INAE Lett (2016) 1:59–64
DOI 10.1007/s41403-016-0009-1

REVIEW ARTICLE

Microfluidics-based Low-Cost Medical Diagnostic Devices: Some Recent Developments

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Received: 4 April 2016 / Accepted: 30 June 2016 / Published online: 2 August 2016

Abstract Here we present a short review of recently developed low-cost microfluidic devices aimed towards medical diagnostic applications. This review specifically focuses on three inexpensive devices, namely lab-on-a-compact-disc, paper-based, and thread-based devices. Here, we present a concise summary of all the recently developed protocols for simple bioassays to complex diagnostics. We also provide a new outlook on how the present practice of pathological diagnostics can be improved with the usage of such recent developments.

Keywords Microfluidics · Inexpensive · Diagnosis

Introduction

In accordance with World Health Organization (WHO)'s report (Observatory 2015), life expectancy of human beings has increased significantly throughout the globe. Thus, it is becoming a tough challenge for each nation to provide quality healthcare to its population. Involved clinical practices often follow tedious laboratory protocols like clinical chemistry, hematology, and immunology etc., which require the usage of expensive chemicals, sophisticated instruments, and trained personnel. In many scenarios, the sample collected from the patient needs to be transferred to a specialized laboratory from a remote place. Even, the patient may need to be physically transferred to a sophisticated healthcare centre for diagnosis. Furthermore, the duration of analysis in traditional diagnostics may range from few hours to days. Thus there are urgent needs of fast, easy and affordable diagnostic devices and/or protocols. In this context, microfluidic technologies have found significant potential in terms of providing low-cost, rapid and yet efficient diagnostics (Pai et al. 2012; Gubala et al. 2012).

Typical microfluidic devices for medical diagnostics can be classified into two broad categories. One category involves the fabrication based on glass, silicon, PDMS or other polymer-based substrates, which often require expensive instruments and clean room facilities; thus may not suit for on-field applications.

Recent developments of lab-on-a-compact disc (CD) (Gorkin et al. 2010), paper-based (Gubala et al. 2012) and thread (specifically made of cotton)-based (Li et al. 2010) microfluidic devices hold the potential to alter the scenario of point-of-care (POC) diagnostics (i.e. diagnostic activities on patient's site, and not in the laboratory). In this letter, we outline the recent developments on all three aforesaid devices for several fluidic functions and complex biological assays.

CD-based Devices

CD-based devices are explored for successful demonstration of several fluidic functions like mixing (Chakraborty et al. 2011; Ducrée et al. 2005), valving, separation (Zhang et al. 2008; Kuo and Li 2014; Kim et al. 2013a), bubble (Chakraborty and Chakraborty 2010)/droplet (Haeberle

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et al. 2007) generation. Operational details of such devices have been elaborately demonstrated by Madou et al. (2006) and its function as a potential bio-medical platform (Gorkin et al. 2010; Ducrée et al. 2007) has been well documented in literature. CD-based devices may be fabricated following different approaches. For example, one may adopt a layer-stacking method (Madou et al. 2006; Kar et al. 2015a), by which three PMMA sheets are attached with two pressure sensitive adhesives (PSA) layers and thus circumventing the usage of expensive instruments and intensive labor. In such devices, micro-channels are engraved within the adhesive layers. Fluid transportation through such devices is mainly guided by rotational actuation and thus diminishes the usage of expensive syringe pumps or other actuation mechanisms.

Complex biological assay like nucleic acid analysis (Kim et al. 2014), enzyme linked immunosorbent assay (ELISA) (Lai et al. 2004) have been explored on CD-based devices by judicious tailoring of rotational speeds. To improve the sensitivity of detection, Kim et al. demonstrated an electrochemical immunosensor (Kim et al. 2013b) which detects the bio-analytes at pg/mL level. Efficient separation of blood plasma (Zhang et al. 2008; Kuo and Li 2014) from whole blood has been illustrated on CD-based platform, simply with the controlled interplay of channel geometry and as well as the rotational speeds. Centrifugally-actuated platforms have been delineated for high throughput screening and multiplexing (Park et al. 2012), and thus hold the potential of performing batch experiments on a single device.

