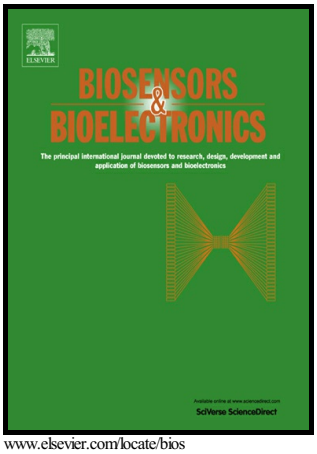

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Automatic Smartphone-based Microfluidic Biosensor System at the Point of Care

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Abstract

Point-of-care testing technique is increasingly important for healthcare management in human being's daily life. However, traditional biosensor systems for health care are relatively expensive, bulky and hard-to-handle, which largely limits their use in point of care testing. The problems mentioned above are successfully addressed with the popularization of smartphone and the development of microfluidic technology for their applications of biosensor, which integrates smartphones, microfluidic components and sensory elements together, paving the way for wide application of smartphone-based microfluidic biomedical sensory system. According to the varieties of analytes, the most common sensing modalities of biosensor systems are divided into imaging analysis to detect cells and bacteria, biochemical analysis to detect blood sugar and blood fat, immunoassay to detect protein specifically bound to antibody, as well as molecular diagnosis to detect DNA and other biomolecules. Bsed on the most common analytical methods, this review article covers five types of smartphone-based microfluidic biosensor systems at the point-of-care detection, i.e., smartphone-based imaging biosensor, smartphone-based biochemical sensor, smartphone-based immune biosensor, smartphone-based hybrid biosensor with more than one sensing modality, and smartphone-based molecular sensor. We lay emphasis on reviewing the structures, analytical methods and sensing modalities about the four kinds of biosensor systems with detailed discussions on their application potentials, aiming at giving the audience an overview of the recent developments of automatic smartphone-based microfluidic biosensor systems, as well as their future prospective.

Keywords: Microfluidic Biosensor, Smartphone, Point of care, Biosensor

1. Introduction

Lacking diagnostic tools in resource-limited settings remains one of the major obstacles to disease diagnosis and treatment (Bélec and Bonn 2011; Sharma et al. 2015). Most of the traditional biosensor technologies are costly, relatively complex, and rely on advanced infrastructure and trained personnel, which restricts their applications in point-of-care diagnostics (Jung et al. 1998; Teich 1998; Wilson 2013). In recent years, microfluidic-based biomedical sensory systems have emerged as a compact and cost-effective analytical platform by combining microfluidic components with sensory elements for cells or biomolecules detection to meet global health and related biomedical challenges. Microfluidics can analyze small sample volumes, minimize costly reagent consumption, automate sample preparation as well as reduce processing time. The integration of microfluidics and biosensor technologies provides new opportunities and promises for POC diagnostics, including high-throughput analysis, portability and disposability (Cetin et al. 2014; Fan and White 2011; Guo et al. 2017; Guo et al. 2016). The published research articles have reported wide biomedical diagnostic topics such as single-cell analysis (Guo et al. 2015b; Guo et al. 2014b; Guo et al. 2014c; Guo et al. 2015f; Guo et al. 2013a; Guo et al. 2013b; Huang et al. 2014; Shi et al. 2015a; Xue et al. 2014), enzymatically catalyzed biochemical sensor (de Marcos et al. 1999; Guo 2016, 2017; Guo and Ma 2017; Ji et al. 2017; White and James Harmon 2002; Zhang and Liu 2016a), immune sensor (Eltzov et al. 2015; Shi et al. 2015b) and molecular diagnosis (Ferguson et al. 2009; Zhao et al. 2016) based on microfluidic systems. The sensing mechanisms extend from physical (Chen and Guo 2015; Chen et al. 2016;

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Chen et al. 2015; Fu et al. 2017; Guo et al. 2015a; Guo et al. 2015e; Guo et al. 2014d; Ren et al. 2016), magnetic (Ungerbock et al. 2014), optical (Guo et al. 2015c; Guo et al. 2015d; Guo et al. 2014a), acoustic (Huang et al. 2017; Li et al. 2003), and electrochemical method (Guo 2016, 2017; Guo and Ma 2017) with excellent sensitivity and specificity. Incorporating with signal processing circuits and systems, the abovementioned microfluidic-based biomedical systems showed good performance in the numerous fields, for examples, biomedical point-of-care testing (POCT) (Guo 2017; Guo and Ma 2017; White and James Harmon 2002), environment monitoring (Ottesen et al. 2006), and food quality analysis (Loutfi et al. 2015).

On the other hand, 2 billion people across the world had a smartphone by the end of 2015, and it is expected to further increase to cover approximately 80% of the world population by 2020 (Fehske et al. 2011), which has paved the way for wide application of smartphone-based biosensor systems. Smartphone-based biosensor systems as the alternatives of conventional bulky biochemical analyser are capable of addressing a variety of healthcare issues such as problems caused by global aging. Especially, smartphones integrated with microfluidic biosensors with the computational power and cost-effectiveness have been endowed great potential to transform traditional uses of imaging, sensing and diagnostic systems, even for POCT applications. The smartphone-based microfluidic biosensor systems can provide fast, portable and easy-to-handle platform to achieve sensitivity and high-throughput real-time analysis of small biological samples, especially for the diagnosis and monitoring of blood samples.

In this work, we will review recent works about smartphone-based microfluidic biosensor system at POC in detail. According to the varieties of analytes, the smartphone-based biosensor systems are divided into five types, which are imaging biosensors to detect cells and bacteria, biochemical sensors to detect blood sugar and blood fat, immune biosensors to detect protein specifically bound to antibody, hybrid biosensor to detect more than various analytes, as well as molecular sensors to detect DNA and other biomolecules. The systems are divided into according to analytical method and sensing modality, including on-chip imaging devices, biochemical parameter sensors, immunoassays, and molecular diagnosis. We will lay emphasis on the structures, functions, sensing principles and properties, for different types of the smartphone-based biosensors. In the conclusion section, a brief discussion is given about the future prospects of this emerging technique.

