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Review

Microfluidic platform towards point-of-care diagnostics in infectious diseases☆

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ABSTRACT

Rapid and timely diagnosis of infectious diseases is a critical determinant of clinical outcomes and general public health. For the detection of various pathogens, microfluidics-based platforms offer many advantages, including speed, cost, portability, high throughput, and automation. This review provides an overview of the recent advances in microfluidic technologies for point-of-care (POC) diagnostics for infectious diseases. The key aspects of such technologies for the development of a fully integrated POC platform are introduced, including sample preparation, on-chip nucleic acid analysis and immunoassay, and system integration/automation. The current challenges to practical implementation of this technology are discussed together with future perspectives.

Contents

☆ A selected paper contributing to the issue in honor of Professor Peichang Lu's 90th Birthday.

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1. Introduction

Infectious diseases are leading causes of mortality and morbidity in developing countries, accounting for more than half of all infant deaths [1]. Standard methods of pathogen detection, including cell culture, nucleic acid amplification, and enzyme-linked immunoassay, are often laborious and time consuming. Due to inadequacies in the health care infrastructure and cost constraints, over 95% of deaths from infectious diseases are caused by a lack of proper diagnostics and treatments [2]. Thus, there is a great need for a new diagnostic tool that will allow rapid and effective diagnosis of infectious diseases. The World Health Organization (WHO) has established a set of criteria to guide the development of diagnostic tools in resource-limited settings, which are: (i) affordable, (ii) sensitive, (iii) specific, (iv) user friendly, (v) rapid and robust, (vi) equipment-free, and (vii) deliverable to end-users, abbreviated "ASSURED" [3]. These criteria can be used to develop suitable diagnostic methodologies for use in low-resource settings.

Microfluidic technologies are emerging as powerful tools that meet the ASSURED criteria. Microfluidics-based devices are on-chip sensing devices that require only a small volume of bodily fluid and can be used for diagnosis and real-time monitoring of infectious diseases at the point of care (POC) [4]. Microfluidic diagnostic devices can analyze diverse clinical samples, including blood, oral fluid/saliva, and urine. Disposable devices can be produced, eliminating the need for washing processes between sample separations and making them easy to use even in remote regions. One of the most effective disposable devices so far is the immunochromatographic strip (ICS), which is currently used in developing countries for the detection of bacterial [5], viral [6], and parasite antigens [7,8]. The cost per disposable microfluidic device can be further decreased by mass production, quality control, and miniaturization [2]. Microfluidics-based devices use channels to transport small amounts of fluid precisely by actuation forces. Multiple approaches to fluidic transport have been developed, and these fall into two categories: passive (e.g., gravity, surface tension, capillary force) [9–11] and active (e.g., micropump, electric force, and centrifugal force) [12–15] approaches. Passive flow approaches require very little equipment for implementation, whereas active flow approaches handle the fluids via pumps or electric or centrifugal force. In addition, several materials are available for constructing effective microfluidics-based devices. These materials fall into three broad categories: inorganic materials (silicon, glass, ceramics), polymeric materials (elastomers, thermoplastics), and paper [16]. The choice of material depends on the function(s) the device will execute, the processing cost, and its compatibility with bulk manufacturing techniques. For example, an electrically conductive material and a simple method of electrode fabrication are necessary to realize integrated electrochemical detection on a POC diagnostic device. All considerations depend on what needs to be achieved with the device.

This review presents an overview of the latest advancements in microfluidic platforms for promising POC testing in the context of infectious diseases. First, the principles of pathogen detection based on miniaturized on-chip nucleic acid analysis and immunoassay are summarized. The key aspects of microfluidic technologies needed for the development of a fully integrated POC platform are then introduced, including sample preparation, on-chip nucleic acid analysis and immunoassay, and system integration/automation. Finally, the current challenges to implementing this promising technology and future perspectives with practical concerns for infectious disease diagnostics are discussed.

2. Advances in microfluidics-based technologies targeting pathogens in POC testing

The specific identification of an infectious pathogen is essential for the diagnosis of any infectious disease. Pathogens are generally recognized on the basis of two main constituents: nucleic acids and proteins (Table 1). Several practical issues are receiving attention in the development of diagnostic devices, including interference with nucleic acid analysis from samples with high salt concentrations, cost, storage, and the selectivity of antibodies. This section summarizes the various steps involved in a microdevice that is to be used for nucleic acid and/or protein analysis and detection modes.

2.1. On-chip sample preparation

On-chip sample preparation is the first step to pathogen detection in infectious disease diagnostics, and this is also a prerequisite for POC devices with sample-to-result capability. This step mainly involves the enrichment of target cells, which is generally required prior to handling complex sample matrices such as blood, saliva, urine, and smears.

Samples used for clinical diagnostic tests usually contain very low concentrations of the target cells. For reliable and reproducible detection, concentration steps as well as cell separation steps are needed to exclude unwanted cell types and matrix fluids from the sample [17]. The physical and mechanical properties (e.g., centrifugation, size, deformability, polarizability) of the different cell populations are commonly applied to cell separation. Conventionally, concentration of the target cells is performed by centrifugation. Although centrifugal microfluidic platforms [14] or centrifugal disks have been developed [18], they are used mainly for fluidic transport during the bioassay. Filtration is a common concentration technique that is based on cell size and rigidity and is implemented in microfluidic devices. Obstacle structures fabricated in microfluidic channels have been used in the isolation and quantification of biologically important analytes, such as swine influenza virus [19], *Escherichia coli* [20,21], and HIV [20]. Zhang and colleagues [21] used a porous polytetrafluoroethylene (PTFE/Teflon^{TM}) membrane to enrich *E. coli*. High enrichment (~85%) was successfully achieved within 15 min (Fig. 1i). Deformability is another intrinsic property commonly applied to cell sorting. Several inertial microfluidic devices with contraction-expansion channels can separate label-free bacteria from blood samples with high efficiency [22]. Hou and colleagues [23] recently demonstrated high-throughput *E. coli* and *Saccharomyces cerevisiae* removal from whole blood, inspired by the *in vivo* microcirculatory phenomenon of leukocyte margination (Fig. 1ii).

Another approach to cell separation and concentration is dielectrophoresis (DEP), which is based on the differences in the ability of polarized cells to migrate. When subjected to a non-uniform electric field, bacterial cells with dielectrophoretic activity will move toward high or low electric field regions. On the basis of the DEP principle, several bacteria cells have been successfully separated, such as *E. coli* [24], *Staphylococcus aureus*, and *Pseudomonas aeruginosa* [25]. Typically, Park and colleagues [24] combined positive and negative DEP to continuously separate and concentrate *E. coli* at a high efficiency of 87.2% in human cerebrospinal fluid and blood (Fig. 1iii).

In addition to the approaches relying on the physical properties mentioned above, cell separation and concentration can be conducted efficiently on the basis of immunoaffinity [26–35]. With this approach, aptamers and antibodies are commonly adopted for heterogeneous cell separation. Aptamers are nucleic acid molecules developed by an *in vitro* process; they can bind to their molecular targets (e.g., small molecules, proteins, cells) with high affinity and