

Microfluidic-based point-of-care testing for disease diagnosis

Ruixuan Wen¹

¹Kunshan International School, 288 Qingyang Middle Rd, Kunshan, Suzhou, Jiangsu, China

Abstract. The early detection of diseases is important to optimize clinical treatment. To meet the increasing requirement of rapid medical diagnosis, point-of-care testing (POCT) has drawn scientists' great attention for its outstanding advantages including simple, efficient, inexpensive and instrument-independent. Recent advantages of microfluidic-based diagnosis have played an important role in the significant technological process of POCT, which can achieve rapid diagnosis and provide high precision testing results for correct clinical decision making. This review focuses on design strategies, detection targets and sensing mechanisms of recent microfluidic-based POCT for disease diagnosis. Further, we provide a perspective of the next generation of POCT devices and their future opportunities.

1 Introduction

Early detection of disease is necessary for it may result in optimized treatment and reducing mortality. Recently, point-of-care testing (POCT), a simple, efficient, user-friendly, inexpensive and instrument-independent portable analytic system, has been developed to achieve accurate and rapid disease diagnosis. [1-4] The diagnosis results can be quickly obtained using POCT system, which enables a more effective disease therapy and a correct use of drug. Therefore, POCT is very useful for disease prevention, diagnosis as well as the control of disease outbreak, especially in countries with low medical resources.

During last two decades, there is a significant technological progress in microfabrication technologies, which has already become an essential research area for microfluidic applications in pharmaceuticals, environment and biomedical engineering research area. [5-7] In particular, biomedical microsystems have already shown an extremely positive potential for POCT application, widely employed for high-precision material synthesis, preparation of biochemical sample, and biophysical analysis. [8-10]

In this review, we focus on the microfabrication techniques, disease biomarkers and the detection strategies to demonstrate the design of microfluidic POCT systems as well as their future perspectives, which will contribute to the development of next-generation POCT system.

2 Microfluidic device fabrication

At present, various microfabrication technologies have been developed to achieve the development of microfluidic devices, which can be fabricated by various

materials including glass and silicon, polymers, and paper.

2.1 Glass and silicon

Glass and silicon wafers were used for the first generation of microfluidic chips. Micromachining and photolithography on glass and oxidized silicon have been well developed during 90s decade, which makes them suitable for biological and biomedical applications. [5,11-13] The fabrication of this type of microfluidic devices is usually achieved in a clean room, which requires complicated equipment. Although the cost is high, glass or silicon based microfluidic devices are very reliable and precise, which can achieve on-chip reactions, capillary electrophoresis, droplet formation and solvent extraction in extreme test. Digital microfluidics is an important class of glass or silicon-based microfluidics. As shown in Fig 1A, Wan et al. has developed a digital microfluidic system using glass to detect specific pathogen through loop-mediated isothermal amplification. [14]

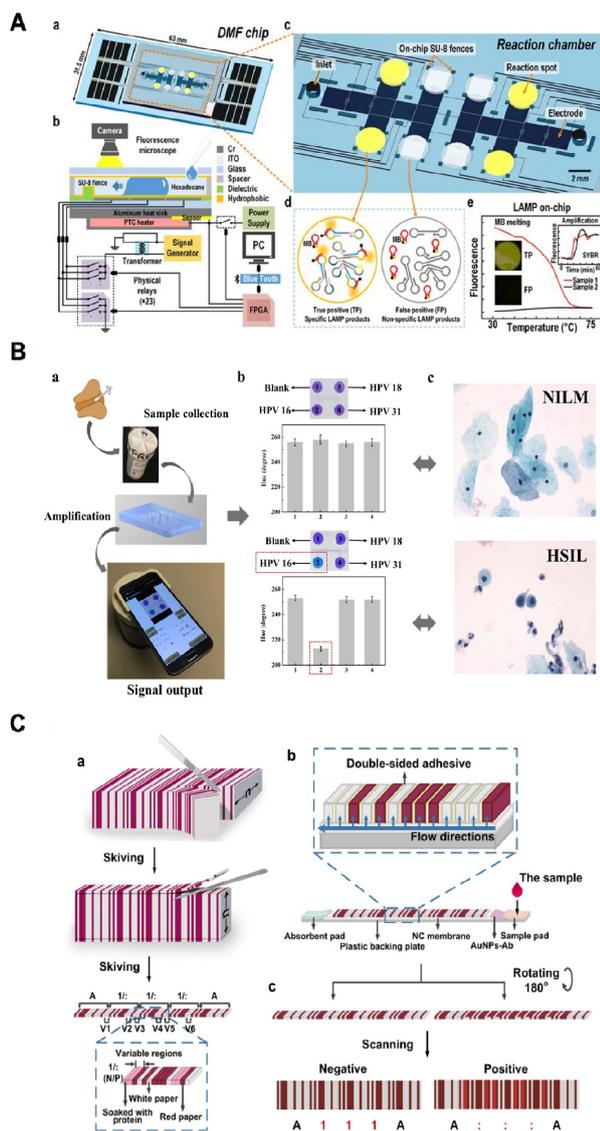


Figure 1. Microfabrication technologies for POCT. A: digital microfluidic system using glass to detect specific pathogen. B: colorimetric molecular detection method using PMMA-based microfluidic device for HPV-associated cancer screening. C: test paper for rapid and multiplexed assay.

