

Aptamer Based Microfluidic Chip for Microbial Detection

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Abstract. Aptamer becomes a novel biosensor for detection of small chemical molecules, biological molecules, and cells. The integrating of aptamer biosensors with microfluidic chip for detection have attracted much attention due to their advantage of miniaturization, sensitivity and high specificity. Microbial detection is very important in the field of clinical diagnosis, environmental analysis and biodefense. It is highly desirable to develop a sensitive and rapid method for microbial detection. In this paper, we develop a novel method using aptamer based microfluidic chip for rapid and specific recognition of *Lactobacillus acidophilus*. It will contribute to the microbial detection portably and rapidly.

Introduction

Microbial infection can bring lots of diseases, and cause great economic losses in the public health all over the world [1]. As a result, microbial detection plays an important role in the prevention and treatment for infectious diseases.

The common method utilized in microbial detection is the conventional culture of microorganisms, such as microscopic observation, biochemical reactions and molecular identification. The process contains many steps including microbial culture, separating, purification and identification, leading to consuming time and high costs [2, 3].

In order to overcome the disadvantages in traditional methods, there are urgent needs to develop novel method to detect the microorganism with rapidness and sensitivity. The rapid development of aptamers and microfluidic chips provides a new approach to solve the problem through integrating aptamer biosensors with microfluidic chips.

Aptamers are nucleic acids that can bind to the target with good specificity and affinity, including small molecules, biomacromolecules and even whole cells [4]. Aptamers are selected from a random sequence oligonucleotide library via systematic evolution of ligands by exponential enrichment (SELEX) in vitro [5]. Aptamers are more appealing because of the unique advantages over antibodies, such as synthesis in vitro, nontoxicity, easier modification, and good maintenance [6]. With the feature of high specificity and affinity to the target molecule, aptamers become popular utilized in the field of biosensors as specific recognition elements [7].

Microfluidic chips are able to handle microliter or nanoliter volumes of fluids, using micro-channels with dimensions of micrometers. Microfluidic devices have the advantage of a greater surface-to-volume ratio, a faster mass and energy transfer, and manipulating and controlling the small volume of fluids precisely and flexibly [8]. Microfluidic chips have been widely applied in analytical chemistry, biotechnology, synthetic chemistry, and so on [9]. An ongoing central issue of microfluidics is integrating various function modules into the microfluidic chip, aiming to Lab-On-a-chip in order to realize the multiple function of the common biological and chemical experiment lab [10].

The infectious disease caused by the harmful microorganism can lead to the mortality of human beings and huge economic losses [11]. Developing rapid and sensitive method for microbial detection is helpful to prevent the infectious disease. The purpose of our study in this paper is to develop a

novel method using aptamer based microfluidic chip for rapid and specific recognition of the typical bacteria stain *Lactobacillus acidophilus*.

Materials and Methods

Bacterial Culture. The typical stain *Lactobacillus acidophilus* was purchased from CGMCC (China General Microbiological Culture Collection Center). The bacteria were cultured in typical MRS medium following the CGMCC's instructions under aerobic conditions at the temperature of 37 °C.

The bacteria were cultured in the medium overnight, and the subculture of bacteria was obtained. The cells were then centrifuged at the speed of 2500rpm and at the temperature of 4°C . After that, the bacteria were washed twice in the buffer (5 mM KCl, 1 mM CaCl₂, 1 mM MgCl₂, 140 mM NaCl, 20 mM Tris-acetate buffered saline, pH 7.4). At last, the bacteria were resuspended in the binding buffer.

Bacteria Preparation . The bacterial cells were stained by fluorescein isothiocyanate (FITC) solutions at the temperature of 37 °C for two hours, and washed twice by the buffer (20 mM Tris-acetate , pH 7.4). FITC was dissolved into acetone and pyridine immediately. And then the bacteria was incubated with the FITC solution for 15 min in the temperature 37°C. At last, the bacterial cells were rinsed three times with PBS solutions.

Fabrication of Microfluidic Chip . The UV-curable adhesive NOA81 (Norland Optical Adhesive) was studied to fabricate the microfluidic chip. NOA81 was cured by UV light exposure to forming a layer of thin film with a micro-structure. And then multilayer films were bonded together to fabricate the microfluidic chip. Next, avidin (1mg/ml) in the Tris-acetate buffer was injected into the microfluidic chip and incubated for one minute and then rinsed with binding buffer three times.

The aptamer labeled by biotin targeted to the bacteria was denatured by heating at 94 °C for one minute. And then the aptamer solution was put on the ice immediately for 10 minutes, following to incubate them for one minute and rinsed with the binding buffer three times.

Microbial Detection in the Microfluidic Chip. Bacterial cell solutions were injected into the microfluidic chip and incubated for one minute, and then rinsed with the washing buffer (Binding Buffer with 0.05% BSA). The fluorescence images of bacterial cells captured by aptamer immobilized in the microfluidic chip were obtained using an Olympus IX71 inverted fluorescence microscope.

