〈Mini-Review〉

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Bacterial Concentration Analysis by Dynamic Guiding in Flow System

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Abstract

In this review, we have summarized the analytical methods for bacterial concentration measurement in the flow system based on dynamic guiding of the target bacteria to the aimed position by external fields such as electric field, magnetic field, pressure, light, *etc*. All the methods commonly increase the efficiency of detection and measurement by guiding bacteria into the observation region. In particular, the analytical method using the photothermal assembling (PTA) enables contactless and remote accumulation of bacteria by laser irradiation. Moreover, PTA allows us to rapidly and conveniently estimate the bacterial concentration in a droplet system even in the absence of a microchannel. By using these methods complementarily, it is expected that the efficiency of bacterial concentration measurement will be dramatically increased.

Keywords Bacteria, counting, detection, microfluidic, flow assay

1. Introduction

In recent years, the damage caused by bacterial infection still remains the leading cause of death worldwide, and the preventive testing of food and drinking water remains highly important [1]. Currently, the low-cost and easy culture methods are the mainstream in the field of detection and measurement of bacteria, but it takes several days for the culturing process to sufficiently increase the number of bacteria before counting [2]. Since this time lag is a serious obstacle in preventing bacterial infections, a rapid bacterial detection method is strongly desired. It is needless to say that simplicity such as that anyone can easily handle the detection system, as well as the cost reduction, is necessary in the clinical test. Therefore, it is necessary to detect the bacteria not only in water but also in liquids containing many dispersed foreign substances and contaminants such as biological fluid (blood, saliva, stomach fluid etc.). The removal and/or extraction of such concomitant are generally needed in order to accurately detect bacteria and measure bacterial concentration. As described above, many demands are imposed on the bacterial detection, and the development of detection methods that can comprehensively resolve them is desired.

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In this minireview, we will introduce some of the latest technologies developed to overcome these various problems. Among the technologies, we have primarily focused on the detection method developed by our group [3], which provides high efficiency analysis by guiding bacteria toward the observation region using a external field-driven "flow". After introducing researches using electric field, magnetic field and pressure in the microchannel [4,5,6], we will introduce our latest achievement using light-induced "flow" in a droplet [3].

2. Various bacteria detection and counting methods using microfluidics

First, we introduce a bacterial detection method of large amount of samples, which is specialized for continuous water quality inspection for a long period of time by combining microflow channel and isotachophoresis [4]. The isotachophoresis is a technique for guiding positive or negative charged substances in a liquid to each corresponding electrode by applying a DC voltage to the microchannel. In this technique, positively charged antimicrobial peptides labeled by a fluorescent dye are guided to the negative electrode by isotachophoresis. A pressure-driven flow is introduced from the negative electrode, where the target can be captured at the position with the balance of the electrostatic force and the force by pressure-driven flow (**Figure 1**). When the dispersion liquid of bacteria flows from the negative electrode side,

bacteria can be guided to the positive electrode side by alternate application of the electrostatic force and pressure driven flow since the surface of bacterium usually has a negative charge [7]. As the bacteria pass through the capture region of the antimicrobial peptide, they were bound with the peptide and stained with the fluorescent dye. Thereafter, by performing fluorescence sensing in the same channel, the bacteria in the liquid was detected and the bacterial concentration was measured from the number of bacteria passing through the microchannel. By using this method, the detection of bacteria in water was continuously carried out for about 1 hour, and the measurement limit of concentration was reached to 10^4 cells / mL.

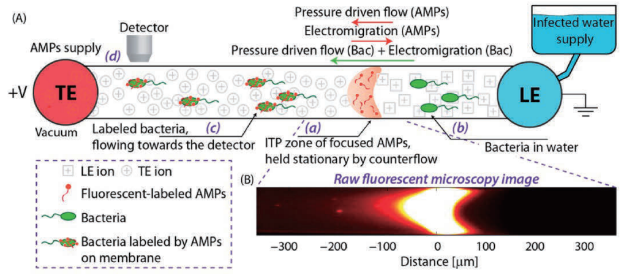

Figure1. Scheme of continuous bacteria detection using antimicrobial peptides and isotachophoresis. Adapted from ref. [4] with permission from ACS.

