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Research review paper

Portable microfluidic and smartphone-based devices for monitoring of cardiovascular diseases at the point of care

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ABSTRACT

Cardiovascular diseases (CVDs) are the main causes of morbidity and mortality in the world where about 4 in every 5 CVD deaths happen in low- and middle-income countries (LMICs). Most CVDs are preventable and curable, which is largely dependent on timely and effective interventions, including diagnosis, prognosis and therapeutic monitoring. However, these interventions are high-cost in high income countries and are usually lacking in LMICs. Thanks to the rapid development of microfluidics and nanotechnology, lots of portable analytical devices are developed for detection of CVDs at the point-of-care (POC). In the meantime, smartphone, as a versatile and powerful handheld tool, has been employed not only as a reader for microfluidic assays, but also as an analyzer for physiological indexes. In this review, we present a comprehensive introduction of the current status and potential development direction on POC diagnostics for CVDs. First of all, we introduce some main facts about CVDs and their standard diagnostic procedures and methods. Second, we discuss about both commercially available POC devices and developed prototypes for detection of CVDs *via* immunoassays. Subsequently, we report the advances in smartphone-based readout for microfluidic assays. Finally, we present some examples using smartphone, individually or combined with other components or devices, for CVD monitoring. We envision an integrated smartphone-based system capable of functioning blood tests, disease examination, and imaging will come in the future.

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1. Introduction

Cardiovascular diseases (CVDs), including heart diseases and strokes, have been the leading causes of death worldwide (Mendis et al., 2011). The deaths caused by CVDs account for ~30% of the 55 million global deaths in 2013 (Naghavi et al., 2015). Of these 17.3 million deaths, around 20% of deaths occurred before the age of 60 (*i.e.*, premature deaths), and the premature mortality rate in low-income countries is ~10 times higher than that in high-income countries (Mendis et al., 2011; WHO, 2011). Moreover, during the past two decades, deaths from CVDs have been increasing in low- and middle-income countries (LMICs) and decreasing in high income countries (Mendis et al., 2011). For the next decade, the mortality rate will continue to increase throughout the world with the greatest increase in Africa (WHO, 2011). In fact, most CVDs are preventable and curable, which is greatly dependent on timely and effective interventions (Mendis et al., 2011). In developing countries, public health services are largely lacking. The limited healthcare resources are always gathered in centered hospitals and mainly served for treatment of diseases (Mendis et al., 2011). A large number of people with high cardiovascular risks are thus misdiagnosed (Mendis et al., 2011). Moreover, some people diagnosed with CVDs show poor clinical compliances since they are lacking in the sense of severity of CVDs (Ouyang, 2014). Therefore, user-friendly and cost-effective techniques are in urgent demand for diagnosis, prognosis, and therapeutic monitoring of CVDs at the point-of-care (POC).

POC testing (POCT), referred to a laboratory assay that can be performed outside of centralized facility, shows great promise for application in LMICs (Scardino and Hay, 2007). It develops fast as microfluidics and nanotechnologies develop. Nowadays, many platforms have been and are being developed for POCT, such as optical microfluidic technologies (Myers and Lee, 2008), lab-on-a-chip systems (Ahn et al., 2004; Tüdős et al., 2001), electrochemical biosensors (Wang, 2006), POC ultrasonography (Moore and Copel, 2011), paper-based devices (Choi et al., 2015; Dou et al., 2015; Hu et al., 2014), and smartphone-based strategies (Xu et al., 2015a). Of these techniques, microfluidics-based devices stand out and have drawn intensive attention from institutes to industries due to their various useful capabilities, such as low cost, simple operations, short turnaround, limited samples and reagents consumption, and capability for simultaneous separation and detection with high resolution and sensitivity (Sackmann et al., 2014; Whitesides, 2006). These advantageous features make them very suitable to create portable POC medical diagnostic systems (Yager et al., 2006). The last three decades, especially the recent decade, have witnessed the rapid development and wide application of microfluidics, especially for chemo/biosensing (Sia and Kricka, 2008), such as isolation and capture of circulating tumor cells (CTCs) (Karabacak et al., 2014; Sarioglu et al., 2015), diagnosis of infectious diseases (*e.g.*, human immunodeficiency virus (HIV) and syphilis) (Chin et al., 2011), and multiplex detection of biomarkers from cell lysates (Song et al., 2012). Moreover, materials for preparing microfluidics have moved from silicon and glass to soft materials, such as polymer (*e.g.*, poly(dimethylsiloxane), PDMS) and paper (Nge et al., 2013). Every material has its strengths as well as weakness. Hybrid devices, for example PDMS/paper composites (Han et al., 2013), are thus expected to combine each material's strengths. To further promote the application of microfluidics based devices at the POC, smartphone-based data collection and analysis are extensively explored. Furthermore, due to the development of software, hardware and serves, smartphones have found widespread applications in monitoring physiological indexes of CVDs as well. In a word, microfluidics and smartphone based devices are expected to improve the healthcare at the POC.

Compared with the themes of global health (Yager et al., 2008), infectious diseases (Niemz et al., 2011; Wang et al., 2010) or cancers (Rusling et al., 2010; Soper et al., 2006), CVDs received less emphases in LMICs. The World Health Organization (WHO) made in-depth and comprehensive investigations about CVDs, providing evidence-based data to assist our knowledge about CVDs as well as detailed instructions from individual level to global level. However, to further address the threat of CVDs, we are still on the way. Some documents have reported cardiac biomarkers (Fathil et al., 2015; Maisel, 2012; Marian and Nambi, 2004) and corresponding detection techniques (Adams and Apple, 2004; Altintas et al., 2014; Fakanya et al., 2013; McDonnell et al., 2009; Mohammed and Desmulliez, 2011), which are limited and not integral; especially about the emerging microfluidics- and smartphone-based devices for monitoring of CVDs, there are rare reports. Therefore, we intend to provide a concise and clear introduction of the status of CVDs and pave a way for the development of POC devices for detection of these diseases to all the stakeholders. In this review, we start from the point of CVD tests, especially focus on the research and development of microfluidics- and smartphone-based devices and their applications in this field. First of all, we give a brief introduction to CVDs and their standard diagnostics, followed by existing commonly-used and commercially-available solutions. Most importantly, we present portable microfluidic devices with promising capability of and potential application in detection of CVDs *via* their biomarkers mainly using chip- or paper-based materials. Their combination or integration with smartphone will be a trend and expected to be widely-used in future diagnostics, thus being shown in this review using several typical examples. Finally, smartphone based devices that have already been used for CVD monitoring based on collecting physiological signals, such as heart rate, blood pressure and electrocardiography (ECG), are discussed in this review as well.

2. CVDs: incidence, diagnosis, and biomarker

2.1. Incidence of CVDs

CVDs, also called heart diseases, are referred to a group of diseases of the heart and blood vessels, including atherosclerosis and hypertension (AHA, 2014; BHF, 2015; WHF, 2015; WHO Regional Office for Europe, 2015). CVDs continue to be the top one killer on the earth (Mendis et al., 2011). In 2013, CVDs killed about 17.3 million people, accounting for about 1/3 deaths worldwide and surpassing the sum of death toll caused by infectious diseases and cancers (Fig. 1A) (Naghavi et al., 2015). Most of these deaths were attributed to ischemic heart disease (heart attack) and cerebrovascular disease (stroke) (Fig. 1A). Heart attack and stroke were responsible for 8.1 and 6.4 million deaths and listed as the top one and two in 240 causes of deaths, respectively (Naghavi et al., 2015). Heart attack and stroke both mainly result from the process of atherosclerosis. Atherosclerosis is a chronic and complex pathological process which can happen throughout the whole circulation system. It is a condition wherein the walls of arteries become thick and stiff after fatty deposits build up on the inner walls of medium- and large-sized blood vessels (arteries). The fatty deposits are called plaques which contain not only fatty materials but also cholesterols. The plaques can form and also rupture. When they form, the lumen of blood vessels becomes

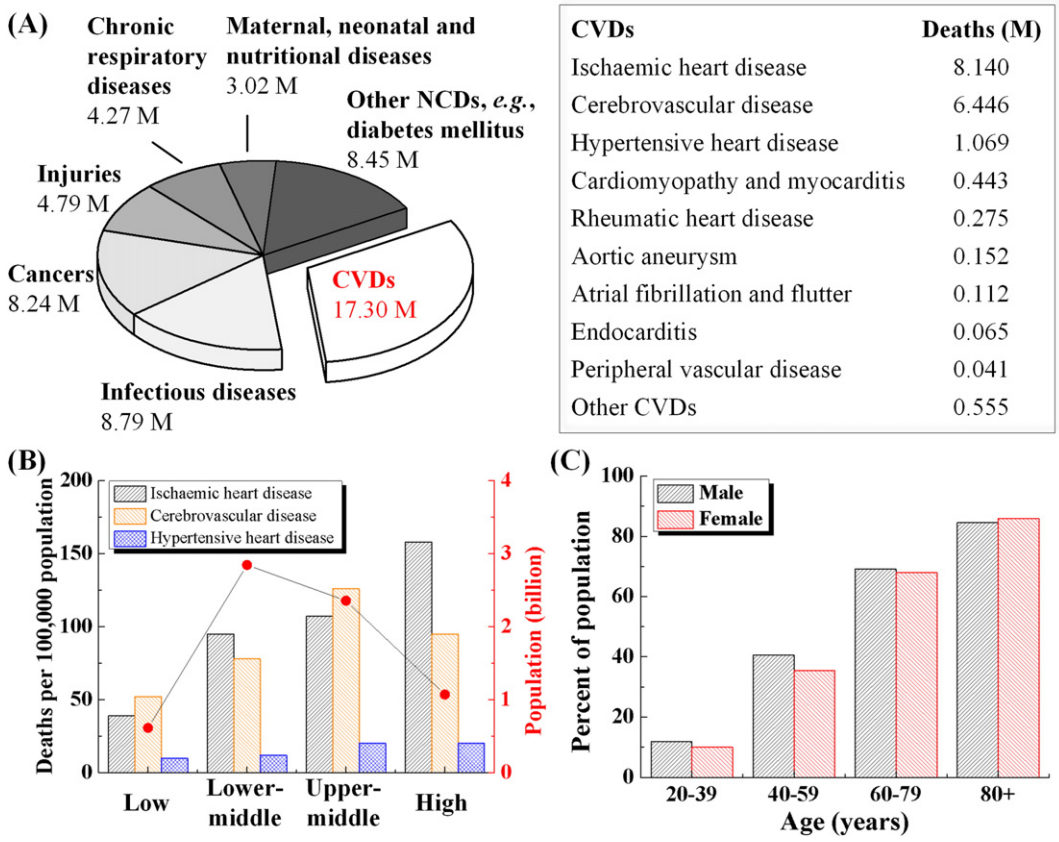

Fig. 1. Some facts about CVDs. (A) Leading causes of death worldwide and the deaths due to CVDs by their types. CVDs, as the number one cause of death throughout the world, were responsible for 17.3 million (about 1/3) of 54.9 million deaths in 2013. Of these deaths, 8.1 million people died of ischaemic (or ischemic) heart disease (heart attack) and 6.4 million cerebrovascular diseases (strokes). Reprinted from Naghavi et al. (2015)) with permission of Elsevier. (B) Three major CVD deaths per 10,000 population in 2012 by country income group with its population in 2014. High income countries own the highest proportion of death caused by CVDs, while over 80% of deaths occurred in low- and middle-income countries (LMICs). Data obtained from the World Health Organization (http://www.who.int/whr/en) and the World Bank (http://www.worldbank.org/). (C) Prevalence of CVDs in adults ≥20 years of age by age and sex in the USA. These data include heart attack, heart failure, stroke, and hypertension. Reprinted with permission of Mozaffarian et al. (2015), Circulation (2015), Copyright © 2015, Wolters Kluwer Health.

