex operation, and there are still limitations in practical application. Therefore, in order to meet the above shortcomings and the growing demand for DNA testing in the era of precision medicine, we urgently need a low-cost, ultra-sensitive and stable DNA testing means.

Liquid crystal (LC) is a kind of ultra-sensitive, quick response and low cost material, which exists between liquid and crystal. Liquid crystals are easy to respond to external stimuli such as electromagnetic fields, pressure, surface effects, optical properties, temperature and chemical analytes, and we use the ordered arrangement and optical anisotropy of liquid crystals to realize the application of biosensing.[13–16] Compared with the detection using the traditional polarizing optical microscope, the liquid crystal based biosensing platform has higher

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sensitivity and lower detection limit, and it also has the advantages of low cost and fast response speed. At the same time, we introduce optical microcavities with whispering-gallery mode (WGM) into liquid crystal biosensing. The combination of LC and WGM laser emission produces an efficient and ultra-sensitive detection spectrum, improving the sensitivity of LC-based biosensors.

In this work, taking advantage of the high Q-factor, small mode volume, and strong evanescent field of WGM microcavity, we developed an LC-amplified resonator for ultra-sensitive biological detection, as shown in Fig. 1. The biomolecules attached to the inner surface of the functionalized microcavity can change the orientation of the liquid crystal molecules, resulting in a change in the resonance wavelength. Moreover, the thin microcavity wall allows the WGM spectrum to capture the interaction between light and matter and the orientation change of liquid crystal molecules, thereby enhancing the biomolecular signal and monitoring the small changes. Here, we realized the detection of DNA molecules with an order of 10 fM, which has a lower detection limit compared to the liquid-crystal based microarray technology.[17] Overall, our platform demonstrates a method for label-free, ultra-sensitive DNA hybridization detection. In addition, based on the widespread use of liquid crystals in the field of biosensing, this platform can also be extended to detect other types of biomolecules by changing the decoration of the inner surface of microfluidics.[18–22]

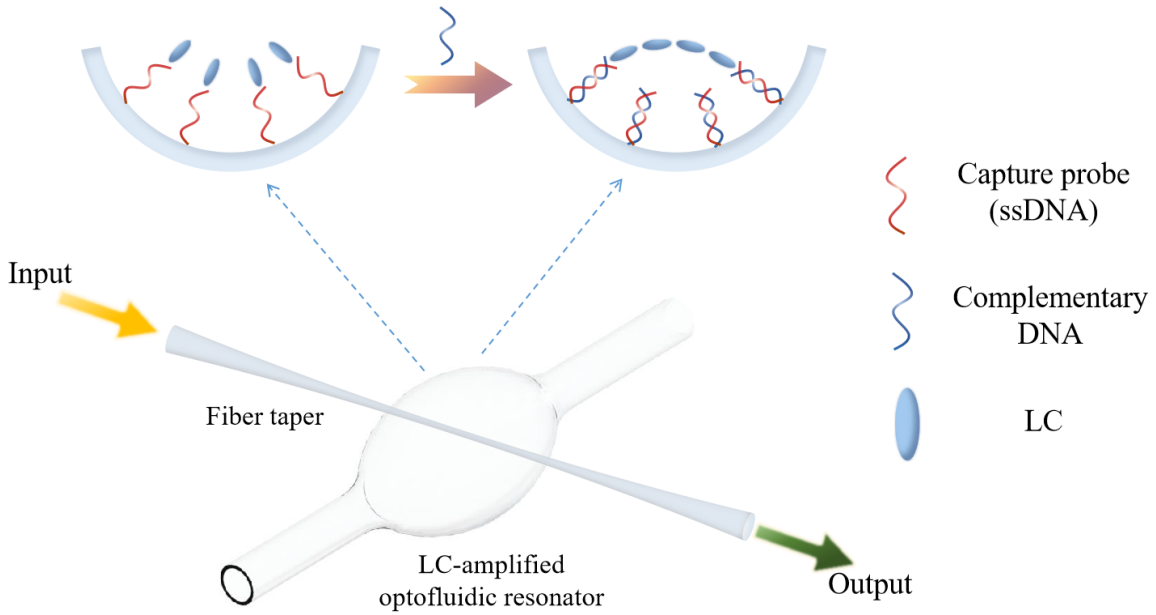

Figure 1. Schematic diagram of experimental principle. DNA hybridization disrupts the arrangement of LC molecules, and the light from the tunable laser was coupled into the LC-based optofluidic resonator through the fiber taper to obtain the WGM spectrum.

2. MATERIALS AND METHODS

2.1 Preparation of microbubble and fiber taper

In this experiment, we make a micro-resonator by microbubble. The preparation of the microbubble first requires the silica capillary tubes to be heated and stretched to a centimeter diameter using a hydrogen-oxygen flame. Then, it switches to a small millimeter-sized flame and simultaneously applies a constant pressure inside the capillary tube, using the surface tension of the silica tube to expand it to form microbubbles. The diameter and wall thickness of the microbubble can be adjusted by adjusting the drawing length, flame size or pressure size. In this paper, a microbubble with a diameter of 245µm is used. In addition, a fiber taper with a diameter of 1-2 µm was prepared based on the heating-stretching technique to excite the WGM.

