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A Paper-Based "Pop-up" Electrochemical Device for Analysis of Beta-Hydroxybutyrate

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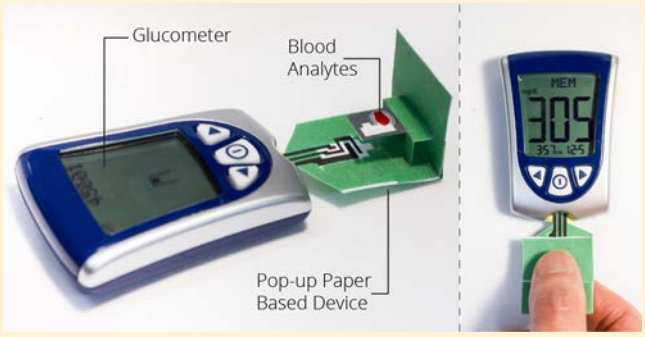
Supporting Information

ABSTRACT: This paper describes the design and fabrication of a "pop-up" electrochemical paper-based analytical device (pop-up-EPAD) to measure beta-hydroxybutyrate (BHB)—a biomarker for diabetic ketoacidosis—using a commercial combination BHB/glucometer. Pop-up-EPADs are inspired by pop-up greeting cards and children's books. They are made from a single sheet of paper folded into a three-dimensional (3D) device that changes shape, and fluidic and electrical connectivity, by simply folding and unfolding the structure. The reconfigurable 3D structure makes it possible to change the fluidic path and to control timing; it also provides mechanical support for the folded and unfolded structures that enables good registration and repeatability on folding. A pop-up-EPAD designed to detect BHB shows performance comparable to commercially available plastic test strips over the clinically relevant range of BHB in blood when used with a commercial glucometer that integrates the ability to measure glucose and BHB (combination BHB/glucometer). With simple modifications of the electrode and the design of the fluidic path, the pop-up-EPAD also detects BHB in buffer using a simple glucometer—a device that is more available than the combination BHB/glucometer. Strategies that use a "3D pop-up"—that is, large-scale changes in 3D structure and fluidic paths—by folding/unfolding add functionality to EPADs (e.g., controlled timing, fluidic handling and path programming, control over complex sequences of steps, and alterations in electrical connectivity) and should enable the development of new classes of paper-based diagnostic devices.



Nearly 400 million people have diabetes, 80% of whom live in low- and middle-income countries (LMICs).[1] Uncontrolled diabetes can lead to the catabolism of fatty acids and the production of so-called "ketone bodies," comprising acetone (2%), acetoacetate (AcAc, 20%), and beta-hydroxybutyrate (BHB, 78%).[2] The buildup of these metabolic acids can cause an acid–base imbalance called "diabetic ketoacidosis" (DKA).[3] DKA can result in vomiting, severe dehydration, confusion, and, occasionally, coma. Emerging evidence shows that pediatric patients with severe DKA show decreased cognitive function compared to their peers.[4] Without early detection and treatment, DKA can be fatal. Despite improvements in insulin therapy from the 1980s to early 2000s, mortality rates for DKA in developed countries has remained stubbornly high (~4% of people with DKA die from it).[2,5,6] In the developing world, although limited medical records make exact numbers difficult to establish, the mortality rate from DKA in diabetics is thought to be even higher.[5,7] The use of point-of-care (POC) diagnostic tools for the early detection of DKA presents an opportunity to identify and treat DKA before it reaches acute levels.[2,3,8] Direct measurement of BHB in blood is the best method for diagnosing DKA.[8,9]

Electrochemical enzymatic detection of BHB has recently been enabled by hand-held devices that use disposable plastic test strips and can be used at the POC.[10−13] These hand-held detectors have the advantages of quantitative detection and simple integration with data-management systems and "the cloud". They are, however, less readily available and more expensive than commercial glucometers. Most glucometers cannot be used to read current test strips for BHB, because the timing for the multistep enzymatic reaction used to measure BHB is different than that used to measure glucose. The test strips used for BHB are also expensive ($5−8 each), in part

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because they require both complex fabrication to enable the multistep enzymatic reaction, and relatively large quantities of expensive biochemical reagents to give short assay times.[11] The requirement that patients purchase a meter specifically to measure BHB and the cost of the strips prevent widespread use even in developed countries.[14] A low-cost strip that could be read by a glucometer could reduce the barriers to BHB monitoring.

