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Microfluidics-Based Lysis of Bacteria and Spores for Detection and Analysis

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Abstract

The disruption of the membrane/coat, or lysis, of bacteria and spores is often a critical step for analyzing the intracellular molecules such as proteins and nucleic acids. In this chapter, we review recent advances in the application of microfluidic devices for lysis of bacteria and spores. We divide existent devices and methods into five categories: mechanical, chemical, thermal, laser, and electrical. We also point out future directions in this field.

1. Introduction

Rapid detection and analysis of pathogenic bacteria/spores has become increasingly important for applications ranging from public health and food safety to biological weapons defense. Traditional methods for the detection of bacteria on foods rely on culturing and plating of the bacteria followed by identification using biochemical or serological assays. These cultural methods are time consuming—taking three days to determine a total viable count and five to seven days to detect specific pathogenic bacteria (Kaspar and Tartera 1990). Recent advances in the development of rapid detection tools have dramatically improved both the speed and sensitivity (Swaminathan and Feng 1994; Ivnitski et al. 1999). Most of such assays are based on the detection and analysis of proteins and nucleic acids. For example, enzyme-linked immunosorbent assay (ELISA) has been used to identify bacteria by detecting their binding to the surface immobilized antibody (Bhunia 1997; Gehring et al. 1998, 2004). Gene probes are also used to recognize and bind to nucleic acid targets. Polymerase chain reaction (PCR) based DNA hybridization methods have shown superb sensitivity and specificity (Tietjen and Fung 1995; Fratamico and Strobaugh 1998; Fratamico 2003).

Although the size of bacteria cells is much smaller than those of animal and plant cells, they have almost the same structure from a morphological point of view. Depending on the type of the bacteria cells, generally, a barrier called a capsule (or outer membrane, cell wall, plasma membrane) composed of polysaccharide and/or proteins protects the bacteria from the extracellular environment. In this barrier the membrane proteins are doped to form one or more sites all over the hydrophobic surface. Most of the proteins and DNAs, which are of interest for detection, exist in the internal structures of the bacterial cells, such as nucleoi, ribosome, storage

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granules, and sometimes, endospores. Cell/spore lysis can disrupt the capsule and release the majority of the intracellular materials for detection and analysis. As an added benefit, such lysis also leads to inevitable cell death, which is desired for sterilization purposes.

A number of methods have been developed for lysis of bacteria/spores during the past decades for assays at bench scale (Harrison 1991). Recent developments in microfluidics have opened up possibilities for new, revolutionary approaches to performing biological and chemical assays at micrometer scale (Verpoorte 2002). Microfluidic chips offer reduction in sample amounts (consuming nanoliter to picoliter volume), portability for field applications, high level of integration and automation, and high throughput. The concept of integrating different steps of the biological assay onto one microchip makes it possible to construct portable devices for point-of-care pathogenic detection (Manz et al. 1992). Effective coupling of lysis with other steps is challenging on a microfluidic platform. As we will review in this chapter, some conventional lysis methods have been adopted in microfluidic systems. Moreover, novel lysis approaches have also been demonstrated taking advantage of versatility offered by microscale device design. Successful detection of pathogenic bacteria on a microfluidic platform relies on a series of procedures including culturing and selection of the bacteria cells, lysis of bacteria cells, as well as separation and detection of intracellular materials using biochemical assays (El-Ali et al. 2006).

The choice of the lysis method largely depends on the target intracellular species and the particular assay following lysis. Although varying on a case-by-case basis, several general considerations often apply. First, one would want to avoid potential interference with the subsequent assay from the lysis step. When intracellular proteins (antigens) are targeted, the physical and chemical conditions (such as temperature, pH value and chemical composition of the buffer) can have dramatic effects on the functions of the assayed molecules. For example, the results of immunoassays can potentially be influenced by the surfactants, such as sodium dodecyl sulfate (SDS), as well as high temperature, which are often applied for lysis. Second, the time required for lysis needs to be considered, especially when the assay involves different steps in series. Rapid lysis decreases the total time for the detection assay. Third, another important factor is the throughput of the lysis method. Depending on the sensitivity of the subsequent assay, a large number of bacterial cells/spores are typically required for detection and analysis. The ability to handle cells/spores with high throughput in a microscale device is often important.

In this chapter we will mainly discuss the recent advances of bacteria/spores lysis based on microfluidics. We will point out the characteristics and potential applications of different mechanisms, such as mechanical, thermal, chemical, laser-based, and electrical lysis. We will also discuss the future directions that this field may take.

2. Bench Scale Methods for Bacteria/Spore Lysis

Cell lysis has been a routine operation and many commercialized techniques and protocols have been developed over the year. Here we will briefly review the conventional methods for bacteria/spores lysis because many of the microfluidics-based assays are based on the same or similar mechanisms as those of the large-scale ones. The conventional methods can be roughly classified into physical and chemical/biological methods (Geciova et al. 2002).

Physical methods dominated the field in the early days. They include manual grinding, freeze/thaw cycles, mechanical disruption, sonication and liquid homogenization.

Manual grinding is relatively simple and commonly used for the disruption of plant cells because the wall of the plant cells could be effectively destroyed in this way (Tonshoff and Raschke 1977). With this approach, the cells are usually frozen in liquid nitrogen, and the