Microfluidic Chips as Point-of-Care Testing for Develop Diagnostic Microdevices

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Abstract Rapid, specific, and reliable diagnostic tests in portable, easy-to-apply systems are of great importance for medical diagnosis, especially in emergencies such as pandemic outbreaks or in environments where resources are scarce. Point-of-care testing platforms are ideal for these purposes, providing fast and timely accurate results. Interest in laboratory-on-a-chip devices has grown rapidly in recent years. Innovative microfluidic devices that have gone through the technology development process have demonstrated the potential to perform unimaginable analyzes using traditional techniques. Advances in the microfluidics chip field have sparked innovative upheavals in various biomedical fields, such as single-cell detection, diagnostic methods, and micro- and nano-size-product manufacturing. Microfluidic chips currently play an important role in multiple biological technologies. Microfluidics have been shown to offer a number of benefits over existing conventional methods, thanks to improved controllability and precision. In this chapter, the authors discussed how point-of-care tests, developed by the integration of numerous nanomaterials into microfluidic chips, play an active role in the diagnosis and diagnosis of many diseases and their potential biomedical applications.

Keywords Microfluidic chips · Point-of-care tests · Diagnosis · Nanomaterials

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1 Introduction

Early detection of infectious diseases plays a critical role in the correct and effective treatment of diseases. The COVID-19 pandemic has shown that it is very important to detect the infectious agent early at the beginning of the infection in order to quickly control the spread of the disease. Early diagnosis and timely treatment are critical not only for infection but also for other types of cancer and serious diseases. Early diagnosis can increase response rates to treatment and reduce treatment costs. Among the tests performed for this purpose, serology, virology, and imaging techniques are among the most preferred medical diagnostic techniques [66].

Serological methods are based on the detection of changes in protein biomarkers related to diseases in the human body. Virological methods consist of techniques for detecting viruses that infect the body, while imaging methods are used to test for structural changes in organs, tissues, and structures in the human body. Expensive test devices used for analysis are available in research laboratories in clinics and require expert personnel due to the complexity of the analysis stages of these methods. Because of the highly costly, long-lasting, labor-intensive nature of detection methods, they may not be sufficiently applicable techniques for the early diagnosis of diseases [75].

Lack of adequate medical facilities delays disease detection, especially for people in underdeveloped countries. Routine medical tests are also not possible for patients living in this region In developing countries such as South-East Asia and Africa, medical facilities such as hospitals, which should be widespread, are very inadequate. Therefore, it is very difficult for countries with inadequate medical resources and health systems to combat bacterial or viral diseases [34]. If a pathogent is present and spread in these regions, the infectious agent may not be detected and isolated in time. In such cases, the infection carries the risk of spreading epidemically. The COVID-19 pandemic is a good example of this issue. The infection agent in a single region has shown a serious spread that can affect all countries of the world as a result of international travel [14].

In countries with inadequate health systems, there are no techniques that enable sensitive rapid detection of diseases in the clinic. Even in rural areas of developed countries, disease prevention services and health screening can be very inadequate [60]. Therefore, it is of great importance to produce inexpensive, portable diagnostic equipment for home self-testing or field testing. When such devices are developed, the workload of existing health systems in underdeveloped regions can be reduced, early diagnosis can be made, and overuse of medical resources can be reduced. Microfluidic technologies have been developed and fabricated in recent years to solve these problems and offer innovative perspectives. The chips developed within microfluidic technologies are small in size, require few reagents, and easy to carry to diagnose the disease. The biggest advantage of microfluidic chips is that they perform detection in a short time compared to conventional detection devices and kits. For this reason, microfluidic chips are technological innovations that can provide fast and effective results in situations where the health system and medical resources are

insufficient, such as in underdeveloped regions. Microfluidic technologies are used in the fields of laboratory tests [57, 65], medical diagnosis [3], and cell analysis due to their small size, short process requirements and portability. The development of microfluidic chips has also contributed significantly to the advancement of point-of-care (POC) test technologies. POC test (POCT) is an analytical test that offers medical diagnosis to patients even in limited and under-resourced healthcare systems [54]. The development and application of POCTs are promising for countries in developing regions in need of medical diagnostic tests [44].

This book chapter includes studies on the necessity and development of technologies for the rapid diagnosis of diseases at an early stage that can be offered to the service of underdeveloped countries. Firstly, we will describe microfluidic chip technologies and introduce their types and advantages. Then, we will then introduce microfluidic POC tests for the early detection of various diseases and describe their current applications.