2.2 Internal surface of the microcavity treatment and functionalization

A solution of DMOAP (N,-N-dimethyl-noctadecyl-3-aminopropyltrimethoxysilyl chloride) with a concentration of 0.5% (v/v) was first filled into the microcavity at room temperature for 10 minutes to produce the original vertical arrangement of LC molecules. After drying, the inner surface was continued to be modified for 1 hour at 55°C with an aqueous solution containing 1% (v/v) TEA and 0.5% (v/v) DMOAP for fixation of the ssDNA probe, and the microcavity was again rinsed with deionized water.

To fix the capture probe to the inner surface of the microbubble, the ssDNA probe(P1) was dissolved in a 20 mM Tris-HCl buffer solution containing 100 mM (pH=7.4). After incubation at 37°C for 2 hours, a buffer solution (2×SSC) containing 0.1% (v/v) SDS was used to eliminate unbound capture probes.

2.3 DNA hybridization

ssDNA targets T1, T2, and T3 were dissolved in 20 mM Tris-HCl buffer solution containing 100 mM (pH= 7.4), respectively. Solution was injected into the modified microbubbles, incubated at 37°C for 2 h, rinsed with buffer solution (2×SSC) containing 0.1% (v/v) SDS, and dried. Finally, LC was filled into the micro-resonator at a flow rate of 0.7 µL/min for DNA hybridization sensing.

Among them, the oligonucleotides used in this paper include 5'-NH_2-$(CH_2)_6$-GGGTATACAT TTGAA CC-CCA AT-3' (P1), 5'-ATTGG GGTTC AAATG TATAC CC-3' (T1), 5'-ATTGG GGTTC AAATG TCTAC CC-3' (T2) and 5'-TCGAA TTGGA CCCAC CGCGA AA-3' (T3) were synthesized by Sangon Biotech Co., Ltd.

2.4 Optical Setup

The tunable laser serves as the pump source and is connected to the micro-resonator by a fiber taper. Depending on the high refractive index of the liquid crystal molecule and the thin wall of the microresonator, the evanescent field can be coupled into the optofluidic resonator and can be used to monitor the change of the liquid crystal.

3. RESULTS AND DISCUSSIONS

DMOAP/TEA (alkane containing) decorated on the surface of the microcavity can form a self-assembled monolayer (SAM) film and can trigger the vertical arrangement of the liquid crystal. The TEA molecule can form covalent Si-O-Si bonds between its silane parts and between its silane parts and silanol groups on the glass surface. Moreover, SAM membrane can also provide aldehyde groups for covalent fixation of amine-labeled ssDNA. In addition, with appropriate chain length and surface coverage,[23] ssDNA can also contribute to the vertical arrangement of LC molecules due to its linear and flexible chain (longer than LC molecules). Therefore, when DNA molecules hybridize, the vertical orientation of LC molecules will change and appear as wavelength shifts in the laser spectrum. Thus, the information contained by the biomolecules in the surrounding environment can be intuitively reflected in the changes in the spectrum directly caused by the change in the orientation of the liquid crystal. Spectroscopy is a more reliable, sensitive and accurate monitoring technique.

We used P1 as a DNA probe to monitor changes in WGM spectra by changing the concentration of complementary DNA (T1).For different concentrations of T1, the spectral responses all showed a red-shift followed by a blue-shift trend, as shown in Fig. 2 (a). This is caused by changes in resonance frequency, which resulted from random absorption of biomolecules and reorientation of liquid crystals. Therefore, the total wavelength shift ((absolute value of red shifts plus absolute value of blue shifts) of the WGM spectrum is used to characterize the concentration of T1. The signal response at different concentrations of T1 (from 3×10^{-14} M to 3×10^{-8} M) was monitored for 6 minutes at a time, as shown in Fig. 2 (b). There is a linear relationship between the total spectral wavelength shift and the concentration of complementary DNA (T1). And the minimum concentration of observable spectral shift is about 30 fM (M), indicating that small changes in the concentration of T1 can be effectively captured by LC orientation transition and WGM spectroscopy.