2. Smartphone-based imaging biosensor

Smartphone-based imaging sensor is one of the most widely applied sensing modality at the POC. With the development of advanced integrated circuit (IC) design and manufacturing technology, the resolutions of the embedded Complementary Metal Oxide Semiconductor (CMOS) image sensor (CIS) cameras in smartphones have now reached more than 20-Mega pixels, and pixel pitch has diminished to ~1μm. Taking advantage of this high pixel density, smartphone-based optical sensor has been widely applied for microfluidic biomedical imaging diagnostics in recent years. Besides, the computational power of the embedded Central Processing Unit (CPU) and Graphics Processing Unit (GPU) in smartphone is rapidly advancing, which allows the biomedical images to be calculated and analyzed in real time. Hence, the morphological features such as object size, shape, as well as brightness or color can be quantified as signal tags for applications such as healthcare diagnostics, flow cytometry, clinical chemistry, drug screening, food analysis, and environment monitoring, etc.

2.1. Bright-field bio-imaging

Different optical bio-imaging modalities have been incorporated with smartphones to provide POC sensing. Among them, one important imaging modality is the bright-field on-chip microscopy. The smartphone-based microscopy usually contains a 3D-printed opto-mechanical attachment unit integrating sample tray, external lens and light source holder, field-of-view (FOV) and focus adjustment, etc. In this mode of imaging, a bio-sample of interest is illuminated uniformly using white light source, such as a broadband light-emitting diodes (LEDs), and the images are captured by the CIS camera of a smartphone. To magnify the sample image for resolution improvement, an external lens may be used together with the internal lens of the smartphone, or image super-resolution processing can be performed for resolution improvement.

The simplest form of bright-field on-chip microscopy is based on the shadow imaging. The pioneering work was done in Yang's group, where one CIS chip was directly integrated below one microfluidic channel in very close proximity (Heng et al. 2006; Zheng et al. 2010). When the bio-samples such as cells or microorganisms flew through the microchannel with white light source illuminating from above, a series of their diffracted shadow images could be captured by the sensor and then processed with super-resolution algorithms for resolution improvement (Huang et al. 2016; Huang et al. 2015a; Huang et al. 2015b). Such system setup needs to incorporate a detected LED light source, which may affect the compactness of configuration. To remove the light source, based on similar imaging principle, in 2014, Lee *et al*. developed a standalone chip-scale lensless microscopy device by adapting a smartphone's camera using ambient illumination as its light source (Lee and Yang 2014), as shown in Fig. 1a. The image reconstruction was performed using a custom-built Android application to reach sub-micron resolution. In its proof-of-concept measurement, the prototype achieved a resolution of 500 nm for portable microscopic imaging of blood smear and freshwater microorganisms, demonstrating promising healthcare and environment monitoring applications for the microscopy devices on smartphone. The key advantages of this technique are its simplicity and robustness. Another example of smartphone based microscopy was the ContactScope shown in Fig. 1b proposed by Navruz *et al* (Navruz et al. 2013). It used a tapered fibre-optical array in contact with the samples of interest above the smartphone camera for contact imaging. The sequence of transmitted light pattern images was then captured and computationally processed for a shift-and-add fusing by a custom-developed Android application on the smartphone. In testing, wide-field blood smear sample image was obtained by the

prototype with well agreement to their corresponding 10X microscope objective images. As another example, D'Ambrosio *et al*. developed a smartphone-based bright-field video microscope to automatically detect and quantify Loa microfilariae in whole-blood samples from a fingerprick without any previous sample preparation or labelling steps (D'Ambrosio et al. 2015). In this design, as shown in Fig. 1c, an external lens module is used, and a LED array illuminates the whole-blood sample in small glass capillaries. A resolution of < 6.5 mm over a 4 × 3.16 mm wide FOV was achieved. By detecting microfilarial motion in whole blood through processing the captured frame series of each FOV of the sample, this device can provide results in less than 2 minutes, much faster than conventional staining for morphology or screening for molecular markers. Field testing of the prototype in Central Africa demonstrated 94% specificity and 100% sensitivity comparing with manual thick smear counts. One possible cause of error of such system is incorrect focus due to accidental bumps, which should be prevented. One more recent study developed a field-portable bright-field microscope based on smartphone for label-free detection of S. haematobium eggs in urine samples in rural areas of Ghana, Africa as shown in Fig. 1d (Bogoch et al. 2017). The smartphone-based microscope used an external lens, and two LEDs as light source. A half-pitch spatial resolution of approximately 0.87 μm and a FOV larger than 4 mm^2 was achieved. A translation stage was designed to scan S. haematobium eggs on microscope slide. Field testing of the prototype showed a modest sensitivity of 72.1%, a high specificity of 100%, a positive-predictive value of 100%, and a negative-predictive value of 57.1%.

The aforementioned examples usually rely on capturing a sequence of images to compute a final output image, which could limit the imaging speed, or testing throughput. Also, these examples of bright-field smartphone-based microscope all used in-coherent light source, hence the imaging sensitivity and signal-to-noise ratio (SNR) of the diffracted images could be limited. Another scheme is that Ozcan's group developed the holographic on-chip imaging using coherent illumination source (Seo et al. 2009), as shown in Fig. 1e. Based on this holographic imaging principle, they reported a mobile phone based digital microscopy without lenses or lasers (Tseng et al. 2010). The captured holographic signatures of the imaged sample were reconstructed to its microscopic image through rapid digital processing. The demonstrated microscope could image various micro-particles as well as bio-samples such as red blood cells, white blood cells, platelets and waterborne parasite such as Giardia lamblia cyst.

