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**REVIEW**

# Advance in Bacteria Chemotaxis on Microfluidic Devices

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**Abstract:** Chemotaxis is the response ability of motile cells to chemicals gradients in environment and the migration toward higher concentration of chemoattractant or lower concentration of repellent. This mechanism is a basic nature of microorganisms to adapt to the environmental changes. The research of microbial chemotaxis is of great significance in utilizing bacteria to solve environment problems, control the pathogen infection, and develop microbial industrial projects. Microfluidic devices can realize qualitative and quantitative detection of bacterial chemotaxis. In comparison with traditional detection methods, microfluidic assay has an accurate control over bacterial microenvironment with higher sensitivity. In the past few years, the study on bacterial chemotaxis based on microfluidic assay was developed rapidly. In this paper, the microfluidic chemotaxis detectors that appeared in recent years were introduced from the aspects of chip structure, working principle and their applications. Finally, we provided insights into the challenges of bacterial chemotaxis and provided future perspectives.

**Key Words:** Microfluidics; Bacteria; Chemotaxis; Microenvironment; Review

## 1 Introduction

Suitable natural environment for bacteria to grow and reproduce must have certain concentration of irons and nutrients, and bacteria can feel a chemical stimulus in their environment and move along the chemical concentration gradient. This kind of response is called chemotaxis[1]. Positive chemotaxis occurs if the movement is towards a higher concentration of the chemical substances like food molecules, whereas negative chemotaxis occurs if the movement is in the opposite direction to flee from poisons. In this way, bacteria can survive by selectively adsorbing on the surface of some algae, crustaceans, shellfish and other plants. Therefore, chemotaxis is an important ecological effect for bacteria to settle in a particular environment. Moreover, chemotaxis of bacteria is very useful in the applications, such as in situ biological decontamination, formation of biofilm, pathogenesis of infection, function of nitrogen fixation, transfer of microorganisms in underground and soil environment, and microbial oil recovery, etc[2].

There are many traditional methods for characterizing bacteria chemotaxis[3], e.g., capillary assay, stopped-flow diffusion chambers, and swarm plate assay, which are easy to operate and well-developed, but can only be carried out as qualitative analysis methods of chemotaxis, other than quantitative detection. Tethering assay and automated tracking of swimming cells can track and observe the movement of a single cell, and provide methods for the studies of bacteria chemotaxis mechanism and theoretical model. But tethering assay spends much more time on the observation process, has poor reproducibility on detection of cell movement, and can not response to the concentration gradient. The automated tracking of swimming cells can only track one cell at one time, and depends on highly complex devices. Hence, it is difficult for the above traditional methods to detect bacteria chemotaxis in a quantitative mode and characterize movement of bacteria precisely.

The advent of microfluidics technology has led to new technologies in many fields[4]. It now becomes one of the hottest frontier technologies because it provides rapid analysis with advantages such as low cost, energy-saving, good reproducibility, high-throughout, multifunction, high-integration

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and running under physiologically relevant conditions. Therefore microfluidics technology provides a new platform for the qualitative and quantitative study of bacteria chemotaxis.

## 2 Materials and structures of microfluidic chips for chemotaxis

Material, structure and fabrication methods of microfluidic chips depend on its function. In the application of bacteria chemotaxis based on microfluidics, it is necessary to generate controllable, stable and quantitative concentration gradient of attractants in the microchannel, and establish micro-environment in the channel to keep bacteria in a normal physiological structure with normal physiological functions, moreover, move freely in the channel. Therefore, in the researches of chemotaxis, we need to decide and fabricate channel structure to form the desired flow state, mixing mode, contract method of bacteria and attractants, and concentration gradients.

### 2.1 Chip material for chemotaxis study

With the development of microfluidic platform applications, the right selection of the material has to consider from the start point to the end. Navigating material principles for microfluidic technology are generally as follows: (1) good chemical and biological compatibility with samples and/or medium; (2) good heat conductor but electrical insulator; (3) excellent optical properties, little or no interference to detection; (4) ease of modification on the surface to generate electroosmotic flow or immobilize biological molecules; (5) ease of fabrication and low cost[5]. In case of characterizing bacteria chemotaxis, the interactions between bacteria and chip material must be considered in addition to the above conditions. Hence, bacteria can maintain their normal biological activity and move freely instead of adsorbing or adhering to the channel surface.

Currently, the materials used in microfluidics include quartz, glass and polymers, such as polymethylmethacrylate (PMMA)[6,7], polycarbonate (PC)[8], polydimethylsiloxane (PDMS)[9] and so on. Quartz and glass are the first materials used for microfluidics because of good electrical permeability and optical quality, good surface absorption and surface reaction ability, as well as chemical resistance. Therefore, they are usually used as substrate for characterizing bacteria chemotaxis. PMMA, also known as acrylic or acrylic glass, displays excellent optical transmittance for visible light, good impact strength and durability, but is not suitable for most biological applications due to low biocompatibility, plastic deformation and difficulty for biochemical modification, which result in bad reproducibility[6,7]. PC is another thermoplastic alternative to glass, because it offers good light permeability, heat and oxidation resistance, excellent material toughness properties, good mechanical properties in the suitable temperature range. However, PC is limited by its intrinsic drawbacks in stability to hydrolysis resistance, abrasion and chemical resistance[8]. PDMS, also called silicone rubber, appears as the preferred material in laboratories. PDMS displays excellent optical properties for convenient fluorescence detection. Owing to its gas permeability, PDMS appears as a candidate material for many cellular studies. It provides soft bonding pathway by plasma oxidation, which does not need adhesive or heating. However, PDMS has own disadvantages such as hydrophobicity, which results in bubble accumulation inside of the channels; absorbing surrounding organic solvent and small molecules, such as bimolecules and some drug molecules in the channel. Surface modification can fix the above issues by using silicon atoms[10], polyelectrolyte multilayer film[11], surfactant-coating[12], or phospholipid bilayer membrane[13].

### 2.2 Special on-chip structures for chemotaxis

Besides materials and temperature, the characteristics of bacterial physiological activity have to be considered. Bacterial chemotaxis studies based on microfluidics in recent years showed that bacteria with different physiological characteristics requireded different experimental conditions, thus structure and inner environment of the channel should be accurately designed and fabricated[14].

Bacteria tend to move towards dead-end channels with a width and height matching to those of the cell body, which results in high bacterial concentration in this region. To explore bacterial behaviors in the micro-structure, Park *et al*[15,16] designed a structure (Fig.1A), in the middle of which there was a 250 μm × 250 μm square reservoir and a 40-μm-wide channel on it to link the inner and outer space. After 3 hours, the density of cells was more than seven times greater inside than outside due to bacterial chemotaxis. Based on the above study, the authors designed another interesting maze structure, by which their analysis indicated that for a small volume connected by a small opening to a large volume, such as their enclosure within a relatively large microfluidic chamber (Fig.1A) or the dead ends in a maze (Fig.1B), because the bacteria were attracted to each other due to their secretion of amino acids, such as glycine, which were chemo attractants. Hence, the characteristics of bacteria movement should be taken into account to avoid the dead corner of channel, resulting in accumulation of bacteria, or to design concentration structure by using this feature to make bacteria getting together. So it is convenient to carry out quantitative analysis of bacteria and effectively improve detection limit.

On the other hand, bacteria can pass a narrow microchannel by growth or changing their own shapes[17,18]. Männik *et al*[18] designed microfluidic chips with submicron channels. As shown in Fig.1C, the bacterial movement from left to right was observed in array structures consisting of multiple