REVIEW ARTICLE

Lab on a Chip for *in situ* Diagnosis: From Blood to Point of Care

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Abstract

As the point of care diagnosis devices are becoming ever more popular, this paper suggest a miniaturized testing device from a drop of blood to diagnosis of disease for the global healthcare. The minimal requirements for the POC blood-testing device are blood microsampling, blood separation, immunoassay, and detection and communication of the signals. The microsampling of the blood can be achieved by specialized needle, which can be connected to the microchip or analytical devices. The sampled blood is then separated using either a filter (weir or pillar type), or by the phenomena unique to microfluidic system. The separated blood should then go through sandwich, homogeneous non-competitive, or competitive immunoassay, which can effectively diagnose diverse diseases. Lastly, the device should detect and translate the immune-signals to readable, and clinically significant signals. The development of such device will play a great role for improving healthcare technology.

Keywords Lab-on-a-chip, Biosensor, Immunoassay, Point of care, Micro-sampling, Microfluidics

INTRODUCTION

Despite recent remarkable progress in medical technology, the challenge of overcoming diverse critical diseases–including cancer, cardiovascular disease (CVD), and Alzheimer's–still remains. Even though the complete treatment of these diseases is difficult, early diagnosis might be one of the crucial clues for the patient survival and successful prognosis of the disease. To date, several techniques have been developed for the early detection of such diseases, but most of these techniques are lab-based, complex, slow, and also require experienced personnel to conduct the analysis. Moreover, the patients need to visit medical service centers, such as hospitals, for the diagnosis. However, the contemporary generation simply does not have time to spare on regular medical checkups, which prevents the early detection of disease. To minimize the patients' inconvenience, the analysis of blood sample, saliva, urine and body fluid at home or office and the direct transfer of data through networks are highly required. Therefore, there has been an ever-increasing need for novel, efficient, easy and personal diagnostic tools for the early detection of diseases, especially at the point-of-care (POC) [1, 2]. However, the technical limits (size, sensitivity, etc.) have hindered the realization of such POC devices. Recent rapid progress in microfluidic technologies has played a pivotal role in handling and processing quite a small amount of liquid, and the integration of biosensors in the microfluidic chips has extended the application of 'lab on a chip (LOC)' to biomedical areas [3-8]. More importantly, size, compact integration of the components, low sample volume consumption, and fast analysis and response time due to short diffusion distances may enhance the portability of device as well as shortening the diagnosis time and enabling the integration of LOC to electronic devices such as smartphone, and personal computers (PC). Supported by LOC technology, the POC device for the ubiquitous diagnosis could be achieved.

In this mini-review, we suggest an overview of LOC-based diagnostic technology, which is currently being actively developed and researched, as well as being readily available for in situ diagnosis. To narrow the focus, we concentrated on the diagnosis from a small amount of blood. For this purpose, blood microscale sampling, blood separation, immunochemical assay, and detection (optical and electrical), and communication to personal device (such as smart phones

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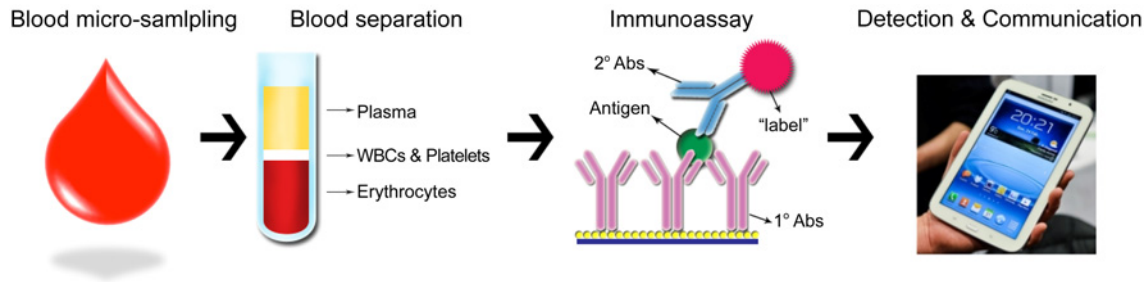E-mail : dbiomed@korea.ac.kr

Fig. 1. Schematics of POC diagnostic device.

or PC) are the essentially required processes (Fig. 1). Especially, the data stored in the PC or smartphone can be transmitted to doctors and nurses for analysis and earliest possible diagnosis. Here, we have described the technical status and trends about these four processes and considered a future prospects. The described LOC technologies for in situ diagnosis can be extensively used even for diagnosis of the extremely contagious and infectious diseases such as severe acute respiratory syndrome (SARS) and avian influenza. The early detection of such contagious diseases will greatly contribute to prevent the rapid spread throughout the global world.