This paper describes the development of a prototype of an inexpensive, paper-based test strip for the detection of BHB that operates using a commercial glucometer. In order to accommodate the multistep reaction to detect BHB, we have developed a new type of device: a reconfigurable three-dimensional (3D) "pop-up" structure inspired by pop-up greeting cards (Figure 1). This structure provides the basis of an electrochemical paper-based analytical device (pop-up-EPAD). The reconfigurability of the flow path in this device that can be achieved by folding and unfolding, as well as the ability to separate and reconnect layers spatially in the pop-up structure, enables time-controlled valving of fluid flow in a simple device made from one sheet of folded paper.

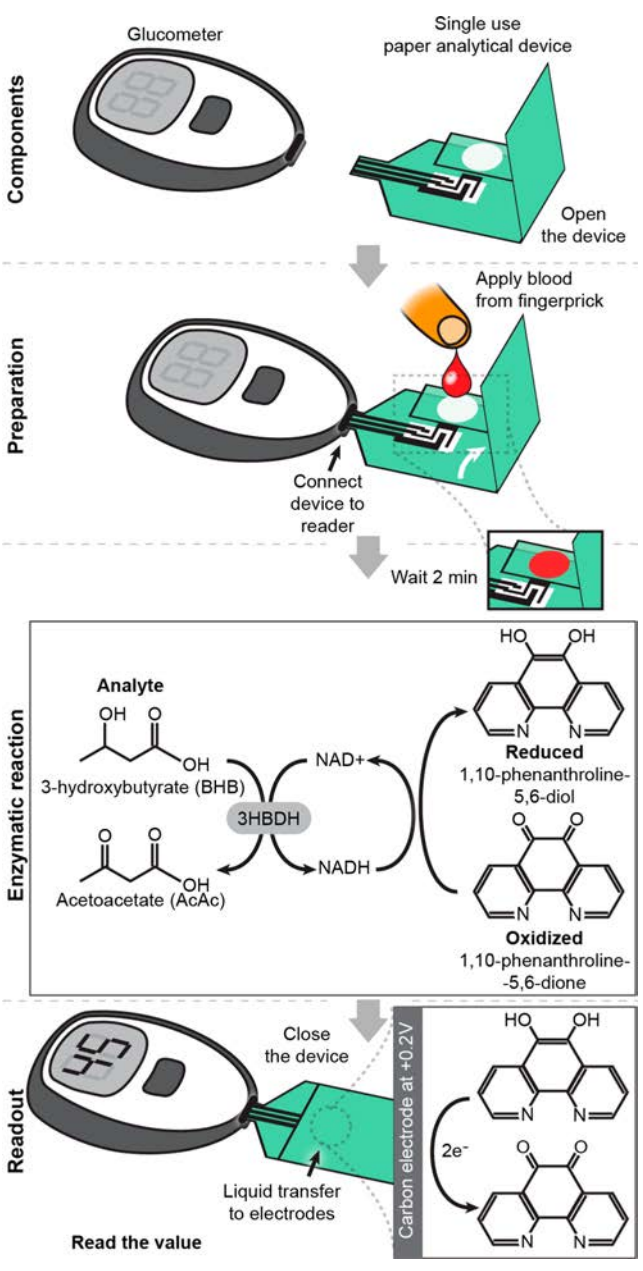

Figure 1. Illustration of the process of testing BHB using "pop-up" paper test strips and a glucometer.

We demonstrate the feasibility of this pop-up-EPAD by testing whole blood spiked with BHB, and we find good agreement between paper devices measured by a reader for glucose and commercial test strips measured by a reader for BHB, throughout the clinically relevant range from 0.1 mM to 6.0 mM. By creating a low-cost device for BHB from a single sheet of paper and by demonstrating that this device can be read by a common, commercial electronic technology (i.e., a glucometer), we hope to improve access to diagnostics for DKA at the POC and thus improve management of diabetes.

Paper Provides an Excellent Platform for Electrochemical Analysis. Paper-based microfluidic devices can be used for performing chemical analyses using hand-held devices.[15−21] Paper devices are less expensive and easier to fabricate than open-channnel microfluidic chips (normally fabricated in polymers), and they do not require pumps and electrical power to manipulate fluids. The reagents necessary for chemical analyses (e.g., enzymes, substrates, and electrochemical mediators or colormetric indicators) can be stored in the pores between the cellulose fibers of the paper either as a dry powder (usually included in a solid stabilizing agent such as dextran or trehalose) or suspended in a hydrogel; there is thus very little manipulation required by the users. To run a complete assay, the user simply adds the sample and a solution of electrochemical mediator (alternatively, the mediator can be stored in the device) to the popped up (unfolded) device, inserts the device into a glucometer, waits for a short time, folds it down, and reads the results. (Paper devices also have disadvantages relative to other type of microfluidic systems—complex paths for flow, limited control of flow rates and volumes, evaporation of water and changes in concentration, an optically scattering medium, and others. The relative merit of paper-based and polymeric devices depends on the application. This paper is intended to address weaknesses in paper devices in timing and control of flow paths.)