narrow and thus restricts the flow of blood. When they rupture, they would trigger the formation of blood clot. If the blood clot was located in a coronary artery, it would cause a heart attack; in the brain, a stroke, and in the legs, peripheral arterial diseases (Mendis et al., 2011). Additionally, there are many other types of CVDs, including hypertensive heart disease, inflammatory heart diseases (*e.g.*, pericarditis, myocarditis, and endocarditis), rheumatic heart disease, congenital heart disease, cardiomyopathies and cardiac arrhythmias (Mendis et al., 2011). The World Heart Federation (WHF) divides CVDs into heart-, brain- and peripheral circulatory system-related CVDs according to the directly affected positions.

Most CVDs are preventable and curable (Division for Heart Disease and Stroke Prevention, 2015). However, the occurrence and outcome of CVDs between countries and population are largely different. First, from a global perspective, the mortality rates of three major CVDs in different country income groups are not the same (Fig. 1B). Briefly, the high income country group owned the highest CVD mortality rate, while LMICs were responsible for more than 80% of CVD deaths. Moreover, over 3 million CVD deaths occurred before 60 years old of whom most took place in LMICs (Mendis et al., 2011). In low-income regions, for example Africa, undiagnosed and untreated high blood pressure (hypertension), as main causes for CVD deaths, affects nearly 1/2 of the people over 25-years of age; the rate is higher than any other continent in the world (Ouyang, 2014; WHO, 2013). Nowadays, CVDs are one of the top causes of death in sub-Saharan Africa in adults over 30 years of age. Due to a misperception that non-communicable diseases (NCDs) are for high-income countries, non-communicable, chronic diseases are increasing fast without timely attention, especially hypertension (Ouyang, 2014). Second, from a national perspective, according to the American Heart Association (AHA) statistical update in 2015, the prevalence of CVDs in adults (≥20 years) increases as age increases in both male and female in the USA (Mozaffarian et al., 2015). Approximately 20% of Americans who died of CVD in 2011 were <65 years of age, and 34% of deaths before the age of 75 years (below 78.7 years, the current average life expectancy). Furthermore, mortality declined for all USA groups during 1969–2011. However, there are faster declines in mortality among higher socioeconomic groups who have benefitted from early intervention.

2.2. Standard diagnosis of CVDs

Intervention, treatment or rehabilitation cannot live without diagnosis, and especially effective intervention cannot be independent of early diagnosis. Currently, mature procedures with many diagnostic tests have been established for CVD diagnosis and prognosis (Antman et al., 2000). First of all, people may feel uncomfortable sometime or somewhere and ask for doctors' help. The doctor may get to know the sufferers' symptoms, medical history and history of disease *via* an inquiry and physical examination, and then choose suitable test(s). In Traditional Chinese Medicine (TCM), this step is performed using four diagnostic methods, *i.e.*, inspection, auscultation–olfaction, interrogation and palpation (Beijing Digital Museum of TCM, 2009; The Hong Kong Polytechic University, 2015). Briefly, the four diagnostic methods are to diagnose diseases by means of human sense and experience. These

four basic procedures are generally for clinical assessment of any disease, including CVDs. Everyone can partially or totally adopt the four diagnostic methods for self-monitoring or monitoring others, for example, using pulse examination to record heartbeat rate. However, these methods are human sense-based and vary person-to-person and time-to-time. Only experienced ones can collect accurate and effective information for aid of disease diagnosis and prognosis. Nevertheless, inspection and interrogation could be a general step to diagnose CVDs, which is still performed in current clinics worldwide.

To improve accuracy and reduce ambiguity in detection of CVDs, instrumentation-based methods have been well-established for measurement of physiological, imaging, biochemical and even pathological indexes so far. They can be divided into non-invasive and invasive tests. Briefly, the measurement of physiological indexes, including heart rate, blood pressure, and even ECG, are based on relatively simple devices, which provides limited clinical value sometimes although the cost is not high. The collection of imaging signals usually requires bulky instrument (*e.g.*, chest X-ray and computed tomography (CT)), professional operation (*e.g.*, catheterization and angiography), and careful analyses. These methods usually can provide very helpful information, but their costs are usually very high. Meanwhile, they are usually not available in resource-limited settings. Therefore, blood tests for cardiac biomarkers using a microplate reader or biochemical analyzer, providing biochemical indexes with an intermediate cost, have been widely used as routine checkup for detection of CVDs.

2.3. Biomarker of CVDs

Biomarkers are referred to those molecules carried with diagnostic and prognostic information. Ideal biomarkers would be directly corresponded to the etiological or pathophysiological information of diseases, but currently widely-used biomarkers are mainly tissue-specific expression or statistical correlation of diseases, including cells, nucleic acids, proteins, metabolic molecules, *etc.* (Sanjay et al., 2015; Sorger, 2008). First biomarkers for monitoring of CVDs were reported over a half of century ago when measurements of enzymatic activities of lactate dehydrogenase (LDH), creatine kinase (CK), or aspartate transaminase (AST) were used to diagnose acute myocardial infarction (AMI) (HyTest, 2015). Since these assays had poor specificity and sensitivity, immunoassays were subsequently developed for detection of biomarkers using polyclonal and further monoclonal antibodies since the 1980s. The past decade has witnessed an explosive growth in the use of biomarkers for various clinical applications, especially for cardiology. Lots of cardiac biomarkers have already been reported and novel biomarkers for CVDs are increasingly emerging as well. Nowadays, these biomarkers include lipids (such as cholesterol, high-density lipoprotein, and low-density lipoprotein), hormones, enzymes, proteins, and genetic biomarkers (such as single-nucleotide polymorphism (SNP) and structural variants). These biomarkers are used to identify inflammation/vascular damage, atherosclerosis/unstable plaque, AMI, heart failure (HF), extracellular-matrix remodeling/fibrosis, *etc.* (Adams and Apple, 2004; Braunwald, 2008; Daniels, 2012). A book entitled Cardiac Biomarkers: Expert Advice for Clinicians (edited by Dr. Maisel (2012) has summarized existing and newer cardiac biomarkers, which is a good reference for further information. For the use of cardiac markers in acute coronary syndromes (ACS, clinical symptoms caused by myocardial ischemia) and HF, the National Academy of Clinical Biochemistry has presented detailed laboratory medicine practice guidelines (Christenson et al., 2007).

Although there are a prolific number of cardiac biomarkers, limited cardiac biomarker tests are routinely used in the clinic, which mainly includes AMI (heart-type fatty acid binding protein (h-FABP), creatine kinase-MB (CK-MB), myoglobin, cardiac troponin I/T (cTnI/cTnT)), heart failure (B-type natriuretic peptide (BNP) and N-terminal prohormone B-type natriuretic peptide (NT-proBNP)), and future heart risk (C-reactive protein, CRP) (Table 1). The biomarkers accepted into clinic care are required to meet several criteria, where three benchmarks have been outlined to do such assessments (Morrow and de Lemos, 2007). First, accurate and reproducible measurements of the biomarker with short turnaround time and reasonable cost profile must be available to the clinician. Second, the candidate biomarker should provide validated clinical value (either adding to or replacing of established ones) that confirms the relationship between the biomarker and the outcome or disease. Finally, knowing the measured results can facilitate the clinician improve patient care. In other words, an accepted cardiac marker should be useful in clinical care and feasible in analytical methods (Kemp et al., 2004). Lots of cardiac biomarkers currently are only used for research but may be available in clinical practice after external validation. The biomarkers listed in Table 1 are just the

Table 1
Representative biomarkers used for monitoring of CVDs.

Biomarker	Clinical level (ng/mL)	Clinical indication	Initial elevation (h)	Time to peak (h)	Return to normal (days)	Specificity
h-FABP (McDonnell et al., 2009; Mohammed and Desmulliez, 2011)	6	Early detection of AMI	2–3	8–10	0.75–1.25	Low
Myoglobin (McDonnell et al., 2009; Mohammed and Desmulliez, 2011)	0.07–0.2	Early detection of AMI and reperfusion	1–3	6–12	1–2	Low
CK-MB (McDonnell et al., 2009; Mohammed and Desmulliez, 2011)	5.0	Early detection of AMI	4–6	12–24	3–4	Medium
cTnI/cTnT (Fathil et al., 2015; McDonnell et al., 2009)	0.001 0.01 0.1 1 10 100	Healthy Stable angina or chronic HF Micro AMI, myocarditis or acute HF Small AMI, myocarditis, or pulmonary embolism Medium sized AMI or severe myocarditis Large AMI	4–6	12–24	6–8 (for cTnI); 7–10 (for cTnT)	High
CRP (Adams and Apple, 2004; Mohammed and Desmulliez, 2011)	<1000 ng/mL low risk >3000 ng/mL high risk	Future heart risk	~1–3	ND	ND	High
BNP (McDonnell et al., 2009; Mohammed and Desmulliez, 2011; Weber and Hamm, 2006)	<0.1 ng/mL HF unlikely >0.5 ng/mL HF likely	HF	ND	ND	ND	High
NT-proBNP (McDonnell et al., 2009; Mohammed and Desmulliez, 2011; Weber and Hamm, 2006)	For age < 50, <0.3 ng/mL HF unlikely, >0.3 ng/mL HF likely; For age > 50, <0.3 ng/mL HF unlikely, >0.9 ng/mL HF likely	HF	ND	ND	ND	High

Note: h-FABP: heart-type fatty acid binding protein; CK-MB: creatine kinase-MB; cTnI/cTnT: cardiac Troponin I/T; CRP: C-reactive protein; hs-CRP: high-sensitivity C-reactive protein; BNP: B-type natriuretic peptide; NT-proBNP: N-terminal prohormone B-type natriuretic peptide; AMI: acute myocardial infarction (heart attack); HF: heart failure. ND: not consensus definition.

representative validated ones today and may be updated in future. In fact, the change is taking place. For example, cardiac troponins, as one of the most specific cardiac biomarkers, have largely replace CK-MB and myoglobin in the USA. High-sensitivity cardiac troponin test that detects the same protein but at a much lower level than the standard test has already been clinically used in Europe and Canada, which is still under research in the USA (American Association for Clinical Chemistry, 2015).

