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feature

Diagnostics for the Developing World: Microfluidic Paper-Based Analytical Devices

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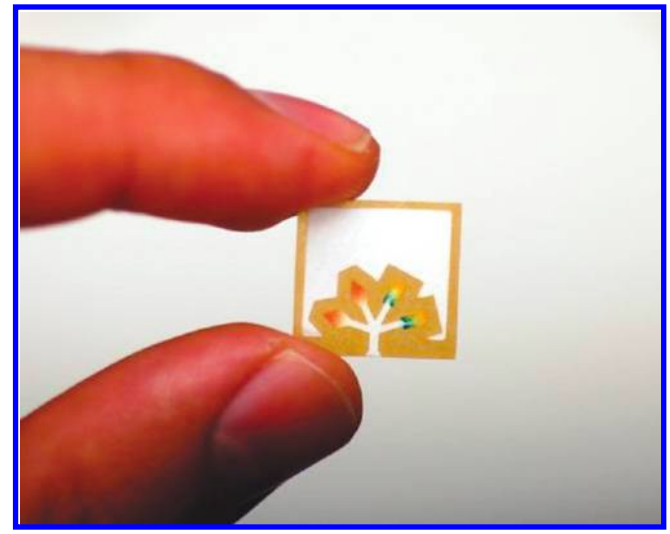
Microfluidic paper-based analytical devices (μPADs) are a new class of point-of-care diagnostic devices that are inexpensive, easy to use, and designed specifically for use in developing countries. (To listen to a podcast about this feature, please go to the *Analytical Chemistry* multimedia page at pubs.acs.org/page/ancham/audio/index.html.)

The first step in proper prevention and treatment of disease is accurate diagnosis, but diagnostic technologies that are successful in the economically developed world often are difficult to use in developing countries. People who live in these countries frequently cannot afford even modestly expensive tests, and basic infrastructure—i.e., reliable power, refrigeration, and trained personnel—is often not available.[1−5] Currently, diagnosis usually requires a trained healthcare worker to recognize symptoms or interpret analyses. By developing effective technologies in health-related diagnostics for developing countries and coupling these technologies to existing communication infrastructures, healthcare in areas without access to trained medical personnel may be possible.

According to the World Health Organization, diagnostic devices for developing countries should be ASSURED: affordable, sensitive, specific, user-friendly, rapid and robust, equipment free and deliverable to end-users.[6] Diagnostic devices made of patterned paper—which we call microfluidic paper-based analytical devices (μPADs)—are a new platform designed for ASSURED diagnostic assays. These systems combine some of the capabilities of conventional microfluidic devices with the simplicity of diagnostic strip tests.[7−11] When fully developed, μPADs may provide bioanalyses that are more rapid, less expensive, and more highly multiplexed than current analyses. They require only small volumes of fluid and little or no external supporting equipment or power because fluid movement in μPADs is controlled largely by capillarity and evaporation.



This Feature summarizes the first steps in the development of a complete system based on μPADs for diagnosing disease in resource-limited settings. We describe methods for fabricating μPADs, colorimetric assays that have been demonstrated on μPADs and reflectance detection for quantifying the results of these assays, and the integration of μPADs with camera-equipped cellular phones as a method for providing low-cost healthcare in the field. We conclude the article with a summary of two new types of μPADs that bring new capabilities to paper-based bioanalyses: multizone plates and 3D μPADs.

PAPER

We and others chose paper as the starting material for fabricating low-cost microanalytical devices for three reasons: (i) it is available everywhere and is inexpensive (~$6/m^2 even for high-quality chromatography paper), so a simple μPAD typically can be

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fabricated for <$0.01 (for the cost of the paper and patterning); (ii) paper wicks aqueous fluids, and this wicking makes passive transport of fluids without active pumping on μPADs practical; and (iii) paper has been used for decades as a platform for analytical chemistry, and thus μPADs can take advantage of existing analytical techniques.

Paper has several additional advantages as a material for making ASSURED diagnostic devices: (i) paper is thin, lightweight (~10 mg/cm^2), available in a wide range of thicknesses (0.07−1 mm),[12] and easy to stack, store, and transport; (ii) paper typically is made of cellulose or cellulose-polymer blends and is compatible with biological samples;[13] (iii) paper can be modified chemically to incorporate a wide variety of functional groups that can be covalently bound to proteins, DNA, or small molecules;[11,14] (iv) paper is usually white (because it scatters light) and is a good medium for colorimetric tests because it provides strong contrast with a colored substrate; (v) paper is flammable, so μPADs can be disposed of by incineration easily and safely after use; (vi) paper is flexible and compatible with a host of existing printing technologies that could, in principle, be used to fabricate μPADs;[11,15] and (vii) "paper" (defined as a thin fibrous sheet) is available in a wide range of highly engineered forms with a very wide range of properties.[12] For example, filter papers with well-defined pore sizes can separate suspended solids from samples before an assay or remove erythrocytes from blood. Papers containing conducting carbon or metal fibers are electrically conducting or magnetically responsive, and paper containing biodegradable polymers may make μPADs biocompatible.