BLOOD MICRO-SAMPLING

Human blood is an important key to point of care diagnosis, as they are easily accessible, as well as containing a lot of important information about our bodies. Therefore, a small sample of blood can reveal a lot about a patients' health status. The blood contains white blood cells (that can be counted for the immune-responses), red blood cells (anemia, sickle cell disease), and platelets (blood clotting activity), as well as plasma that contain the possible bacteria and virus that can affect the bodily functions. Thus, blood sampling in a microscale amount, and loading the sample to diagnostic

device is important. Traditional sampling method is a simple collection of blood using the syringe. A rather large amount of blood was drawn using the needle of the syringe, and then discharged into the prepared test tubes containing the additives such as Ethylenediaminetetraacetic acid (EDTA) and citrate. Sometimes, the blood had to be collected multiple times if a patient needs to be tested for multiple diseases. This method of blood sampling had many shortcomings, as the whole process was time consuming as well as providing a lot of room for error.

Sampling of blood and body fluid using microfluidic technology has emerged in recent few decades, and such methods allowed for diagnosis with small amount of sample, and with more accuracy. Li et al. have developed a system that can sample blood from a laboratory mouse in the ~ 1 L range by eliminating the large dead/ transfer volumes of conventional approaches (Fig. 2) [9]. The system draws blood sample through the needle and into a biocompatible steel reservoir that could be interchanged for a microanalytical chip. In the microfluidic sampling system, the needle must at least penetrate through the stratum corneum layer of the skin into the dermis layer, which indicates the importance of the needle [10]. Several blood or body fluid sampling systems that are simple, biocompatible and stable have been developed. S. Aoyagi proposes a biodegradable polymer (Poly Lactic Acid (PLA)) needle with a trench for collecting blood [11].
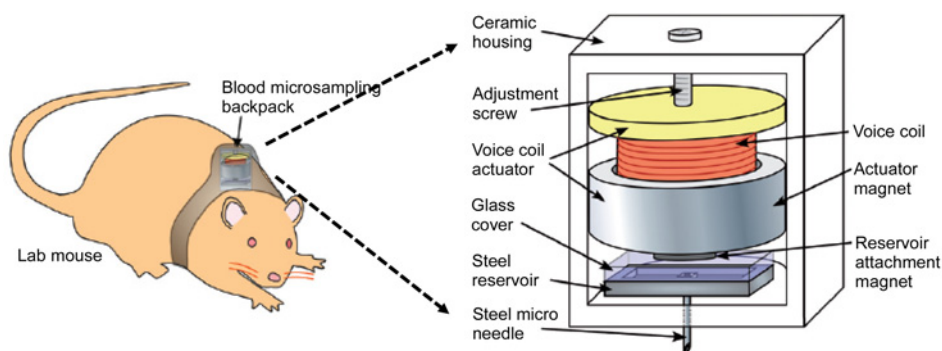

Fig. 2. (Left) Diagram showing application scheme of the blood sampling microsystem for pharmacokinetic studies, (Right) Schematic diagram of the proposed system from front view. Adapted from "A Blood Sampling microsystem for pharmacokinetic applications: Design, fabrication, and initial results" by Li, T., et al., 2009, Lab on a Chip, with permission of the The Royal Society of Chemistry.

Because hollow needles are difficult to fabricate, etching a trench on the needle surface has been proposed as an alternative. E.V. Mukerjee et al. have developed micro-needle array for the effective puncturing of human skin and extracting and delivering interstitial fluid (ISF) to the microchannels [12]. Besides, diverse micro-pumping and valve systems can be combined with microneedles to enable delivery and extraction of blood and body fluids of accurate amount.

ON CHIP BLOOD SEPARATION

Second step in on chip diagnosis using blood is separating the collected blood sample into cells including RBC and WBC, platelet and plasma. The cells can be separated from the blood based on their sizes, and weights. The most widely used methods of today are physical filtration, fluorescence-activated cell sorting (FACS), and magnetically actuated cell sorting (MACS). Conventionally, the physical membrane-based filtration has been widely used because it is simple and relatively efficient. However, the high cellular fractions lead to membrane clogging and compromise the separation efficiency. The FACS and MACS are methods that sort cells with high precision. But the technical requirements for these methods are too expensive and complicated to be used for a point of care diagnosis, and the machines required for these are not mobile at all, being bulky and heavy. In addition, these macro-scale cell-sorting methods have their limitations in complicated sample preparation procedures, target cell loss, requirement of skilled technician and introduction of artifacts, which are highly undesirable for the point of care diagnosis.

Recent progress in microfluidic technologies has enabled the blood separation to overcome aforementioned problems. Due to the microscale effect [13], microfluidic chip has demonstrated many appealing characteristics compared to the larger scale blood separation systems. In addition, the small size and simple sampling procedure of microfluidic system reduce the introduction of the unwanted artifacts, as well as allowing for dramatic reduction of target-cell loss, which enables even the detection of wanted rare cells such as circulating tumor cells (CTCs) [14].