Fluid flow can be controlled in a paper-based microfluidic device by wax barriers (i.e., channels) patterned using a solid-wax printer. We and others have used paper-based microfluidic devices for a wide range of biological and environmental applications.[19−27] More recently, several research groups have developed valving strategies to incorporate new functionalities (i.e., timing, programming, and multistep processing) into paper devices. Our group incorporated single-use buttons designed to control the flow path by manually pressing paper buttons.[28] Phillips and co-workers developed methods for controlling the timing of an assay by changing the permeability and/or wetting properties of paper using paraffin wax[29,30] or phase-switching depolymerization.[31,32] Yager and co-workers have created time delays of the fluidic path by engineering unique channel geometries and by incorporating dissolvable sugar barriers.[33−36] Other valving methods include using hollow-channels,[37] tunable cellulosic shunts,[38] dissolvable bridges,[39] folding casings,[40] and magnetic timing valves.[41] Paper-based devices are now of increasing interest for the electrochemical detection of analytes,[42−45] in part for the ease with which their results can be transferred to the cloud, or to a distant clinic or doctor's office. Electrochemistry offers three advantages as the basis for bioanalysis: (i) It provides

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quantitative measurements. (ii) It is independent of lighting and color (both good lighting and a colorless solution are usually required for colorimetric and spectrophotometric assays). (iii) It allows easy interfacing with electronic medical-records systems.

We and others have developed electrochemical paper-based analytical devices (EPADs) by stencil-printing carbon or silver onto wax-printed paper to make working, counter, and reference electrodes.[42,44,46−50] We have also incorporated ion-selective membranes into these devices to make ion-selective electrodes.[51] These EPADs can perform a wide range of electrochemical methods (e.g., potentiometry, voltammetry, chronoamperometry, and coulometry) to detect a variety of analytes.[15,37,48,51−58]

In order to apply EPADs in POC settings, we have integrated the devices with a commercially available glucometer,[15] and we have also developed a much more versatile, portable, electrochemical reader capable of a wide variety of electrochemical measurements with transmission of data over the audio channel of any cell-phone, with any mobile network.[59]

One of the limitations of previous EPADs is that the enzymes and other reagents are stored on or near the electrodes. When the solution of sample or mediator contacts the electrodes and the EPAD is connected to a commercial glucometer (or other commercial device), the electronics immediately start the measurement (sometimes after only a 5−10 s reaction period). This automatic (and immediate) electrical response limits the time allowed for a reaction. In order to generate a measurable signal within these time limits, we and others, have increased the concentration of the enzyme stored in the device, but using more enzyme also increases cost.[15,18,43] For some applications, there are no enzymes that can react rapidly enough to work with conventional EPADs. To overcome these challenges, a device must be able to decouple the enzymatic reaction from the specific timing sequence for analysis imposed by commercial glucometers.

EXPERIMENTAL DESIGN

Design and Fabrication of the Pop-up-EPAD. We were inspired by the ability of pop-up greeting cards and children's books to change the topography and topology of 3D structures quickly and easily. With this inspiration, we have developed a new class of EPADs with a pop-up, 3D structure. The pop-up-EPAD (Figure 2 and Figure S1) includes a sample port, a reaction zone where enzymes can be stored, and a detection zone that is spatially separated from the first two zones. The detection zone interfaces with a glucometer, through three stencil-printed electrodes: (i) a working electrode, (ii) a common counter and reference electrode, and (iii) an indicator electrode. The pop-up structure acts as a reversible, mechanical valve to change the fluidic connectivity of the system. When the device is "closed" using a modest mechanical pressure (i.e., when squeezed between the thumb and forefinger or placed on a flat surface with a weight on top), the valve goes from an "off" to an "on" state because the contact between the separate paper components allows a fluid connection, with liquid flowing from one sheet to another (Figure 2c). This connection is insensitive to the applied pressure within the range of 0.07 N/cm^2 to 0.17 N/cm^2 (Supporting Information, Figure S2)—a range conveniently maintained either by sandwiching the device between two kitchen magnets or with the gentle pressure of two fingers, because it requires, primarily, fluidic contact and capillarity to establish the fluidic path between the layers of the paper, regardless of whether the pressure was supplied by a finger or solid object. Once a continuous fluidic channel is generated, the system is relatively insensitive to pressure. When placed in the "open" state, the pop-up-EPAD maintains its structure until it is manually closed.