Paper is already used extensively in analytical and clinical chemistry.[16] Paper chromatography, developed in the 19th century, has been used to separate and identify mixtures of small molecules, amino acids, proteins, and antibodies.[14,17] Urinalysis dipsticks and litmus paper are the most commonly used paper-based diagnostic devices, but paper is also used as a support for qualitative spot tests for analytes with applications covering clinical diagnostics, organic and inorganic chemical analysis, environmental and geochemical analysis, and pharmaceutical and food chemistry.[16−19] Lateral-flow immunoassays are a highly engineered and broadly used form of paper-based diagnostic assays; they can provide "yes/no" detection of a wide range of analytes using labeled antibodies or analytes.[20−23] Paper also is used in other areas: e.g., in chemistry for synthesis of peptides and small molecules and as a platform for macroarrays.[24−27]

We used Whatman No. 1 chromatography paper for most of our work because it is a clean paper, is made out of pure cellulose, and has relatively uniform thickness and wicking properties. We have also worked with two other types of paper useful in μPADs: paper towels because of their low cost ($0.15/m^2) and ITW Technicloth ($1.20/m^2) because of its high wicking speed.[10] As new applications for μPADs are developed, we expect that other types of paper will be useful in fabricating devices with specific capabilities.

PAPER-BASED MICROFLUIDIC DEVICES

Traditional microfluidic devices are fabricated by etching or molding channels into glass, silicone, PDMS, or other polymers or plastics.[28] Fabrication of μPADs is based on patterning sheets of paper into hydrophilic channels (paper) bounded by hydrophobic barriers (Figure 1); the resulting channels either can be left open to the atmosphere or sealed to thin polymer sheets.[8−10,29] A number of methods are now available to accomplish this patterning (Table 1 and Figure 2) with different trade-offs among cost, convenience, and resolution. The patterning process defines the width and length of paper-based microfluidic channels; the thickness of the paper defines the height of the channel. The

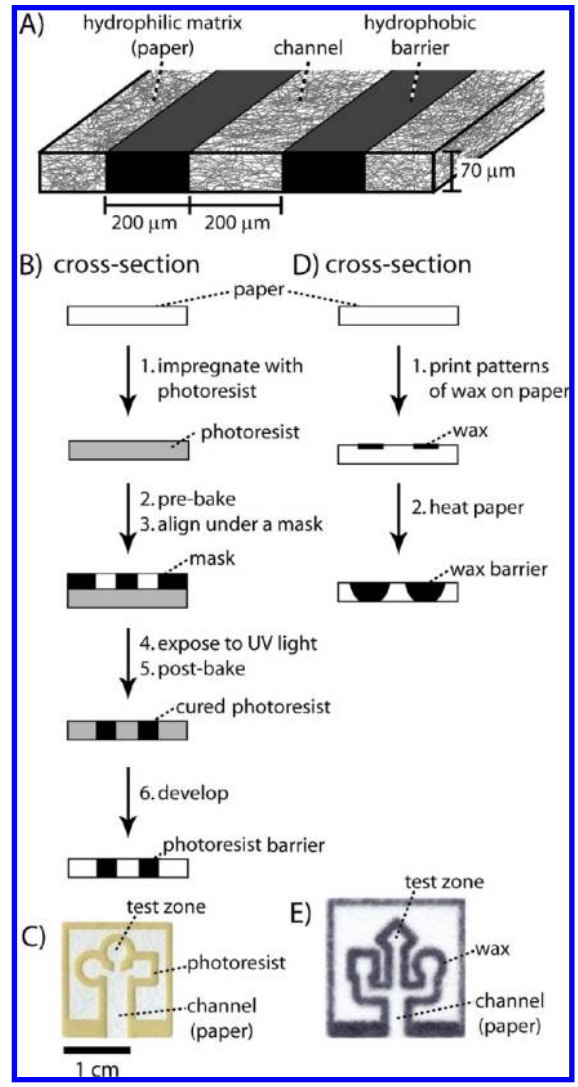

Figure 1. A) Schematic of a paper-based microfluidic channel. The channel comprises a porous matrix of hydrophilic cellulose fibers that wick fluids along the path defined by the channel. The sides of the channel are bounded by hydrophobic barriers, and the top and bottom of the channel are open to atmosphere. The height of the channel is defined by the thickness of the paper. The resolution of the hydrophobic barriers is defined by the method of patterning. B) Photolithography on paper requires six steps and produces well-defined hydrophobic barriers of photoresist that extend through the thickness of the paper. C) An example of a device fabricated by photolithography with a central channel that wicks fluids into three independent test zones. D) Wax printing requires two steps and produces hydrophobic barriers of wax that extend through the thickness of the paper. When the paper is heated, the wax melts and spreads both vertically and laterally into the paper. The vertical spreading creates the hydrophobic barrier. The lateral spreading lowers the resolution of the method and produces barriers that are much larger than the original printed pattern. E) An example of a device fabricated by wax printing with a central channel that wicks fluids into three independent test zones.