Blood separation method using microfluidic chip can be categorized as follows: separation by size and weight, and by hydrodynamic mechanisms. The separation using filter or mesh is most common, and integration of such small filter in the microfluidic chip is one of popular methods in blood separation. In a microfluidic chip, diverse shapes of filters are used for blood separation, and among them, the comb, pillar, weir-type and microparticle based filters are popular (Fig. 3a and Fig. 3b). VanDelinder and Groisman devised weirs of gap size 0.5 m that can weed out the WBCs, RBCs, and platelets while letting the plasma pass through [15]. The

device can operate continuously for an hour extracting up to 8% of plasma at the flow speed of 0.65 L/min and with less than 0.1% hemolysis. Moorthy and Beebe came up with a microporous filter within the microchannel using the emulsion photo polymerization to filter through the whole blood that can be utilized to extract the plasma from the sample [16]. Crowley and Pizziconi also utilized the microfilters of planar shape to separate the plasma from the whole blood. They demonstrated the design and operation of passive microfilter devices applicable to the separation of plasma from whole blood in miniaturized clinical diagnostic devices [17]. Chen suggested pillar- and weir-type filtration microchips, and optimized cell concentration and length of separation channels to enhance the separation efficiency, and 95% RBC can be removed from the initial whole blood, while 27.4% WBC can be obtained [18].

Yang et al. has suggested plasma separation by designing a simple microfluidic network based on the Zweifach-Fung effect (Fig. 3c). The effect states that at the branching points of capillaries, or microchannels, the particles will always enter the channel with higher flow-rate [19]. The microfluidic network was designed using an analogous electrical circuit and the experimentally determined plasma selectivity with respect to blood hematocrit level was almost 100% [20]. Some researchers also developed a microfluidic device capable of separating WBCs from the whole blood without any pretreatment and lysing the separated WBCs in a continuous and near real-time fashion. Choi et al. have developed isolation method of WBCs using a microfluidic device composed of slanted obstacles and filtration obstacles, and the device isolated WBCs with 210-fold enrichment within a short filtration time of ~ 0.3 s [21]. This proved that
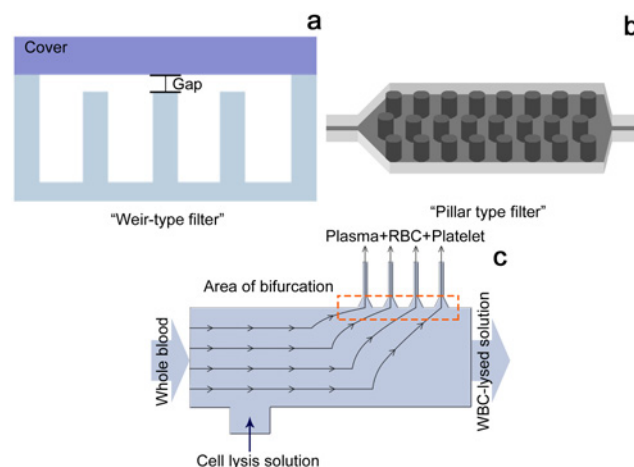

Fig. 3. (a) The schematic of the weir-type barrier. (b) The schematics of pillar-type barrier. (c) A microfluidic device for continuous WBC separation and lysis from whole blood. Consists of a blood inlet, a cell lysis solution inlet, a bifurcation outlets (composed of microchannels), and a WBC-lysed solution outlet.

the traditional centrifuge-based blood separation method could be replaced by microfluidic technology.

IMMUNOASSAY

After blood separation, immunoassay should be performed, and perhaps this is the most important step in blood-based diagnosis. The term "immunoassay" refers to a quantitative measurement, or a detection that depends on recognizing an analyte through the use of antibodies or highly purified antigens. The significance of immunoassay lies in detection of very small amount of analyte in any fluid, as immunoassays are highly sensitive and specific. Through immunoassay, not only the measurement of drugs, specific hormones, tumor markers, and many more analytes of extremely minute volume are possible, but also the qualitative detection of viral hepatitis, HIV and Lyme disease is possible.

The effectiveness of the immunoassays is maximized when done in micro-scale. The most distinguished aspects of the micro-scale immunoassays are its simplicity, efficiency, and the speed. First of all, the on-chip immunoassay allows for extreme simplification of the whole procedure. The simplicity comes from the integration of all immunoassay steps on one chip. The traditional immunoassay procedures require sampling of the whole blood, separation of the cells, and many other steps before the actual immunoassay takes place. Also, data needs to be collected in a separate apparatus, and analyzed before the results can be presented. However, the on-chip immunoassay integrates all those preceding steps, detection, and analysis into one, which increases the mobility of it. This makes the micro-scale immunoassay highly desirable for POC, or single use assays that can be widely distributed to the public. The on-chip immunoassay allows the increase of precision by decreasing the volume of the analytes. The small size of it not only increases the mobility and precision of this diagnostic tool, but also allows multiple assays to be done in simultaneously: the micro-scale immunoassay widens the scope of the analysis [22]. Lastly, the on-chip immunoassay is kinetically efficient. By reducing the distance the molecules need to travel, the assay can be done in a speedier manner without affecting its results [23].