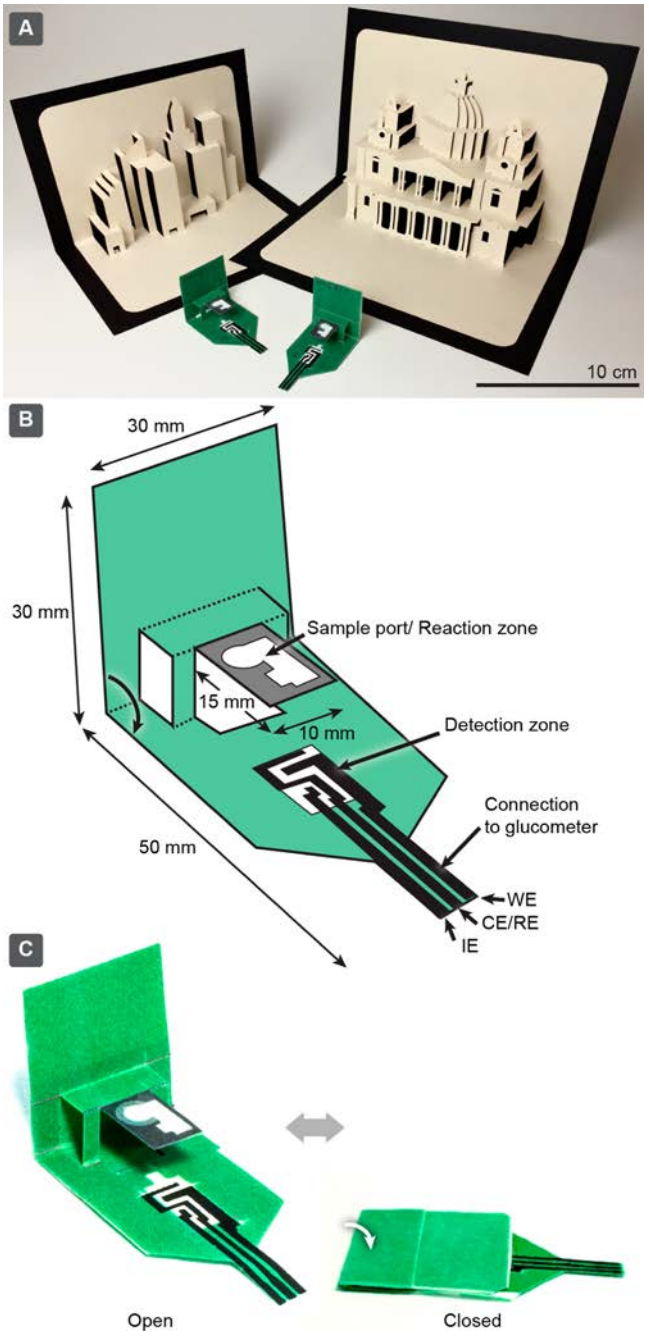

Figure 2. (a) Photographs of pop-up greeting cards (Source: http://www.popupology.co.uk/. Copyright 2005 and 2013 by Popupology, reproduced with permission), and a pop-up structure of the type used in the EPAD described here. (b) A schematic of the pop-up-EPAD in the "open" format. Enzymes are stored in the reaction zone. The detection zone contains printed electrodes designed specifically for the commercial electrochemical reader used in this study. IE: indicator (filling) electrode; CE/RE: common counter and reference electrode; WE: working electrode. (c) Photographs showing the valve capabilities of the pop-up-EPAD when it is open and closed.

The fabrication of a pop-up-EPAD requires six steps: (i) wax-printing microfluidic channels on chromatographic paper

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(Whatman 1 Chr),[60] (ii) stencil-printing electrodes and circuits using graphite ink, (iii) pipetting a solution of enzyme and cofactor into the reaction zone, (iv) drying the system for 6 h at 4 °C in the dark, (v) cutting the paper with a razor along printed lines, and (vi) folding the pop-up paper device (Figure S3 and Figure S4). The first five steps of the fabrication process can be performed on a single sheet of paper, and could be carried out on a reel-to-reel printing line or digital craft-cutting plotters.[61] After the individual test strips are separated, a series of "through cuts" (i.e., a cut through the entire thickness of the paper) and "half cuts" (i.e., a light cut that does not go through the entire thickness of the paper but enables easy folding) are performed over guiding lines. The simple and reproducible fabrication procedure ensures that the devices that are produced have consistent overlap between the reaction zone and detection zone. The Supporting Information includes a step-by-step video of the entire fabrication process, and a PDF giving the design—including a guide to cutting and folding the pop-up structure—used to print the devices.