Table 1. Comparison of the Published Methods for Patterning Paper. The Channel and Barrier Widths are the Minimum Dimensions Reported

Method (References)	Channel (μm)	Barrier (μm)	Advantages	Disadvantages
Photolithography (10)	186 ± 13	248 ± 13	Can pattern a wide variety of papers up to 360 μm in width.	Hydrophilic areas exposed to polymers and solvents.
Plotting (30)	~1000[a]	~1000[a]	Hydrophilic channels not exposed to polymers or solvents; hydrophobic barriers are flexible.	Requires a customized plotter.
Inkjet etching (31)	420 ± 50	—[a]	Reagents can be inkjet printed into the test zones using the printer.	Requires a customized inkjet printer; hydrophilic areas exposed to polymers and solvents.
Plasma etching (32)	~1500[a]	—[a]	Useful for laboratories equipped with a plasma cleaner that wish to make many replicates of a few simple patterns.	Hydrophilic areas exposed to polymers and solvents; metal masks must be made for each pattern; cannot produce arrays of free-standing hydrophobic patterns.
Cutting (29)	1000[b]	700[b]	Hydrophilic channels not exposed to polymers or solvents.	Devices must be encased in tape; cannot produce arrays of free-standing hydrophilic patterns.
Wax printing (33,34)	561 ± 45	850 ± 50	Rapid (~5 minutes); requires only a commercially available printer and hot plate; hydrophilic channels not exposed to polymers or solvents.	The design of the patterns must account for the spreading of the wax in the paper.

[a] The minimum dimensions were not reported. [b] The method was demonstrated using polyester-backed nitrocellulose membranes.

hydrophilic cellulose fibers of paper enable aqueous fluids to wick along the channels. The rate of the wicking depends on the dimensions of the channel, the characteristics of the paper, and the characteristics (temperature and relative humidity) of the environment. The cellulose matrix also provides a solid support for reagents, for filtering samples, or for performing chromatographic separations.

METHODS OF PATTERNING PAPER

Patterning a piece of paper into hydrophilic channels demarcated by hydrophobic barriers requires that these barriers extend through the entire thickness of the paper. Most simple printing technologies are not suitable for patterning the full thickness of the paper because they are designed to deposit inks on the *surface* of paper. Our first successful approach to patterning paper used photolithography.[8,10] We impregnated an entire sheet of paper with photoresist (SU-8, SC) and selectively polymerized the photoresist by exposing it to UV light through a transparency mask (black ink printed on the transparency film). We then removed the unexposed photoresist from the paper by washing (Figure 1B and C). This method produced surprisingly sharp lines (edge roughness was ~15 μm; minimal width of line to produce a barrier was ~200 μm) because the paper and photoresist have similar indexes of refraction, and a paper sheet containing photoresist is translucent and scatters light much less than does paper. The method has the disadvantage that it requires organic solvents to remove unused photoresist and leaves a layer of hydrophobic organic scum on the paper (which can be made hydrophilic or removed entirely by exposure to an oxygen plasma).

We have improved this photolithographic process by lowering the cost of the photoresist and reducing the amount of time required for the fabrication process.[10] The photoresists we now use are homemade versions of commercial photoresists used for microelectronics, but the quality and cost are both lower. Paper impregnated with photoresist can be dried and stored in the dark for months; thus, large batches (20 sheets) of photoresist-impregnated paper can be prepared and stored for later use. Photolithography on paper can generate hydrophobic barriers as

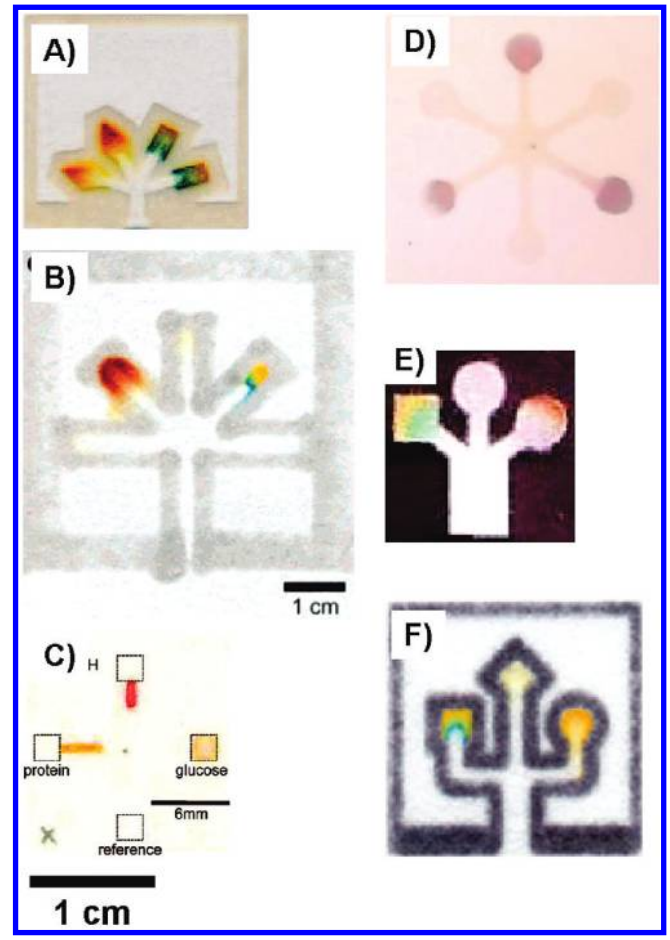

Figure 2. Devices fabricated by A) photolithography detecting glucose and protein. (adapted from ref. 7); B) plotting detecting glucose and protein (adapted from ref. 30); C) inkjet etching measuring protein, pH, and glucose (adapted from ref. 31); D) plasma etching detecting alkaline phosphatase (adapted from ref. 32); E) cutting detecting protein and glucose (adapted from ref. 29); F) wax printing detecting protein, cholesterol, and glucose (adapted from ref. 34). All the devices are shown on the same size scale except for image B, which is scaled down by 50% compared to the other images.