Although the on-chip micro-scale immunoassays possess these obvious advantages as opposed to the macro-scale immunoassays, they are still in the development stage. Many studies are being actively conducted as we speak. Here, three types of on-chip immunoassay methods will be discussed.

Immobilization (the "sandwich type" immunoassays)
The first and the most common type of the immunoassays is the "sandwich type"–also known as, noncompetitive heterogeneous–immunoassay. This assay utilizes primary–or capture–antibody, a target antigen, and a labeled secondary antibody. This assay provides the highest sensitivity and specificity. The "immobilized" antibodies are coated onto a surface, then the antigens are bound to the immobilized antibodies, and the labeled secondary binds to the antigens, making a "sandwich" [24].

One method of immobilization is surface based immobilization. In 2011, Beck and his coworkers devised an on-chip cluster of differentiation 4 (CD4) counting kit using the timed release of antibodies [25]. They first pre-coated the microfluidic chamber of 25m height with the antibodies and other reagents, and let the coating dry. Then, the whole blood was sampled and incubated in the chamber for 10-30 minutes. Finally, two images of the chamber–one with red excitation light, and the other with blue–were taken. The CD4, or the target cell was counted after digitally summing up the two images. The immobilization in this case, has been done on the hydrogel, and the activation was achieved through diffusion, making the timed release of the antibodies possible. While diffusion may take long in the case of non-microscalse chambers (as long as a month), the microchamber of miniscule height made the assay possible in this case. Darain et al. also successfully immobilized primary antibodies onto the polystyrene (PS) surface based on the covalent bonding through a self-assembled monolayer of the thiol [26].

Apart from surface based immobilization, the bead-based immobilization is also common. This allows for maximum surface area, thus improving the performance of the immunoassays. Ko et al. developed a microfluidic electro-immunosensing chip for simultaneous determination of cancer biomarkers. They used the combination of antibody-conjugated PS beads for immobilization, gold nanoparticles for labeling, and silver enhancer for signal amplification. PS beads were coated with streptavidin for conjugation with biotinylated capture antibodies. These antibody-conjugated PS beads were trapped in the reaction area with the PDMS pillars, then the sample fluid containing the cancer markers were introduced, followed by the gold nanoparticles, and the silver enhancers. The whole assay was done in 55 minutes [27].

One site-noncompetitive assay (microfluidic reactor)
Another type of immunoassay is one site-noncompetitive assay. Unlike the "sandwich" type immunoassay, the antigens of interest each only bind to one labeled antibody. Then, the unbound labeled antibodies are washed out, leaving the bound labeled antibodies for measurements and analysis. This type of immunoassays is usually done on electrophoresis-based microchips, and requires many concentration strategies to stack the target analytes for detection and measurements [28].

Meagher and the coworkers developed a microfluidic apparatus with a photo-polymerized membrane and

polyacrylamide gel-filled microchannels. The former is for pre-concentration and the latter for sample loading and separation of left over antibodies from the complexes. First, the sample was loaded onto the chip and went through the membrane to trap the preconcentration. Then, the dye-labeled antibodies were loaded and were allowed to mix. After mixing and conjugating, the remaining antibodies and the complexes were separated in the channels. Finally, measuring the ratio of unbound and bound immune-complex using sensitive laser-induced fluorescence detection provides quantitation of analyte in the sample [29].

While this assay was relatively simple, it required separation channels. Reichmund et al. developed a microfluidic chip-based immunoassay without the separation channels. This apparatus only needed a chamber and a polyacrylamide membrane. Whereas Meagher et al. utilized the membrane only for pre-concentration, Reichmund and the coworkers utilized it for both concentrating the viral particles, and separating the labeled complexes from the unbound antibodies. The assay was done in following steps: first, the sample was mixed with the fluorescently labeled antibodies. Then, the antibody-virus complexes were concentrated on the membrane. The excess antibodies were removed by electrophoresis through the membrane, and the complex was then detected downstream. The total assay time was 6 minutes, while successfully detecting the inactivated influenza virus with less than 50m sample volume[30].

Competitive assay

The last type of immunoassay technique is the competitive assay. There are two types of competitive immunoassay–homogeneous competitive assay, and heterogeneous competitive assay. In both cases, the labeled analytes compete with the unlabeled analytes in the sample to bind to the antibody. The homogeneous competitive assay measures the labeled unbound analytes, which would be proportional to the unlabeled analyte of interest, whereas the heterogeneous competitive assay measures the labeled bound analytes.

Lee et al. used the competitive immunoassay to measure the amount of hippuric acid (HA) in human urine. The system put HA and the ferrocene-hippuric acid complex (Fc-Lys-HA) in competition to bind to HA antibody coated onto

polybeads which generated electric signals proportional to the HA concentration. The total assay only took 1 minute, which is much faster than the traditional electrochemical HA immunoassay (20 minutes) [31].