Choice of Glucometer: Precision Xtra Meter. We used the Precision Xtra Blood Glucose Monitoring System (Abbott Laboratories Inc.) as the electrochemical reader that combines the ability to measure glucose and BHB. This glucometer has three attractive characteristics: (i) it is relatively low in cost ($35 for the device) and easy to use; (ii) it is one of only two commercial glucometers that also measures the concentration of BHB; and (iii) it has been widely used in hospital and field tests, adequately characterized in the literature, and demonstrated to have reliable performance in human and animal studies.[62,63] The Precision Xtra meter uses different test strips for glucose and for BHB; the device recognizes the type of strip automatically based on a recognition electrode on the back side of the BHB strip. That is, the glucose strips have no electrodes on the backside; the BHB strips have a recognition electrode patterned onto their backside (see images of deconstructed electrodes in Figure S5).

We aimed to create a device for BHB that could be read by simple glucose meters without requiring this type of recognition of strips; we, therefore, designed our paper devices without the recognition electrode on the backside. The pop-up-EPADs for detecting BHB mimic the configuration of commercial glucose strips in term of the configuration at the junction between the strip and the meter and thus trick the meter into operating as a glucometer. We also designed strips that could be used with a commercial glucometer (CVS Truetrack glucometer), which do not include a mode for measuring BHB (Figure S6 and Figure S7). In both cases, the number displayed by the reader is a representation of the actual BHB concentration value and not the exact concentration; the true value of BHB can be calculated from the reading on the meter using a calibration curve, or the reading for a pop-up-EPAD can be read against an empirical scale. Neither is satisfactory for a fully developed device, but for a prototype, the design we used is sufficient. In a device intended for POC use, the conversion could be easily accomplished by the electrochemical reader.

Although we only demonstrate readings with two different commercial meters in this work, the pop-up-EPADs could be used with other glucometers or electrochemical detectors with simple modifications of the electrodes and other design aspects. Using the Precision Xtra, we were able to make a direct comparison of our measurements of BHB, made with paper devices, to those made with commercial, plastic test strips using the same reader in different test modes.

Pop-up-EPAD Enables Controlled Valving and Timing. The pop-up structure provides spatial separation in the "open" configuration; it enables the operator to wait for the enzymatic reaction to reach completion before changing the path of the fluid (i.e., "closing" the pop-up) and enabling the fluid flow that triggers the initiation of the electrochemical measurement sequence by the glucometer.

One challenge in lowering the cost of POC detection of BHB is the high cost of the enzyme, 3-hydrozybutyrate dehydrogenase (3-HBHD, EC 1.1.1.30; ∼ $5−7/U); the smaller the amount of enzyme used in the reaction, the longer the time required for the assay to reach completion. Previous work in our lab and elsewhere using EPADs to detect blood glucose used ≥0.5 U of glucose oxidase/device compared to ≥0.03 U/device for commercial strips.[15,18,43] Glucose oxidase is, however, much less expensive than 3-HBHD ($0.0058/U vs ∼ $5−7/U). The pop-up-EPAD structure enables the controlled timing of the enzymatic reaction. We can, therefore, reduce the total amount of the 3-HBHD required by allowing a longer reaction time and thus reduce the cost of each test strip (with the trade-off being that the analysis is slower). This design also allows reagents to be stored in the paper and to be activated by the fluid flow; no premixing of the components is needed.

Enzymatic Reaction Principle. We used a commercially available BHB assay kit (Randox Laboratories, Inc.) that contains 3-HBDH and NAD^+. Using this kit, we designed the amperometric assay for BHB on the pop-up-EPAD to have three steps (Figure 1 and Figure S8): (i) 3-HBDH catalyzes the oxidation of BHB (present in the sample) to acetoacetate (AcAc), with a corresponding reduction of NAD^+ to NADH; (ii) the NADH produced donates two electrons to the electron-transfer mediator, 1,10-phenanthroline-5,6-dione (1,10-PD), and generates the reduced form of 1,10-PD;[10] (iii) the working electrode oxidizes the reduced form of 1,10-PD at a potential of +0.2 V (set automatically by the hand-held reader), and the resulting current is displayed as a numerical value on the electrochemical reader. We varied the quantity of enzyme and the cofactor NAD^+ to ensure that the signal for appropriate BHB concentrations would correspond to the linear range of the glucose output; if a concentration were out of this range, the reader would display an out-of-range error message rather than a number.

