Sensors and Actuators B 245 (2017) 695–701

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

Microchip device with parallel operation for bacterial chemotactic analysis

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ARTICLE INFO

Article history:
Received 31 August 2016
Received in revised form 7 January 2017
Accepted 25 January 2017
Available online 26 January 2017

Keywords:
Microchip
Bacteria
Capacitance change
Chemotaxis

ABSTRACT

In this study, we developed a novel microchip device system that enables the analysis of a large amount of bacterial chemotaxis data, which is one of the most fundamental behaviors of bacteria. Chemotaxis involves sensing of chemical gradients and behavioral adjustment by bacteria. In the proposed system, we conducted parallel analysis of bacterial chemotaxis without microscopic observation. We first confirmed that a simple microchannel could be used to analyze bacterial chemotaxis by microscopy. Next, we developed a system involving the photolithography technique and sputtering, and counted bacterial numbers without microscopic observation by measuring the capacitance between the two microelectrodes. Furthermore, we developed an integrated microchip for bacterial chemotactic analysis and successfully analyzed motile bacteria with single and parallel operation. This device may achieve large-scale analysis by parallel operation and may be useful for high-throughput analysis of unknown bacterial chemotaxis for identifying new attractants and repellents.

1. Introduction

Many bacteria are motile and can sense chemical gradients. Therefore, bacteria can move towards attractants and travel away from repellents. This behavior is known as chemotaxis [1–3]. Chemotactic analysis is important for applications in infection defense against pathogenic bacteria [4], contributes to the understanding of an ecosystem [5,6], and allows for the isolation of useful bacteria for use as biosensors [7,8].

Recently, studies utilizing and controlling bacteria using a micro-robot have been conducted. A technique for transporting drugs to an affected area was proposed as a drug transport robot [9–13], which involved binding of the bacteria to a carrier that encapsulated a drug. Chemotaxis attracts much attention as a method that can be used for transport control [14–16]. It has also been reported that infection efficiency can be increased using chemotaxis in pathogenic bacteria [5]. In contrast, bacteria that produce useful substances have been reported from the perspective of green technologies such as bioremediation [17], and it is possible to use chemotaxis as a separation and control method for these novel useful bacteria. Measuring the chemotactic response of bacteria can be used for preventing infection by pathogenic bacteria by placing a repellent in the affected area and for separating useful bacteria.

Methods for analyzing the chemotactic response typically include the agar plate culture method [3,18], two-chamber culture method [19], and micro-video recording method [20]. In order to analyze bacterial chemotaxis, counting of bacterial numbers is necessary. The agar plate culture method and two-chamber culture method require long periods for counting of bacterial numbers since culture time is needed. The micro-video recording method could analyze without long culture time, though this method could not exam several condition at the same time. To overcome this limitation, several microchip devices were proposed. The microchip devices have a potential for analyzing chemotac-

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http://dx.doi.org/10.1016/j.snb.2017.01.159
0925-4005/

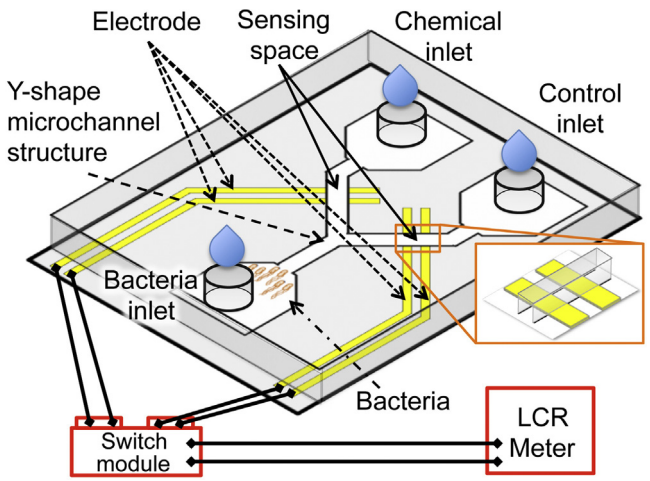

Fig. 1. Schematic illustration of the proposed microchip.

tic behavior directly (without long culture) in multiple condition. Mainly, the devices depend on flow-based and diffusion-based methods; in the case of the flow-based method, mixed laminar flows generated a gradient [21,22], and thus the system can be used to precisely control the gradient. External equipment, such as syringe pumps and tubes, are needed and cause difficulties in preparation and operation. In the diffusion-based method, a non-flow environment can be created [23–25]. Therefore, the system does not influence cell behavior. The system requires less external equipment and is easier to prepare. However, in both cases, microscopic observation is required, increasing equipment costs even if the diffusion-based method is used. Furthermore, it is difficult to miniaturize such equipment, and therefore parallel operation of the microchip device and mobile device are impossible (or limited). New methods are important for improving the microchip device.