2 Microfluidic Tests

2.1 Introduction

Microfluidic device is a portable, analyte capture and identification system that can perform sample detection steps. In these innovative systems, reagents and samples are used in very low quantities. In addition, the efficiency and analysis speed of the system is very high. Furthermore, the analysis process can be automated to eliminate human error. Devices developed with microfluidic technology are frequently preferred in physics, biology, chemistry, biomedical sciences, and engineering. Devices manufactured with microfluidic technology are known as portable devices. Because the analytic process is carried out in small-sized devices that are completely portable [64]. The use of small amounts of reagents for analysis in microfluidic devices offers advantages for under-resourced regions in harsh conditions. The small size and small amount of reagent consumption significantly reduces the cost of the analysis [25]. POCTs are portable devices that enable analysis and detection in various regions and for various purposes outside of clinical laboratories [55].

POCT technology can be used not only for humans but also for the detection of animal diseases. In a study, Pascual-Garrigos and coworkers developed the loop-mediated isothermal amplification (LAMP) assay. The purpose of the test is early stage diagnosis of respiratory system diseases [56].

Microfluidic technology has made a significant contribution to the application of POC tests in disease diagnostic systems. Microfluidic devices provide fast results and high sensitivity. Therefore, microfluidic devices integrated with POC tests are the cheapest and easily portable devices that can be preferred to provide rapid and sensitive detection [62].

2.2 Benefits of Microfluidic Tests in Clinic Laboratory

Identifying biomarkers plays a critical role in disease diagnosis. The most preferred method for diagnosis is the ELISA test. The ELISA assay produces measurable signals in the presence of a sufficient amount of analyte. ELISA test uses different enzymes such as horseradish oxidase, alkaline enzyme, and β-galactosidase. Substrates are used to react with the enzymes and produce colorimetric results [20]. ELISA is the most preferred technique for detecting protein-based molecules. Because it is very sensitive in detecting the presence of protein. Detect proteins as well as many pathogens, including viruses and bacteria [70]. ELISA has been used to identify and quantify many viruses such as coronavirus [1, 6], Zika virus [52], dengue virus [49], influenza virus [59]. Conventional ELISA needs to be improved to provide the high sensitivity required for protein identification and quantification [29].

Mass spectrometry (MS) can detect different biomarkers such as proteins [2]. Integration of microfluidic chips into the MS (μchip-MS) has the potential to meet the needs of clinicians. Thus, new methods may be revealed in monitoring stages of diseases other than infectious diseases including cancer, diabetes, and other chronic diseases [26, 50, 58]. The combination of MS and a microfluidic chip platform offers an innovative perspective for microfluidic chips. Compared to conventional immunoassay tests and techniques, μchip-MS has been shown to have a higher specificity and sensitivity in analysis, and also provides faster results with less labour. Surface plasmon resonance (SPR) is another optical detection method. SPR detection method is used in virus detection and detection of cancer biomarkers due to its many reasons for preference such as high accuracy, low production cost, and sensitivity [5, 47]. Liu et al. used microfluidic technologies and nanoparticles together to detect the target protein by the SPR technique. Since the results were better than the method with SPR alone [40], it shows that combining microfluidic technologies with existing technologies will lead to advantageous results.

Many traditional methods have started to be integrated with microfluidic POC devices. Especially for patients in underdeveloped countries, these integrated devices can detect proteins in the serum and provide excellent analysis results [38, 39].

2.3 Equipment Varieties for Microfluidic Tests

The basic components of the first microfluidic system developed were chemical etching technology and photolithography [68]. Subsequently, microfluidic devices made of polydimethylsiloxane (PDMS) materials were also developed and today, most of the microfluidic chips are made of PDMS polymer [46].

In recent years, paper-based technologies and three-dimensional (3D) printing methods has been used to reduce production cost [9, 77]. These materials can be used for POC analysis, especially in undeveloped regions. One of the best examples of combining POC technologies with microfluidics is mobile sensors where microfluidic systems are integrated into smartphone applications [79].

2.3.1 Microfluidic Devices Produced by 3D Printing Techniques

The development of 3D printers has directly influenced the advancement of microfluidics [7]. These devices work automatically and are not dependent on a person, thus eliminating the need for human resources required to produce PDMS-based microfluidics [8]. Moreover, 3D printing techniques provide serious high potential and prototypes can be developed with this technique. Rapid production can increase the efficiency and frequency of experiments and thus, accelerate the commercialization of experimental methods [15, 71].