Next, we explain bacterial detection method which can rapidly respond to the low concentration pathogenic bacteria contained in food *etc*. with magnetic nanoparticles and a cylindrical microchannel prepared by 3D printing technique [5]. The magnetic nanoparticles modified with the antibody were attached to the inner wall of the flow channel *via* the magnetic interaction with the permanent magnet inserted in the hollow cylindrical microchannel (**Figure 2**). Thereafter, by injecting the bacterial dispersion into the flow channel, only the specific bacteria were collided with and adhered to the magnetic nanoparticles, where they continued to stay in the flow channel. After injecting all the sample solution into the channel, the trapped bacteria were released into the liquid with the adhered magnetic nanoparticle after detaching the permanent magnet. Thereafter, the bacterial dispersion was recovered from the outlet and the ATP emission intensity was measured using luciferase to detect bacteria and to measure their concentration (only 1 mg of luciferase is several hundred dollars). The authors stressed that a 10 mL of solution sample extracted from the food could be measured within 10 minutes by this method, and that the limit of concentration of which was as low as 10 cells / mL. However, this method requires the measurement with a luminometer in addition to taking out the aggregated magnetic nanoparticles with bacteria. The problem is the complexity of the post-treatment and cost of these process. Before the detection using the luminometer, ATP of the *Salmonella* bacteria was extracted by putting the aggregates of magnetic nanoparticles with bacteria into the benzalkonium chloride solution. After that, the solution of extracted ATP was mixed with lyophlized luciferin and luciferase powder to observe the luminescence intensity. Also, the specific detection of a target bacterium (*Salmonella* bacteria) was performed in the case of mixed condition with 10^5 cfu / mL of other bacteria (*Vibrio* bacteria or *Escherichia coli*). This research group also performed the selection of bacteria with magnetic nanoparticles using other cylindrical microchannels and Dean drag force as a fluidic effect [8].

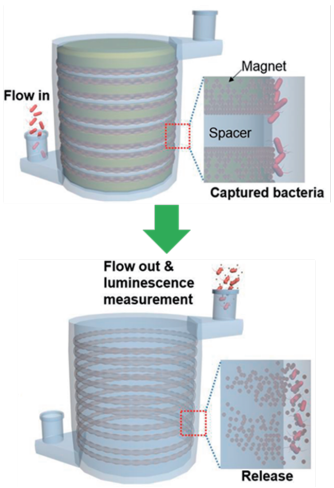

Figure2. Scheme of the high-capacity efficient magnetic separator. Adapted from ref. [5] with permission from ACS.

Finally in this section, we show the other research example with a microchannel for 1 cell/mL bacterial detection similarly to a flow cytometry [9], where an enzyme exhibiting fluorescence that stains only the specific bacteria and eluates from them was enclosed in a water-in-oil emulsion (**Figure 3**) [6]. This method was basically developed for the analysis of blood sample and, therefore, it could be applied to practical clinical examination. The reason why the emulsion was used is that the fluorescence intensity can be enhanced by confining the eluate from the bacteria within a narrow region to promote the reaction with the enzyme. The fluorescence from each generated emulsion can be examined using the 3D particle counter developed by this research group [10]. Assuming that 1 cell of bacteria was confined in an emulsion and emitted fluorescence, it is possible to detect and measure bacteria from 1 cell at minimum. In addition, only a specific bacteria can be detected by carrying out similar comparison experiments with plural types of bacteria. However, the measurement time is slightly long since it takes 1.5-4 hours to analyze several mL of a liquid sample.

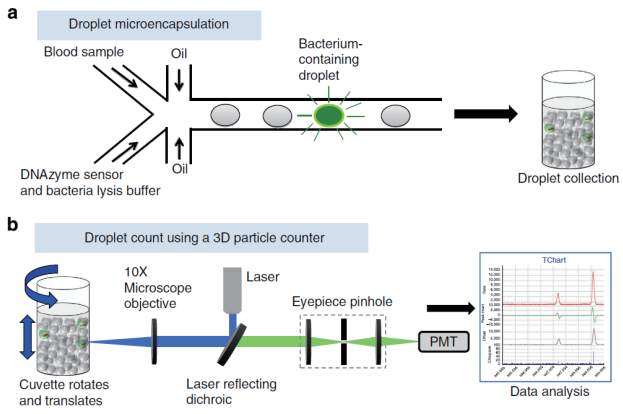

Figure3. Blood samples and DNA enzyme sensors are mixed and then encapsulated of millions of micrometer-sized droplets. Adapted from ref. [6] with permission from NPG.