DETECTION

Depending on the label (or label-free), several different detection methods have been developed. For detection, transducers play an important role in signal transduction of the immune-recognition event to electrical signals. The electrochemical (amperometry, potentiometry, conductimetry/impedimetry), optical (colorimetric, fluorescence, luminescence, interferometry), calorimetric (thermistor), mass change (piezoelectric/acoustic wave) or magnetic events are the major immune-recognition events. However, detection with high sensitivity still remains a challenge. In addition, a detection strategy with highly effective signal transduction and multiplexed analysis is another important factor for high quality immune-sensor. The detection methods can be categorized into 3 groups: optical, electrical and mechanical detection. Recent microtechnologies enable these 3 categorized detection methods with high accuracy and fast processing time.

Optical immune-sensing is the simplest and highly popular method among the immunoassay methods, and several labels (e.g., a fluorescent label, enzyme, or metallic particle) facilitate optical signal enhancement and increase detection sensitivity. Recently, lots of optical microfluidic immune-sensing systems have been reported, and they have extensive potential applications in clinical diagnostics such as POC due to their versatility in functions. Optical detection methods can be categorized into five types based on the detection signals: fluorescence, luminescence, absorbance (colorimetry), surface plasmon resonance (SPR), and surface-enhanced Raman scattering (SERS).

Fluorescence detection is most popular method and suitable labels conjugated with the antibodies or antigens are excited by a laser or light-emitting diode light source (Fig. 5 (left)). The emitted light from the labeled molecules is detected by the photodetector. Hu et al. utilized the aqueous-

Fig. 4. Schematics of "Sandwich" type immunoassay.

phase-synthesized quantum dots (aqQDs) as fluorescent markers to develop highly sensitive (femtomolar sensitivity) microfluidic platform for detecting the cancer biomarkers [32]. Luminescence detection, on the other hand, measures the spontaneously generated luminescence intensity without the excitation light source because an enzymatic reaction between luminescent substrates and enzyme reagents causes photochemical emission. In 2007, Battacharyya et al. developed a microfluidic chip for detection of C-reactive protein (CRP) using chemiluminescence-based immunoassay. The assay results were presented on an on-chip instant photographic film, which was then read by an imager that can detect the chemiluminescent signals [33]. In colorimetry, colored stains resulting from the immunoassay is measured by a spectrophotometer. Despite the poor sensitivity of colorimetry compared to that of other optical methods, the simplicity and potential for miniaturization of colorimetry provide several advantages. Yu et al. developed a dextran-functionalized microfluidic immunosensor that can demonstrate colorimetric detection of several biomarkers (IL-5, hepatitis B surface antigen, and immunoglobulin G) in sera through spectrophotometer or naked eyes. The dextran modification on the microfluidic channel surface enhanced the hydrophilicity and the efficiency in protein immobilization, distinguishing this particular platform from the other colorimetric platforms with a high sensitivity (detection limit of up to 100pg/ ml) [34]. SPR is one of the useful biosensing systems to investigate biomolecular interactions and has diverse features including label-free, real-time, high-throughput, and sensitive analysis. SPR is also the most common optical evanescent wave biosensor. This type of detection uses the variation of the reflectivity on a metallic layer, which when closely in contact with a dielectric media, reflects the concentration of bound target (Fig. 5 (right)). Kurita et al. successfully detected a cardiac marker B-type natriuretic peptide (BNP) with the detection level of 5 pg/ml – 100 ng/ml. This was achieved by monitoring the real-time SPR angle shift while accumulating the thiol compound (generated by the enzymatic reaction during the immunoreaction) on a gold thin film on the microfluidic platform [35]. SERS was first introduced in order to enhance the sensitivity of Raman spectroscopy. The method incorporates metallic nanostructures or surfaces to improve the sensitivity from 10^6 to 10^{14}. SERS provides molecular information and its unique features are non-destructive, non-invasive, works in-situ and in-vitro for biological samples. In addition, SERS can operate irrespective of temperature and pressure. The molecules adsorbed on rough metal surfaces enhance Raman scattering and enhancement factors can be over 10^{10}, which are sufficient to allow even single molecule detection. Chon et al. used SERS of hollow gold nanospheres on a microfluidic chip to successfully detect the target marker. The limit of detection on the specific target marker they used (rabbit immunoglobin, IgG) was estimated to be $1 - 10$ ng/ml [36].