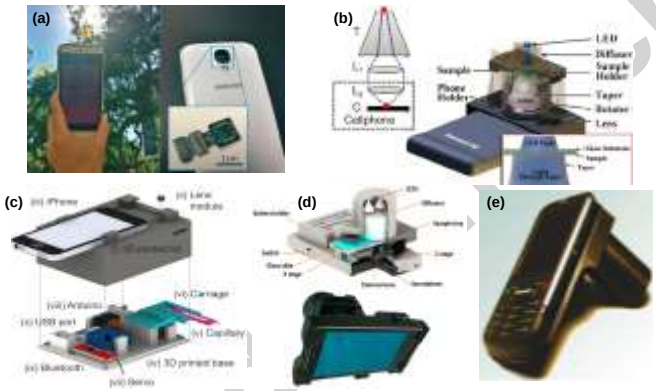

Fig. 1. Examples of smartphone-based bright-field bio-imaging sensor: (a) chip-scale lensless microscopy device using ambient illumination, reproduced from ref. (Lee and Yang 2014) with permission from the Royal Society of Chemistry; (b) Schematic diagram of the cellphone attachment of the Contact Scope, adapted from ref. (Navruz et al. 2013) with permission from the Royal Society of Chemistry; (c) bright-field video microscope to automatically detect and quantify Loa microfilariae, adapted from ref. (D'Ambrosio et al. 2015) with permission from the American Association for the Advancement of Science; (d) bright-field microscope for label-free detection of Schistosoma haematobium eggs, adapted from ref. (Bogoch et al. 2017) with permission from the American Society of Tropical Medicine and Hygiene; (e) holographic on-chip imaging based microscope, adapted from ref. (Seo et al. 2009) with permission from the Royal Society of Chemistry.

2.2. Fluorescent bio-imaging

Besides the aforementioned bright-field bio-imaging modality, another most commonly used microfluidic imaging modality is smartphone-based fluorescent microscopy. Different from the bright-field prototypes, the 3D printed opto-mechanical attachment in such microscopy design generally requires an excitation filter and/or an emission filter, and LEDs or laser diodes to form the excitation source.

Smartphone-based fluorescent bio-imaging sensor has been widely used for the detection of biological targets such as cells, bacterium and viral antigens, proteins, nucleic acids, and mycotoxins (Arts et al. 2016; Barbosa et al. 2015; Cevenini et al. 2016; Knowlton et al. 2017; Koydemir et al. 2015; Park et al. 2016; Priye et al. 2017; Rajendran et al. 2014; Wargocki et al. 2015; Wei et al. 2014; Wei et al. 2013; Zhu et al. 2011). For example, Zhu *et al*. designed a mobile phone-based fluorescent microscope as an imaging flow cytometer for analyzing blood samples and quality of drinking water samples (Zhu et al. 2011). In this prototype as shown in Fig. 2a, the fluorescent tags were excited using LEDs butt-coupled from the side, and a series of images when fluorescently-labelled particles flowing through disposable microfluidic channels were captured by the smartphone camera underneath. The measurement results of white blood cell density showed a decent match to a commercially available hematology analyzer.

Another smartphone-based fluorescent microscopy example can rapidly and sensitively detect and automatically count the waterborne parasites such as Giardia lamblia cysts in large volume water samples (e.g., 10-20 ml) using machine learning based

image-processing algorithms as shown in Fig. 2b (Koydemir et al. 2015). This compact and cost-effective platform demonstrated a limit of detection of about 12 cysts per 10 ml with a G. lamblia cyst, classification accuracy of approximately 95% for various water samples. The total time to result is about 1 hour, including sample collection, labelling, filtration, optical detection, data transfer, and automated counting at the server, thus providing a promising solution for water quality monitoring in resource limited settings.

To further improve the limit of detection to nanoscale so that bio-samples such as bacteria or deoxyribonucleic acid (DNA) can be detected for molecular diagnostics, Wei *et al*. designed one smartphone attachment where a laser diode-based illumination with a highly-oblique incidence angle (e.g., ~75°) was employed for excitation of target fluorescent tags and particles as shown in Fig. 2c (Wei et al. 2013). Such method enabled a strong rejection of the excitation beam owing to the low numerical aperture of the smartphone camera system. This design made it possible that isolated 100 nm fluorescent particles and human cytomegalo viruses were imaged by disposable microfluidic devices or simply between two sealed cover glasses. Another similar smartphone-based design can image and measure the lengths of individual double-stranded DNA molecules with a sizing accuracy of <1000 base pairs (Wei et al. 2014). In this study, the samples to be tested were labelled and stretched by disposable chips.

Another recent example is a smartphone-based cell sorter via magnetic focusing and fluorescence imaging (Knowlton et al. 2017). This device uses two permanent magnets together with the imaging processing capability of the smartphone to analyze the spatial distribution of cells in the magnetic field and to realize density-based cell separation as shown in Fig. 2d. It can be used to identify cell type such as cancer and blood cell, and image the cells in either bright-field, dark-field, or fluorescent imaging modes using the built-in CMOS image sensor.

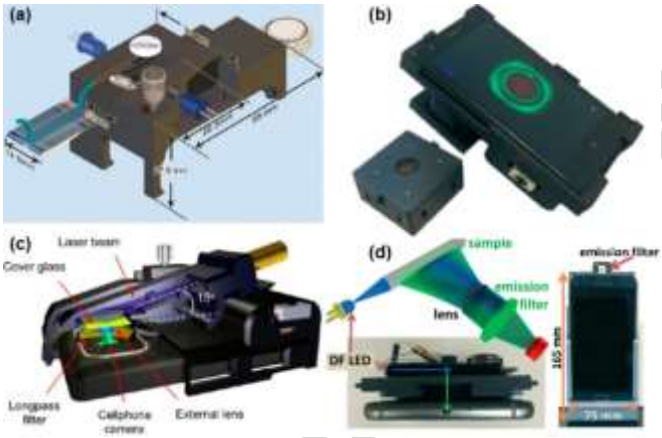

Fig. 2. Examples of smartphone-based fluorescent bio-imaging sensor: (a) smartphone-based fluorescent microscope as an imaging flow cytometer, adapted from ref. (Zhu et al. 2011) with permission from the Royal Society of Chemistry; (b) smartphone-based fluorescent microscopy to detect and automatically count the waterborne parasites, adapted from ref. (Koydemir et al. 2015) with permission from the Royal Society of Chemistry; (c) smartphone-based fluorescent microscopy to detect nanoscale bio-samples, adapted from ref. (Wei et al. 2013) with permission from the Royal Society of Chemistry; (d) smartphone-based fluorescent imaging cell sorter using magnetic focusing, reproduced from ref. (Knowlton et al. 2017) with permission from the Royal Society of Chemistry.

