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Paper-based point-of-care testing in disease diagnostics

“Further advances in the design of paper-based platforms due to new technologies coupled to fabrication optimization will continue to embolden their integration as instruments to understand a wide array of global problems currently impacting mankind.”

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Statement

Paper-based microfluidics presents a unique opportunity for diagnosis of various diseases due to their affordability, ease of fabrication, reproducibility, sensitivity and low limits of detection.

Clinical diagnostics play an important role in healthcare in both developed and underdeveloped countries. The incidence rate of communicable and noncommunicable diseases including cancer, cardiovascular and diabetes is much higher in the developing world than in the developed world. According to the WHO, cardiovascular diseases (CVDs) caused about 31% of global deaths in 2013 with 20% occurring before the age of 60 [1]. The mortality rate in low-income countries was approximately ten-times higher than that in high-income countries [2]. After CVDs, infectious diseases are a major leading cause of death worldwide [3]. Approximately, 4.3, 1 and 5 million deaths are caused by acute respiratory infection, malaria and tuberculosis and AIDS combined per year in the developing world, respectively [4].

Along with infectious diseases, the cancer rate has also increased in developing countries and now accounts for 65% or 5.3 million deaths worldwide per year [3]. It is estimated that the annual number of new cancer cases will increase from 14 million in 2014 to 22 million by 2030. Similar to CVDs and infectious diseases, cancer incidence is highest in developing nations [5].

Roughly, 5.8 billion people worldwide live in low- and middle-resource settings and lack access to expensive medical instruments required for disease detection [6]. Hence, the development of low-cost, simple and portable point-of-care (POC) diagnostic devices is needed to improve patient access to better healthcare delivery with concomitant rapid diagnosis.

POC testing, which goes beyond conventional diagnostic tests in the clinical laboratory setting and that are near patient settings, provides physicians with timely diagnostic information, thereby, allowing them to make better informed decisions regarding diagnosis and treatment [7]. The global POC diagnostics market is expected to increase to US\$27.5 billion by 2018 and at an estimated compound annual growth rate of 9.3% from 2013 to 2018 [4].

Currently, there is great interest in using paper-based systems for POC devices due to their simplicity, affordability and ease of use [8,9]. Since the seminal work of Whitesides and colleagues in 2007 describing the first microfluidic paper-based analytical devices (μ PADs) [10], a myriad of applications in bioanalysis, healthcare and disease screening have been demonstrated. The combination of the capabilities of microfluidic systems with the flexibility of lateral flow test strip technologies has yielded valuable POC diagnostic platforms [11–13]. Paper-based microfluidics presents a unique opportunity for diagnosis

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of various diseases due to their affordability, ease of fabrication, reproducibility, sensitivity and low limits of detection. These systems have been applied in the detection of a number of analytes in blood, urine and saliva [6]. Recent work in paper systems has shifted away from the traditional 'single-strip' format, thereby, allowing for a wider range of applications [8,14]. These features make this type of test appropriate for the primary care level and in remote settings where a lack of medical infrastructure may exist.

“Although several studies have focused on using paper-based assays to detect glucose and cancer biomarkers, a few reports have employed this method for detection of cardiovascular diseases.”

Noncommunicable disease (cancer, CVD & diabetes)

Early diagnosis is well known to improve the outcomes of patients, however, conventional approaches are frequently too costly or complex to be implemented in resource-limited settings with little to no medical infrastructure. To overcome this problem, different efforts have been devoted to develop inexpensive, simple paper-based devices as useful alternatives for detection of noncommunicable diseases such as cancer, CVD and diabetes. For example, Wu *et al.* reported a paper-based electrochemical immunoassay for the simultaneous measurement of four cancer biomarkers in serum samples. The signal amplification was achieved by precipitation of a reduced graphene oxide solution onto the working electrode and through silica nanoparticles as a tracing tag. This strategy provided high-throughput, simple and inexpensive POC immunoassays of cancer biomarkers [15].