Cardiac biomarkers have various concentrations (from pg/mL to μg/mL), show different stabilities (from several hours to several days), own diverse specificity (from low to high level), and indicate distinct stages of diseases (Table 1). To measure these biomarkers, the techniques require the capability of serial measurements (to detect the same biomarker at different time) and multiplex detection (to detect different biomarkers at the same time). In the clinic, general lab tests, including blood gases, metabolic panel, electrolytes and complete blood count, are routinely used along with cardiac biomarkers to evaluate patients' general health status. Moreover, biomarker tests are performed along with ECG and imaging techniques as multimodal approaches for monitoring of CVDs. CVDs are so complicated that we may need to diagnose and evaluate them from all the aspects of pathology, biochemistry, ECG, and imaging by putting different techniques together (Antman et al., 2000; Daniels, 2012). Integrated multimodal approaches can overcome the disadvantages of each technique by providing even more comprehensive information of the diseases, which actually happens in centered laboratories but poses more challenges in resource-limited settings.

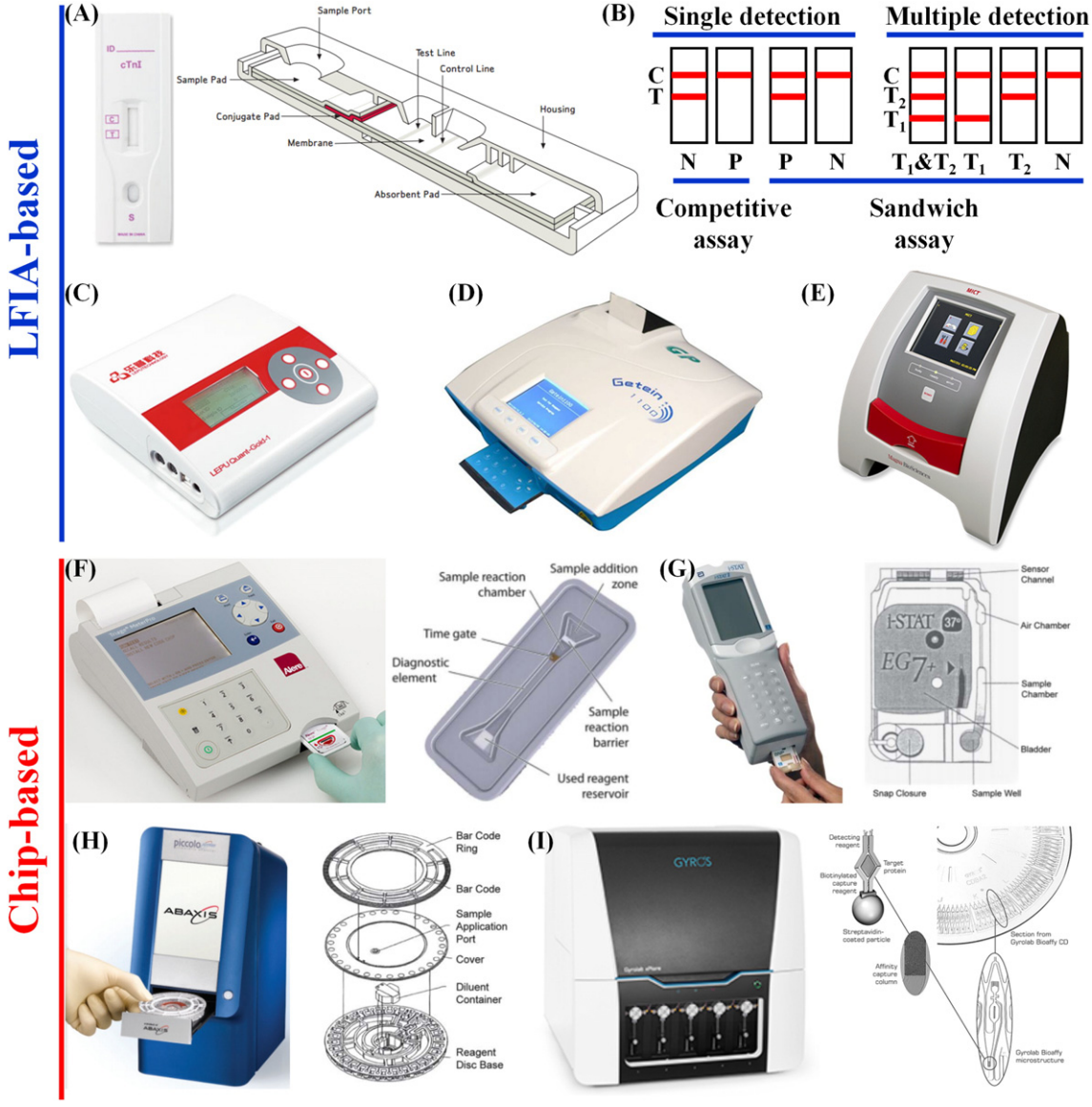

Fig. 2. Typically commercially-available point-of-care blood tests, including lateral flow immunoassay and microfluidic chip-based immunoassay, for detection of cardiac biomarkers. (A) Image of a representative lateral flow test strip (http://www.lepumedical.com/) and its schematic (EMD Millipore, 2013). Copyright © 2013, EMD Millipore Corporation. (B) Single and multiple detection based on lateral flow immunoassays. Typical portable devices for quantification of lateral flow immunoassay results based on (C) colorimetric (Lepu Quant-Gold 1, Dimensions: 178 × 165 × 46 mm, Net weight: 620 g, http://www.lepumedical.com/), (D) fluorescent (Getein 1100, Dimensions: 385 × 345 × 155 mm, Net weight: 2.1 kg, http://www.bio-gp.com.cn/), and (E) magnetic (MICT® Bench-Top System, Dimensions: 330 × 250 × 230 mm, Net weight: 3.3 kg, http://www.magnabiosciences.com/) signals. Images and schematics of selected microfluidic chip-based immunoassays: (F) Alere Triage® MeterPro (Dimensions: 225 × 190 × 70 mm, Net weight: 700 g without batteries, http://www.alere.com/) and the schematic of its test strip, and (G) Abbott i-STAT System (Dimensions: 234.8 × 76.8 × 72.4 mm, Net weight: 635 g, https://www.abbottpointofcare.com/) and the schematic of its test cartridges. Reprinted with permission of Chin et al. (2012)), Lab Chip (2012), Copyright © 2012, Royal Society of Chemistry. (H) Abaxis Piccolo Xpress® (324 × 152 × 203 mm, Analyzer: 5.1 kg, Power adapter: 0.7 kg) and its reagent disk. (I) Gyrolab xPlore™ (Dimensions: 540 × 580 × 640 mm, Net weight: 80 kg, http://www.gyros.com/) and Gyrolab Bioaffy CD for sandwich immunoassay. Reprinted with permission of Inganäs et al. (2005), Clinical Chemistry (2005), Copyright © 2005, P.B. Hoeber Publisher.

3. Portable microfluidic-based diagnostic devices for CVDs

The requirements of diagnostic techniques in resource-limited settings are quite different from those in developed countries. For example, enzyme-linked immunosorbent assays (ELISA) for cardiac biomarker tests taken granted in developed countries are generally not feasible in resource-limited settings due to two main constraints (Hu et al., 2014; Yager et al., 2008). One is staffing constraints. In resource-limited settings, the quantity of qualified healthcare providers is limited and their ability is limited as well. In some settings, some assistant medical officers run entire rural district hospitals and are just able to handle some easy-to-use assays. The other is laboratory infrastructure and resources. First, the laboratory capacity is limited and not integral. Second, the procurement of supplies is unreliable and unstructured, and the quality of purchased supplies is usually poor. Finally, the laboratory suffers from physical constraints, including limited clean water, unreliable power sources, and vast temperature and humidity variations. All the above factors combined together pose poor clinical sensitivity and specificity, which not only waste time and fund but also may cause serious medical accidents. Therefore, the WHO has outlined guidelines for diagnostic systems used in resource-limited settings, *i.e.*, ASSURED criteria: affordable, sensitive, specific, user-friendly, rapid and robust, equipment-free and deliverable to end-users (Martinez et al., 2010; Peeling et al., 2006; Yager et al., 2008).

3.1. POC products available in the market

Currently, the most successful portable microfluidic devices should be rapid immuno-chromatographic test strips, also known as lateral flow immunoassays (LFIAs) (Wong and Tse, 2009). The first commercially-available LFIA is human chorionic gonadotropin (hCG) test for home pregnancy (EMD Millipore, 2013). Nowadays, LFIAs are widely used in biomedical, phytosanitary, veterinary, food safety, and environmental settings for qualitative, semi-quantitative and quantitative monitoring (Posthuma-Trumpie et al., 2009). A representative lateral flow test strip is made of six components, including a sample pad, a conjugate pad, a nitrocellulose membrane, an absorbent pad, a backing pad and a cassette, in which the conjugate pad is deposited with modified labels, and the nitrocellulose membrane is immobilized test and control lines (Fig. 2A). The LFIA is not only used for detection of simplex target but also multiple targets (Fig. 2B). For simplex detection, it has sandwich and competitive formats. In the sandwich assay, the target binds both detection probe on modified labels and capture probe at test line, while in the competitive assay, either the target or modified labels bind with capture probe at test line (Hu et al., 2014). Therefore, in the sandwich assay, the test line showing positive signal indicates the existence of the target or the target over a cut-off value (qualitative) and the stronger the positive signal, the more target (semi-quantitative). In the competitive format, the test line showing signal indicates there is no target or the existence of target below cut-off value. In both sandwich and competitive formats, the control line is designated for the confirmation that the test strip works well. Moreover, the competitive format is usually used for detection of small molecules since they cannot provide two binding position for two molecules (*i.e.*, detection and capture probes) simultaneously (Sajid et al., 2015). For the detection of multiple targets, it is totally based on the sandwich assay, in which the appearance of certain test line directly indicates the existence of corresponding target of interest, and all the test lines share the same control line. Nowadays, LFIAs for detection of CVDs are mainly used in hospital and used together with a device for quantification of the analyses. These devices are categorized for colorimetric, fluorescent, and magnetic immunoassays based on the labels used.