RESULTS AND DISCUSSION

Ensuring Performance of the Carbon Electrodes. In order to test the performance of stencil-printed carbon electrodes, we first performed cyclic voltammetry on a solution of the mediator, 1,10-PD. We scanned the voltage between −0.3 and 0.6 V, at a scan rate of 50 mV/s, in the presence of different concentrations of NADH. Figure S9 shows a concentration-dependent increase in the height of the anodic peak in a mixed solution of 1,10-PD and NADH. The dependence of peak current on the concentration of NADH demonstrates that stencil-printed carbon electrodes on paper behave similarly to the screen-printed electrodes on plastic test strips.

Integration of the Pop-up-EPAD with a Glucometer. We developed an EPAD with a "pop-up" format that allows an enzymatic assay for BHB to be read with a commercial glucometer. First, we prepared the pop-up-EPADs having the

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enzyme and cofactor reagents stored on the devices. An enzyme/cofactor solution was prepared to a final concentration of 2 U/mL of 3-HBDH and 42 mM NAD^+ in Tris-buffer (pH 8.0), spotted onto the reaction zone of pop-up-EPADs, and dried at 4 °C for 6 h in the dark (Supporting Information). The volume of the enzyme/cofactor solution was chosen to be 45 μL by titration to ensure that the signal for appropriate BHB concentrations would correspond to the linear range of the glucose output (Table S1). Figure S10a–c shows the operation of the device with the glucometer. First, we inserted the dry pop-up-EPAD into the glucometer in the open configuration and waited for the reader to indicate it recognized the device. Then we loaded the sample (BHB in buffer) and a separate mediator solution (2.5 mg/mL 1,10-PD) onto the reaction zone in the top layer of paper device. The sample fluid was retained in the top layer of paper, and the bottom layer remained dry (because there is no fluidic connection between the two zones—top and bottom—in the "open" configuration). After the enzymatic reaction was completed (at a specified time based on the level of enzymatic activity in the devices), we changed the fluidic connectivity by simply closing the device. The liquid from the reaction zone could then wick into the detection zone. Once the sample reached the electrodes, the glucometer initiated the amperometric measurement at a potential of +0.2 V and displayed a number for the measured analyte.

We demonstrated the use of the Precision Xtra reader in glucometer mode to analyze the concentrations of BHB in Tris buffer (100 mM Tris-HCl, pH 8.0). The concentration of BHB in healthy individuals is less than 0.5 mM, and diabetics with a BHB concentration greater than 3 mM are advised to seek medical attention immediately.[2,3] The curve for the measurement of BHB shows that the values displayed were linearly proportional to the BHB concentrations in the range of 0.1–6.0 mM (R^2 = 0.98, Figure S10d). These data demonstrate that the electrode structure of the pop-up-EPAD can distinguish different concentrations of BHB in Tris buffer. Importantly, the enzymatic assay used in this study is time-dependent[64] and requires a defined time for reaction development and/or signal readout. We evaluated the effect of premature or delayed closing of our device on the value displayed on the glucometer (Supporting Information, Figure S12). For closing times within the range of 90 to 150 s, the mean value displayed on the device was within one standard deviation of the mean of the value measured at the standard closing time of 120 s (425 ± 35, n = 7). Measurements within the time range of 90–150 s also produced a low relative standard deviation (RSD, defined as the ratio of the standard deviation to the mean of the distribution, expressed as a percentage) of <12% (n = 7). If the device was closed after only 60 s, on the other hand, the mean value was lower (389 ± 55, n = 7) than the value for the standard closing time. This difference is expected, as the enzymatic reaction continues to proceed. If the reaction was allowed to continue for a longer time (i.e., delayed closing after 150 s), the display value decreased and produced a large RSD (>23%, n = 7).

Off-Chip Validation of the Electrodes of the Pop-up-EPAD with BHB Measurements Using Whole Blood Samples. Conponents present in whole blood can have confounding effects on electrodes and on the accuracy of electrochemical assays.[43,65] To evaluate this potential interference, we thus tested BHB-spiked whole blood mixed with reagents off-chip, and we found a linear response (Supporting Information, Figure S13).