In this study, we built a novel chemotactic analysis system that is compact and parallelizable for simultaneous multi-sample. We integrated chemotactic analysis using a simple microchannel involving diffusion-based methods and cell counting by measuring the capacitance between two electrodes. We first confirmed that the simple microchannel could be used to analyze bacterial chemotaxis by microscopy. Next, we built a chip system involving microelectrodes and the photolithography technique. In our constructed system, we counted bacterial numbers without microscopic observation by measuring the capacitance between two microelectrodes. Furthermore, we demonstrated bacterial chemotactic analysis using the proposed device and successfully analyzed single and parallel operations.

2. Materials and methods

2.1. Design of micro device with microchannel

Fig. 1 shows a schematic illustration of the proposed microchip. This device was mainly composed of two parts. One is a Y-shape microchannel structure with tree chambers, which is used to analyze bacterial chemotaxis. The other is a pair of electrodes on glass substrate, which is used for sensing bacteria number. The pair of electrodes sandwiched the sensing space and were used to measure the capacitance. The pair was placed on the glass substrate and located to the bottom of the Y-shape structure. First, bacteria in motility-medium were introduced to the bacteria inlet (left side), while chemical (serine (Ser), aspartic acid (Asp)) and buffer solutions were introduced to chemical inlet and control inlet (right side), respectively. Bacterial number in the sensing space was measured as the capacitance between the electrodes.

2.2. Fabrication of microchannel for chemotaxis assay

The micro channel was fabricated in polydimethylsiloxane (PDMS; SILPOT 184, Dow Corning Toray Co., Tokyo, Japan) by soft lithography. PDMS is a suitable material for constructing a structure that is compatible with living organisms because it is non-toxic, transparent, and permeable to oxygen and carbon dioxide [26].

The mold for the microchannel was fabricated on a silicon substrate using SU-8 3050 (Microchem Co., Westborough, MA, USA). The mold was a Y-shape convex for channel with three chambers. The channel size is 100 μm in width and 100 μm in height for easy handling of solutions and effective observation of micro channel (If a narrow channel is used, high presser is needed to introduce solutions. Furthermore, the maximum viewable field is around 100 μm^2 in our observation system.) (Fig. 2A). The PDMS (ratio of base polymer to cross-linker of 10:1) was poured onto the mold substrate and cured at room temperature for 24 h. After curing, the PDMS replica was peeled off from the master mold and cut into the piece containing a pattern, the Y shape microchannel. Finally, the replica was attached to the glass substrate on which the electrode was fabricated (Fig. 2B). For this bonding process, 3 min plasma hydrophilization treatment (PIB-10 with hard mode, Vacuum device, Japan) was applied to both PDMS and glass substrate surfaces.

2.3. Fabrication of electrode pair

A pair of electrodes was fabricated in chromium (Cr) and gold (Au) on a glass substrate using the lift-off method; next, the following fabrication process was used. First, the photoresist AZ 5214 (AZ Electronics Materials, Luxembourg, Luxembourg) was coated on the glass substrate. Second, the photoresist was exposed under UV light to form the electrode pattern. Third, the photoresist was developed using an AZ 300MIF developer (AZ Electronics Materials) and then the pattern was formed on the glass substrate. Fourth, Cr was sputtered (8 min), after which Au was sputtered (4 min). Finally, the photoresist was removed by an ultrasonic cleaner, and then Cr and Au electrodes remained on the glass substrate. The electrodes on the glass substrate were connected to wires by silver (Ag) paste. For precise fabrication and durability, the electrode was 600 μm wide and 176 nm in height. A gap of 300 μm was used to measure bacterial numbers between the electrodes (Fig. 2A and B)

2.4. Bacterial strains and culture

Escherichia coli strain RP437 [27] and *Vibrio alginolyticus* strain VIO5 [28] were used. *Escherichia coli* were grown in tryptone broth (1% tryptone and 0.8% NaCl) and the chemotaxis buffer contained Na+-motility medium (10 mM potassium phosphate (pH 7.5), 0.1 mM ethylenediaminetetraacetic acid (EDTA), and 85 mM NaCl). Bacterial cells were grown overnight in growth media with shaking, then diluted to 1/50 in fresh growth medium and cultured again. After 3 h, the cells were harvested by centrifugation. Next, the cells were suspended in TMN 300 buffer to an optical cell density of OD_{660} = 10. For culturing *V. alginolyticus*, the growth media was VPG (1% (w/v) polypeptone, 0.4% (w/v) K_2HPO_4, 3% (w/v) NaCl, 0.5% (w/v) glycerol) and the chemotaxis buffer was TMN (TMN 300 buffer; 50 mM Tris-HCl (pH 7.5), 5 mM $MgCl_2$, 5 mM glucose, 300 mM NaCl). All chemicals were dissolved in deionized distilled water and the pH was 7.