small as ~200 μm in width and hydrophilic channels as small as ~200 μm in width that wick fluids.[10] Photolithography using SU-8 as photoresist can be used to pattern a wide variety of papers with thicknesses up to 360 μm. The transparency masks for photolithography can be printed with an inkjet printer, a photocopying machine, or drawn by hand, and the exposure can be performed with sunlight if a UV lamp is not available.[10]

We and others have described several other methods for patterning paper since the development of photolithography (Table 1 and Figure 2). Bruzewicz *et al.* used a modified desktop plotter to define patterns of PDMS in paper.[30] Abe *et al.* used inkjet etching to remove a hydrophobic coating of polystyrene deposited on a sheet of paper.[31] This work also demonstrated the use of inkjet printers to deliver reagents in the test zones of μPADs. An alternative method for removing a hydrophobic coating on paper using plasma oxidation through a metal mask was described by Li *et al.*[32] Fenton *et al.* described the use of a knife plotter to cut paper into patterns of microfluidic channels.[29] The cut patterns were encased in tape to provide structural support to the paper and facilitate manipulation of the devices.

The procedure that presently seems best for large-volume, moderate-resolution μPADs was first described by Lu *et al.*[33] and uses a solid wax printer (an inkjet printer in which the ink is supplied as a solid low-melting wax, which is melted before being ejected from the print head, but solidifies immediately on paper). Lu *et al.* and Carrilho *et al.* used this relatively inexpensive printer (Xerox Phaser 8560, ~$800) to first print patterns of solid wax on paper. Next, the paper was heated to re-melt the wax, which penetrated the paper to generate complete hydrophobic barriers (Figure 1D and E).[33,34] Wax printing has lower resolution than photolithography: the smallest hydrophobic barriers we have consistently generated are ~850 μm in width with an edge roughness of ~60 μm, and the smallest hydrophilic channels are ~560 μm in width. Solid wax printing has five main advantages: (i) it takes <5 minutes to pattern a letter sized sheet of paper; (ii) the hydrophilic channels and test zones are never exposed to photoresists or other polymers that could contaminate them, and the μPADs do not require external processing steps to develop the patterns; (iii) no exposure to solvents or drying is involved; (iv) it easily accommodates printing different colors (a capability useful in many circumstances); and (v) sheets of paper patterned by wax printing can be fed directly into an inkjet printer for delivery of reagents into the test zones of the devices.[28]

PREPARATION OF μPADs FOR BIOANALYSES

After patterning paper, the final step in the fabrication of μPADs is the addition of reagents for assays to the paper-based microfluidic device. Most of the work to date has emphasized colorimetric assays, which are well suited for paper-based platforms and are easily read by medical personnel or patients by eye (other types of readout are possible as well). Thus far, all the assays demonstrated on μPADs are based on enzymatic reactions or small molecule dyes (e.g., pH indicators). Solutions of the reagents for the assays were spotted by hand or inkjet printed in the test zones and dried.[8,31] The reagent volume requirements of the devices depended on the size of the test zones. For most devices 0.1−1 μL of reagent solutions were sufficient to saturate the test zones.[7] Once the reagents were dry, the μPADs were ready for use. Thus far, colorimetric assays for glucose, proteins, pH, and alkaline phosphatase have been demonstrated on μPADs (Figure 2).[7,31,32]

μPADs FOR URINALYSIS

The first μPAD-based bioanalytical device that we created was for urinalysis.[7,8] We carried out assays for glucose and protein in artificial urine on a simple μPAD with a central channel for sample introduction, which branched into three test zones into which the reagents for each assay had been spotted and dried during fabrication of the device (Figure 3). The user dips the bottom of the entrance/distribution channel into a drop of the sample, capillarity wicks the sample into the test zones, and the results of the assays develop as colors over 30 minutes. The assays were based on well-known chemical and enzymatic reactions that have been used in urinalysis dipsticks.[8]

The results from urinalysis assays on μPADs demonstrated several characteristics that make them attractive platforms for ASSURED diagnostics: (i) μPADs are compatible with colorimetric assays that use either enzymes or small-molecule dyes; (ii) multiple independent assays can be carried out simultaneously in different test zones on a device without cross-contamination of the reagents (easy multiplexing is one of the potential advantages of μPADs over simpler paper-based assays); (iii) μPADs require small volumes of sample (~5 μL), and a particular design will wick reproducible volumes of sample (reproducible delivery of the same volume of sample to the test zone is required for quantitative results, and an attractive feature of μPADs is that the user does not have to measure out given volumes of sample); and (iv) the intensity of the color that develops in each test zone is a function of the concentration of the analyte, which enables quantitative measurement of analytes based on reflectance detection of the intensity of the color in the test zone.

