CONCENTRATING URINARY BIOMARKERS BY COMPRESSIVE EVAPORATION ON A PAPER MICROFLUIDIC PLATFORM

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ABSTRACT

We report a strategy to concentrate milliliter volumes of a biological fluid to enrich for a target analyte on a paper-based platform. Sample concentration is achieved without the use of centrifuges, vacuum pumps, and other expensive laboratory equipment by simultaneously heating and compressing the paper substrate. This technology was developed to concentrate a urinary biomarker of Tuberculosis (TB) that could ultimately be incorporated into a point-of-care device for TB diagnosis. Using bromophenol blue (BPB) in water as a model system, we concentrated BPB by up to 34-fold in just 20 minutes.

KEYWORDS: Sample concentration, paper microfluidics, tuberculosis, diagnostics

INTRODUCTION

Inspired by the need for simple and low-cost point-of-care (POC) diagnostics for the developing world, we report a method to concentrate milliliter volumes of a biological fluid to enrich for a target analyte that can be performed without centrifuges, chromatography equipment, vacuum pumps, and other expensive laboratory equipment. The purpose of our technology is to enhance the diagnostic sensitivities of in-line, downstream detection assays. Our method employs heat and compression to concentrate a biomarker within urine on a paper-based microfluidic platform and that is compatible with diverse downstream detection methods (e.g., immunodetection, spectroscopy, etc).

Development of our technology was motivated by the global health burden caused by tuberculosis (TB). In 2011 alone, 8.7 million people were infected with TB and 1.4 million died of the disease [1]. Despite being a largely curable disease, the TB epidemic remains out of control due in large part to low detection rates. Currently the most widely-used methods for detecting and diagnosing TB are sputum smear microscopy, bacterial culture, and chest radiography [2]. These methods require physicians and trained technicians to perform and results can take weeks to obtain. Moreover, these methods are only available in centralized laboratory facilities located in urban settings where only 40% of suspected TB cases reside [3]. The remaining 60% of suspected TB cases live in rural areas, where medical attention is initially provided at rural health clinics. The resources in these clinics are extremely limited – specifically, no test is available to diagnose TB. As such, this underserved population goes undiagnosed and untreated, spreading the disease to others in their community and further exacerbating the global crisis. There is thus a clear and urgent need for a simple-to-use, inexpensive, rapid, and accurate POC TB diagnostic test that can be used in the most resource-constrained tier of the health care system of the developing world [4].

THEORY

One approach towards developing a POC TB diagnostic is to detect for mycobacterial antigens present in the urine of persons infected with TB, to serve as surrogate biomarkers for infection with the *Mycobacterium tuberculosis* (MTb) bacterium [5]. Presently, the most promising biomarker for TB diagnosis is lipoarabinomannan (LAM), a lipopolysaccharide found on the cell wall of MTb. This biomarker is released from metabolically active or degrading mycobacteria and is believed to enter the circulation and subsequently filtered into the urine [6]. Therefore, detection and quantification of urinary LAM provides an estimate of the bacterial load, metabolic activity and/or bacterial degradation rate [7]. There is currently only one device on the market that diagnoses TB based on the detection of LAM – the Determine™ TB LAM Ag (Alere) lateral flow dipstick test. While initially promising, this test does not currently have sufficient sensitivity to detect LAM in a general population of people with presumptive TB. In fact, recent clinical evaluations of this diagnostic have overwhelmingly demonstrated that it is

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only sensitive enough to diagnose patients who are co-infected with HIV and TB, since their TB LAM levels are higher [8]. This sub-group represents approximately 13% of the TB-infected population. For the remaining 87%, urinary LAM levels are too low to be accurately detected by the Determine™ TB LAM Ag test. However, when urine from this population is pre-concentrated by centrifugation, LAM is indeed detectable by the same immunodetection method [7, 9]. We are therefore developing a method to enrich LAM to concentration levels high enough for accurate downstream immunodetection within urine without the need for sophisticated laboratory equipment. This strategy was inspired by the use of the Determine™ TB LAM Ag test, but is independent of the actual assay used to detect LAM.

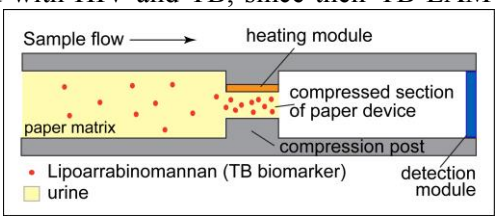

Figure 1: Schematic illustration of our compressive evaporation technology for sample concentration

Due to LAM's inherent thermal stability (since it is a glycolipid and not a protein), we developed a method that utilizes heat to concentrate LAM in urine. The method is performed on a paper-based platform, which enables us to modify the fluid flow rate of urine by simultaneously compressing the paper's cellulose fiber network through which the urine wicks. The paper-based platform was selected for several additional reasons - 1) liquid samples can wick through paper's cellulose fiber network via capillary action, thereby abrogating the need for external pumps to drive fluid flow; 2) cellulose paper is thermally stable at temperatures up to 300 °C [10]; and 3) our sample concentration technology may be readily incorporated in-line with a lateral flow assay design with sample input upstream and detection module downstream of our technology. We hypothesized that heat and mechanical compression of the paper fibers, herein referred to as compressive evaporation, would cause urine to evaporate and LAM to accumulate and concentrate at the heat source, respectively. Heat also accelerates the overall concentration process. (Figure 1)