Microfluidic POCT chips produced with 3D printers have been used in experimental studies. Song and coworkers develop a new approach for sensitive detection of viruses in the platform developed with microfluidic technology. The box prepared for detection was produced by 3D printing technology and only a saliva sample was used to detect the Zika virus [63]. Furthermore, Kadimisetty and coworkers designed a low-cost microfluidic POC test based on the nucleic acid amplification method for the diagnosis of infection [32].

Thanks to three-dimensional printing technology, the commercialization of microfluidic POCT techniques has gained great speed. With 3D printing technology, it has become very easy to rapidly manufacture and produce microfluidic equipment. Therefore, 3D-printed microfluidic POC tests offer a serious advantage for use in undeveloped countries.

2.3.2 Analyzing Microfluidic Chips with Smartphones

Since the early 21st century, the continuous development of microelectronics has resulted in the production of smartphones. Smartphones have the potential to be an alternative to computers for data collection and processing in underdeveloped countries [24, 74, 76]. As a result of these developments, a new generation of mobile sensing techniques has emerged by combining smartphones with microfluidic devices. Combinations of microfluidic devices and smartphones are very useful for regions with inadequate and weak healthcare systems.

Researchers have developed a paper-based microfluidic test to detect Zika virus through reverse transcription cycle-mediated isothermal amplification (RT-LAMP) technique. In this study, ZIKV RNA triggers a color change in the microfluidic system and the results can be obtained within minutes and can be analysis with a smartphone [31]. In another study, Jalal and coworkers fabricated a microfluidic chip consisting of polycarbonate (PC) plastic material and reagent paper to detect chemical molecules in human urine. Using a smartphone, the resulting colorimetric results are recorded with the phone camera [30]. The systems in which microfluidic chip and smartphones are integrated are easy to use. It gives reliable results without requiring specialized personnel. These integrated systems are a promising technology.

3 Microfluidic Tests for POC Diagnosis of Infectious Diseases

3.1 Introduction

Coronavirus diseases affects countries all over the world, causing widespread deaths and straining national economies [35]. Early diagnosis of COVID-19 and timely and correct treatment of patients is one of the most important measures to be taken. Accurate and rapid detection is critical in the fight against infection. In this direction, ELISA, RT-PCR, colloidal gold immunochromatographic assay are among serological methods [16]. However, ELISA and RT-PCR may limit their use in less developed countries. Consequently, POCTs offer an innovative approach in the production of inexpensive and rapid tests for respiratory system diseases.

Compared to conventional tests, microfluidic devices can measure biomarkers and antibodies accurately and sensitively. Microfluidic technologies can be integrated with other conventional methods used to provide efficient test results. Microfluidic chips therefore have promising potential for the detection of SARS-CoV-2.

3.2 Applications for Diagnosis of Infectious Diseases

Immunoglobulin G (IgG) and immunoglobulin M (IgM) antibodies are revealed and play an active role in the defense against viral disease agents. Therefore, the progression and treatment of infectious diseases are determined by the detection of antibodies. In a study, Lin and colleagues developed a diagnostic kit for COVID-19 diagnosis by integrating a diagnostic microchip and a POC test that can detect IgM, IgG, and other biomarkers with portable fluorescent detectors [39].

However, microfluidic devices have shortcomings in diagnosis of COVID-19, such as requiring a long incubation time, and further development is needed for rapid detection of SARS-CoV-2 antibodies [67].

Although nasopharyngeal specimens are often preferred for diagnosis of COVID-19, human saliva can also be usable as a test sample [69]. Patients have the advantage of being able to collect their own samples and there are studies showing that saliva samples are also suitable for detection. Wang and coworkers developed a POC test using RT-LAMP technique for diagnosis of COVID-19 in human saliva. This test provides colorimetric results, although the assay only requires a heat source. Due to this feature, it can be used in countries with limited healthcare [73].

4 Microfluidic Tests for POC Diagnosis of CVDs

4.1 Introduction

As reported by the World Health Organization, CVDs cause 17.9 million deaths worldwide each year [28]. High CVD rates also show that health services in developing regions are very inadequate compared to developed regions [4].