QUANTITATIVE DETECTION OF ANALYTES USING μPADs

Quantitative colorimetric detection of analytes using μPADs is possible by reflectance detection when the intensity of the color that develops in the test zones is a function of the concentration of the analyte. Reflectance detection is based on the measurement of the light reflected off of the surface of the test zone.[35,36] The reflected light can be captured by a desktop scanner or a digital camera. By measuring the intensity of the color in a digital image of the test zone, it is possible to calculate the concentration of the analyte by comparing its intensity to a calibration curve (Figure 3E and F).[7] Calibration curves for reflectance detection typically follow nonlinear functions of the form $y = a\ ln(bx)$.[35,37] The nonlinearity at high concentrations of analytes is the result of color saturation.[35]

Reflectance detection is well suited for use with μPADs because it is easy to perform and requires simple equipment (i.e., a desktop scanner). The challenges with reflectance detection are that it is compatible only with colorimetric assays, and, for digital cameras, the results are dependent on the quality of the image and the lighting conditions. Desktop scanners provide more reproducible results because the focus of the image and the lighting conditions are constant.

Even though we focused our initial work on reflectance detection of colorimetric assays, μPADs are compatible with other

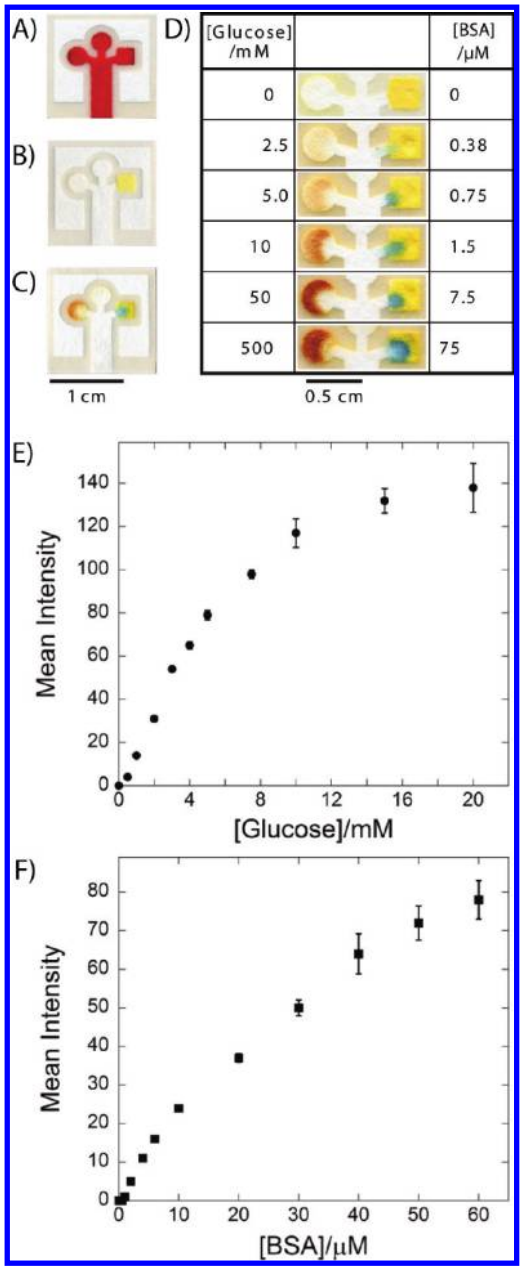

Figure 3. μPADs for analysis of glucose and protein in urine. A) Patterned paper after distributing 5 μL of Waterman red ink to show the integrity of the hydrophilic channel. The darker lines are cured photoresist; the lighter areas are unexposed paper. B) Complete μPAD after spotting the reagents. The square zone on the right is for a protein test, and the circular zone on the left is for glucose analysis. The circular zone on the top was used as a control. C) Positive assays for glucose and protein using 5 μL of a solution that contained glucose (20 mM) and BSA (75 μM) in an artificial urine solution. D) Results of paper-based glucose and protein assays using a range of concentrations of glucose and BSA in artificial urine. E) and F) Analytical calibration plots for the concentration of glucose (E) and BSA (F) in artificial urine measured using μPADs. The mean intensity for each data point was obtained using the histogram function in Adobe Photoshop by selecting the area of the test zone for each assay in a digital image of the μPAD that was obtained with a desktop scanner. Each datum is the mean of twelve assays; error bars represent the relative standard deviations of the measurements. (B), (C), and (D) adapted with permission from ref. 8.

methods of detection. We have shown that quantitative fluorescence and absorbance measurements can be obtained from paper-based devices using a microplate reader.[35] Most recently, Dunchai *et al.* demonstrated the detection of glucose, lactate, and uric acid by electrochemistry on μPADs.[38] Other methods of detection that should be compatible with μPADs are radiolabeling and electrochemiluminescence.