Results and Discussions

Fabrication of Microfluidic Chip. The adhesive NOA81 was investigated as the material to fabricate the microfluidic chip. The black mask was designed as shown in Fig. 1 for fabricating the microfluidic chip by NOA81 for microbial detection using aptamer. The microfluidic chip has four micro-channels consisting four spots in which aptamers were immobilized on the glass surface in order to capture the microorganism specifically.

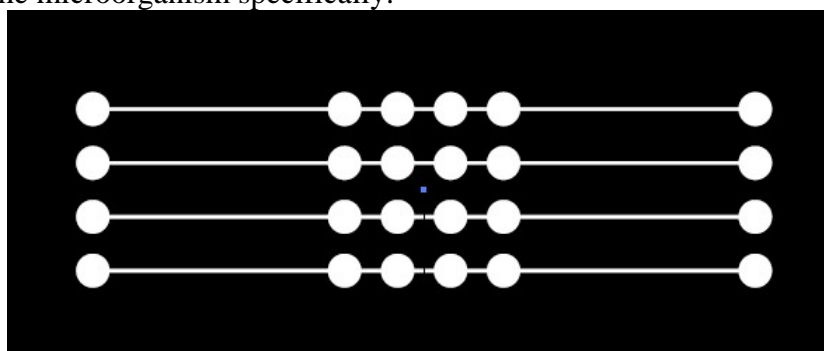


Fig. 1 Design of black mask and photograph of microfluidic chip

The diameter of detection spot was 500 μm , and the array spots were used to display the fluorescence signal of the bacteria captured by the aptamer. The width of micro-channel in the microfluidic chip is 50 μm . The aptamer targeted to the bacteria was modified with a poly T linker in order to keep the specific conformation of the aptamer targeted to the bacterial cell when it was immobilized on the glass surface of the microfluidic chip. The aptamer based microfluidic chip was fabricated as shown in Fig. 2.

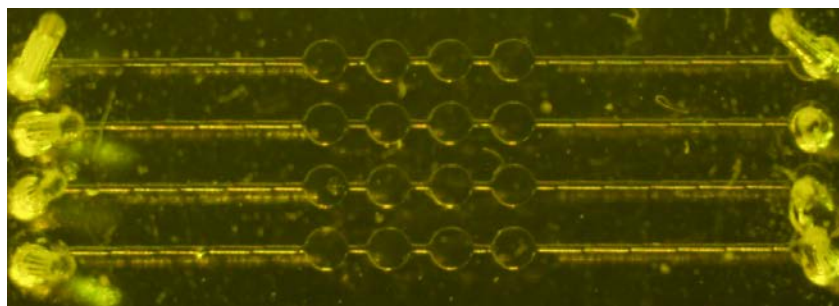


Fig. 2 Photograph of aptamer based microfluidic chip

Microbial Detection in the Microfluidic Chip. The solution of bacteria labeled the FITC was injected into the microfluidic chip. After washing three times, the non-target bacteria will be rinsed off, and *Lactobacillus acidophilus* will be captured by the aptamer immobilized in the microfluidic chips due to the specific affinity between the aptamer and the bacterial cells.

The fluorescence microscope was utilized to obtain the fluorescence signal of the bacteria captured by the aptamer in the microfluidic chip. As is shown in Fig. 3, the green fluorescent signal was obtained from the horizon of the fluorescent microscope in the microfluidic chip. And the fluorescent signals were distributed evenly in the array spots. It is inferred that the aptamer based microfluidic chip was able to detect the bacteria *Lactobacillus acidophilus* rapidly.

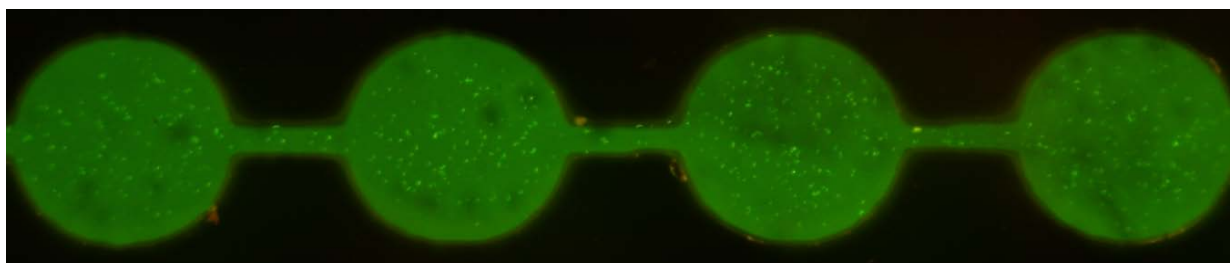


Fig. 3 Fluorescence image of *L. acidophilus* captured by aptamer in the microfluidic chip (20 \times)

Conclusions

In this paper, the aptamer was used as the specific recognition element integrated into the microfluidic chip for microbial detection. The results showed that the aptamer based on microfluidic chip was able to detect *Lactobacillus acidophilus* by the specific affinity between aptamer and bacterial cells. It is helpful to develop the portable, rapid, and sensitive device for microbial detection.

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