When using μ PADs, it is often difficult to eliminate all nonspecific binding. Liu *et al.* have described a novel washing strategy in the detection of carcinoembryonic antigen using a ring-oven technique integrated onto the μ PAD that washes all nonspecific binding protein from the region of detection ensuring a low background reading [16]. The paper device is similar in size to the ring-oven and yields a LLOD (0.03 ng/ml) by ELISA. The washing efficiency was greater than that found in traditional techniques. Recently, Zhou *et al.* constructed a paper dot immune-graphene-gold filtration assay using photothermal properties of graphene/gold nanocomposite for specific detection of MCF-7. Due to the simple, rapid and relatively low detection limit of 600 MCF-7 cells, this method is capable of cancer diagnosis in hospitals [17].

In addition to cancer biomarkers, glucose, the most important analyte to monitor and diagnosis of diabetes, has also been investigated using POC diagnostics.

Gonzalez *et al.* developed thread/paper-based analytical devices in both flat- and cone-shape platforms to detect glucose via a colorimetric assay. The thread/paper-based analytical devices were fabricated from nylon thread and circular zones of chromatography paper that were previously spotted with glucose. Solutions of glucose oxidase (GOx), horseradish peroxidase (HRP) and potassium iodide were transported to the analysis sites where a yellow color was realized, detected and analyzed [18].

Although several studies have focused on using paper-based assays to detect glucose and cancer biomarkers, a few reports have employed this method for detection of CVDs. Lin *et al.* developed a simple and inexpensive paper-based device for the detection of C-reactive protein (CRP) using by a latex agglutination test. The paper was initially patterned via the wax-printing method to create three fluidic channels. The wax-printed paper was then modified with different concentration of commercial latex. A blood sample was then added onto the center of each channel to initiate the agglutination reaction with CRP. Finally, CRP levels were determined by serum stain length within the channel [19].

Dong *et al.* developed a technique for rapid and inexpensive detection of CRP from blood by integrating a paper-based microfluidic immunoassay with a smartphone. The method was based on sandwich immunoassays in which CRP was captured by gold nanoparticle-conjugated anti-CRP antibody. Here, the CRP-bounded particles are captured by antibodies generating a colorimetric signal. The linear detection range of the CRP-chip is up to 2 μ g/ml and is capable of detecting 54 ng/ml within 15 min [20]. The technique has the potential to improve chronic kidney disease by examining combinatorial multimarker simultaneously and incorporating more sensitive detection technologies.

Communicable disease (infectious diseases)

The major infectious diseases including HIV, dengue, malaria, tuberculosis and hepatitis C virus are particularly attractive in the context of healthcare POC diagnostics. Choi *et al.* constructed a paper-based device by agarose-incorporated lateral flow test strip for detection of dengue virus (the fastest spreading mosquito-borne diseases). This method improved the sensitivity of a modified test strip by approximately tenfold compared with an unmodified strip due to the increased interaction between gold nanoparticle-detector probes and target [21]. Recently, a new type of μ PAD (the 3D slip-PAD) was developed to detect infectious human norovirus. The assay can be conducted in a single step based on a moveable slip design. A 3D fluidic network is designed by a layered construction of wax-patterned papers, resulting in the attainment of variable fluidic paths. The ability to release and mix minute volumes

of solution allows for an enhancement in reagents and, therefore, an improvement in sensitivity and detection limits (9.5×10^4 copies mL^{-1} for human norovirus) [22].

Paper-based devices will continue to emerge as a multiplexable platform for disease diagnostics and particularly in resource-challenged settings where there is frequently limited access to expensive and state-of-the-art instrumentation. These techniques provide a number of advantages including small sample volumes, low cost, flexible properties, ease of integration with other fluidic or analytical devices and compatibility with a plethora of biological systems. The facile fabrication of paper-based devices systematically allows their integration into an array of industries (e.g., agriculture, biomedical, wastewater treatment), thereby making them an almost ideal platform for a number of applications. Further

advances in the design of paper-based platforms due to new technologies coupled to fabrication optimization will continue to embolden their integration as instruments to understand a wide array of global problems currently impacting mankind.

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