Electrochemical immune-sensing is another commonly used analytical technique for the detection of biomarkers in a sample, followed by optical detection. This method of detection is highly sensitive as well as rapid and selective in determining the analytes, and they can be easily incorporated into the miniaturized POC devices. The electrochemical immunosensors detect the electric signals that arise from the immunoreactions that occur near the surface of the electrodes. The two main types of electrochemical reactions—redox, and non-redox–that can be detected by the immunosensors are discussed here.
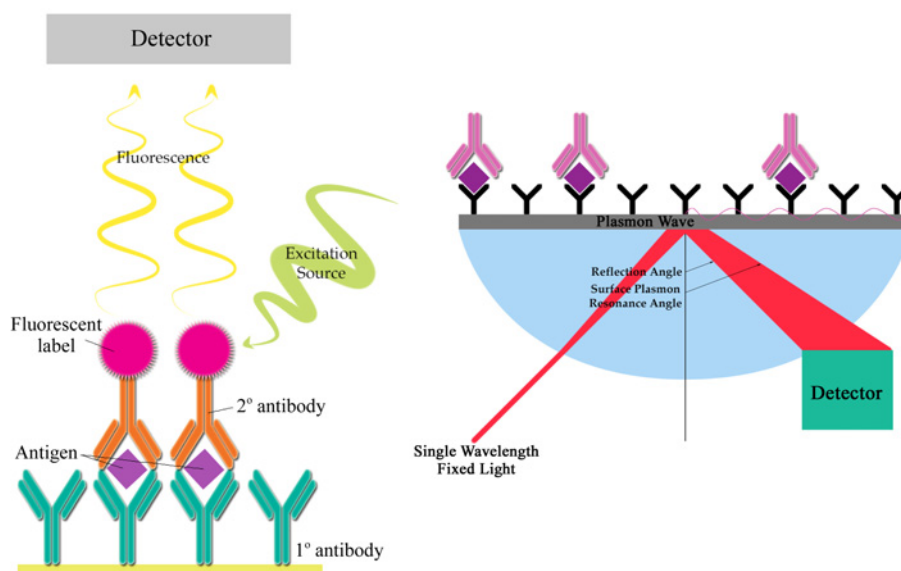

Fig. 5. Schematics of fluorescent immune-sensor (left), and surface plasmon resonance immune-sensor (right).

Most redox-based detection relies on the electrochemical signals that the conjugated enzyme generates in enzyme-labeled assays. Commonly, the redox species such as Prussian blue (PB) are added to monitor the electron transfer in the enzymatic reaction [37]. Rossier and Girault presented ELISA with electrochemical detection on a microchip with the horseradish peroxidase-secondary antibody conjugate (HRP-conjugate) as the enzymatic mediator (to catalyze electron transfer reaction). They incorporated the electrode inside a 40 nL microchannel that can detect the redox active enzyme substrate directly in the microchannel. The apparatus detected $0.1 - 100$ nM of D-Dimer with the sandwich immunoassay, which was the relevant concentration level for clinical settings [38]. Meanwhile, enzymatic reactions that convert an electrochemically inactive substrate into active product can be another signal that can be electrochemically detected. Jang et al. used the alkaline phosphate (AP) labeled secondary antibody to convert the electrochemically inactive substrate into active p-aminophenol, which was measured by the oxidation peak current during electro-oxidation into p-quinoneimine (PQI) at the working electrode. Using this method, Jang and her coworkers were able to detect with the limit as low as 485 pg/mL with 95% accuracy [39]. The electrochemical signals can also be generated by the non-redox reactions. Zhao et al. reported a novel non-redox based electrochemical immunoassay. They used the Cadmium-Sulfur (CdS) quantum dots (QDs) on the electrode surface, allowing for the photoelectrochemical sensing. The HRP conjugated with the antibody allowed for the precipitation on the CdS-QD electrodes, which was directly proportional to the target concentration [40].

Detecting for mechanical signals is another way of immunosensing. Mechanical detection provides highly sensitive and multiplexed analysis in a short period of time. This method usually involves immobilized antibodies on a sensing surface, and the binding of the target antigens onto these antibodies, which causes physical disruption of the equilibrium state, and ultimately, a mass change. This change in mass can be detected by the use of microcantilevers (MCs) or acoustic wave sensors. These offer highly sensitive detection of the mass changes, and do not require labels, thus deeming suitable for the POC diagnostic devices. The use of microcantilevers in immunosensors provides sensitive and label-free detection. The deflection mode of detection is based on the change in the mechanical deflection of the MC that occurs as a result of antigen binding. In 2005, Backmann et al. reported a MC-based immunosensor that operates on a deflection mode with a sensitivity level comparable to the SPR. Using the single chain Fv antibody fragments as the receptor molecules, Backmann and the coworkers were able to attain the sensitivity of ~1 nM [41]. MCs working in the resonance mode are generally used to measure the change in resonance frequency resulting from the target binding. These are generally called the "piezoelectric" microcantilever (PEMC). Hwang et al. utilized the PEMC in a microfluidic immunosensor. The device allowed for dynamic detection and measurement of prostate-specific antigen (PSA) without using the labels [42].

