Lab on paper†

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Introduction

Lab-on-a-chip (LOC) devices, which are suited to portable point-of-care (POC) diagnostics and on-site detection, hold great promise for improving global health, and other applications.¹⁻⁸ While their importance and utility are widely acknowledged and extensive research has been conducted in the laboratory on device manipulation and proof-ofconcept demonstration, there are few commercialized LOC products that are fabricated using clean-room based technologies.8 This is partly due to the fact that LOC devices, although much more simplified than conventional analytical instruments, are still not readily accessible to average users, particularly those in developing countries.8 On the one hand this is due to the fact that the lithographybased clean-room infrastructure for the construction of LOC devices where channels, pumps and valves are created on a plastic (or glass, silicon) substrate is complex and expensive,8 while on the other hand regulatory approval of POC devices is a time consuming process. It is important to realize that many of the existing POC tests currently in use make use of porous substrates such as fleeces, membranes and meshes,² and that in fact many such devices deserve the name 'labon-a-chip' because of their small size. These substrates have in common that their porous nature takes care of one of the primary tasks of any diagnostic tests using body fluids, fluid transport. Recent developments suggest that bioassays on paper-based substrates may be another interesting alternative for both existing porous substrate devices and solidsupport "classical" labs on a chip.9-12 Paper, normally made of cellulose fibers, is abundant, inexpensive, sustainable, disposable, easy to use, store, and transport, easy to modify chemically and familiar to the public,9-12 but perhaps its most important advantage is that an

enormous variety of inkjet printing techniques is available for its functionalization. While the use of paper for bioanalysis and diagnostics is not a new concept,6,13 it has never attracted as much attention as it does now. Two large research networks, the Sentinel: Bioactive Paper Network in Canada¹⁴ and VTT Technical Research Centre in Finland, have been focusing on the development of paper-based bioassays (or "bioactive paper" termed by both networks) in the past several years. A milestone event is the first international conference on bioactive paper that was held in Espoo, Finland in June, 2008. Meanwhile, several research groups elsewhere including Whitesides's group at Harvard are also conducting research towards the development of paper-based diagnostic tools.¹⁰⁻¹² This Focus article attempts to highlight the recent development of paper-based bioassays, and will show there is great potential for the use of such assays in a large variety of applications.

Paper strip tests

Paper-based bioanalysis dates back to the early 20th century, and a big breakthrough is the invention of paper chromatography for which Martin and Synge were awarded the Nobel Prize in chemistry in 1952. The development of diagnostic and biodetection tools using the paper strip test started almost at the same time.¹³ It was driven by the fact that paper strip tests for biologically relevant species (e.g., glucose in urine), which would be as simple to use as pH paper, has been long desired.13 Decades of development has made paper and paper-like materials (i.e., nitrocellulose membrane) the most widely used substrates for practical POC diagnostics.⁶ The most prevalent example is immunochromatographic tests (also called lateral flow or dipstick test),6,7 and a well-known example is the pregnancy test strip. Typically, these tests are based on a strip of paper (or membrane) immobilized with capture antibody specific to an antigen of interest. When the sample is applied, the antigen binds to another

antibody (conjugate antibody) which is conjugated to a signal indicator (e.g., colloidal gold). The formed antigen/ conjugate antibody complex flows along the paper matrix, which is driven by the capillary force, and is subsequently captured by the capture antibody on the paper strip. A color signal can be visualized in a few minutes, which indicates the presence of the target antigen.^{6,7} Many paper strip tests have been commercially available for POC diagnostics such as diabetes and pregnancy tests, and detection of biomarkers of pathogens and infectious diseases.^{6,15} These strip tests are advantageous because of their simplicity and low cost, but often suffer from the fact that they are not quantitative, not sufficiently sensitive to certain biomarkers, and lack the ability for multiplex analysis.6,15

Recently, there has been an emerging trend of adapting new developments in nanotechnology, biotechnology and materials science to paper-based assays, which not only addresses some of the above-mentioned issues associated with the conventional paper strip tests, but also provides new opportunities in the development of practical diagnostic devices.⁹⁻¹²

An excellent example is the introduction of gold nanoparticle (AuNP)-based colorimetric sensors^{16,17} onto paper or paper-like substrates. Like other nanoscaled materials (e.g., quantum dots, nanowires and nanotubes, etc.),18 AuNPs have very unique physical properties which are dependent on their size, shape and the interparticle distance.¹⁹ The AuNP-based colorimetric biosensing assays take advantage of the fact that AuNPs appear red or blue (or purple) in color, depending on whether they are in dispersed and aggregated states, respectively. In other words, biological analytes that can induce the aggregation process of small AuNPs or can redisperse AuNP aggregates can be detected by the color change (Fig. 1A).^{16,17} Owing to its extremely high extinction coefficient (which is >1000 fold higher than that of common dyes), AuNP-based assays