TELEMEDICINE

However simple and user-friendly, μPADs still require a trained healthcare provider to interpret the data they provide and to prescribe any necessary treatments. Trained personnel are a limited and valuable resource in developing countries because transporting them to remote settings is impractical and expensive.[1] Telemedicine is an attractive alternative system for providing healthcare in developing countries because it allows a relatively untrained person to provide useful healthcare in remote settings. Telemedicine has the potential to use the time of highly trained healthcare workers fully and efficiently and gives experts in "First World" countries the opportunity to provide their services to clinics in remote settings.

To develop a complete system that provides diagnostically useful information in remote settings, we have integrated camera-equipped cellular phones with μPADs[7] to provide a form of telemedicine.[39−41] In our proposed system, a paramedical technician with limited medical training would test a patient using a μPAD, photograph the results with a camera phone, and transmit the image to a central laboratory. An expert would then analyze the image and respond with a phone call or text message to prescribe an appropriate treatment. Portable scanners or inexpensive devices such as credit card scanners may also be useful tools for telemedicine and might be integrated with a PDA (personal digital assistant) for wireless communication.

One challenge of telemedicine is to transmit images that can be accurately interpreted at a remote site. The intensity of the colors in an image will vary based on the lighting conditions, the resolution of the camera, and the focus of the picture. Many of these user-dependent conditions may be accounted for by printing a calibration chart directly on the μPAD. A solid-wax printer is particularly attractive for this purpose because it can integrate color standards with fabrication of the microfluidic channels.

PAPER MICROZONE PLATES

A slightly different approach to paper-based diagnostic devices uses paper microzone plates as alternatives to plastic microtiter plates (Figure 4).[35] A paper microzone plate is made by patterning a sheet of paper into an array of circular or square test zones with the same dimensions and distribution as the wells in a plastic plate. We have demonstrated both 96-zone plates and 384-zone plates made out of paper.

Paper microzone plates have many advantages over plastic plates. Paper microzone plates are inexpensive (~$0.05/plate);[35] easy to stack, store, transport, and archive; and compatible with the technology developed for plastic plates, including 8-channel pipettes and plate readers. Paper microzone plates are fabricated easily in such a way that they incorporate complex microfluidic channels, interconnecting zones, or zones of unusual geometry. These systems improve the distribution and manipulation of samples on the plate—a capability that is impractical to accomplish