Our research group initially focused on exploring several fluidic functions like capillary filing dynamics (Chakraborty et al. 2009), mixing behavior (Chakraborty et al. 2011), and generation of micro-bubble (Chakraborty and Chakraborty 2010), droplets to serpentine threads (Kar et al. 2015b) on CD-based platforms by exploiting the inter-playing physical forces. Currently, we are exploring this particular device for diagnostic applications. Our recent study reveals that on a rotating platform, the fluidic parameters are significantly altered with the change in the hematocrit value of blood (Kar et al. 2015a). In our case, we have characterize the blood dynamics in terms of two fluidic parameters namely burst frequency (f_b) (i.e. the minimum rotational speed which is required to overcome the surface tension force or below which the fluid column will not burst into the channel from the inlet reservoir) and volumetric flow rate (Q). In our experimentation, the used device has the dimension of length $(L) = 2.9$ cm, height $(H) = 100$ µm, whereas width (W) varies from 200 to 700 µm. For the characterization of blood dynamics, rotational speed of the device has been controlled in the range of 100–500 rpm. In our experimentation, alteration in the hematocrit value of blood, in turn, alters the rheological behavior of blood to a significant extent. This affects the fluidic parameters (Fig. 1 shows that the burst frequency is increasing with the increase of hematocrit value of blood). In another recent study, we have illustrated the effect of rotational stress on RBC's morphology (Kar et al. 2015c) (i.e. at cellular level). To characterize the RBC's morphological alterations, we define a parameter, named 'deformability index (DI)' (i.e. the ratio of major and minor axes of the ellipse while the RBCs are mapped as an ellipse). Hemoglobin, being an internal constituent of the RBCs, has significant influence on dictating the RBC's morphology (see Fig. 2). When the hemoglobin content is less than the standard value, then the cytoplasmic viscosity is expected to be less, which is manifested from the lesser value of DI. From our investigation, we envision that such alteration of fluidic parameters, or measurement of deformability indices can be used as inexpensive alternative to the conventional diagnostic approach for diagnosing hematological disorders.

Paper-based Devices

With an aim of providing 'zero cost diagnostics' Whitesides and co-researchers have first illustrated the fabrication and functions of paper-based devices (Martinez et al. 2010). Paper devices are fabricated by creating hydrophobic barriers, which direct the transportation of fluids in desired direction. To fabricate hydrophobic barriers on paper substrates, several techniques [like usage of

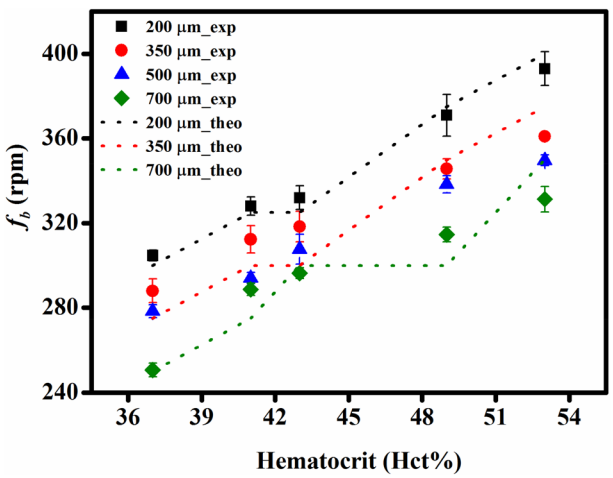

Fig. 1 Effect of hematocrit on burst frequency. The theoretical and experimental findings are merged in the same figure. *Scattered data points* represent the experimental findings whereas the *dotted lines* represent the theoretical results. The *error bars* indicate standard deviation (SD) of the results from repeated set of experiments (reproduced from Ref. Kar et al. (2015) with due permission from Royal Society of Chemistry, the article can be found http://pubs.rsc.org/en/content/articlehtml/2015/an/c4an02020k)

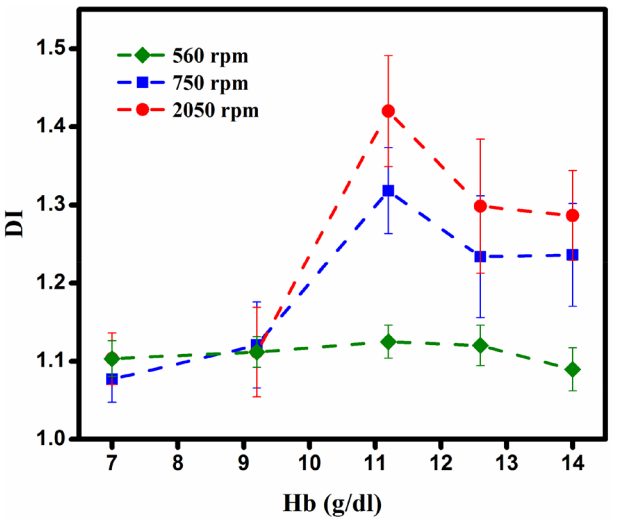

Fig. 2 Deformation characteristics as a function of hemoglobin content of the blood samples for different rotational speeds. The *error bars* represent the standard deviations (SD) of the results obtained from four repeated sets of experiments (reproduced from Ref. Kar et al. (2015) with due permission from Royal Society of Chemistry, the article can be found http://pubs.rsc.org/en/content/articlehtml/2015/lc/c5lc00968e)