2.2 Polymers

Polymeric and organic materials are most widely used to fabricate microfluidic devices for biomedical application. Compared with glass or silicon-based microfluidics, the fabrication process of polymer-based microfluidic device is based on replication. Therefore, the fabrication of microfluidic devices using polymers is faster and less expensive, which is more suitable for mass production. Additionally, polymer-based microfluidics are easy processing, low-cost, flexibility and meantime owns a wide range of chemical and mechanical properties, which is an attractive alternative to glass and silicon. [15] The most popular materials for polymer-based microfluidic systems are poly (methylmethacrylate) (PMMA) and poly(dimethylsiloxane) (PDMS). As shown in Fig. 1B, Yin et al., has developed a colorimetric molecular detection method using PMMA-

based microfluidic device for HPV-associated cancer screening. [2]

2.3 Paper

Paper is a type of useful substrate for microfluidic device fabrication. Paper-based microfluidic device is flexible, low-cost, disposable and adaptable to large-scale manufacturing. [16] Typically, the microfluidic analytical devices using paper do not require external power because of the capillary effect of cellulose fibers in paper. Considering that cellulose fibers sometimes are partially filled in channels of closed-channel paper-based microfluidic devices, some open-channel devices have been developed using embossing, folding, engraving and embossing cellophane methods, which can achieve similar functions with conventional polymer-based microfluidic devices. As shown in Fig. 1C, Yang et al. has developed a test paper for rapid and multiplexed assay. [17]

3 Disease biomarkers

The specific identification of biological biomarkers is the key point for the detection of pathogens. The recognition of targets is usually based on two different ways: 1) recognize the membrane specific epitopes using antibody and its alternatives; 2) recognize the specific nucleic acid.

3.1 Antibody and its alternatives

For the detection of pathogens, antibody-based system is one of the major analytical methods (Fig. 2A). [18,19] Despite labor-intensiveness, this technique has proven to be very important in the high-affinity and specific detection of disease biomarkers. Whole cell detection and specific protein detection are the two most widely used antibody-based methods for disease diagnosis. However, traditional antibody-based detection technique has several drawbacks, including expensive cost, poor physical and chemical stability, and large size, which limits its application for certain disease biomarkers. To address these drawbacks, some alternatives to antibody have been developed such as molecularly imprinted polymers (MIPs) and aptamers. MIPs can be produced at low cost with high reproducibility and stability, which have been used for the detection of human rhinovirus serotype 2 and tobacco mosaic virus. [20,21] Aptamers are another recently-developed alternatives to replace antibodies. [22] Aptamers are usually isolated by the systematic evolution of ligands by exponential enrichment (SELEX) technique. [23] They are molecules of nucleic acid developed by an in vitro process, which can recognize their targets (e.g. proteins, cells, etc.) with high specificity and affinity, which can achieve better limit of detection compared with traditional antibodies.

3.2 DNA/RNA

Compared with traditional antibody-based approach, DNA/RNA-based detection methods have outstanding advantages because of their capabilities for rapid, specific and sensitive detection of target nucleic acids (Fig.2B). PCR (polymerase chain reaction) is the most widely used molecular technique for DNA amplification. It has already played an important role in disease diagnosis. However, PCR process includes three temperature steps, which is not easy to develop point-of-care devices. Therefore, isothermal amplification has drawn great attention because the reaction can be achieved at one temperature, which means that it is simpler and energy efficient. The loop-mediated isothermal amplification (LAMP), recombinase polymerase amplification (RPA), rolling circle amplification (RCA) and helicase-dependent amplification (HDA) are some established isothermal amplification methods. [24-27]

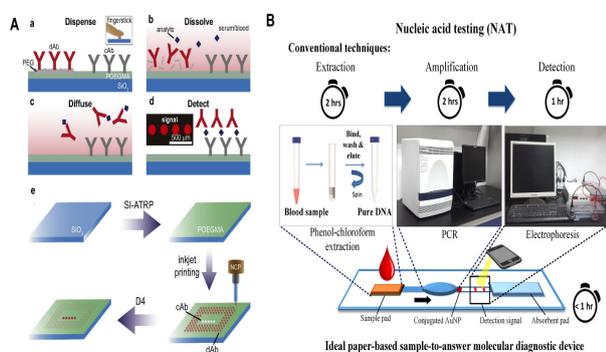


Figure. 2 The point-of-care testing for disease diagnosis based on the recognition of antibody (A) and nucleic acids (B).