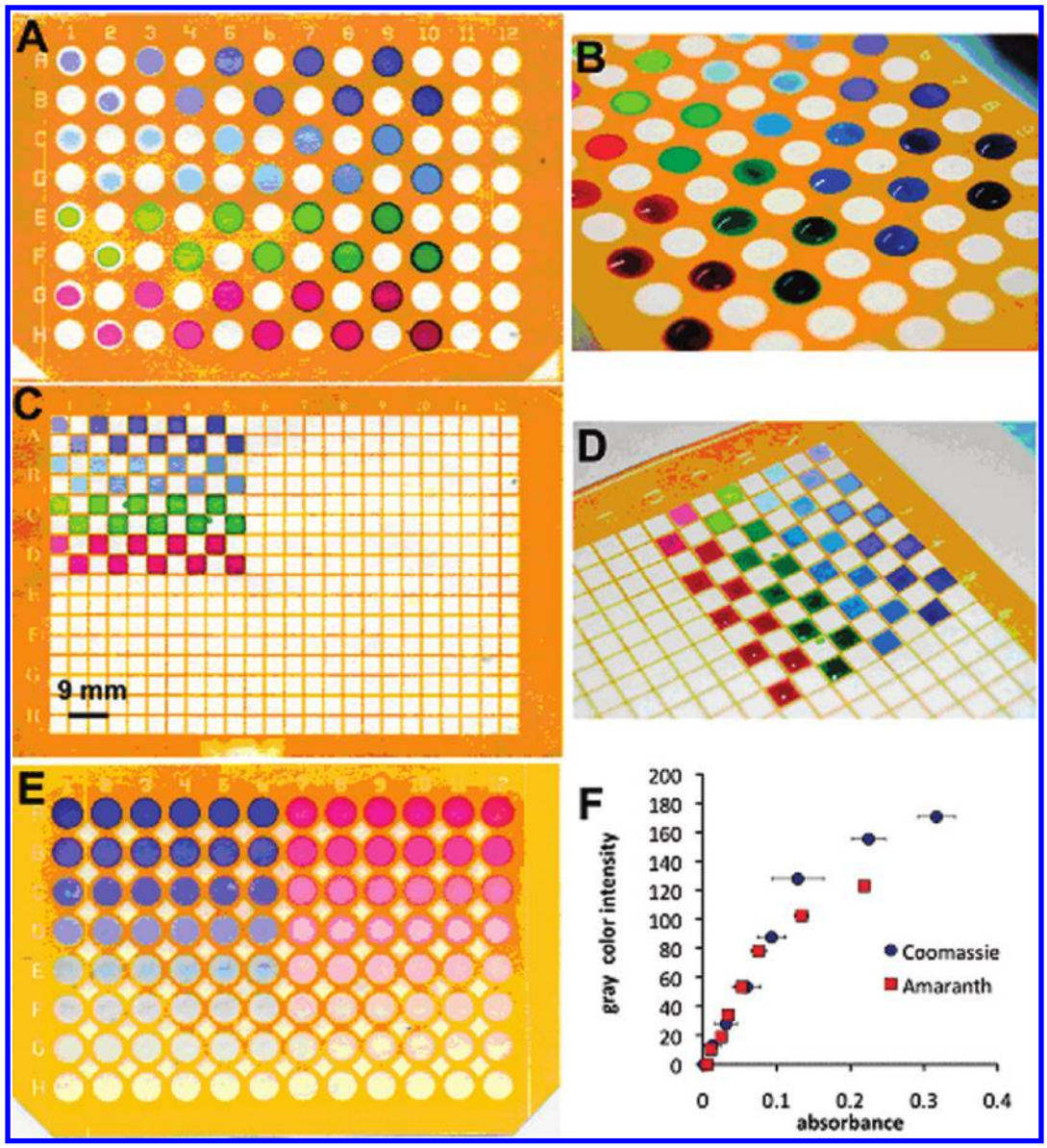

Figure 4. A) Image of a 96-zone plate after application of a range of volumes (1−55 μL) of solutions of aqueous dyes (Coomassie Brilliant Blue R250, Coomassie Brilliant Blue G250, mixture of Erioglaucine and Tartrazine, and Amaranth) in alternating zones. This image demonstrates the fluidic isolation of the zones. B) Image showing the 96-zone plate with volumes of liquid up to 55 μL that were completely contained by the hydrophobic barrier. C) Image of a 384-zone plate after application of 1−10 μL of the same solutions as in (A). D) Image showing the 384-zone plate with volumes of fluid up to 10 μL that were contained by the hydrophobic barrier. E) Image of a 96-zone plate with a serial dilution (by 2×) of 10 nmol of Coomassie Brilliant Blue R250 and 14 nmol of Amaranth. F) Correlation of the absorbance values from a microplate reader and the gray scale values from an image acquired using a desktop scanner for the paper plate shown in (E). The plate reader measured the absorption values from each sample at the maximum absorption wavelength for each dye (580 nm for Coomassie, 530 nm for Amaranth, and 750 nm as reference). The program ImageJ provided the average gray scale value over the area of each microzone after subtraction of the blank value. (A), (B), (C), (D), and (E) adapted from ref. 35.

with plastic-well systems. Paper microzone plates also bring a new layer of flexibility in design, and by reducing the quantity of material used in the test device, they diminish the environmental impact of manufacturing and disposal.

Like the wells in conventional plastic plates, each zone in the paper plate can be used to run an independent assay, and the design of the plate facilitates parallel processing of large numbers of samples. Because the test zones in paper plates are open to the atmosphere and have a high surface to volume ratio, solutions added to paper plates concentrate rapidly by evaporation; this increases sensitivity in detection by ~40×. As is true for other μPAD systems, colorimetric assays on paper microzone plates can be read and quantified using an inexpensive scanner when a plate reader is not available.

We anticipate that paper microzone plates will be useful both for laboratories in developing countries and for high-volume applications in the developed world. They also may enable new types of manipulations that are useful for collection and transport of samples from the field to a laboratory.

THREE-DIMENSIONAL μPADs

μPADs made from a single layer of paper generate 2D systems of channels and test zones. As was demonstrated in conventional microfluidics, the development of 3D microfluidic devices