Colorimetric analysis devices are the most popular ones for quantification of results in LFIAs with gold nanoparticles as the labels, such as Roche Cobas h 232 POC System (http://www.cobas.com/), Getein FIA 8000 Quantitative Immunoassay Analyzer (http://www.bio-gp.com.cn/), and LEPU Quant-Gold-1 (http://www.lepumedical.com). These devices are very compact and even handheld, for example, Quant-Gold-1 (Fig. 2C). The system is mainly composed of a camera that optically records the reflectance signal of the test strip. Test and control lines are identified by a pattern recognition algorithm. Since gold nanoparticles are easy to prepare and modify, colorimetric assays are relatively low cost. However, although some gold nanoparticle based LFIAs provide an ultra-sensitive cut-off, for example, 0.1 ng/mL in Roche CARDIC Trop T Sensitive test, most of them own a limited sensitivity and even does not meet the clinical limit of detection (LOD), thus hindering their further applications in detection of low concentration of targets, such as BNP. Therefore, fluorescent and magnetic particles are introduced in LFIAs as labels for replacement of gold nanoparticles in order to improve LOD. Since these particles are not good for visual observation, the corresponding LFIAs are required to adopt devices for result readout, which is to stimulate and quantify fluorescent or magnetic signals of corresponding labels (Fig. 2D-E). Although fluorescent or magnetic assays provide a relatively high sensitivity compared to colorimetric assays, they increase the cost and are mainly served in clinics and hospitals.

On the other hand, microfluidic chip-based immunoassays have been found in the market as well. For simplex detection, there are two selected examples: one is Alere Triage® system based on fluorescent assays (http://www.alere.com/, Fig. 2F) and the other is Abbott i-STAT® system based electrochemical assays (https://www.abbottpointofcare.com/, Fig. 2G). Both systems are based on sandwich ELISA which shares the similar principle with sandwich LFIA. The main difference is to employ enzymes (*e.g.*, horseradish peroxides and alkaline phosphatase enzyme) instead of gold nanoparticles, and further to add corresponding substrates for generation of colorimetric, fluorescent (Lin et al., 2012), chemiluminescent, or electrochemical signals for readout. For example, i-STAT test cartridge just makes use of a sandwich ELISA coupled electrochemical measurement. Briefly, a couple of antibodies specific for a human cardiac biomarker (*e.g.*, cTnI) are deposited on two different positions on silicon chip-based electrochemical sensor. One of the two antibodies is conjugated with alkaline phosphatase enzymes. After the whole blood or plasma sample is brought into the silicon chip, it will contact with the sensors and make the antibody/enzyme conjugate dissolved. During about 7 min of incubation, cTnI within the sample will combine with both two antibodies, *i.e.*, finally be labeled with alkaline phosphatase enzymes and captured on the surface of the electrochemical sensor. The sample, together with excess enzyme conjugates, will be washed off the sensors using the enzyme substrate solution. With catalytic effect of the immobilized enzyme, the substrate releases an electrochemically detectable product. The handheld i-STAT (amperometric device) just measures this enzyme product to obtain the concentration of cTnI within the sample. Recently, Alere developed Heart Check System for quantitative measurement of BNP from fresh capillary whole blood based on electrochemical signal analysis as well. However, the test strip is required to store in the refrigerator and incubate at room temperature for at least 20 min, and the meter is required to keep free of vibration during tests. In a word, compared with LFIAs, microfluidic chip based immunoassays are much more sensitive. However, since they involve even more reaction steps, microfluidic chip based immunoassays are much more difficult to design and realize, thus increasing their cost.

For multiplex detection, two companies have developed centrifugation-based microfluidic compact disk (CD) and corresponding analyzers: one is Abaxis Piccolo Xpress® for analysis of absorbance (Fig. 2H) and the other is Gyrolab xPlore™ for analysis of fluorescence (Fig. 2I). The Piccolo processes blood sample into multiple aliquots of precisely diluted plasma and reports results of up to 14 tests, including Na^+, K^+, Cl^-, Ca^{2+}, total CO_2, AST, alanine transaminase (ALT), total bilirubin, alkaline phosphatase, blood urea nitrogen, creatinine, albumin, total protein, and glucose on a single-use, 8 cm diameter Comprehensive Metabolic Reagent CD in about 12 min. The reagent CD contains all the required liquid diluent and lyophilized regents (Fig. 2H, right). Using capillary action and centrifugal forces, the system automatically performs plasma separation, fluid mixing, and spectrometric measurement on the CD. Gyrolab Bioaffy CD engineered with nanoliter microfluidics is employed

to realize fluorescence-based sandwich ELISA (Fig. 2I, right) and Gyrolab xPlore™ automatically processes parallel immunoassays by using centrifugal and capillary forces and then performs fluorescence detection. This system can save sample, reagents, and time (hands-on time and assay time) since it does not require incubation and wash steps and shortens run time by miniaturizing and automating the immunoassays at nanoliter scale. It can record up to 112 data points in less than one hour from one Bioaffy CD. Currently, Gyros' platforms have successfully analyzed some biomarkers of CVDs. Moreover, different from the aforementioned microfluidic chip-based ELISA, this system is an open platform adapted to customers' assays. For further information, Table 2 just compares and summarizes the performances of the representative commercially-available products in monitoring of CVDs.

3.2. POC prototypes under development in the laboratory

3.2.1. Lateral flow immunoassays

Although various LFIAs for CVD tests have already been commercially available, it doesn't mean they fully meet clinical demands, leaving large room for improvement. In general, there have been two main drawbacks in LFIAs, *i.e.*, poor LOD and limited detection range, which limits the clinical utility of LFIAs (Zhu et al., 2011b). To some extent, these problems are also the barrier for the applications of CVD LFIAs at home like pregnancy test ones. Nowadays, tremendous efforts have been made to improve them and generated several novel prototypes of which some have become products.

For one example, the concentration of high-sensitivity (hs-) cTnI for diagnosis of acute myocardial infarction (AMI) is at ng/L, below the lower limit of conventional LFIAs. To address this issue, Xu et al. (2009) developed LFIA using super-paramagnetic nanobeads (SPMNBs) as labels for detection of cTnI with MICT® System as the magnetic assay reader. The developed LFIA has a LOD of 10 ng/L with a wide detection range of 5 orders of magnitude; the performance is greatly improved compared with commercially-available magnetic LFIA, MICT® c-TnI Heparin plasma test. On the other hand, Ryu et al. (2011) increased the analytical sensitivity in magnetic LFIA using magnetic beads with orientation-controlled antibodies as the labels. Different from direct immobilization of antibodies on labels, they first combined protein G and then anti-cTnI antibodies on magnetic beads, which made the LOD of cTnI down to 10 ng/mL as well.

Just as previously mentioned, magnetic assays and other non-colorimetric assays require involvement of reader, which is difficult to be applied at home for self-administration. To address this issue, on the basis of conventional colorimetric LFIAs, some studies report using dual gold nanoparticle (AuNP) conjugates as labels to magnify extremely low signals (Choi et al., 2010; Zhu et al., 2011b). Taking the work of Zhu et al. (2011b) as an example, in this work, one AuNP label is smaller AuNP (13 nm) conjugated biotinylated single-stranded DNA and anti-hs-cTnI antibody, and the other is larger AuNP (41 nm) with streptavidin. Since the larger-sized AuNP migrated slower than the smaller one, therefore, after the sandwich among smaller AuNP, hs-cTnI and another anti-hs-cTnI antibody is formed at test line (just like in conventional LFIAs), an additional reaction between the larger- and smaller-sized AuNPs would happen due to the combination between streptavidin and biotin. The developed assay shows LOD of 1 ng/L for hs-cTnI, which is two orders of magnitude lower than that obtained in conventional sensitive colorimetric LFIAs. Additionally, the developed assay simultaneously detected myoglobin (another biomarker for early detection of AMI, but with a high concentration) without signal amplification, and the linear measurement range for both two biomarkers has five orders of magnitude. To further evaluate the accuracy of the modified LFIA in diagnosis of AMI, they performed a comparison between the modified and a commercial LFIA for detection of clinical samples using an electrochemiluminescence immunoassay (ECLI) as reference, and found that the modified LFIA shows a consistent agreement with ECLI and a higher sensitivity than the commercial LFIA, demonstrating its feasibility for detection of AMI (Zhu et al., 2013). They have already got patent licenses both from China and USA (Zhu, 2012). To make the licensed technology translated into commercialized products, however, some more work is required. Actually, the collaboration between Chan et al. and a company for improvement, production and distribution of their developed h-FABP LFIA is a good model (Chan et al., 2003). Meanwhile, they both produce hospital-use (CardioDetect®med) and home-use (CardioDetect®self) ones (http://www.renesa.de/). Hu et al. (2013) further simplify the preparation of labels using oligonucleotide-linked AuNP aggregates, and are developing prototypes for detection of a cardiac marker, BNP.

For another sample, high-sensitivity CRP (hs-CRP), as a well-known marker of acute inflammation, has been widely used for prediction of CVD risk, with a rather high concentration than other cardiac markers, such as cTnI and myoglobin (Oh et al., 2013). The normal CRP level is up to 3 μg/L, and the abnormal over 5 μg/L, above the upper limit of conventional LFIAs. Focusing on this issue, Kim et al. (2014) prepare a nitrocellulose (NC) coated metal clad leaky waveguide (MCLW) sensor for one-step and label-free detection of CRP, which shows a detection range of 0.1–10 μg/L in diluted human serum samples. However, this method requires an additional wash step and a MCLW sensor system for readout. On the other hand, Leung et al. (2008) develop a barcode-style LFIA for semi-quantitative detection of CRP without the aid of any expensive reader. Although the reported detectable CRP level is at mg/L, using multiple test lines in replacement of single one for detection of an individual target is a good idea for semi-quantitative or wide range detection. Besides LFIAs, a novel vertical flow immunoassay (VFIA) is developed for rapid and one-step detection of hs-CRP (Oh et al., 2013). Similar with LFIAs, the VFIA is composed of a sample pad, conjugate pad, and I-shaped NC membrane. All the components are vertically instead of laterally assembled. The NC membrane with test and control

Table 2
A comprehensive comparison of performances of selected commercially-available techniques for monitoring of CVDs.