CONCLUSION

For the one-step assay from small amount of blood sample to in-situ analysis, four procedures including blood microscale sampling, blood separation, immunochemical assay and detection should be performed on a single platform. In this review, we overviewed the microfluidic technologies that are required per procedure and are currently being actively developed and researched. The developed microfluidic technologies surpass the limit of conventional immunosensing systems by enabling the reduced consumption of reagents and samples, and reducing the assay time, cost, power consumption, and size. In addition, the versatile technologies facilitate the sensitive multiplexed immunosensing and increased realistic clinical applications for POC diagnostics. We expect that the microfluidic immunosensing system on a single microfluidic platform is relevant for use by primary care physicians and outpatient clinics even in developed countries, because in-situ diagnosis can lead to higher rates of correct treatment and lower rates of unnecessary overtreatment [43]. Furthermore, in developing countries or on-going battlefields, the proposed microfluidic immunosensing system will play a great role to save lives and prevent the disease breakouts, such as highly infectious bird flu.

CONFLICT OF INTEREST STATEMENTS

Lee J declares that s/he has no conflict of interest in relation to the work in this article. Lee SH declares that s/he has no conflict of interest in relation to the work in this article.

REFERENCES

1. Sorger PK. Microfluidics closes in on point-of-care assays. Nat Biotechnol. 2008; 26(12):1345-6.

2. Webb SA. Returning to diagnostic basics. BioTechniques. 2010; 49(1):491-3.

3. Grayson AR, Shawgo RS, Johnson AM, Flynn NT, Li Y, Cima MJ, Langer R. A BioMEMS review: MEMS technology for physiologically integrated devices. Proc IEEE. 2004; 92(1):6-21.

4. Jeong GS, Chung S, Kim CB, Lee SH. Applications of micromixing technology. Analyst. 2010; 135(3):460-73.

5. Ng J, Shin Y, Chung S. Microfluidic platforms for the study of cancer metastasis. Biomed Eng Lett. 2012; 2(2):72-7.

6. Park JY, Takayama S, Lee SH. Regulating microenvironmental stimuli for stem cells and cancer cells using microsystems. Integrative Biol. 2010; 2(5-6):229-40.

7. Quake SR, Scherer A. From micro-to nanofabrication with soft materials. Sci. 2000; 290(5496):1536-40.

8. Whitesides GM, Ostuni E, Takayama S, Jiang X, Ingber DE. Soft lithography in biology and biochemistry. Annu Rev Biomed Eng. 2001; 3(1):335-73.

9. Li T, Barnett A, Rogers KL, Gianchandani YB. A blood sampling microsystem for pharmacokinetic applications: design, fabrication, and initial results. Lab Chip. 2009; 9(24):3495-503.

10. Kim MT, Son J, Cho CN, Park CM, Kim KG. Quantitative analysis of applied force on biopsy needle insertions. Biomed Eng Lett. 2012; 2(4):249-54.

11. Aoyagi S, Izumi H, Isono Y, Makihira K, Fukuda M. Biodegradable polymer needle having a trench for collecting blood by capillary force. Micro electro mechanical systems. Int Conf IEEE MEMS. 2006. 1:450-3.

12. Mukerjee E, Collins S, Isseroff R, Smith R. Microneedle array for transdermal biological fluid extraction and in situ analysis. Sensor Actuat A-Phys. 2004; 114(2):267-75.

13. Beebe DJ, Mensing GA, Walker GM. Physics and applications of microfluidics in biology. Annu Rev Biomed Eng. 2002; 4(1):261-86.

14. Kim ES, Kim S, Choi KY, Han KH. Micro-/nanotechnology-based isolation and clinical significance of circulating tumor cells. Biomed Eng Lett. 2012; 2(2):78-87.

15. VanDelinder V, Groisman A. Separation of plasma from whole human blood in a continuous cross-flow in a molded microfluidic device. Anal Chem. 2006; 78(11):3765-71.

16. Moorthy J, Beebe DJ. In situ fabricated porous filters for microsystems. Lab Chip. 2003; 3(2):62-6.

17. Crowley TA, Pizziconi V. Isolation of plasma from whole blood using planar microfilters for lab-on-a-chip applications. Lab Chip. 2005; 5(9):922-9.

18. Chen X, Cui DF, Liu CC, Li H. Microfluidic chip for blood cell separation and collection based on crossflow filtration. Sensor Actuat B-Chem. 2008; 130(1):216-21.

19. Doyeux V, Podgorski T, Peponas S, Ismail M, Coupier G. Spheres in the vicinity of a bifurcation: elucidating the Zweifach–Fung effect. J Fluid Mech. 2011; 674:359.

20. Yang S, Ündar A, Zahn JD. A microfluidic device for continuous, real time blood plasma separation. Lab Chip. 2006; 6(7):871-80.

21. Choi S, Song S, Choi C, Park JK. Continuous blood cell separation by hydrophoretic filtration. Lab Chip. 2007; 7(11):1532-8.