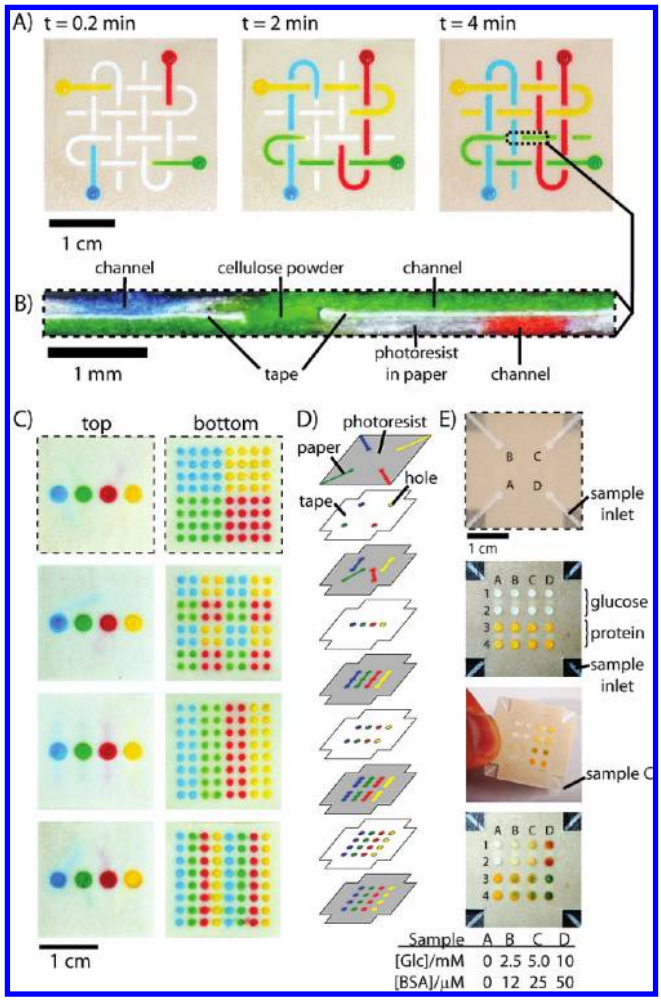

Figure 5. A) A 3D μPAD with four channels that cross each other multiple times in different planes without mixing their contents. B) Cross-section of the device shown in (A). The device is made from two layers of patterned paper and one layer of double-sided adhesive tape. Channels patterned in the paper wick fluids in the plane of the paper, and holes cut through the tape provide contact points between adjacent layers of paper. The holes in the tape are filled with cellulose powder to allow fluids to wick between adjacent layers of paper. C) Three-dimensional μPADs that distributed four samples added to the fluid inlets on the top of the device into arrays of 64 test zones on the bottom of the device. The channels in the middle layers of the device determine which sample will fill each test zone. D) Schematic representation of the layers of paper and tape used to assemble a device designed for testing four samples for the presence of glucose and protein. E) Three-dimensional μPAD that can test four different samples for glucose and protein. The front face of the device has four fluid inlets at each corner of the device that can be dipped directly into the sample. The back face of the device has an array of 16 test zones that were prespotted with the reagents for the assays. The results of the assays are displayed side by side for easy comparison. The concentrations of glucose (Glc) and BSA in each sample are indicated below the device. All adapted with permission from ref. 9.

can enable many new and useful applications.[42,43] We believe that 3D μPADs will also enable applications for paper-based devices that would not be possible with 2D μPADs. Three-dimensional systems of channels can be fabricated in paper by stacking alternating layers of patterned paper and double-sided adhesive tape patterned with holes (Figure 5A).[9] The patterns of holes in the tape provide connections between channels in adjacent layers of paper (Figure 5B).

Three-dimensional μPADs offer several potential advantages over 2D devices. They can incorporate intricate networks of channels connected to large arrays of test zones (Figure 5C), and each layer in a 3D μPAD can be made of a different paper, so multiple functionalities provided by different types of paper can be combined into a single device. For example, the top layer of the device can be a filter to remove red blood cells from a blood sample. As the sample wicks through the middle layers of the device, it can react with light-sensitive assay reagents contained within before wicking through to the bottom, where test zones would display the results. The complexity of the systems of channels that can be generated in a 3D μPAD is—in our experience—larger than can be practically produced in PDMS.

We have used glucose and protein assays to demonstrate colorimetric analyses using 3D μPADs (Figure 5D and E).[9] The 3D μPAD shown in Figure 5 was able to assay in duplicate up to four different samples for glucose and protein. The test zones were arranged in a side-by-side configuration for easy comparison of the results. The device was designed to incorporate a fluid inlet at the corner of the device, so that the device could be dipped into a drop of each sample.

CONCLUSIONS

Their low cost, simplicity, and flexibility make μPADs a promising starting point for the development of new solutions to the problem of health-relevant assays in developing economies. So far, we and others have developed prototype μPAD systems for clinically relevant bioanalyses that use colorimetric assays and telemedicine. When fully developed, we hope μPADs will provide a platform with several new capabilities for bioanalysis, such as (i) the ability to multiplex assays; (ii) the ability to store, mix, and combine reagents; (iii) the ability to filter samples and separate mixtures into individual components for analysis; (iv) the ability to automatically analyze controlled volumes of sample starting from unknown volumes of sample; and (v) the ability to analyze multiple samples using a single device.

This work and this field are in their infancy, and there are many opportunities to research ways to make μPADs practical and genuinely useful and to expand their capabilities. These include (i) a detailed study of capillary wicking in paper-based microfluidic devices that could help improve the design of the devices and may suggest new capabilities for these devices; (ii) the development of new methods for fabricating μPADs and the development of new components such as electrodes, valves, filters, mixers, and coatings for μPADs that will expand their capabilities; (iii) the development of new diagnostic assays for disease that are compatible with the μPAD platform; (iv) the development of methods for stabilizing reagents stored in μPADs so that they can be distributed without relying on refrigeration; (v) the development of new technologies for measuring the results of paper-based assays that could enable the use of new types of assays with μPADs; and (vi) testing the devices in the field. All of these projects will likely require the involvement of chemists, biologists, engineers, and medical doctors.

A great advantage of μPADs is that they can be fabricated easily, and the processes do not require complex equipment or

extensive training. This simplicity should enable scientists with limited experience in device fabrication to start independent experimentation and adaptation of μPADs. To use wax printing, a laboratory can buy all the equipment necessary for fabricating μPADs in very large numbers for ~$1000 and can start making prototype devices within a day of setting up the equipment. We believe wax printing to be capable of practically generating hundreds of thousands of devices without further adaptation.

The applications of μPADs are not limited to diagnostics for developing countries. They should be useful as diagnostic assays for use by first responders and by the military in operations in harsh environments and may also find applications in clinical laboratories and home healthcare in developed countries by providing low-cost alternatives to current diagnostic technologies. μPADs may be sufficiently inexpensive to be applicable to agriculture, water, food, and environmental markers. As with any new technology, μPADs may also find new applications in basic research.

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