Representative product	Specimen	Sample pretreatment	Multiplex detection	Detection result	Featured assay	Time turnaround	Cost
Lepu Leccurate™	Whole blood	No	Yes	(Semi)qualitative and quantitative	cTnI: 0.5–50 ng/mL; CRP/hs-CRP: 3–50 μg/mL	10 min	~$40/strip; ~$3000/analyzer
Getein Fast Diagnostics	Whole blood	No	Yes	Quantitative	cTnI: 0.1–50 ng/mL; hs-CRP: 0.5–200 μg/mL	15 min	~$30—40/strip; ~$15,000/analyzer
MagnaBioSciences MICT® test	Heparin plasma	Yes	No	Quantitative	cTnI: 0.2–30 ng/mL	15 min	NA
Alere Triage® system test	Whole blood in EDTA	Yes	Yes	Quantitative	BNP: 5–5000 pg/mL	15–20 min	~$50/chip; ~$7000/analyzer
Abbott i-STAT test cartridge	Sodium or lithium heparin whole blood	Yes	No	Quantitative	BNP: 15–5000 pg/mL	15–20 min	~$50/chip; ~$8000/analyzer
Abaxis centrifugal-based reagent CD	Whole blood	No	Yes	Quantitative	Blood chemistry tests	12 min	~$50/chip; ~$16,000/analyzer
Gyrolab centrifugal-based Bioaffy CD	Whole blood	No	Yes	Quantitative	Open platform	60 min	NA

Note: cTnI: cardiac Troponin I; CRP: C-reactive protein; hs-CRP: high-sensitivity C-reactive protein; BNP: B-type natriuretic peptide; EDTA: ethylenediaminetetraacetic acid; CD: compact disk. NA: available. Herein, the technique that can work for whole blood as specimen can work for serum and plasma as well.

lines, and conjugation pad with antibody modified AuNP, are the same as those used in LFIAs. However, compared with LFIAs, this VFIA widens the detection range with reduced hook effect in the tested range and reduces the turnaround time (less than 2 min, faster than LFIAs). In the VFIA system, some additional components play a vital role, including flow control film (FCF), flow through holes (FTH) and asymmetric membrane (ASPM). These components are used for flow control in order to improve the performance of VFIAs, mainly about signal intensity, precision and dynamic range. FCF absorbs AuNP-Ab conjugates and improves their release from the conjugation pad. ASPM, with different pore sizes in its two sides and smaller pore face upwards, facilitates the aqueous solution uniformly distributing in the horizontal direction first and then wicking in vertical direction. The FTH promotes the reaction between the sample solution and the antibodies in a small area due to concentration effects. In a word, the VFIA can detect hs-CRP concentrations from 0.1–10 μg/L, a broader range than that from LFIA. They further introduce the ASPM in LFIA system and realize automatic chemiluminescent ELISA based on the delayed-release effect of ASPM (Joung et al., 2014); the working principle is similar with Abbott i-STAT. They obtain an even wider detection range for detection of hs-CRP, *i.e.*, 1–10,000 μg/L.

The above or some more examples have greatly improved the performance of LFIAs by refined design of labels or delicate modification of structure. Here, we would like to introduce two more studies for further discussion about the exploration of LFIAs. One is about the signal readout. As is known to all, AuNPs have been widely-used in LFIAs due to excellent visual contrast. However, Qin et al. (2012) employed thermal contrast instead of visual contrast to improve sensitivity in LFIAs using photothermal effects of AuNPs. AuNPs efficiently generate heat upon laser irradiation, and this phenomenon is largely related with the shape of the nanoparticles and will be strongly enhanced during plasmon resonance (Govorov and Richardson, 2007). Therefore, in the study, for the result readout, they used a 532 nm CW laser beam to irradiate the test line for 1 min and used an infrared camera to measure the temperature change remotely during laser irradiation. The maximum temperature changes as the signal instead of optical density, producing a 32-fold signal enhancement. To use low-absorbing backing pad and even higher-absorbing nanomaterials can further increase the sensitivity. This method may appeal to central laboratory performing thermal contrast readings. The other is about biomarker design. Considering that naturally-occurring, disease-specific endogenous markers are lacking, engineering injectable synthetic biomarkers specific to thrombosis (blood clot) using nanoscale exogenous agents is reported (Lin et al., 2013; Warren et al., 2014). The synthetic biomarkers are iron oxide nanoworms (NW) conjugated with a thrombin-sensitive in tandem with a ligand-encoded reporter. Once a patient with blood clots is injected the synthetic biomarker, the intact NW agents survey the vasculature for the sites of clot formation where thrombin activity cleaves and releases reporters into urine for LFIA, which allows for non-invasive and low-cost diagnosis of blood clot at the point-of-care in resource-limited settings.

3.2.2. Microfluidics based immunoassay

Microfluidics, referred to the science and technology for processing or manipulating small amounts (nL to aL) of fluids using channels at scale of nano/μm, have already found widespread applications in biomedical fields and will be used even more and better (Gervais et al., 2011a; Hitzbleck and Delamarche, 2013; Sackmann et al., 2014; Whitesides, 2006). Compared to LFIAs with passive control of fluid flow, microfluidic devices, controlling fluid flow by means of either passive or active behaviors, provide much more capability and flexibility in achievement of immunoassays. According to the materials for microfluidics and the control of fluid flow, we divide these assays into microfluidic chip-based (mainly based active fluid flow) and paper-based (mainly based passive fluid flow) immunoassays. Here, the microfluidic chip is referred to the platform consisted of the materials out of paper, including silicon, glass, silicon, *etc.* (Nge et al., 2013), while the paper referred to the porous substrate mainly made of cellulose fibers (Pelton, 2009).

3.2.2.1. Chip-based devices

3.2.2.1.1. Conventional optical and electrochemical assays. Like LFIA, microfluidic chip based capillary systems as prototypes for detection of cardiac markers using fluorescent signal have been developed as well. In 2004, Wolf et al. (2004) developed a Si-based microfluidic chip with self-regulating microfluidic networks (μFNs) that could simultaneously detect CRP and other cardiac markers based on micromosaic immunoassays (μMIAs) using patterned PDMS. However, this platform requires manual, multiple-step operation. Subsequently, following the principle of LFIA, one-step sandwich immunoassay for detection of CRP using microfluidic chip has been realized as well (Gervais and Delamarche, 2009; Gervais et al., 2011b; Zimmermann et al., 2009). Basically, the whole chip is microfabricated on 4-inch Si wafers with carefully designed structures as different functional elements, including sample collector, delay valves, flow resistors, deposition zone, reaction chamber, capillary pump and vents. The deposition zone is used for storage of fluorophore-labeled anti-CRP-C6 antibody as detection antibody (dAb) (Gervais and Delamarche, 2009), in which this step is greatly simplified compared to the first report (Zimmermann et al., 2009). The reaction chamber contains planar PDMS patterned with lines for anti-CRP-C2 antibody (capture antibody, cAb) as test lines and CRP as control lines. Therefore, after the addition of sample, the microfluidic chip triggers a sequent of fluid flow based on capillary forces and initiates corresponding sandwich immunoassay to form dAb/CRP/cAb complex, which is very similar with LFIAs. Finally, the readout is using a fluorescence microscope in the study, and the detection range is 1 μg/L–1 mg/L for CRP. Recently, they improved this platform to be capable for realizing multi-parametric immunoassays (Gervais et al., 2011b). Additionally, some more microfluidic chips using optical (*e.g.*, fluorescence) (Gunda and Mitra, 2013; Tsaloglou et al., 2014) and chemiluminescent (Bhattacharyya and Klapperich, 2007) and electrochemical (Abad et al., 2012) readouts, have been reported for detection of cardiac markers, greatly enriching the platforms for well use of these two most commonly-used readout.

3.2.2.1.2. Some emerging assays. Beyond optical and electrochemical readouts, surface acoustic wave (SAW) (Mitsakakis and Gizeli, 2011) and surface plasmon resonance (SPR) (Kurita et al., 2006) based parameters have been introduced in microfluidic immunoassays for detection of CVDs as well. Their underlying working principles are based on the interaction between electromagnetic wave (acoustic or optical) and substances, which causes a change in the phase of acoustic wave or an angle shift of the SPR, respectively. Further, the degree of these phase changes increases in proportion to the amount of substance. To be specific, the SAW based platform is a multi-channel microfluidic module integrated with a surface acoustic wave device. The acoustic device is sequentially assembled by a quartz piezoelectric substrate (for signal input and output), gold patterned interdigital transducer (IDT) electrodes (for signal transformation between the electric and acoustic ones), a thin PPMA layer (as a waveguide for sensitivity increase), and a gold film (for sensing). The gold film with the sensing area for antibody adsorption is covered by the microfluidic module. The signal is real-time recorded using network analyzer when different solutions are sequentially injected by syringe pump. The setup can sensitively detect four examined biomarkers, including CK-MB, CRP, D-dimer, and pregnancy-associated plasma protein A (PAPP-A), and selectively detect CRP and PAPP-A from a mixture. On the other hand, a trace level (15 fg within 3 μL) of BNP can be monitored by the real-time SPR angle shift (Kurita et al., 2006). In this study, an alike competitive enzyme immunoassay is realized using a T-shape microfluidic device combined with a portable SPR system. Briefly, during the immunoassay, both the sample BNP and immobilized BNP can combine with the acetylthiocholine-labeled anti-BNP antibody conjugate, and only unbound conjugate will be trapped on BNP-immobilized film. After phosphate-buffered saline (PBS) rinses, thiocholine will be released from the conjugate and accumulated on the bare gold film where the SPR angle shift is monitored. The detectable range is 5 ng/L–100 μg/L within 30 min. Nevertheless, the multiple operation steps limit its application at the POC. To reduce the assay steps and

increase the assay sensitivity as well, cationic isotachophoresis (ITP) is introduced in poly(methyl methacrylate) (PMMA) microfluidic chip for preconcentration of cardiac marker, cTnI (Bottenus et al., 2011). The microfluidic chip is designed with a 5× reduction in depth first and then 10× reduction in width. By performing ITP effect in the 2D reducing microfluidic chip, the concentration of cTnI can gradually increase from area A *via* B to C, and the concentration factor over 10,000 is obtained. The whole experiment time is less than 20 min and can potentially be coupled to existing platforms for immunoassays. However, it requires external device for applying voltages.

3.2.2.1.3. Selected examples holding promising capability for CVD applications. As introduced in the above, currently developed microfluidic chip based immunoassays of cardiac markers do not fully meet the demands for detection of CVDs at the point of care. In fact, what developers of technology, especially in universities, are interested in usually does not address well with the concerns of users of such technology (Whitesides, 2013). One party would like to develop technically cool devices, while the other party prefers to use such devices cheaply and simply — solving problems. Considering lots of microfluidic chip based platforms have been reported and even some of them are on the way to enter the market, we thus select and provide some examples for reference here.