EXPERIMENTAL

Initial feasibility studies were performed with bromophenol blue (BPB) in deionized water as a model system for LAM in urine. Our experimental setup is illustrated in Figure 2, where an aqueous solution of BPB, at an initial concentration of 100μM, was concentrated within an 80 x 50 x 0.8 mm strip of Whatman GB003 blotting paper. The resistive heater (OMEGA Engineering, Inc.) and the distal 1-cm tip of the paper strip were sandwiched in between two custom-made aluminum plates and pressed together with a medium-sized binder clip. The paper was heated to 220°C by a DC power supply with 8.3W. After sample concentration, the distal 1-cm tip of the paper strip containing the concentrated BPB was placed in a 0.6mL microtube, with a hole punctured at the bottom, which was placed in a 1.7mL microcentrifuge tube. The tube-within-a-tube was spun at 10,000*xg* for 10 minutes to recover the liquid in the saturated paper for subsequent quantification via spectrophotometric analysis at 590 nm. Evaporated volumes were estimated as the volume difference between the initial BPB solution (10mL) and the volume of initial solution remaining plus volume in the paper strip, which was determined by weight.

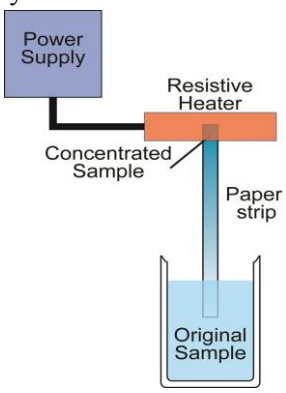

Figure 2: Experimental setup of our compressive evaporation method

To evaluate the feasibility of miniaturizing the heating technology to a battery-powered, self-contained format, we sought to replace the resistive heater with a gold heating element. 50 nm of gold was sputter coated onto Scotch® tape, cut into 5 x 10 mm strips and adhered to 40 x 8 mm strips of Whatman GB003 blotting paper. The coated tape was heated by a DC power supply connected via alligator clips. The temperature of the gold film was recorded with a fine gage, bare wire thermocouple that was mechanically juxtaposed to the film.

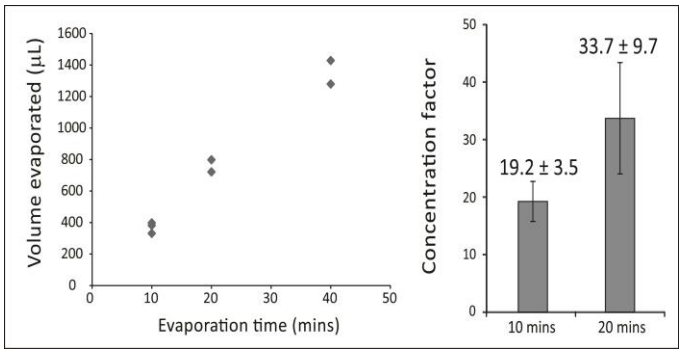

Figure 3: (A) Volume of water evaporated after 10, 20, and 40 minutes. (B) Fold factor that BPB was concentrated after10 and 20 minutes of compressive evaporation

RESULTS AND DISCUSSION

In this simple experimental setup, we evaporated up to 1.4 mL of liquid in 40 minutes. (Figure 3A) After 10 and 20 minutes of concentration, BPB was concentrated by approximately 20-fold and 35-fold, respectively. (Figure 3B) We next evaluated the feasibility of miniaturizing the heating technology to a more portable, battery-powered platform. With less than 1W of power, the temperature of the gold film reached over 200°C. With these encouraging results, we will evaluate the effects of concentrating LAM in urine using this strategy and adapt our method onto a battery-powered, self-contained device with sample concentration and lateral flow detection fully integrated (Figure 4).

CONCLUSION

In conclusion, we designed, built and demonstrated a technology to concentrate a target analyte from within a liquid solution on a paper-based platform by up to 34-fold in 20 minutes. This technology will be initially used towards developing a POC device for diagnosing individuals suspected of having TB. However, the technology can be easily adapted to other clinical applications that require concentrating heat-stable biomarkers that are present at low concentrations in urinary samples to enhance downstream detection.

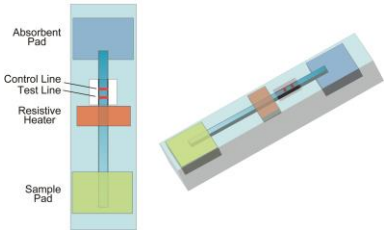

Figure 4: Top and orthogonal views of future integrated diagnostic device

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