3. Smartphone-based biochemical sensor

Electrochemical amperometric biochemical biosensor is based on quantities of transferred electrons generated between the enzyme modified working electrode and the corresponding counter electrode due to the oxidation or reduction of the electroactive substances during the enzyme catalyzed biochemical reaction, when applying the specific electrical potential to the working electrode. The generated electrochemical current is linearly proportional to the concentration of the biochemical molecules to be characterized (Guo 2016, 2017).

Amperometric biosensors connected with smartphone for remote health management were reported and believed to be the promising solution to the issues caused by global aging due to the large amounts of the smartphone users. Amperometric biosensors are constructed by different working principles, for examples, chronoamperometry (CA), cyclic voltammetry (CV), differential pulse voltammetry (DPV) and square wave voltammetry.

It was firstly reported that the utilization of amperometric biosensor with the combination of the smartphone was applied (Guo 2017; Guo and Ma 2017; White and James Harmon 2002). The proposed miniaturized system was composed of the disposable microfluidic chip integrated with biosensor electrodes, weak signal processing circuit and system. The amperometric module was designed with highly compact size and low cost which makes the system applicable at the POC spot. The micro-universal serial bus (USB) port located on the smartphone was capable of providing the electronic interface between the miniaturized amperometric system and the smartphone for medical data transmission and upload.

As another example, Deng *et al*. demonstrated a smartphone-based electrochemical test strip for POC gender verification, which is key factor for forensic analysis (Deng et al. 2016). The miniaturized system was developed with amperometric

characterization module and a smartphone application. In this following section, we will introduce the function of smartphone as the electrochemical analyzer and medical data platform for biochemical sensing.

3.1. Smartphone as an analyzer

In the proposed research work, Guo successfully demonstrated the medical-level smartphone as the miniaturized electrochemical analyzer station by cooperating with the electrochemical test strip for POC measuring uric acid in finger pricked whole blood (Guo 2016). The proposed medical smartphone is cost effective and with price tag less than 60 US dollar. The photograph of the medical smartphone is as Fig. 3 illustrated. One disposable enzymatic uric acid (UA) test strip was inserted into the amperometric module embedded with the smartphone through the specifically designed interface: a slot located at the edge of medical smartphone. The medical smartphone is believed to be a promising solution for fulfilling the rigid demand of the mobile health management.

Another typical example, smartphone-powered medical dongle as a compact amperometric analyzer cooperated with an enzymatically beta-hydroxybutyrate test strip for precise measurement of blood ketone in finger pricked whole blood at the POC, was reported by the Guo's group (Guo 2017). The proposed system was capable of providing a solution for the characterization and treatment of Diabetic ketoacidosis (DK) and diabetic ketosis acid (DKA).

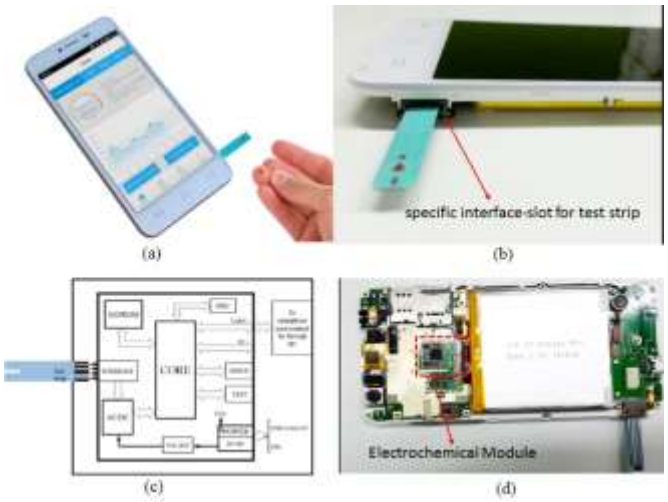

Fig. 3. The photograph of the proposed medical smartphone in which a electrometer has been pre-buried, the side photograph of the proposed medical smartphone: slot is the interface for insertion of UA test strip, schematic structure view of the electrometer, a micro controller for resolving the electrochemical current formed in the test strip, the top view of the layout of the medical smartphone: the electrochemical module is integrated with the main print circuit board of the smartphone, reproduced from ref. (Guo 2016) with permission from American Chemical Society.

3.2. Smartphone as a medical data platform

As Fig. 4a shows, Erickson et al. integrated a smartphone Cholesterol Application for Rapid Diagnostics System that can quantify cholesterol levels according to colorimetric changes of cholesterol reacting enzymatically on a test strip (Oncescu et al. 2014). The system was composed of a smartphone accessory to acquire image of the test strip and an app as medical data platform to analyse parameters of the test area, and measured the cholesterol levels directly on a smartphone. The accuracy of measurement was within 1.8% in the relevant physiological range (140 mg dL^{-1} to 400 mg dL^{-1}). And the maximum difference between the predicted value for user testing and the measured value using the CardioChek PA system were less than 5.5% in all cases. The system can identify erroneous readings and have certain repeatability. In addition, a disposable electrochemical blood ketone strip was associated with the medical dongle (Fig. 4b) which is powered by the smartphone through an OTG (On-The-Go: a kind of device communication standard) (Guo 2017). The blood ketone level was evaluated by measuring the electrochemical current. According to the signal-to-noise ratio (SNR) level, the limitation of detection (LOD) by the proposed medical dongle can approach to 0.001 mmol/L. The linear detection range of blood ketone was located from 0.001 to 6.100 mmol/L.