To simplify the procedures of preparation, operation and adoption of microfluidics, Chin et al. (2011) integrated novel procedures to manufacture devices, manipulated assays and detected signals in a mobile microfluidic chip for immunoassay on protein markers (mChip). The developed mChip can completely realize all steps of ELISA and perform detection of multiple targets. Performed in practice for simultaneous diagnosis of HIV and syphilis at the POC, this mChip assay shows enough sensitivity and specificity compared to bench-top assays. From this study, three main innovations may benefit the future prototype development: (1) high-throughout (1 chip every ~40 s) and low-cost ($ 0.10) manufacturing of high-quality microfluidic cassettes using injection molding strategy with careful control of process parameters; (2) automatic loading of necessary reagents for each step of ELISA using passive reagent delivery; (3) silver staining based signal amplification and inexpensive compact optical device for signal detection. In another example, just like a thermometer, a multiplexed volumetric bar-chart chip (V-Chip) embedded with red inks for quantitative result display as bar charts is reported (Song et al., 2012). The V-Chip is developed based on SlipChip, but employs modified flow paths and innovative signal readout (Du et al., 2009; Li et al., 2009). Samples or reagents are first preloaded in drilled holes and then diffuse for ELISA when the chip slips to a designated configuration, which is similar with previous SlipChip. Differently, the enzyme is catalase and the substrate is H_2O_2; when they meet, O_2 will be generated, released and drive the advancement of red ink, which is directly related to the concentration of sample. The V-Chip based assay inherits the advantage of SlipChip, *i.e.*, without pumps or valves. Additionally the signal readout does not require any instrument for data reading or data analysis. Finally, it is very convenient to perform multiple detection (up to 50) on the V-Chip. Recently, Pt nanoparticles with stable chemical properties and good catalytic capability have been investigated, and can be used instead of catalase (Song et al., 2014). On the other hand, Au@Pt nanoparticle encapsulated target-responsive hydrogel has been introduced, eliminating the dependence on SlipChip technology (Zhu et al., 2014). All the advantages and the progressive improvements enable V-Chip being a good candidate as blood tests for CVDs at the point-of-care. In fact, Li et al. just made full use of V-Chip for qualitative detection of BNP in which the LOD is below 5 pM. Meanwhile, in the study, the result is expressed a 6-bit binary number as readout, which is very convenient (Li et al., 2015a).

Out of ELISA, some more diagnostic principles can be adopted for rapid and accurate measurement of biomarkers. One good example is self-amplifying proximity assay using magnetic nanoparticles (Lee et al., 2008), whose underlying mechanism is the change of spin–spin relaxation time during the transformation of monodispersed magnetic nanoparticles (T_1) to self-assembled clusters (T_2). To be specific, when the sample containing target molecules is introduced, a few magnetic nanoparticles will bind with the target molecules through affinity ligands, and form soluble nanoscale clusters. Since the clusters are more efficient at dephasing nuclear spins of many surrounding water protons, T_2 is thus less than T_1 and further is proportionally related with the amount of the target molecules in a certain range. Correspondingly, a miniaturized diagnostic magnetic resonance (DMR) system that can perform the self-amplifying proximity assay is developed. Using NMR signal as readout, the DMR system requires few or no processing for samples and performs the detection in a rapid, simple and high-throughput manner. Various targets, including protein biomarkers, have been detected using this system. Considering that this system consumes a small sample volume (5–10 μL), it may require preconcentration of sample or utility of micrometer-sized magnetic particles for some cardiac marker detection. However, since proteins may degrade fast in sampled blood, it requires not only effective but also immediate measurement of plasma proteins. An integrated blood barcode chip (IBBC), functioning a reliable on-chip plasma separation and *in situ* measurement of a panel of plasma proteins within a short enough turnaround (Fan et al., 2008), may represent an ideal reference to develop clinical diagnostic platform for CVDs. Briefly, the IBBC separates proteins from a finger prick of blood based on the Zweifach–Fung effect and measures protein *in situ* based on multiple DNA-encoded antibody library (DEAL) arrays patterned within the plasma-skimming channels. Finally, a typical sandwich reaction takes place among the targeted plasma proteins, DNA-antibody conjugates, and biotin-labeled detection antibodies, and different targeted proteins allow this reaction happen in corresponding DNA codes (such as A, C). The signal is indicated using fluorescence detection, and the green bar (such DNA code B) is designated as a control. The total assay is within 10 min.

3.2.2.2. Paper-based devices. Paper-based devices refer to a kind of microfluidic devices made of paper. These devices not only own some capabilities of chip-based microfluidics, but also retain some performances of lateral flow test strips (Martinez et al., 2009). Currently, various methods have been developed to prepare both two-dimensional (2D) and three-dimensional (3D) paper-based devices, such as wax printing (Carrilho et al., 2009), layer-by-layer stacking (Martinez et al., 2008b), paper origami (Liu and Crooks, 2011), *etc.* Further, they have found widespread applications in POC diagnostics mainly with optical and electrochemical sensing mechanism (Yetisen et al., 2013). Although there are rare reports about using paper-based devices for detection of CVDs, lots of research groups have made and are making great efforts to transform them from prototypes to products; some of them even have created companies focusing on the promotion of commercialization and application of paper-based devices, such as Diagnostics for All (http://www.dfa.org/), a nonprofit company initiated by the Whitesides Research Group at Harvard University. Here, we briefly introduce some potential examples that not only may be used for detection of cardiac markers, but also provides some clues for future development of prototypes.

Although colorimetric assays may be not so sensitive for some applications, they are always the first choice, especially for the paper substrate. The successful examples include commercially-available pH test strip (stands for dipstick assay) and home pregnancy test (stands for LFIA) (Hu et al., 2014). For paper-based microfluidic analyses, the representative may be a micropatterned paper device for liver function test by measuring serum transaminases, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) from whole blood at present (Pollock et al., 2012; Vella et al., 2012). The test device is just a 3D device assembled by a polyester film (as laminated cover), a plasma separation membrane and a wax-patterned paper. The wax-patterned paper contains five separate zones: two detection zones (for measurement of AST and ALT, respectively) and three control zones (AST positive control, AST negative control and ALT negative control). Each zone is deposited with corresponding chemical mixtures to ensure a specific measurement condition. The assay can perform the measurement directly from a drop of whole blood within 15 min and the assay result is reflected as the color-coded changes. For result interpretations, a color

read guide is used for reference. This convenient, rapid and semi-quantitative measurement is very suitable for POC assay in resource-limited settings. However, it remains challenging to realize such mature color reaction on AST assay (caused by sulfonation) or ALT assay (induced by oxidation) for another marker, especially for cardiac markers, which requires lots of efforts input. Therefore, this study provides a lesson about the design of paper-based device, but some more general sensing mechanisms are needed to consider.

On the one hand, semi-synthetic bioluminescent sensors may be useful. In fact, these sensors are reported for monitoring of therapeutic drug at the POC by Cheng et al. (2010). Briefly, these sensors are based on luciferase-based indicators of drugs (LUCIDs). LUCIDs consist of three components: a receptor protein for target drug, a luciferase and a synthetic molecule containing a fluorophore and a ligand for the receptor protein. Before assays, since the ligand combines with the receptor protein, the fluorophore is brought close to the luciferase, which allows bioluminescent resonance energy transfer (BRET). During assays, the targets are displacing the ligand from the receptor protein, thus reducing BRET efficiency. Finally, the ratio of light emitted from the luciferase (blue) and the synthetic fluorophore (red) is then a quantitative signal related to the amount of the target, which can be recorded by a simple point-and-shoot camera. These sensors have been engineered to quantify various drugs, including antiarrhythmics, which is also a diagnostic method for CVDs. Meanwhile, it can make use of Förster resonance energy transfer (FRET) as well and is extrapolated to be capable of proteins, including cardiac markers.

On the other hand, paper-based ELISA is promising. ELISA, as a standard immunoassay, is widely used in biochemical fields. However, these assays are typically carried out in microplates, small vials, or microfluidic chips (Cheng et al., 2010; Chin et al., 2011). Recently, it has been realized in LFIA system as well (Joung et al., 2014). However, it is only for a single assay. In fact, paper-based devices patterned with a 96-microzone plate and capable of performing high-throughput ELISAs have already been developed (Cheng et al., 2010). The whole protocol is similar with conventional ELISA but decreases all the costs, including reagent consumption (1/25), assay time (1/4), and uses a low-cost detection device (1/200). However, sensitivity is about an order of magnitude higher than that of conventional ELISA, which is a disadvantage. At the same time, the multiple step operation also remains a challenge. To solve these problems, some more studies have been reported (Hsu et al., 2014; Liu et al., 2011; Murdock et al., 2013; Wang et al., 2012). However, their feasibility for detection cardiac markers are needed to explore and evaluation.

4. Smartphone-based diagnostic devices for CVDs

From the above introduction, we may have a brief summary: for either LFIA or microfluidics-based immunoassays, a reader or analyzer is an essential tool for quantitative results; for non-colorimetric assays, it cannot even obtain qualitative results without the aid of corresponding readers. Conventional analytical systems are usually very expensive, large, complicated, and require highly-regulated and quality-assessed infrastructure and skilled medical personnel. Due to the technological development, lots of miniaturized, compact and even handheld readers have been developed and commercialized, such as, Cobas h POC system and i-STAT. However, their costs are usually very high, limiting their applications at home. Meanwhile, they are not compatible with each other since different assays require different devices, hindering their application scopes. In other words, i-STAT cannot quantify Roche CARDIC Trop T Sensitive test results. Therefore, a cost-effective and universal reader is in urgent need.

Nowadays, mobile phone, as an affordable, portable, and compact device, is almost owned by every person (Martinez et al., 2008a,b; Ozcan, 2014). According to the latest Ericsson Mobility Report, worldwide mobile subscriptions are 7.4 billion in 2015, and will reach up to 9.4 billion in 2021. In the meantime, smartphone subscriptions stand at 3.4 billion on the earth, but will explode to 6.4 billion by 2021, which is mainly resulted from increasing affordability in developing markets such as Asia Pacific, the Middle East, and Africa (Ericsson, 2015). Driven by rapid development of hardware and software, mobile phone, especially smartphone, is actually a handheld computer. Moreover, beyond the computational power, abound peripheral devices, customer-designed accessories, various physical sensors, and multiple-system compatible logarithms/applications transform the smartphone into a cost-effective and yet extremely powerful platform to perform both biomedical assays, physiological examination, and imaging (Ozcan, 2014; Vashist et al., 2014a; Vashist et al., 2014b; Xu et al., 2015a). We have presented advances in smartphone-based POC diagnostics as *in vivo* and *in vitro* tests (Xu et al., 2015a). Here, we shall further discuss the applications and potential applications of smartphone in detection of CVDs.

4.1. Optical and electrochemical assays

Nowadays, smartphone applied in biomedical assays are mainly in optical and electrochemical assays. It should be noted the examples chosen here may not be focused on the CVD applications with two aspects of reasons. On the one hand, to our best knowledge, there are limited examples of smartphones used in biomedical assays for monitoring of CVDs. As mobile health develops, however, these examples will arise more and more. It is thus necessary to present the advance in smartphone-related biomedical assays. On the other hand, this section, not limited to the specific examples, but from the perspective of the strategy and methodology that how to apply smartphone into CVD applications and even beyond, is intended to provide some references to those who have interest in this issue. Due to availability, computation, communication, networking and imaging capabilities, smartphone will deploy microfluidics technology in resource-limiting settings as well as developed countries (Erickson et al., 2014).