CVD, also known as cardiovascular system-related diseases including hypo- and hypertension, coronary heart disease, and cerebrovascular disease (stroke) [19]. In recent studies, various biomarkers have been used for the detection of CVDs. Early stage diagnosis of CVD is the main factor that reduces treatment costs and mortality. POC tests have the potential to detect CVD biomarkers rapidly and sensitively [13].

4.2 Applications for Diagnosis of CVDs

There are risks that using a single biomarker to diagnose CVD may lead to misdiagnosis as it may be associated with other diseases. Therefore, it is critical to detect multiple CVD biomarkers at the same time for accurate and sensitive diagnosis. Thus, more reliable, high-specificity results can be obtained. In addition, these techniques reduce the cost and time of analysis [27]. Various platforms have been designed that can detect multiple CVD biomarkers simultaneously [53]. Clinicians advocate the use of these techniques because simultaneous analysis of multiple biomarkers provides more comprehensive and accurate results [18]. Most microfluidic POC tests capable of detecting multiple biomarkers are currently in use [43].

AMI is the most of dangerous diseases. For accurate and timely detection of AMI, multiple biomarkers need to be detected simultaneously [23, 78]. In a study, Li and coworkers developed a 3D printing paper-based microfluidic test (μPAD) that detects numerous biomarkers with three sensing zones. The μPAD can simultaneously measure cTnI, H-FABP, and copeptin using chemiluminescence (CL) emissions. The device has the potential to greatly facilitate early stage AMI diagnosis. Figure 1 shows the design of a 3D μPAD [36].

In another study, Boonkaew and coworkers developed a POC test for the detection of three different CVD biomarkers simultaneously, procalcitonin marker, cTnI, and C-reactive protein. This microfluidic device contains multiple working electrodes and multiple detection sites that can detect different CVD biomarkers in a single human sample [10].

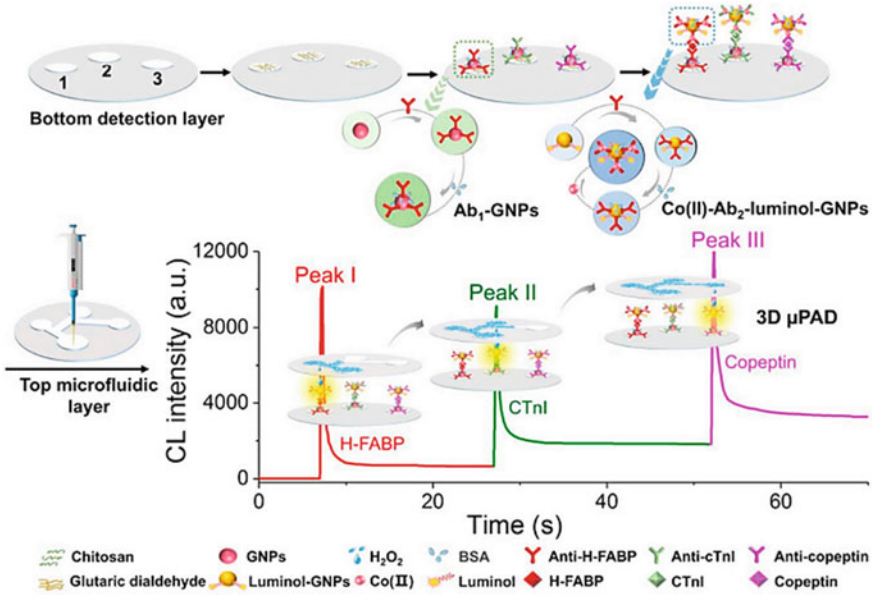

Fig. 1 Schematically illustration for the fabrication of the 3D μPAD for multiplexed CL immunoassay of H-FABP, cTnI and copeptin. Reprinted with permission from [36]. Copyright 2020 Elsevier

5 Microfluidic Tests for POC Diagnosis of Tumors

5.1 Introduction

Cancer diseases are caused by cells that multiply uncontrollably. Cancer disease that is difficult to diagnose, treatment, and follow-up [41]. Cancer is becoming increasingly common and mortality rates are increasing day by day. Cancers of the breast, lung, stomach, and prostate are among the most common varieties of cancer [12]. Symptoms of cancer in the early stage may not be recognized. However, early stage detection of cancer is critical for effective treatment [72]. Current conventional diagnostic techniques for cancer diagnosis such as magnetic resonance imaging, ultrasound tomography are not suitable for routine examinations due to reasons such as cost and radiation exposure. Among the cancer diagnostic methods used in clinics, haematology tests are typically used. Cancer screening with markers found in human serum is widely used to detect cancer at an early stage, reducing patient harm and medical costs [17]. In addition, protein measurements are crucial biomarkers for early stage cancer diagnosis and monitoring disease progression and treatment [48].