4 Detection strategies of POCT

It is crucial to develop a low-cost, user-friendly, and efficient diagnostic system with high accuracy and sensitivity. Up to now, a variety of POCT technologies have been developed for the early disease diagnosis, which can contribute to preventing the outbreak of many pandemic diseases.

4.1 Optical POCT devices

Taking advantages of various optical instruments such as lenses, diffraction gratings, light sources and filters, optical based POCT devices can realize colorimetric, fluorescence, luminescence-based detection.

4.1.1 Colorimetric POCT

Colorimetric detection measures the absorbance variations induced by plasmon resonance phenomena or structural shifts. The colour difference can be directly recognized by naked eyes or detected by spectrophotometer. For example, a POCT device has been developed to simultaneously detect CHIKV, ZIKV,

and DENV based on multiplexed RT-LAMP assay (Fig.3A). [28]

4.1.2 Fluorescence POCT

Fluorescence testing has been widely used in the field of chemistry, biomedicine, and biotechnology because of its outstanding advantages of high sensitivity, ease of manipulation and non-invasiveness, etc. As shown in Fig. 3B, a cost-effective and field-portable POC nucleic acid testing platform has been developed based on fluorescence-based assay. [29]

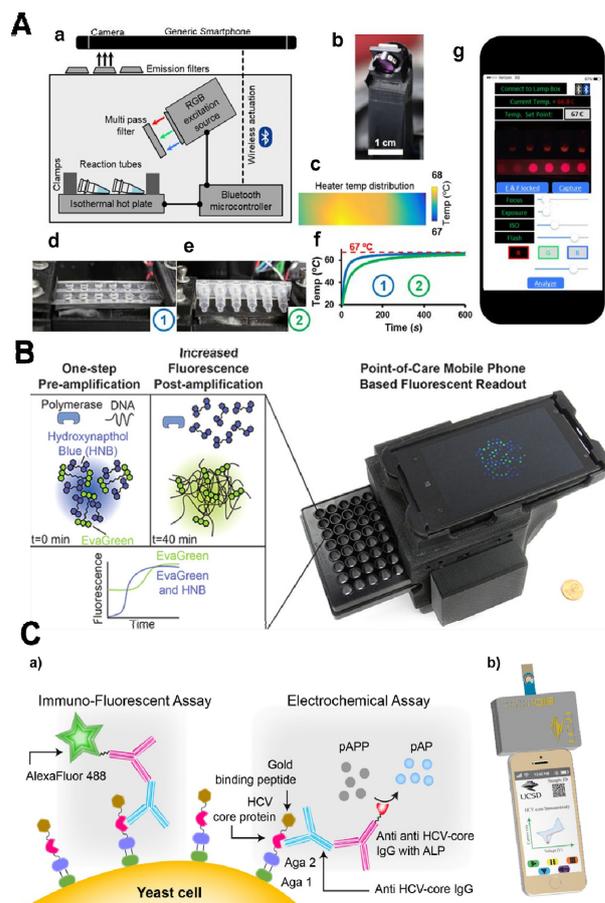


Figure. 3 Different detection strategies of POCT. A: colorimetric-based POCT; B: fluorescent-based POCT; C: electrochemical-based POCT.

4.1.3 Luminescence POCT

Luminescence-based POCT usually owns higher sensitivity than those based on colorimetric reading. At the same time, they can be successfully operated without light source. However, luminescence POCT is time-dependent decaying of a luminescent reaction, which will lead to some limitations for consistent analysis.

4.2 Electrochemical POCT devices

Electrochemical POCT can provide higher reproducibility and sensitivity than optical POC diagnosis methods. As shown in Fig. 3C, Aronoff et al. has established an integrated approach to detect hepatitis

C virus antibodies using electrochemical POC platform, which can also be applied for other antigens such as detect malaria. [30]

5 Conclusion and future perspectives

Microfluidic POCT possesses outstanding advantages over traditional diagnosis methods. For example, microfluidic POCT can reduce the consumption of clinical samples and reagents. Additionally, they are operational automatic and low-costed. Moreover, the high surface-to-volume ratio of microfluidic POCT can inhale mass transport, resulting in greater reliability and shorter trial times and greater reliability.

To achieve better detection, the reaction signal can be further enhanced by some advanced material including golden nanoparticle and fluorescence probes. Additionally, the detection signal can be transferred to smartphone-based devices for further result evaluation. The development of connected electronic devices and worldwide communication system may lead to the next generation of POC diagnosis.

The location labeling testing method can be used for the monitoring and detection of diseases with the help of smartphone-based POCT system. In addition, 3D printing technology can enhance the performance of microfluidic POCT device because of their outstanding advantages including fast and one-step fabrication, which does not require complex laboratory equipment. With the help of 3D printing and smartphone sensing technology, sensitive and low-cost detection devices can be developed. Briefly, the next generation of microfluidic POCT platform with the help of advanced materials, 3D printing technology, smartphone system, and internet clouds will have a huge potential to revolutionize the global health care system.

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