Another example is a smartphone-based passive and wireless near field communication (NFC) biochemical sensor combining a smartphone data reader and a passive NFC tag responder shown in Fig. 4c (Xu et al. 2017). The smartphone could wirelessly power the sensors and receive the detection results by inductive coupling, and transfer data through signal modulation. The system can easily achieve accurate semiquantitative detection of different analytes constructed sensor arrays by adopting rheostats in the resonant circuits of the sensors. The results showed that the tag sensors could discriminate analytes in solution such as ions and bacteria with different concentrations, unfolding new dimensions for biochemical sensing with smartphones at point of care.

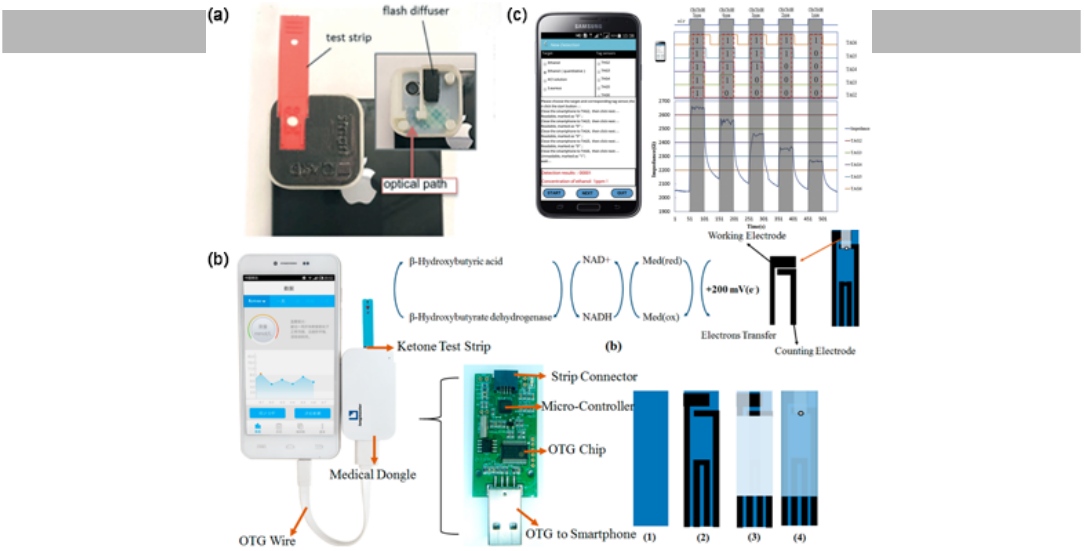

Fig. 4. (a) Picture of the smartCARD (smartphone Cholesterol Application for Rapid Diagnostics) accessory and the test strip; the inset shows the inside of the accessory with the diffuser and the optical path of the flash used to illuminate the strip, reproduced from ref. (Oncescu et al. 2014) with permission from the Royal Society of Chemistry; (b) Photograph of the medical dongle with OTG connecting to smartphone; (b) the working mechanism of blood ketone test strip; the printed circuit board (PCB) and electronic elements in medical dongle; the fabrication procedure of the blood ketone test strip, reproduced from ref. (Guo 2017) with permission from American Chemical Society; (c) the semi-quantitative detection of ethanol from 1 ppm to 5 ppm by the sensor array and smartphone. With the different readabilities of the five sensors in the detections, different concentrations were successfully discriminated, reproduced from ref. (Xu et al. 2017) with permission from Elsevier.

4. Smartphone-based immune biosensor

Smartphone-based immune sensor combines immunoassays with microfluidic systems to construct a promising platform for detecting analytes in disease diagnoses, food security monitoring and environmental monitoring, because of its properties of portability, reliability, simplicity and money-saving (Caliendo et al. 2013; D Gan and Patel 2013; Dou et al. 2016; Zhang and Liu 2016b). Based on quantification techniques used in immunoassays, smartphone-based immune sensor offered another alternative for POC detection to make conveniences for medical diagnostics, especially for people in remotes areas (Arts et al. 2016; Fang et al. 2016; Giavazzi et al. 2014). According to the different roles that smartphones play, the smartphone-based immune sensor could be classified into the following two types.

4.1. Smartphone as a power supplier

In recent days, dual HIV/treponemal-syphilis tests have been exploited, but these tests count on lateral-flow or immunofiltration technologies, which may limit their performance and ability to multiplex (Hung et al. 2004). To overcome this problem, Laksanasopin *et al*. reported a full laboratory-quality immunoassay that could be carried out on a small diagnostic accessory made of a dongle attached to a smartphone providing all necessary power (Fig. 5a) (Jang et al. 2017). Notably, in this design, the smartphone served as not only a passive interface to receive sensing information and but also an active interface to control test procedures and the dongle performed POC enzyme-linked immunosorbent assays (ELISA) quality tests. Compared with laboratory-based diagnostic devices, this device obtained patients' preference for quick and accurate diagnostic results for HIV and syphilis only with a single fingerprick, which could be operated by any population with smartphones.

Then, Hall's group developed a smartphone-based electrochemical biosensor system for POC diagnostics and wellness tracking that leveraged the audio port on the smartphone for communication and even power (Laksanasopin et al. 2015). As shown in Fig. 5b, this system was composed of a low-cost electronic module including a low-power potentiostat, which could harvest power from all kinds of smartphones through the audio port efficiently. In this study, the harvesting efficiency was up to 79% owing to active impedance matching, the consuming peak power of the module is 6.9 mW except for loses from supply regulation and rectification, and <1 nA bidirectional current could be measured. The prototype could be performed within the available power budget of smartphones and produce data well matching with that of expensive laboratory instruments. They proposed that via an electrochemical sandwich assay on disposable screen-printed electrodes, the platform could be utilized in tracking the concentration of secretory leukocyte protease inhibitor (SLPI) in its physiological range. SLPI acted as a biomarker to monitor lung infections and the limit of detection was about 1mM, which is helpful to improve the quality of life for cystic fibrosis patients.