4.1.1. Smartphone for analysis of optical assays

Optical assays are the most commonly-used detection methods (Borisov and Wolfbeis, 2008), and can be divided into colorimetric assays, chemiluminescent assays, fluorescent assays, *etc.* In colorimetric assays, such as AuNP-based LFIA and microchip ELISA (Wang et al., 2011), we can observe signals by the naked eye and also can quantify the signals with the aid of a reader. The underlying mechanism of the quantification is mainly based on the optical density of test zones. Taking AuNP-based LFIA as an example, the signal depends on the amount of AuNPs (*i.e.*, targets) accumulated on the test zone. Similarly, a camera phone or smartphone can totally perform the similar work (Xu et al., submitted for publication). To obtain a high-quality result, it is necessary to unify the imaging condition. Therefore, an accessory is required to hold the test strip steady and it should be photophobic to eliminate the interference of ambient light. To further consider the cost and manufacture, resin is a good choice as the material for the smartphone accessory which can be used to fabricate a customer design by a commercially-available 3D printer. Moreover, 3D printing technique enables an easy modification of the prototype to match well with different types of smartphones. Subsequently, an application running on the smartphone facilitates image acquisition and analysis. To manage the results, some functions are also needed to consider, such as account management, history management, and report and share. Since all the steps are performed within the smartphone, real-time result analysis is allowed. Benefiting from the smartphone capability, the result is able to store and transmit to a centralized laboratory for further analysis and medical advice. For instance, just making use of a smartCARD accessory and an developed algorithm, total cholesterol levels in blood is quantified by colorimetric analysis (Oncescu et al., 2014). Recently, a handheld and cost-effective smartphone-based colorimetric microplate reader is even created, which can present diagnostic results of a 96-well plate within about 1 min using a machine learning algorithm (Berg et al., 2015).

On the one hand, Chen et al. (2014) employed power from the mobile phone to electrolysis micropumps for driving ELISA on the microfluidic chip using a micro-universal serial bus (microUSB) cable. On the other hand, based on their previous work (*i.e.*, mChip), Laksanasopin et al. (2015) further developed a low power consumption dongle that can run a triplex ELISA using power from a smartphone. Innovatively, the dongle makes use of the audio jack connector that generates power-free vacuum pressure as the driving force for the assay and audio-based power from the smartphone lighting the embedded light-emitting diodes (LEDs) for colorimetric analysis. The diagnostic results can be obtained in 15 min from the triplex test. Compared to gold standard laboratory-based assays, the sensitivity and specificity of the test meet current clinical needs. From this work, we can learn from integrating microfluidics with electronics that can make a difference in development of POC diagnostics.

Although the above study is about colorimetric assays, it generally works for any other kind of assays, for example, chemiluminescence-based LFIA. In the chemiluminescent LFIA, the chemiluminescent signal is acquired using a free, professional camera application, Camera FV-5 Lite, which enables digital single-lens reflex camera-like manual controls of phone camera in fingertips (http://www.camerafv5.com/) (Zangheri et al., 2015). The professional application helps user take photos much more flexible and controllable compared to the original approach for operating camera. However, it only uses the camera function of the smartphone, thus post-process is required. For fluorescent measurement, the key point is to develop the accessory components, since fluorescent substances release signal upon proper exciting light. For instanceZhu et al. (2011a), developed a compact accessory component that uses battery-powered LEDs to excite the specimen and an embedded lens to image the fluorescent emission. This system can provide wide-field fluorescent and dark-field imaging for various fluorescent micro-objects in double colors with an about 20 μm-scale resolution, which should meet the requirement for some fluorescent assays, for example, LFIA. Of course, some modifications are needed prior to use. Interestingly, based on some improvement, the smartphone based field-portable fluorescent microscopy platform can measure fluorescence of nanoscale objects (Wei et al., 2013). In fact, the smartphone-based fluorescent readout system has been reported to measure quantum dot barcode signal in microwell chip for multiplexed diagnosis (Ming et al., 2015). All the explorations have laid a good fundament on smartphone-based optical assays of diseases, including CVDs.

4.1.2. Smartphone for analysis of electrochemical assays

Due to high selectivity and sensitivity, electrochemical assays have been extensively explored as well (Li et al., 2015b), in which the most successful example is blood glucose metering (Wang, 2008). Lillehoj et al. (2013) developed an external system combined with mobile phone for rapid and quantitative biomolecule diagnostics based on electrochemical detection. The external system contains an integrated electrical component for signal processing and data analysis, and a disposable microfluidics for sample treatment and detection. The sample processing and sensing on the chip is driven by capillary force, and the smartphone displays graphical step-by-step instruction making the whole operational clear and simple. After the measurement finishes, the smartphone automatically saves the data and displays quantitative results on the screen. Further, Nemiroski et al. (2014) developed a universal electrochemical detector that can operate with various electrode formats with on-board sample mixing and audio-based data transition. Since the audio cable is one of the basic components of current mobile phones, this design thus ensures that the detector works well with all the levels of mobile phone (from feature phone to smartphone). The measured digital data is then transmitted over a live voice connection, guaranteeing it works in any available cellular network (2G, 3G, or 4G). Moreover, the universal detector can provide a flexible electroanalytical technique according to practical cases. Therefore, smartphone and even low-end mobile phone based electrochemical detection is possible. A corresponding electrochemical detection of cardiac markers in microfluidic platform is expected to realize. Or on the other hand, to modify the detector makes it compatible with microfluidic chip.

Additionally, smartphone has also been used as digital microscopy and flow cytometry by using its camera for investigation of biological specimens (*e.g.*, cells, bacterium, and parasites) at the POC (Vashist et al., 2014a; Xu et al., 2015a, submitted for publication). They are mainly used for analysis of blood cells and monitoring of infectious diseases or the relevant, which may be beyond CVD applications. In summary, a smartphone is mainly used as a reader or analyzer in optical or relevant assays, while in electrochemical assays smartphone or mobile phone functions as a helper: to guide operation of the assay or to transfer the data. The former mainly makes use of the imaging techniques while the latter makes use of data transfer and communication capability. In the present smartphone-based electrochemical assays, the smartphone is always used along with a compact electrochemical analyzer for data processing.

Integrated smartphone and microfluidics technologies will enable healthcare delivery. However, there are two concerns here. On the one hand, it is about the research and development. The number of mobile phones is large but the levels of mobile phones and cellular network vary greatly. This means the performances of the smartphone-based microfluidic assays are subject to the parameters of the mobile phones. How to standardize the hybrid platform or eliminate the potential mobile phone-induced variations is a question. On the other hand, it is about commercialization and applications. Currently, conventional bench-top based biomedical assays have found widespread applications in industrial, clinical and research settings (Vashist et al., 2014a). They own well-established supply chains, loyal customers, and experienced after-sale services. Moreover, they are becoming more and more advanced and user-friendly, automating operation and providing high accuracy (including sensitivity, specificity and reproducibility). It is a big challenge to consider where to apply and how to commercialize the smartphone-based microfluidic assays.

4.2. Physiological examination

Beyond serving as a tool to read, analyze and transfer data about cardiac markers from microfluidics, smartphone is playing a vital role in interfacing physiological indexes as well (Fig. 3). In general, the role is realized through the following three aspects, *i.e.*, information-based readout (Fig. 3A-C), internal component-based readout (Fig. 3D-F), and external device-based readout (Fig. 3G-I).

4.2.1. Information-based readout

As mentioned earlier, four diagnostic methods, totally or partially, can be the first choice to obtain the state of CVDs. However, without professional knowledge, it is difficult for common people to know how to collect effective information, how to make use of such information, and how to take action to address potential problems. Due to the development of information technology, interactive websites or specific applications with helpful information, professional guidelines and themed questionnaires may fill the gap to some extent. For example, *Healthy Heart Quizzes* (http://www.heart.org/) with abundant modules (including *Heart Attack*, *Stroke*, *Physical Activity*, *etc.*) are designed to help users to pay attention to CVDs and other diseases related symptoms through questionnaires. Another interactive tool, *Your Disease Risk* (http://www.yourdiseaserisk.wustl.edu/), is used for estimating risk of coronary heart disease and providing personalized tips for prevention to users. However, these interactive websites require internet access, which may not be accessible sometime or available somewhere. Therefore, some specific applications (apps) related to CVD healthcare are developed for smartphone, for example mobile phone physical activity level questionnaire (MobilePAL, Fig. 3A) (Pfaeffli et al., 2013) and *My heart, my life* (https://myheartmylife.org.au, Fig. 3B). It is demonstrated that MobilePAL is a relatively reliable and valid method for cardiac rehabilitation in CVD cohort (Pfaeffli et al., 2013), indicating that mobile phone can be an effective self-report tool and in-time data collector. In contrast, *My heart, my life* app functions even more. This app can help users learn about warning signs of heart attack and what to do. Additionally, it can also be used to manage medicines, manage

Fig. 3. Representative smartphone based strategies for CVD-related physiological indexes. Information-based readout: (A) Screenshot of questionnaire and answer categories of the MobilePAL smartphone. Reprinted from Pfaeffli et al. (2013) under the terms of the Creative Commons Attribution License. (B) Screenshot of *My heart, my life* app (https://myheartmylife.org.au/). (C) Smartphone-based remote monitoring *via* information transmission. Reprinted from Worringham et al. (2011)) under the terms of the Creative Commons Attribution License. Internal sensor-based readout: (D) *Instant Heart Rate* app (http://www.azumio.com/). (E) *Instant Blood Pressure* app (http://www.instantbloodpressure.com/). (F) Galaxy S5 with a built-in heart rate sensor (http://www.samsung.com/). External device-based readout: (G) Wireless Blood Pressure Monitor (http://www2.withings.com/). (H) AliveCor Mobile ECG (http://www.alivecor.com/). (I) Mobisante Ultrasound (http://www.mobisante.com/).

health stats (*e.g.*, weight, blood pressure and cholesterol), *etc.* Although it is free, however, it is not accessible for some devices or in some areas. Moreover, it does not provide medical advice, and most health information, like blood pressure, cannot be known without measurement. Therefore, a remote monitoring system consisted of a miniature heart and exercise monitor, a miniature global positioning system (GPS) receiver, and a programmed smartphone, is developed, which can be used for monitoring exercise in cardiac rehabilitation (Fig. 3C) (Worringham et al., 2011). During usage, the monitor records user' ECG and heart rate, and the receiver collects data about elapsed route, current location and walking speed of user. All the data are transmitted to the smartphone, then to the server, and displayed in-time, and the server-side expert provides the users with advices immediately if necessary. Compared to interactive web page or specific application, the information provided by corresponding monitor or sensor may be not complete, but must be more effective and accruable. Just like the monitor used in Fig. 3C, more and more sensors or devices have been developed, which can be divided by their relationship with smartphone as the internal and external peripherals.