Validation of the Pop-EPAD with BHB Measurements Using Whole Blood Samples. The gold-standard method to detect BHB in blood is a colorimetric enzymatic assay—based on the same reaction we use for our electrochemical assay—performed on a venous blood draw. Several reports have shown good correlation between the concentration of BHB in whole blood from a venous draw and capillary blood from a finger prick,[63,66,67] and for this reason, we only compared the data collected using the pop-up-EPAD with results from commercial test strips using the same electrochemical reader. We next tested the complete pop-up-EPAD system with dried enzyme and cofactor reagents stored in the device. We spotted 45 μL of the enzyme/cofactor solution containing 2 U/mL of 3-HBDH and 42 mM NAD^+ in Tris-buffer onto the reaction zone. The devices were ready for use after the solution dried for 6 h at 4 °C in the dark. To perform an assay, we added 15 μL of BHB-spiked blood and 35 μL of a separate mediator solution (2.5 mg/mL 1,10-PD) onto the reaction zone of the chip, where the reaction was allowed to proceed for 2 min, after which the device was closed. In order to ensure complete and reproducible wetting of the electrodes by the viscous whole blood, the device was held closed for 15–20 s with modest pressure (>0.07 N/cm^2). Figure 3 shows a linear response for BHB concentrations on both the pop-up-EPADs and the commercial test strips. The pop-up-EPADs display a good linear fit in the clinically relevant range of 0.1 to 6.0 mM (R^2 =

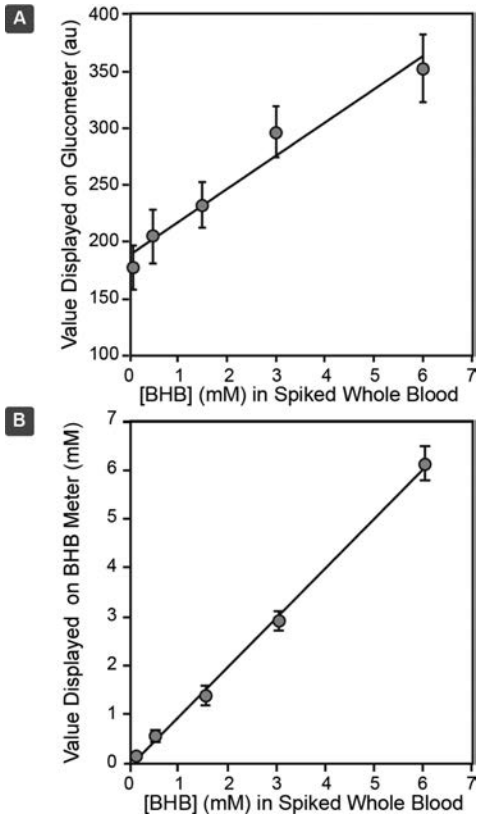

Figure 3. (a) Measurements of BHB spiked into whole blood with a pop-up-EPAD and a glucose meter. The solid line represents a linear fit to the experimental data: $y = 30x + 185.6$ (R^2 = 0.96) and error bars depict the standard deviation of replicate measurements (n = 7) (b) Measurements of BHB in human whole blood samples using commercially available test strips (Abbott, Precision Xtra Blood Ketone Test Strip, lot no. 75001, n = 7, $y = 1.01x + 0.03$ (R^2 = 1.00).

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0.96). The limit of detection (LOD) was calculated to be the concentration that produced a display value three standard deviations above the mean of the value displayed for a blank sample. While the commercial test strips result in a smaller standard deviation than our paper devices, the LOD values of our devices for BHB (0.3 mM) were comparable to those of commercial test strips (0.12 mM, Table 1). Unlike the commercial test strips that were made in a manufacturing environment, the pop-up-EPADs were fabricated by hand in a laboratory. With additional automation and quality systems for manufacturing, the standard deviation for measurements with different test strips should decrease. Note that the value displayed by the glucometer was higher for measuring whole blood than it was for BHB in buffer. The increase in conductivity that caused this has been shown by many researchers,[68,69] and is likely due to extraneous redox reactions between the mediator, 1,10-PD, and electro-active components of whole blood (e.g., endogenous NADH-dehydrogenase, hemoglobin, glutathione, other proteins, ascorbic acid, uric acid, and a mixture of charged amino acids, etc.).[10]

Table 1. Comparison of the Performance of Pop-up-EPADs with Commercial, Plastic BHB Test Strips

substrate	commercial test	pop-up-EPADs
linear dynamic range (mM)	0 to 7	0 to 6
limit of detection (mM)	0.12	0.3
minimum volume of sample (μL)	~2	15−30
waiting time (seconds)[a]	10	120

[a]The time interval between application of a sample to the test strip and reading the meter.