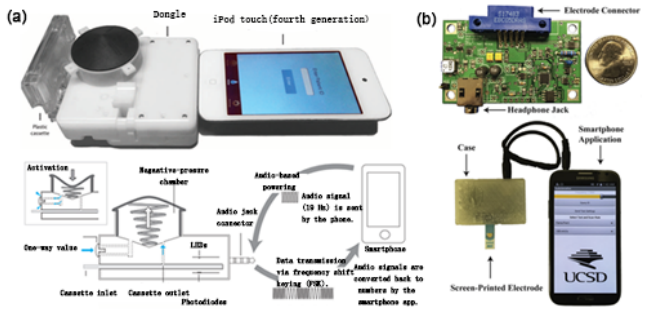

Fig. 5. Smartphone-based immune biosensor at POC. (a) Images of the dongle with a microfluidic cassette connected to an iPod touch and schematic diagram of dongle highlighting a power-free vacuum generator using the audio jack connector for audio based powering and frequency shift keying (FSK) data transmission to a smart-enabled device, reproduced from ref. (Jang et al. 2017) with permission from Elsevier; (b) Photograph of the PCB module prototype and the module being powered by and communicating with a Samsung Note 2, reproduced from ref. (Laksanasopin et al. 2015) with permission from Elsevier.

4.2. Smartphone as a detector

Diagnostics based on amplification of nucleic acid is an effective means to detect and monitor progression of infectious diseases with rapid, sensitive and specific performance (Park et al. 2015). Seo *et al*. realized the first exemplification of pathogen monitoring for smartphone-based healthcare via internet-of-things (IoT)(Seo et al. 2016). In their study, CMOS image sensor (CIS) was utilized to detect the signal produced from a smartphone-based immunosensor system to resolve the difficulties in healthcare monitoring via IoT. The system was developed to pocket-sized dimensions and then adopted to mensurate a food-borne pathogen in real samples. The smartphone in this work was used to control and monitor the analysis process, which was also used to upload the results to the internet server to share the data with the public. However, the hinder for its application is relatively expensive cost including preparation of samples, specific laboratory equipment and training for specific researchers (Rateni et al. 2017). To solve the problem mentioned above, Liao *et al*. designed a smart cup (Fig. 6a), a smartphone-based device, for rapid and quantitative molecular diagnostics of pathogens (Seo et al. 2016). The smart cup supplied heat for nucleic acid with isothermal amplification triggered by water through exothermic chemical reaction. During the amplification process, the flashlight of smartphones as the optical detector is used to excite the fluorescent dye and the phone camera is used to record real-time fluorescence emission. Besides, smartphones installed smart cups could concurrently monitor multiple amplification reactors and analyze the recorded data in a variety of places because smartphones are portable. The smart cup was successfully utilized in amplifying and quantifying herpes simplex virus type 2 (HSV-2) with loop-mediated isothermal amplification (LMIA) assay in custom-made microfluidic diagnostic chip and the detection process could be repeated. Another recent example is a device integrated by a smartphone and a custom-designed cradle designed by Giavazzi *et al*. (Liao et al. 2016). The device was installed with a disposable sensing cartridge, a tiny magnetic stirrer and a few passive optical components. In this work, Reflective Phantom Interface based on measuring the intensity of light was reflected by the surface of an amorphous fluoropolymer substrate, which immobilized antibodies on the same chip. The smartphone's camera installed flash LED could be used as the illumination source.

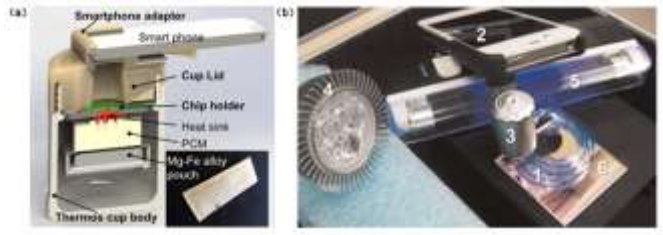

Fig. 6. (a) Exploded view of smart cup for minimally-instrumented, point-of-care molecular diagnostics, reproduced from ref. (Rateni et al. 2017) with permission from Elsevier; (b) Main components of MCF phone (1) Microcapillary Film (MCF) (2) Smartphone (3) Magnifying lens (4) Blue LED (5) UV black light for fluorescence detection, light source for chromogenic detection (6) dichroic filter, reproduced from ref. (Barbosa et al. 2015) with permission from Elsevier.

Also, Barbosa *et al*. have designed a portable detection system, Microcapillary Film (MCF) phone, for colorimetric and fluorescence quantitative sandwich immunoassay of prostate specific antigen (PSA) (Barbosa et al. 2015). The MCF phone (Fig. 6b) is integrated with a magnifying lens, a simple light source and a miniaturised immunoassay platform, the MCF. MCF possesses excellent performance for transparency and flat geometry of fluoropolymer, which is beneficial for quantitation of PSA in whole blood samples. The MCF phone has been proved the capability of fluorescence tests with high sensitivity, colorimetric quantity and good recovery, which further demonstrated the major breakthrough on the integration technology of low-cost and portable microfluidic devices, commercial immunoassay reagents and smartphones.

4.3 Smartphone as a processor

To realize rapid immunosensing for POC testing, Dou *et al*. improved the smartphone-based electrochemical biosensor with an electric field-driven acceleration strategy (You et al. 2017). They have developed a immunosensor (Fig. 7a) made of an embedded circuit in smartphone for signal processing and a screen-printed carbon electrode (SPCE) modified with multi-walled

carbon nanotubes (MWNTs) and goat anti mouse-immunoglobulin G (IgG) sensing layer (MWNTs-I-layer) for rapid and sensitive immunosensing detection of clenbuterol (CLB). The CLB was detected by the competitive immunoreaction on the electrode between horseradish peroxidase-coupled CLB (CLB–HRP) and free CLB in the samples to CLB monoclonal antibody bound to the surface confined antibody. In their work, the competitive immunoassay was finished in 6 min by electric field-driven acceleration and the detection limit was 0.076 ng·mL^{-1}, which exhibited the potential as POC platform for rapid and sensitive detection of all food security-related species.