4.2.2. Internal sensor-based readout

Today, every smartphone has an internal camera as well, which is contributed to the embedded complementary metal-oxide semiconductor (CMOS) image sensor and flashlight. Lots of researchers just make use of this camera to develop applications for CVD monitoring, for example, *Instant Heart Rate* for measuring heart rate (http://www.azumio.com/, Fig. 3D) and *Instant Blood Pressure* for measuring both blood pressure and heart rate (http://www.instantbloodpressure.com/, Fig. 3E). The protocols to use them are the same: 1) place fingertip over the camera lens, 2) run the app in the phone, and 3) hold the gesture steady until the phone completes the measurement. The whole operation generally takes less than 1 min. The working principle of this rapid and simple operation is called photoplethysmography (Pappas, 2015). The underlying mechanism is as follows: each heart beat sends a pulse of blood to the tiny capillary vessels of the fingertip, and the capillary vessels become larger and redder (Pappas, 2015). With the aid of the flashlight illumination, the CMOS sensor can capture the color changes. The application is just an integrated algorithm to initiate the process, record and analyze the data, and present the result. Some of these applications are free (*e.g.*, *Instant Heart Rate*), while some take a certain fee (*e.g.*, *Instant Heart Rate Pro* and *Instant Blood Pressure*). The fee may be generated for a requirement of additional, professional medical advices. However, there are some problems for these applications. First, they cannot provide continuous monitoring and enough sensitivity that external monitors can provide. For another measure, the measurement is not continuous since it cannot be made until the former one finishes the whole step. Moreover, just like the developer of *Instant Blood Pressure* claims, the app cannot replace or substitute blood pressure monitor right now since it requires some more evidenced-data support. Second, they are not robust enough. If users' finger does not cover the camera well, either too close or too loose, the application may stop working (Lee, 2015). Finally, they may not be compatible with all the smartphone system, *e.g.*, *Instant Blood Pressure* is only available for iOS. Therefore, specific sensors are introduced to smartphone, for example, Galaxy S5 (http://www.samsung.com/, Fig. 3F), the first smartphone embedded with a heart rate sensor. The heart rate sensor is on the back of the smartphone and close to the flashlight, and the method to use this sensor is similar to use *Instant Heart Rate*. Differently, this sensor is to capture pulse for measuring the heartbeat rate. The result is finally stored in Samsung's S Health app. Compared to CMOS image sensor based measurement, heart rate sensor based one records

data much faster, but does not add too much value for heart rate monitoring (Miles, 2014). Of course, it is not necessary to buy a Galaxy S5 for heart rate monitoring. Nevertheless, it is a good idea to introduce healthcare tools in smartphone.

4.2.3. External device-based readout

Since it is not easy to integrate sensors with smartphone, it is an alternative to develop devices combined with smartphone for CVD monitoring. Fig. 3G-I are just selected examples to show some smartphone-device combined systems for CVD monitoring. In these combined systems, the devices are used for monitoring signals from users, and the smartphones are used for data storage, display, analysis, and transmission. Generally, these external devices are developed and modified based on their counterparts, including digitalization, miniaturization, *etc.* For example, wireless blood pressure monitor (http://www2.withings.com/, Fig. 3G) looks like conventional cuff blood pressure monitor. After the cuff is slipped on, however, the wireless one launches its application in the smartphone and sends results to the application automatically, which is much simpler and more convenient. Compared to *Instant Blood Pressure* application, the wireless monitor is medically approved and its corresponding application provides professional feedback on measured results immediately. Moreover, the application stores all history data, presents them in a straightforward way, and allows users to share the result to doctors. However, its cost is not cheap, which may limit its spread application.

Another example is AliveCor Mobile ECG (http://www.alivecor.com/, Fig. 3H). ECG recording has more than one century history (Antman, 2015). It is the emerging mobile health technologies that make a breakthrough to record, store and use ECG (Antman, 2015; Mitchell and Le Page, 2015). AliveCor Mobile ECG is a representative product that makes it possible for users to record and transmit single-lead ECG (Antman, 2015; Mitchell and Le Page, 2015; Walsh et al., 2014). From some experiences, the Mobile ECG is demonstrated to be much more effective to find abnormalities compared to standard diagnostic methods, such as 12-lead ECG, ambulatory ECG (Antman, 2015). This ability is very critical for early and effective decision-making since misdiagnoses greatly increase the risk to be much more serious health condition, for example, from atrial fibrillation to stroke (Antman, 2015). However, there are some drawbacks for AliveCor Mobile ECG. First, since ECG only can be recorded during users' operation, it is just an intermittent ECG, which cannot be used for long-term purpose. Second, it is easy to generate noise if the operation is not appropriate, such as lack of adhesive electrodes. Compared to ECG, imaging, as a standard diagnostic technique, can find even more widespread applications in diagnostics (Mertz, 2012). However, imaging machines are often unavailable in resource-limited settings (Mertz, 2012). Mobisante Ultrasound (http://www.mobisante.com/, Fig. 3I) is affordable, portable, and easy to use, which is a good start to fill the gap. The system consisted of a smartphone and a compact ultrasonic transducer with a USB host port (Mertz, 2012). To acquire a good ultrasound image and interpretation, it requires some training, but is easily grasped by medical staff.

Besides the above examples, there are some more smartphone-based applications and devices in the market as well as other wireless devices (Vashist et al., 2014b; Walsh et al., 2014). Most of them are related to monitoring or management of CVD-related physiological parameters. Compared to professional and standard physiological examination in hospitals, these strategies may not yet reach the same performance levels (Vashist et al., 2014a). However, they can provide cost-effective healthcare without the aid of skilled personnel and high-end facility and then realize personalized monitoring and/or self-management of diseases regardless of time and place. As a consequence, smartphone-based and wearable devices can serve as complementary solutions to regulatory guidelines and well-established standards and find applications in some specific demands (Vashist et al., 2014b). Moreover, with advances in cloud computing, smartphone-based strategies can enable telemedicine opportunities in resource-limited settings. It's believed that smartphone and other wearable devices together may revolutionize the way cardiology is practiced. However, there are also some challenges that lie ahead. First, the functions of current smartphone based applications and devices are limited and are restricted to a specific phone. Second, the safety, efficacy and cost-effectiveness of measured data are required to validate. Finally, data processing and clinical and predictive meaning is needed to provide.

5. Perspective and conclusion

This article presents a comprehensive review about CVDs and their diagnostics. Both representative products and prototypes are selected and carefully introduced, including lateral flow immunoassays, chip-based assays, and paper-based assays. Moreover, smartphone-based strategies, not only for biochemical assays, but also for physiological examination, are discussed in details as well. Since CVDs will continue to be the top one killer in the next two decades, the relative research will be highlighted, especially the development of POC diagnostics for resource-limited settings. To develop POC devices meeting the ASSURED guideline requires input of various stakeholders, including the institute, the industry, the hospital, the community, *etc.* In fact, some good references have been made in both microfluidics- and smartphone-based devices; but there still exist some challenges. For the developers, the challenges are: 1) to increase performances of diagnostic platforms, including specificity and sensitivity; 2) to decrease their cost, including time, sample and reagents; and 3) to make the platform robust and simple to operate and the result easy to understand. For the users, the challenges mainly include: 1) to carefully follow the manufacturer's instruction; 2) to carefully obtain and utilize the results; 3) if required to long-term adoption, just follow and pay attention to changes. It is believed that smartphone will change the way of healthcare delivery and further facilitate personalized medicine by cultivating next-generation imaging, diagnostics and measurement tools (Ozcan, 2014; Vashist et al., 2014a, 2015). In the near future, an integrated smartphone-based system for simultaneously biochemical assays, physiological examination and imaging may be developed for monitoring of CVD at the POC.

Vashist et al. have highlighted the recent advances in smartphone-, chip-, and paper-based technologies for next-generation POCT as well as future trends and main challenges (Vashist et al., 2015). Herein, we briefly present some more detailed hurdles and limitations in these emerging microfluidics- and smartphone-based technologies and propose corresponding solutions to address them.

First, it is about issues of non-specific binding (causing negative positive results) in and reproducibility (related to both sensitivity and specificity) of microfluidic devices. The non-specific binding may be controlled well using standard samples, but is challenging to avoid in detection of clinical samples because patient samples are complicated containing potential non-specific substances. Especially in those assays for detection of several similar biomarkers at the same time, the possibility of non-specific binding greatly increases. Non-specific binding may also be caused by the quality of capturing molecules (*e.g.*, antibody) and the material performances of devices; all the reasons can induce poor reproducibility as well. Moreover, poor stability of samples and capturing molecules also affect the reproducibility. For example, during the development of immunoassay for BNP, it is not easy to get repeatable results, which is mainly related to BNP instability. To address this, single epitope sandwich (SES) immunoassay for BNP is developed by HyTest Ltd. In SES assays, the antibody is specific to the stable ring structure only, which (optimizing and improving sensing molecules and then assay formats) may be an example to solve the issue of reproducibility, increase sensitivity, and may improve specificity as well. The second solution is to optimize the materials and structure of the devices. Integrating sample pretreatment and quality control parts may be the third solution, which may increase the cost of the assay and can be adopted in some cases. Not all the assays have high requirements and qualitative detection already satisfies most settings, which may decrease the pressure from specificity and reproducibility.

Second, it is about problems of using smartphones in the healthcare field. Prior to marketing biomedical devices, there are cumbersome, costly and time-consuming regulatory approval processes (Ozcan, 2014). For example, medical devices can only be sold in the USA after being registered with the USA Food and Drug Administration (FDA, http://www.fda.gov); in the European Economic Area (EEA), the devices are required to get CE (abbreviation of Conformité Européenne, meaning European Conformity) marked. As the consumer electronics market develops rapidly, the number of mobile phones and smartphones is increasing worldwide, but their performances may vary largely because of model differences. Such non-standard or unstable qualities of mobile phones definitely affect their imaging, sensing and diagnostics tools and technologies, which is a barrier for their commercialization in biomedical device industry (Ozcan, 2014). One solution to address this problem may rely on a standardized and regulated supply of certain mobile phone (Ozcan, 2014; Vashist et al., 2014a). Another solution is to take a strategy of modularization during the development of smartphone based biomedical devices. Briefly, it makes smartphones separated from but adapted to the sensing parts. The sensing parts as an integrated complete biomedical device is to get FDA-cleared or CE-marked and smartphones are performed as an analyzer *via* data processing, like calculation, communication, network, *etc.* For those technologies subject to changes, like imaging, the sensing part can be embedded with negative and positive controls for quality control.

Last but not least, it is about regulations pertaining to cloud computing and data safety. Recent years have witnessed a fast growth in cloud computing because it saves infrastructure costs (Vashist et al., 2014b). Large amount of data is stored in the cloud now. However, this involves potential issue of data safety since the cloud contains large amount of data concerning personal privacy. Currently, most nations establish laws to guide the data storage and many cloud computing service suppliers take action to ensure data safety (Vashist et al., 2014b). Cloud computing, integrated with microfluidics- and smartphone-based devices, will enable personalized medicine. All the healthcare data is uploaded to cloud storage, different platforms (*e.g.*, personal computer) and parties (*e.g.*, personal doctor) then can download for further evaluation.

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