In addition, we further investigated the high sensitivity and specific biosensing capabilities of the proposed method by repeatedly hybridizing ssDNA probes (P1) with complementary DNA (T1), single-base mismatch DNA (T2), and non-complementary DNA (T3). We exposed the P1 modified microcavity to T1 solution, T2 solution, and T3 solution with a concentration of 3nM respectively. And we found that only the T1 group

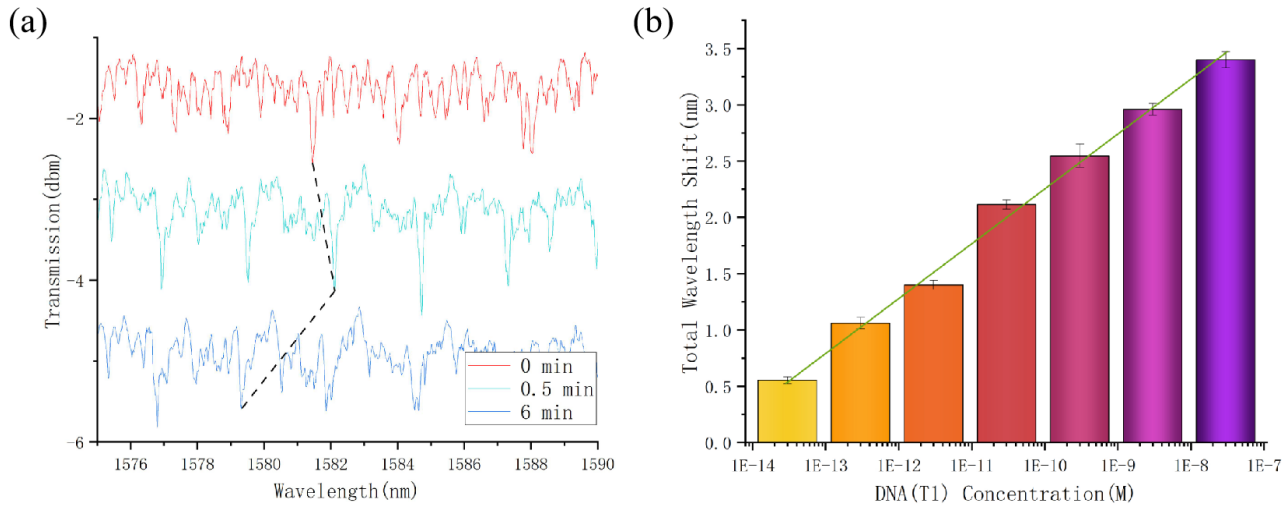

Figure 2. (**a**) The WGM spectrum of the LC-based optofluidic sensor under 3×10^{-8} M ssDNA T1. (**b**) The overall wavelength shifts under different concentrations of ssDNA T1 (from 3×10^{-14} M to 3×10^{-8} M).

showed a significant wavelength response, as shown in Fig. 3.Therefore, this indicates that this detection method is highly selective and can distinguish complementary DNA from other non-complementary DNA well. In addition, introducing nanoparticles such as AuNPs into this biosensing system can further amplify the alignment transition of LCs and the change of the signal in the WGM spectrum.[24]

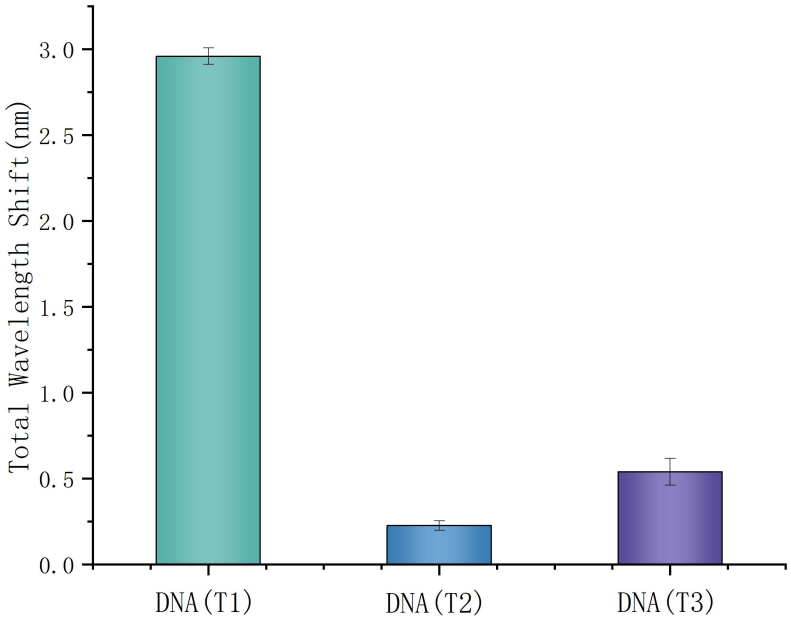

Figure 3. Relative spectral response of the specific binding of LC-based WGM biosensor against different substances. Total wavelength shift responses when LCs were exposed to 3 nM T1, T2 and T3.

4. CONCLUSIONS

We proposed a DNA detection platform based on a liquid crystal (LC)-amplified optofluidic whispering gallery mode (WGM) resonator. It can amplify changes triggered by the DNA hybridization, and the concentration of target DNA can be evaluated via spectral wavelength shifts. In this experiment, we used amine-labeled ssDNA to detect target DNA, and have obtained a lower detection limit compared to traditional DNA detection methods. Moreover, we have also verified the good selectivity of this biosensor platform. This LC based WGM microbubble sensor can detect different types of DNA molecules, which provides a new solution for the ultrasensitive label-free DNA detection.

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