Then, Wang's group designed another improved efficient biochemical analysis method to detect marine toxins with a smartphone-based portable system—bionic electronic eye (Bionic e-Eye) (Zangheri et al. 2015). Compared with conventional methods for detection of marine toxins including microtiter plate reader (MTPR), Bionic e-Eye possesses the advantage of in-field fast measurement and real-time on-line analysis with the comparable precision, dynamic range, detection limit and sensitivity. Bionic e-Eye was supported by software named iPlate, which integrated images capture and deeper date processing. In Bionic e-Eye two color models of HSV and RGB were used for evaluation of bioanalytical performance in saxitoxin (STX) assay and okadaic acid (OA), which were the standard representations of Paralytic shellfish poisons (PSP) and diarrheic shellfish poisons (DSP), respectively. Bionic e-Eye was of great potential to be a novel detection method with a field-test platform rather than conventional methods of off-site detection.

4.4 Smartphone as a transducer

Besides, novel immunosensor based on smartphone which was installed with an immunoblotting assay and a built-in illumination sensor (Fig. 7b) was developed by Yoon's group(Zhang et al. 2016). Due to smartphone-embedded components, the optical biosensing system could be built easily and effectively including a white LED and an illumination sensor, which were designed to be the light source and optical receiver, respectively. Compared with conventional optical sensors, the illumination sensor responded to the variations of external light intensity over a wide range of wavelengths which made it more sensitive. In illumination sensor, smartphone was utilized as a signal transducer in the optical system. Besides, the immunoblotting technique was introduced into the new system so as to change the intensity of light uniformly. The specific mechanism for conduction of immunosensor was that the horseradish peroxidase-induced insoluble precipitate interferes with the penetration of incident light with facilitating the variation of applied light intensity. And then light passed through the biosensing channel and was analyzed by a lux meter in the mobile application. In fact, illumination was a promising candidate for testing of quantitative diagnosis and POC.

The main disadvantages of the above system are that the smartphone must be integrated with other devices, such as dongles, electrical circuits and so on, which means additional expend and more complex systems. What's more, excessive accessories attached to the smartphone lead to the results and analysis easily affected by the conditions of them.

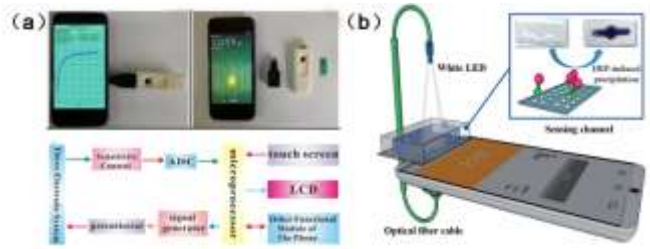

Fig. 7. (a) A photograph of the smartphone-based immune sensor, A picture of the smartphone, USB port, chipbox and screen-printed carbon electrode, An electrical circuit frame diagram of the mobile electrochemical device (ADC: analog to-digital converter. LCD: liquid crystal display.), reproduced from ref. (You et al. 2017) with permission from Elsevier; (b) Configuration of the illumination sensor, reproduced from ref. (Zhang et al. 2016) with permission from the Royal Society of Chemistry.

5. Smartphone-based hybrid biosensor

Hybrid biosensor is an emerging sensor system, which integrate multi-function in one. A biochemical-immunological hybrid biosensor (Fig. 8) based on two-dimensional chromatography was developed for on-site sepsis diagnosis, which could perform the immunoassay and the enzymatic assay in the same sepsis-based sample (Kim et al. 2017). In this work, an enzyme-reaction zone was installed on the signal pad of the typical immuno-strip for the rapid two-dimensional (2-D)-chromatography test. A pre-determined membrane site was etched in a pattern and mounted with a biochemical-reaction pad, which let loaded sample flow in and stay in the pad to produce a colored-signal during the course of an immunoassay, thus minimizing the mutual interference in the hybrid assays. The system was used to analyse a serum sample with the vertical direction flowing along the strip, which provided lactate for the biochemical-reaction zone and protein markers for the immunological binding area pre-coated with capture antibodies. Then the enzyme-signal tracers for the immunoassay and the substrate solution were sequentially furnished with a horizontal direction to trace the immune complexes. The color signal produced from each assay was detected and quantificationally analyzed at a pre-determined time.

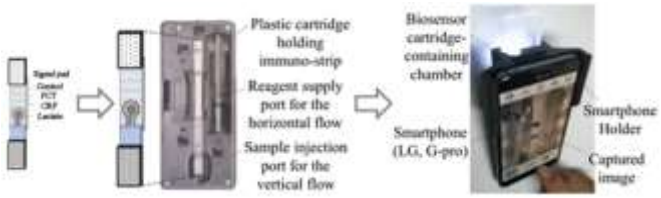

Fig. 8. Construction of hybrid immuno-strip, simultaneous analyses of protein and biochemical markers using 2-D chromatography and Signal detection, reproduced from ref. (Kim et al. 2017) with permission from American Chemical Society.

6. Smartphone-based molecular sensor

Currently available molecular diagnostic technologies are expensive, time-consuming and relatively complex, which limit their use (Jiang et al. 2014). To make low-cost integrated miniaturized devices for molecular diagnosis at the POC come true, Liu *et al*. have designed a reaction-diffusion based method to test HIV viral load monitoring by end-point quantification of target nucleic acids undergoing enzymatic amplification (Lin et al. 2010). In this work, the nuclemeters were housed on a single chip and imaged synchronously for real-time monitoring of multiple amplification processes. Similar to reading temperature in a mercury thermometer, the number of target molecules could be inferred from the position of the reaction-diffusion front. The quantitative detection of HIV viral load could be achieved by taking single fluorescence image at the end of the enzymatic amplification course through a programmed smartphone. A handheld flow genetic analysis system (FGAS) (Fig. 9a) including a thermal-gradient microreactor, a microflow actuator, and smartphone fluorescence imaging was also designed (Liu et al. 2014).