5.2 Applications for Diagnosis of Cancer

Research has been made on the effective use of microfluidic devices for cancer detection. Wang and coworkers developed a microfluidic system capable of DNA methylation analysis. The test time takes 3 h including all stages and this technique allows early diagnosis of cancer [72]. CA-125, another cancer biomarker, provides information about the progression of cancer at varying concentrations. Nunna and coworkers developed a POC testing system that combines a biochip with a microfluidic system aiming to measure CA-125 concentration in human serum obtained from a finger prick, similar to glucose measurement in diabetes [51].

Lung cancer (LC) is the leading deaths due to its high mortality rate and significant spread in all over the world [42]. Exosomes have been used as a novel biomarker for early detection and treatment of lung cancer. Yang and coworkers developed a microfluidic device with adjustable membrane pore size to identify biomarkers in human urine samples. This technique is a promising study for the detection of patients with early lung cancer [80]. Microfluidic POC assays can detect different biomarkers in various cancer-related diseases. Prostate cancer (PCa) is one of the most common cancers in men and its diagnosis is very important [21]. Prostate-specific antigen (PSA) is the biomarker of preference in the analysis [22]. In serum samples taken from healthy men, PSA concentration is in the degree of 0–4 ng/mL. People with PSA concentration higher than 4 ng/mL, which is critical in cancer detection. For this reason, if a developed test can detect PSA levels lower than 4 ng/mL in a cost-effective and rapid manner, it will make a significant contribution to the diagnosis of prostate cancer patients [11]. Mandal and coworkers developed a system combination of graphene FETs, dielectrophoresis (DEP), and a microfluidic chip for early detection of prostate cancer [45].

Since cervical cancer is one of the most common cancers in women, early diagnosis is much more important than other cancers [61]. Because cervical cancer that can be detected at an early stage can be completely healed. Karakaya and coworkers developed a microfluidic test that enables early stage diagnosis of cervical cancer by testing the presence of HPV 16 and HPV 18 in less than 40 min [33]. In another study, Lim and coworkers designed a system that integrates exosomatic mRNA sensors and 3D-nanostructured hydrogels into a microfluidic chip. Thanks to this system, exosomal ERBB2 in breast cancer-associated blood can be further detected and the validity of the system in breast cancer diagnosis can be proved [37].

6 Conclusions and Prospects

Medical diagnosis is very difficult in regions with weak health systems and inadequate health infrastructure. Microfluidic POC tests developed for medical diagnostics that can provide a solution to this problem give fast results, are easy to use, and are very low cost.

Microfluidic devices are mostly produced by utilizing paper, PDMS, or 3D printing technology. Integrating smartphones into microfluidic systems is a highly effective solution for POC applications. Although there are microfluidic POC test equipment with different characteristics, low-cost ones are mostly preferred as the main purpose is to be applicable in harsh environments. For this reason, microfluidic POC test components are small in weight and volume and are suitable for fast results.

In clinical laboratories, one of the most important steps in disease detection is the analysis of disease-related biomarkers. Microfluidics technologies can meet the requirements of medical tests in clinical laboratories because they are sensitive, inexpensive, and portable. Microfluidic POC tests have been used effectively in many fields such as CVD detection, detection of infectious diseases, tumor detection. Microfluidic devices can be integrated into many optical and serological techniques. They can detect biomarkers with high sensitivity and accuracy, which are the most critical parameters in disease diagnosis. Integration of existing technologies into microfluidic devices has provided features such as very low detection limit and high specificity.

With microfluidic POC tests, specialized personnel are not required for disease diagnosis and results can be obtained quickly. However, several disadvantages need to be overcome before POCT systems can be used as standard clinical tests. Microfluidic technology does not comply with industrial standards and guidelines. This lack of industry standards can be considered as a barrier to the commercialization of microfluidic devices. After standardization, application to industry can become easier. Thus, the cost and time of the production procedure can be reduced. Finally, for the integration of microfluidic technologies into the industry, investments in biomedical applications should be increased and microfluidic devices should be used more in clinical laboratories.

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