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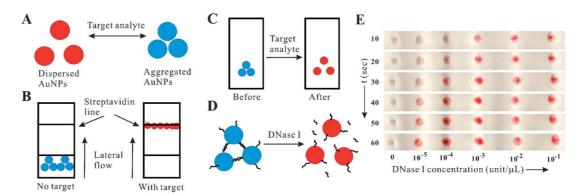


Fig. 1 (A) Schematic illustration of AuNP-based colorimetric biosensing assay. Target analyte that can induce the aggregation of AuNPs (red color) or can redisperse the AuNP aggregates (blue color) can be monitored by the color change.¹⁷ (B) AuNP-based lateral flow device. In the absence of target, AuNP aggregates are too big to migrate on a cellulose membrane upon fluid flow. When target analyte, which can break AuNP aggregate into well-dispersed AuNPs, is present, dissociated small AuNPs (modified with biotin) can migrate along the membrane and are captured by a coated line of streptavidin on the membrane. A red line therefore indicates the presence of target analyte.²⁰ (C) Paper-based spot test using AuNPs. Paper coated with AuNP aggregates generates a red color when target analyte solution is directly applied onto the dried aggregate spot.⁹ (D) One example of the system in (C). DNA crosslinked AuNP aggregates are broken into well-dispersed AuNPs when DNase I, an enzyme that cleaves DNA crosslinkers, is added. (E) Experimental results of the red color development as functions of assay time and DNase I concentration, respectively (adapted from ref. 9 with permission).

normally have relatively high sensitivity.19 While the use of this sensing platform for solution-phase detection has been wellestablished in the last decade,17 the development of practical strip tests is very recent.9,20 Lu and coworkers first developed a lateral flow device where AuNP aggregates, upon addition of a specific target analyte, dissociate into welldispersed AuNPs.20 These dispersed AuNPs which are modified by biotin flow along a cellulose-based membrane and are captured by a coated line of streptavidin on the membrane (Fig. 1B).²⁰ A red color that appears on the streptavidin line indicates the presence of the target analyte. Inspired by this work, Zhao et al. have demonstrated the feasibility of using a AuNP-based sensing platform on paper strips. In a proof-of-concept demonstration,9 AuNP aggregates, which are assembled by interparticle DNA hybridization, are first spotted onto paper strips and allowed to dry. When target endonuclease DNase I, an enzyme that cleaves double-stranded DNA crosslinkers, is applied onto the AuNP sensor spot, AuNP aggregates are broken into redcolored dispersed AuNPs. Significantly, it was found that the paper strips coated with the AuNP aggregate sensor can be dried (even at elevated temperatures (i.e. 90 °C) and stored long-term (at least several months at room temperature) without significant loss of biosensing functions.9 Given that the AuNP-based

biosensing platform is highly generic,¹⁷ one could readily apply this strip test platform for the detection of many other targets.

The above-mentioned system serves as one example of how new developments in nanotechnology can be applied for the construction of paper-based diagnostic kits. Given the significant advances in nanotechnology-based diagnostics in the last decade,²¹⁻²³ one could imagine that many of the nanomaterial-based biosensing platforms could be transformed onto paper strips towards practical applications.

Paper-based microfluidics

Conventional paper strip tests have gained great success in POC diagnostics due to their simplicity, but they are normally not capable of doing multiplex and quantitative analysis.6,15 These issues can be potentially addressed by the recently paper-based microfluidic developed devices.¹⁰⁻¹² Whitesides and coworkers recently introduced the concept of using patterned paper substrate as a microfluidic platform for multiplex analyte detection.10-12 In their studies, hydrophobic polymers (e.g., poly(dimethylsiloxane)) are photolithographically patterned¹⁰ or printed using a desktop plotter¹¹ onto hydrophilic paper so that the millimeter-sized fluidic channels can be well-defined in the paper matrix.

Significantly, owing to the natural capillary action, biological fluids can flow along these channels without the external pumps that are required in the conventional microfluidic devices. The use of these patterned paper platforms not only minimizes the required sample volume,

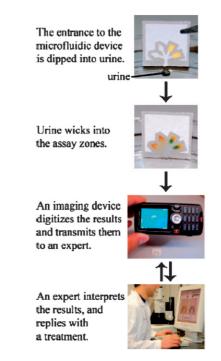


Fig. 2 Paper-based microfluidic device for multiplex and quantitative analysis. See main text or ref. 12 for details. This figure is adapted from ref. 12 with permission.