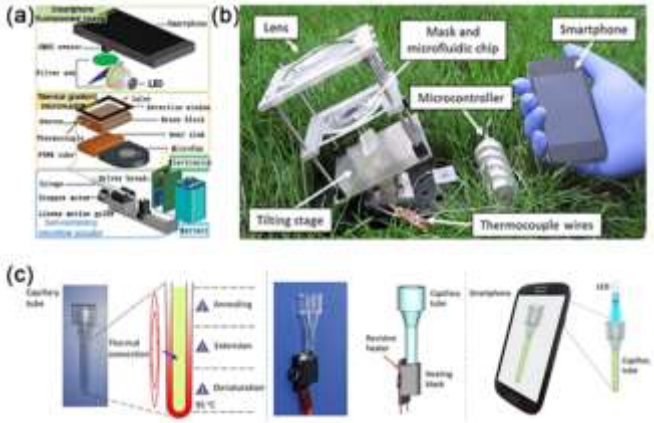

Fig. 9. Smartphone-based molecular diagnosis. (a) Photograph of the expanded view of the design of the FGAS, reproduced from ref. (Liu et al. 2014) with permission from the Royal Society of Chemistry; (b) Solar thermal PCR system: the benchtop component holds the lens, the PCR chip and a rotational stage, reproduced from ref (Qiu et al. 2016) with permission from Springer Nature; (c) Capillary tube PCR reactor for DNA amplification, capillary tube heating with a resistive heater and a simplified schematic illustration of fluorescence detection with a smartphone camera, reproduced from ref (Qiu et al. 2017) with permission from Springer.

Jiang *et al*. have created another smartphone-assisted molecular diagnostics system (Fig. 9b) for polymerase chain reaction (PCR) and integrated solar thermal microfluidics to dispel the requirements of thermal cycling power (Qiu et al. 2016). This system was used for analyzing human skin biopsies infected with Kaposi's Sarcoma herpesvirus with solar thermal PCR, extracting HotSHOT DNA and smartphone fluorescence detection, which could be powered by a standard 5.5 Wh iPhone battery for 70 h. The smartphone-based fluorescence imaging system could capture all PCR channels of the microreactor in one single snapshot.

Additionally, Qiu *et al*. have developed a smartphone-based POC molecular diagnosis of influenza A (H1N1) virus with a microfluidic convection polymerase chain reaction (PCR) in a capillary tube (Qiu et al. 2017; Shu et al. 2015). As is shown in Fig. 9c, a simple heater was designed to heat the capillary tube from the bottom at a constant temperature to form a stable temperature gradient across the tube to generate a continuous circulatory flow for rapid nucleic acid amplification, which was achieved when the reagent was spontaneously and repeatedly transported through different temperature zones associated with the denaturing, annealing, and extension stages of PCR. In their study, a light-emitting diode on the top of the capillary tube was used to excite the reagent, the camera of a smartphone was used to obtain the fluorescent images for real-time fluorescence detection and a custom algorithm on the smartphone developed with Java was used for image analysis to interpret the diagnostic result.

7. Conclusions and future perspectives

In this review article, typical smartphone-based microfluidic biosensors reported in recent years are discussed, with specific focuses on their structures, analytical methods and sensing modalities. These reported research works demonstrate that smartphone-based microfluidic biomedical sensory system can provide rapid and accurate POC detection, with powerful capability of addressing many problems for conventional biomedical analysing instruments, such as huge cost, bulky size and demands of professional operation. In addition to the intrinsic advantages of portability and extreme large popularity, the main

merit of the smartphone based microfluidic biosensor system is that the smartphone can be integrated with many other attachments, such as miniatured camera, optical sensors, dongles, electrical circuits and so on, which provides vast possibilities for additional expansion on their functionality, achieving complex sensory systems. And the versatility of sensory systems has been validated by the increasing number of the detected analytes, from small molecules, proteins and nucleic acids even to bacteria and viruses. Smartphone-based hybrid biosensor has recently appeared as an emerging trend, integrating multi-function in one to make a breakthrough in simultaneous detection of more than one analyte. Overall, the special properties of smartphone-based microfluidic biosensors still are of great interest and hold great significance in the field of biomedical sensing, especially for their promising potentials in the application of POCT.

However, there are still several challenges necessary to overcome in the integration and design of the smartphone-based microfluidic biosensor systems. On the one hand, excessive accessories attached to the smartphone might not only affect the conveniences and flexibilities of the smartphone-based microfluidic biosensor systems, but also perplex the diagnostic process, leading to loss of accuracy. On the other hand, miniatured sensors might also sacrifice the sensitivity and accuracy of the sensing system compared to conventional lab-setting analysing instruments. In addition, some of integrated chips are too expensive to popularize and too complex to fabricate. And the integration of hybrid biosensors has been not fully exploited yet, limiting also their operability in synchronous detection. Now that most of smartphone-based microfluidic biosensor systems are still in the proof-of-concept stage and performed with standard tests in the laboratory, there is still a long road ahead for technology transfer and commercialization.

Therefore, we envision that the future development of smartphone-based microfluidic biosensor for POC diagnostics will focus on the following aspects. New techniques should be adopted to simplify attachments for smartphone by optimizing their structures, as well as smartphones with advanced computational capacities can avoid the loss of accuracy to some extent. Moreover, novel microfluidic biosensor techniques should be developed to improve the analytical performance of miniatured sensors. It is a good way to utilize available materials, such as paper, as supporting substrates to fabricate wireless, built-in function module, resulting in miniatured functional sensors with effective cost and better mobility, which can be embedded into smartphones for quick and accurate POC detection. Future efforts also should be contributed to combining current smartphone-based microfluidic biosensor systems with advanced analytic techniques, including surface-enhanced Raman scattering technology, etc., and developing the complete integration of hybrid biosensors to further improve POCT. We can prospect that more simplified and accurate sensor systems will be developed for POC samples analysis with the developments of smartphone industry, microfluidic chips and advanced sensing technologies.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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