To elimilate the step of adding a separate mediator solution (a key consideration for practical use in the field), we developed an alternative approach to store the 1,10-PD mediator by spotting a solution of the mediator on the detection zone, based on previous literature.[10,15,70] We spotted 70 μL of an aqueous solution of 0.5 mg/mL 1,10-PD onto the detection zone, and we allowed the device to dry for overnight at room temperature in the dark (1,10-PD in solution is sensitive to light). Next, 45 μL of the enzyme and cofactor solution containing 2 U/mL of 3-HBDH and 42 mM NAD^+ in Tris-buffer were added to the reaction zone. The devices were ready for use after the solution dried for 6 h at 4 °C in the dark. With all of the reagents stored on the chip, the user simply adds 30 μL of sample (BHB-spiked buffer or whole blood) onto the reaction zone, waits for 2 min, and closes the device to trigger the electrochemical analysis. The results based on this laboratory prototype were comparable with those obtained with commercial plastic test strips (Figure S14). By further optimizing the dimentions of the reaction/detection zones, electrode configuration, mediator/enzyme/cofactor concentrations, reaction timing and electrode sensitivity to hematocrit levels,[43,71] it should be possible to reduce the volume of sample and improve performance. Recent advances in computer-guided modeling and simulation using COMSOL may enable accellerated optimization of these devices in the future.[72]

CONCLUSION

This paper describes a prototype of a new type of paper-based device—a "pop-up-EPAD"—and demonstrates its application in measuring BHB at the POC using a commercially available glucometer. The integration of a pop-up structure into a paper device opens new opportunities in the design of paper-based diagnostic chips. The reconfigurable 3D structure makes it posible to change the fluidic path during the course of an analysis and thus to control the timing of various steps. By controlling timing, we are able to reduce the concentration of enzymes, and the cost, compared to values reported previously.[15,18,43] For certain applications, the pop-up structure might improve the dynamic range of measurements (relative to 2D test strips) by allowing controlled timing for reaction development and delivery of reagents in a multistep programmed process.[26,41,73]

Measuring glucose in blood is the most common diagnostic measurement in the world; the average diabetic uses 764 test strips per year.[74] While the number of patients with diabetic ketoacidosis (DKA) continues to increase,[75] the availability of BHB meters to diagnose DKA is still small, especially in LMICs, in part, because of the high cost of the commercial test strips. By integrating a pop-up structure into paper-based microfluidics, we have demonstrated a device that can, in principle, substitute more complex operation (in the unoptimized and incompletely developed prototype we describe) for greater accessibility and generality, and that can be used, with minimal modifications, with ubiquitous glucometers.

For high-risk patients in LMICs and moderate-risk patients everywhere, glucose meters are common, but BHB-meters are not as widely used. As an inexpensive test that can plug into an existing glucometer, a pop-up-EPAD for BHB might provide a means to monitor DKA using meters that are already accessible to many patients with diabetes.

Integrating a pop-up structure into paper-based diagnostic devices provides more freedom and flexibility in design and use than previous devices made using the principle of origami.[27,52,76−78] The 3D structure allows the path of the liquid flow and of the electrical conductivity to be reconfigured by spacially separating the layers by folding and unfolding the device. The concept of using a 3D pop-up structure provides five key functions for EPADs: (i) controlling timing and enabling multistep processes; (ii) providing good registration and repeatability upon folding; (iii) interfacing with commercially avaliable hand-held glucometers for greater accessibility; (iv) reducing the total amount of enzymes required (and, thus, reducing the cost) with the trade-off of increasing the time needed to make a measurement; and (v) triggering the electrochemical measurement by folding.

In the future, more elaborate versions of pop-up structures can be made that include, for example, arbitrary fludic paths (e.g., multistep fluidic programming during the course of an analysis) or other sensing components that respond to changes in electrical connectivity, optical and mechanical properties, magnetic fields, or chemical signals when folded/unfolded—and thus enable the development of new classes of paper-based devices.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.analchem.6b00568.

Detailed Materials and Methods section; detailed analysis of the component of the plastic commercial test strips for the Precision Xtra meter and a CVS Truetrack glucometer; additional data showing the measurement

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of BHB in buffer using the pop-up-EPAD; and a table estimating the itemized cost per device.(PDF)
Device schematic (PDF)
Video showing fabrication of a pop-up-EPAD (MPG)

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Notes

The authors declare no competing financial interest.

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