3. Photothermal assembling of bacteria

Our group has also developed a method of measuring bacterial concentration using light-induced "flow". The method has a great advantage in the respect that it can guide bacteria to the observation site remotely in a droplet system without using a microchannel (**Figure 4**) [3]. The "flow" used here is photothermal convection arising from the local heat which is generated by irradiating a gold thin film with laser light. A submillimeter bubble was also generated during the irradiation. The bubble was used as a stopper for the dispersed small objects flowed by the photothermal convection (**Figure 5**).

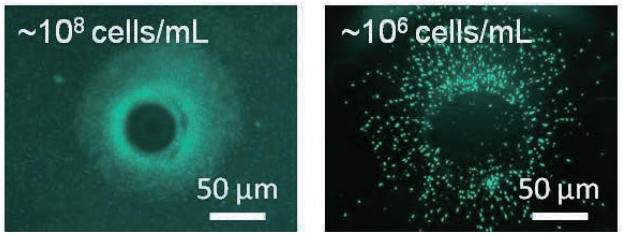

Figure4. Preliminary experiment of photothermal assembling of bacteria with fluorecent dye (SYTO9-stained *E. coli*). Adapted from ref. [3] with permission from OSA.

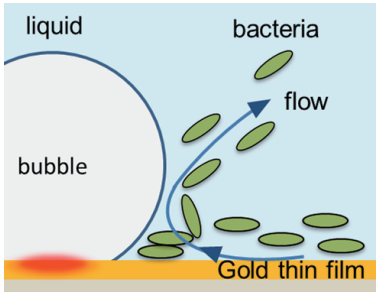

Figure 5. Scheme of photothermal assembling of bacteria in a droplet.

Now, we explain the principle of bacterial concentration measurement in this method. The concentration of the dispersion in the liquid gathered by the photothermal assembling (PTA) method can be estimated from the preliminary obtained assembling rate of similar-sized microparticles. Such a principle can be used for the estimation of bacterial concentration in the liquid. As a preliminary result, the highest aggregation efficiency was estimated as 2% with test microparticles by optimizing the laser power and the laser irradiation time. Using this value, bacterial concentrations were estimated from the number and volume of the assembled bacteria around the bubble by light-induced convection. The

estimated values were compared with the results obtained by a conventional culture method, and the PTA results agreed well with the cultivation results with 95.7% accuracy. (**Figure 6**). The time required for the measurement *via* PTA is only a few minutes. The advantage of this method is that the operation is simple and label-free without fluorescent dye.

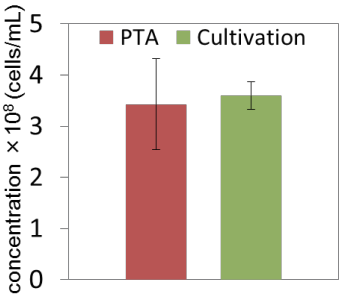

Figure 6. Main experiment of bacterial counting without fluorescent dye (*Pseudomonas aeruginosa* was used as the target). Comparison between photothermal assembling (PTA) method and cultivation method. Adapted from ref. [3] with permission from OSA.

5. Summary and prospects

In summary, we outlined several detection and concentration measurement methods based on dynamic bacteria guiding methods using external fields. At present, detection methods capable of comprehensively covering all fields such as food, water quality, and clinical testing have not been established, where the specialized method was individually developed for each application field.

As introduced in the four examples using electric field, magnetic field, pressure, and photothermal effect, the detection of bacteria using water or blood as a dispersion medium has been able to meet various demands such as the detection speed, the quantitativeness, the measurement limit of concentration, and the selectivity. Particularly, the label-free detection technique with photothermal assembling enables very rapid bacterial counting with a few minutes while the conventional culture method takes a few days. Furthermore, this method exhibits the high quantitativeness comparable to the culture method.

In addition, the extension of the limit of concentration measurement has been greatly improved according to the development, and the measurements on the order of 10^4 cells/mL bacteria have been possible with various methods. Meanwhile, although 1 cell/mL detection has been available in the specialized system, but the measurement time over an hour is the current important problem. Also, the selectivity can be increased by using enzyme or antigen-antibody reaction for detecting only specific bacteria, and the size selection can be also performed by using hydrodynamic driving force such as Dean drag force in a microchannel.

Although the methods introduced in this paper are only a part of the latest bacterial detection methods with the flow system at the laboratory level, in the future, it is expected that the continuous development based on these fundamental results will lead to a practical application of rapid, simple, and low cost bacterial detection method replacing the culture method. We hope that our review would give a chance to readers thinking about the future prospects of the bacterial counting and detection technology using flow system by overlooking the methods for guiding bacteria *via* the external field.

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