epoxy-resin material, inkjet-printing, wax-printing (Carrilho et al. 2009), laser-cutting etc.] have been employed. Recently, we have developed a simple fabrication methodology (Dey et al. 2015), simply by using an office printer and a hot-plate. Through that particular modality of fabrication, necessary design can be fabricated by taking printing on both sides of the paper, followed by a heating at 200 °C. This leads to the melting of toner particles deposited over the paper surfaces. These particles eventually impregnate within the paper, and thus form the hydrophobic barrier. Accordingly, fluid flow is directed along a desired path, primarily by capillary action.

Paper matrix is composed of enormous number of cellulose fibers. The fluid imbibes through such porous networks due to the capillary force. Such tortuous networks of fibres provide rudimentary control over the fluid flow. To achieve better reproducibility and excellent control over the fluid transportation through paper matrix, researchers have introduced several external actuation mechanism like surface acoustic waves (SAW) (Rezk et al. 2012), electric fields (Dey et al. 2015; Mandal et al. 2012), and centrifugal force (Hwang et al. 2011) etc. Considering the inexpensiveness and ease of fabrication of the paper-based devices, our group has shown the use of graphite electrodes simply by rubbing a tip of a pencil on paper, in an effort to have rapid capillary transport with the aid of electric field in a low cost paradigm. It is shown that the fluid transportation rate can be significantly enhanced with the introduction of external electrical actuation on 'paper-and-pencil' devices (Mandal et al. 2012). Thereafter, we have demonstrated controlled mixing of two analytes on a zigzag 'paper-and-pencil' device with the combined interplay of capillary and electrokinetic effect (Dey et al. 2015).

Paper-based devices have been widely used for several investigations ranging from diagnosis to quality control of foods (Jokerst et al. 2012) and drinking water. Colorimetric approach (Dungchai et al. 2010), fluorescence-based approach, electrochemical (Rattanarat et al. 2012; Dungchai et al. 2009) and electro-chemiluminescence (Zhang et al. 2013) approach have been employed to detect level of analytes. Amongst all the aforesaid approaches, colorimetric approach received wide acceptability due to its simplicity and involvement of minimal instrumentation. There are reports where people have explored the detection of bromide ions from natural water (Loh et al. 2015) and other heavy toxic metal ions from the drinking water (Zhang et al. 2013) on simple paper-based platforms at ppm/ppb level. Another notable feature of this specific device is that it can easily be designed for diagnosing multiple analytes simultaneously on a single platform. It has been used for ELISA (Cheng et al. 2010), DNA hybridization, protein assay, anti-microbial activities (Kalsi et al. 2015), 'H-filter' devices for separation (Osborn et al. 2010) and lateral flow strip assays etc.

To avoid complexities while designing the devices, spot diagnosis is always favoured with colorimetric approach. One of the major issues while working with the colorimetric approach is that the intense red colour of the blood interferes in colorimetric detection. Thus, it is essential to separate the RBCs to diagnose any bio-analyte from plasma itself. Researchers have demonstrated efficient separation of blood plasma using a particular membrane (Songjaroen et al. 2012) (which specifically allow RBCs to adhere on its surface) and agglutination reagent (Yang et al. 2012). Very recently, we have demonstrated a simple paper-based 'H-channel' device for separating plasma (shown in Fig. 3) where we have reported an inexpensive modality for efficient separation of blood plasma using the capillarity-driven transportation of blood (Kar et al. 2015d). Using our approach, we have achieved ~75 % separation of plasma. A recent work delineates plasma separation using a 'salt-functionalized' paper (Nilghaz and Shen 2015) where the adhesion properties of red blood cells (RBCs) were augmented due usage of salt, and thus plasma gets separated from blood.

The colorimetric approach is globally used for qualitative and quantitative detection. To enhance the sensitivity of the detection, nanoparticles impregnated paper (Fu et al. 2011; Fu et al. 2010) have been used. Later on, researchers have attempted to employ 'Bioplasmonic paper (i.e. paper impregnated with nanoparticles having plasmon resonance)' for kidney biomarker detection (Tian et al. 2012).