but more importantly, makes multiplex analysis possible. In a prototype demonstration, Whitesides and coworkers were able to detect glucose and protein simultaneously on a single patterned paper.^{10,11} In a more recent work,12 Whitesides and colleagues introduced another central component to the system, an imaging device (camera phone or portable scanner) that makes quantitative analysis possible. The imaging device is used for digitizing the intensity of color associated with each colorimetric assay. The digital information is transferred from the assay site to an off-site laboratory for analysis by trained personnel. The diagnostic results will then be returned to the on-site healthcare provider (Fig. 2).12

The paper-based microfluidics, which combine the simplicity of paper strip tests and the complexity of the conventional LOC devices, hold a lot of potential for POC and on-site diagnostics. What is convenient about using paper as a microfluidic substrate is the fact that high speed coating and printing techniques have long been available for paper substrates.24 For example, inkjet printing²⁵ can be applied to deposit and pattern a variety of materials (e.g., polymers, inorganic particles, and biological species, etc.) on paper.^{26,27} Pelton and coworkers have successfully coated biomolecule-attached microgel inks on paper by inkjet printing.27 They found that microgel, which can serve as a universal linker between biomolecules and paper substrates, help the biomolecule to maintain their biological functions after printing.27 Mass production, such as roll-to-roll manufacturing, is also being developed for biologically functional paper products.24

Advantages and hurdles

Paper-based diagnostic devices have many advantages: (1) they are ease-to-use (particularly suited to nontechnical personnel), inexpensive, low volume, easily adaptable, and are capable of rapid on-site detection. (2) Paper is made of naturally abundant materials (*i.e.*, cellulose), and is biodegradable. Moreover, both simple paper strip tests and more complex paper-based microfluidic devices (where paper itself carries separation and "pumping" functions) require no or little external power sources. Taken together, paper-based diagnostic devices are advantageous from the energy-saving and sustainable development standpoints. (3) Paper products can be easily manufactured on a large scale by the well-established coating and printing techniques,²⁴ which can further lower the cost of the final products. (4) From business and marketing perspectives, paper-based diagnostic kits require a relatively small investment and can get into the market easily, which can facilitate its wide use in practical applications. Above all, the advantages that really make paper substrate distinguishable from the traditional solid-support LOC devices and the existing porous substrate devices are perhaps the fact that the fibrous nature of paper provides intrinsic (capillary) pumping,28,29 and that today's advanced printing techniques^{24,25} enable realization of sophisticated microdevices incorporating more functionality (such as bio-coatings, chemical modification and integrated metal electrodes) without the need for expensive infrastructure (e.g. cleanroom).

Nevertheless, some issues remain. Although the limitations of the traditional paper strip tests can be addressed to a certain extent by the above-mentioned new developments, future paper-based kits may still not be as compelling as the traditional analytical instruments and the conventional LOC devices in terms of sensitivity, accuracy and quantitative and multiplex analysis capabilities. Moreover, the assay stability (or shelf life) might become an issue when these kits are designed to be transported and stored for a long time at room temperature.³⁰ Storage-stable biomolecules will have to be used under certain circumstances. Furthermore, the science behind the fluid flow in a paper matrix, biomolecule immobilization and biorecognition on paper is complex and not yet fully understood.^{27,30} Comprehensive research and optimization have to be conducted to address these issues before paper-based kits can reach their full potential. Last but not least, using paper as a substrate may also limit the possibility of exploiting new nano-scale effects and structures, such as new nanofluidic phenomena for sample enrichment and separation,31-34 and nanowires for ultrasensitive detection.35 It will be a great challenge to develop inkjetbased technologies that are capable of substituting or incorporating the topdown and bottom-up nanotechnologies currently used³⁵ to realise such nanoscale structures on paper substrate.

Applications and perspectives

Paper-based kits will find a large number of applications. They have been, and will continue to be, a key player in POC diagnostics for global health care, particularly considering the wide and urgent need in developing countries, where advanced, expensive cleanroom infrastructures, complex instrumentation and trained personnel are not widely available.^{4,7} They will also find employment in many other fields such as environmental monitoring, water and food safety, filtration systems, biosafety and biodefense, military and homeland security.^{10–12,14}

Given that a wide variety of materials (*e.g.*, polymers, metals, insulators, nanoparticles, biological species, *etc.*) can be readily printed or patterned on paper by the advanced printing techniques,^{24,25} the future paper-based devices will possess more functions (*e.g.*, separation, purification, (bio)chemical reactions, detection, signal communication, *etc.*) than the conventional paper strip tests. This may eventually make paper-based devices, which are served as useful alternatives to other LOC devices, suitable for more complex conditions such as high-throughput, multiplex bioanalysis.

Finally, both scientific and industrial communities will benefit from the study and development of paper-based kits. The study of biomolecule and paper interfaces and the physics of fluid flow in a paper matrix become very interesting fundamental sciences.³⁰ Meanwhile, by introducing value-added paper products, the development of paper-based kits also offers an opportunity for the paper industry to compete in the markets.³⁶

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