22. Lee S, Lee S. Micro total analysis system (μ-TAS) in biotechnology. Appl Microbiol Biot. 2004; 64(3):289-99.

23. Wild DG. The immunoassay handbook: theory and applications of ligand binding, ELISA and related techniques. Sci. 2013.

24. Wehmeyer KR, Halsall H, Heineman W. Heterogeneous enzyme immunoassay with electrochemical detection: competitive and "sandwich"-type immunoassays. Clin Chem. 1985; 31(9):1546-9.

25. Beck M, Brockhuis S, van der Velde N, Breukers C, Greve J, Terstappen LW. On-chip sample preparation by controlled release of antibodies for simple CD4 counting. Lab Chip. 2012; 12(1):167-73.

26. Darain F, Gan KL, Tjin SC. Antibody immobilization on to polystyrene substrate—on-chip immunoassay for horse IgG based on fluorescence. Biomed Microdevices. 2009; 11(3):653-61.

27. Ko YJ, Maeng JH, Ahn Y, Hwang SY, Cho NG, Lee SH. Microchip based multiplex electro immunosensing system for the detection of cancer biomarkers. Electrophoresis. 2008; 29(16):3466-76.

28. Lin CC, Wang JH, Wu HW, Lee GB. Microfluidic immunoassays. J Lab Autom. 2010; 15(3):253-74.

29. Meagher RJ, Hatch AV, Renzi RF, Singh AK. An integrated microfluidic platform for sensitive and rapid detection of biological toxins. Lab Chip. 2008; 8(12):2046-53.

30. Reichmuth DS, Wang SK, Barrett LM, Throckmorton DJ, Einfeld W, Singh AK. Rapid microchip-based electrophoretic immunoassays for the detection of swine influenza virus. Lab Chip. 2008; 8(8):1319-24.

31. Yoo SJ, Choi YB, Ju JI, Tae GS, Kim HH, Lee SH. Microfluidic chip-based electrochemical immunoassay for hippuric acid. Analyst. 2009; 134(12):2462-7.

32. Hu M, Yan J, He Y, Lu H, Weng L, Song S, Fan C, Wang L. Ultrasensitive, multiplexed detection of cancer biomarkers directly in serum by using a quantum dot-based microfluidic protein chip. Acs Nano. 2009; 4(1):488-94.

33. Bhattacharyya A, Klapperich C. Design and testing of a disposable microfluidic chemiluminescent immunoassay for disease biomarkers in human serum samples. Biomed Microdevices. 2007; 9(2):245-51.

34. Yu L, Li CM, Liu Y, Gao J, Wang W, Gan Y. Flow-through functionalized PDMS microfluidic channels with dextran derivative for ELISAs. Lab Chip. 2009; 9(9):1243-7.

35. Kurita R, Yokota Y, Sato Y, Mizutani F, Niwa O. On-chip enzyme immunoassay of a cardiac marker using a microfluidic device combined with a portable surface plasmon resonance system. Anal Chem. 2006; 78(15):5525-31.

36. Chon H, Lim C, Ha SM, Ahn Y, Lee EK, Chang SI, Seong GH, Choo J. On-chip immunoassay using surface-enhanced Raman scattering of hollow gold nanospheres. Anal Chem. 2010; 82(12):5290-5.

37. Guan JG, Miao YQ, Chen JR. Prussian blue modified amperometric FIA biosensor: one-step immunoassay for -fetoprotein. Biosens Bioelectron. 2004; 19(8):789-94.

38. Rossier JS, Girault HH. Enzyme linked immunosorbent assay on a microchip with electrochemical detection. Lab Chip. 2001; 1(2):153-7.

39. Jang Y, Oh SY, Park JK. In situ electrochemical enzyme immunoassay on a microchip with surface-functionalized poly (dimethylsiloxane) channel. Enzyme Microb Tech. 2006; 39(5):1122-7.

40. Zhao WW, Ma ZY, Yu PP, Dong XY, Xu JJ, Chen HY. Highly sensitive photoelectrochemical immunoassay with enhanced amplification using horseradish peroxidase induced biocatalytic precipitation on a CdS quantum dots multilayer electrode. Anal Chem. 2011; 84(2):917-23.

41. Backmann N, Zahnd C, Huber F, Bietsch A, Plückthun A, Lang HP, Güntherodt HJ, Hegner M, Gerber C. A label-free immunosensor array using single-chain antibody fragments. Proc Natl Acad Sci USA. 2005; 102(41):14587-92.

42. Hwang KS, Lee JH, Yoon DS, Park JH, Kim TS. In-situ quantitative analysis of a prostate-specific antigen (PSA) using a nanomechanical PZT cantilever. Lab Chip. 2004; 4(6):547-52.

43. Gubala V, Harris LF, A. Ricco J, Tan MX, Williams DE. Point of care diagnostics: status and future. Anal Chem. 2012; 84(2):487.