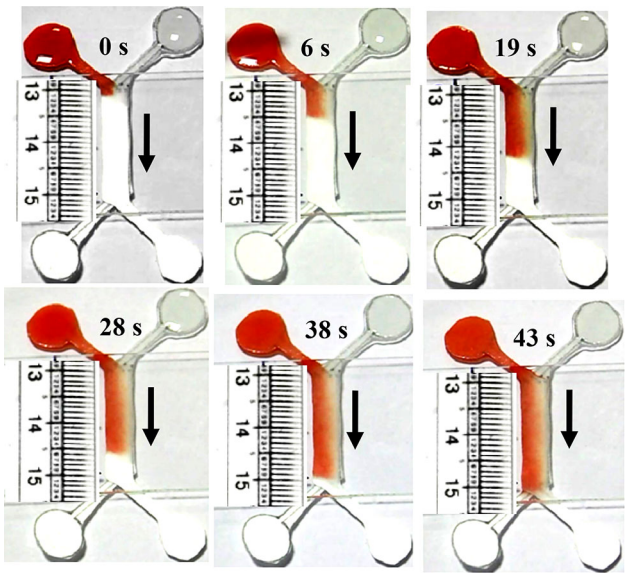

Fig. 3 Real time image sequences showing how the blood stream diffuses into the buffer stream and accordingly the blood strain covers the entire width of the paper channel (reproduced from Ref. Kar et al. (2015) with due permission from Royal Society of Chemistry, the article can be found http://pubs.rsc.org/en/content/articlehtml/2015/an/c5an00849b)

With this approach, they have diagnosed Hepatitis B virus DNA using electrochemical paper sensor (Li et al. 2015). Yildiz et al. have demonstrated a simple paper-based biosensing platform for colorimetric detection of lung cancer associated miRNA (Yildiz et al. 2013).

Three dimensional (3D) paper devices have been fabricated by using paper and tape (Martinez et al. 2008) and also following an origami-based protocol (Liu and Crooks 2011; Scida et al. 2013), and same has been utilized for several applications (Scida et al. 2013; Liu et al. 2012; Ge et al. 2012). There are other reports which discuss about hollow paper channels (Renault et al. 2013), which have also been used for bio-analyte detection.

It is important to note here that the applications of such paper-based devices are not only limited in the field of diagnostics, but have also been extended to address diverse applications like titration (Myers et al. 2015; Karita and Kaneta 2014), power generation (Arun et al. 2014; Veerubhotla et al. 2015) (fuel-cells), and the development of flexible electronics (Kurra et al. 2013; Kurra and Kulkarni 2013) etc.

Thread-based Devices

In this section, we are going to briefly discuss about thread-based (made of cotton) inexpensive platform. Versatility of the thread-based devices in the context of developing diagnostic devices has been established by Li et al. (2010). Thereafter in 2011, Ballerini et al. have demonstrated the usage of thread-based devices for mixing, and acid–base titration (Ballerini et al. 2011). There are reports which show several fluidic functions (Taylor et al. 2012) by using yarns and knots (Safavieh et al. 2011) with the thread. Recently, complex functions like immune-chromatographic assay on threads (Zhou et al. 2012) has been illustrated by Zhou et al. Human ferittin has been successfully diagnosed by performing immunoassay on cotton-threads (Mao et al. 2015).

Conclusions

In this brief review, we have summarized some recent developments on low-cost microfluidic devices for point-of-care medical diagnostics. All the three devices discussed here, namely lab-on-a-CD, paper based devices, and thread based devices are easy to manufacture, portable, multiplexable (i.e. multiple analytes can be simultaneously diagnosed on a single platform), easily disposable, affordable for the mass population, easily operated from resource limited places, and are therefore appropriate for on-field trials. These types of affordable diagnostic platforms can be very useful for on-spot diagnostics and as well as for continuous monitoring for a particular disease condition. Though the several complex bioassays have been demonstrated on simple platforms but such devices are yet to be popularized in mass scale, especially in developing nations. We envision that these devices hold the potential of replacing the traditional laboratory-centric, expensive, and time intensive paradigm of medical diagnostics. One primary bottleneck towards that is an appropriate upscaling of the these devices from the laboratory scale to the commercial scale, as well as establishing the reliabilities of the read-outs from such devices to all the stake holders under concern. Further, one may integrate the low cost hardware of these devices with Smartphone technology (mobile apps) for integrating with the telemedicine facilities available at resource-limited locations. Efforts towards that direction are currently under way.

Acknowledgments SK greatly acknowledges to Council of Scientific and Industrial Research (CSIR), India for his research fellowship.

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