QUANTIFICATION OF AMINO ACIDS IN BLOOD USING DIGITAL MICROFLUIDICS

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ABSTRACT

We report a new integrated microfluidic system incorporating a digital microfluidic platform for on-chip blood spotting and processing, and a microchannel emitter for direct analysis by mass spectrometry. The method is fast (analysis time ~1h), robust (does not require capillary junctions and plumbing), precise (CVs range from 5-11%), and is capable of screening samples for common congenital disorders such as homocystinuria, phenylketonuria, and tyrosinemia.

KEYWORDS: Metabolic disorders, digital microfluidics, tandem mass spectrometry, newborn screening

INTRODUCTION

Amino acid (AA) disorders are a class of inherited diseases characterized by the inability to metabolize AAs and if left untreated, these diseases have serious consequences including mental retardation and death [1]. Because these symptoms occur early in life, many countries have instituted newborn screening programs (NSP) to test infant patients in the first days after birth. Despite the success of NSP, challenges to such methods remain, including many hours of laboratory-time, significant costs, and tricky integration with mass spectrometry (MS). To overcome these challenges, we have developed an integrated microfluidic system incorporating a digital microfluidic platform for on-chip blood spotting and processing, and a microchannel emitter for direct analysis by MS. In DMF, droplets of reagents in micro-to nano-liter volumes are manipulated (i.e., moved, merged, mixed, and dispensed from reservoirs) by applying a series of electrical potentials to an array of electrodes coated with a hydrophobic insulator [2]. DMF has recently become popular for biochemical applications [3] because of the benefits of reduced sample size and analysis time and the potential for multiplexed analysis.

Here, we report a method to process dried blood samples for rapid, low-volume amino acid quantification by tandem mass spectrometry. This method has significant advantages relative to the conventional techniques in terms of reagent use and analysis time. Moreover, this technology is liberated from reliance on robots and complicated capillary-plumbing. We speculate that the new method can contribute to a new generation of screening techniques for AA disorders and other diseases, which could have a major impact on the bottom line for newborn screening programs.

EXPERIMENTAL

DMF devices were fabricated in the University of Toronto Emerging Communications Technology Institute (ECTI) cleanroom facility as described in detail elsewhere [4]. Nanoelectrospray ionization mass spectrometry (nESI-MS) solutions were formed by dissolving extracted samples in 70 µl of acetonitrile/water (4:1 v/v) and injected by (nES) into an LTQ MS operating in positive ion mode with a flow rate of 0.8 µL/min, with an applied voltage of 1.7-1.9 kV. Some samples were analyzed by nESI-MS/MS in-line on hybrid DMF-microchannel devices [5] bearing an integrated nESI emitter. A spray was generated by applying 2.5-3.0 kV to a platinum wire inserted in the access hole (see Fig. 4a).

RESULTS AND DISCUSSION

Figure 1 depicts a prototype DMF device developed to analyze 5-µL blood samples. As shown, blood samples are spotted onto device, sample is extracted into methanol and solvent is dried, extract is then derivatized, and product isolated by allowing solvent to dry.

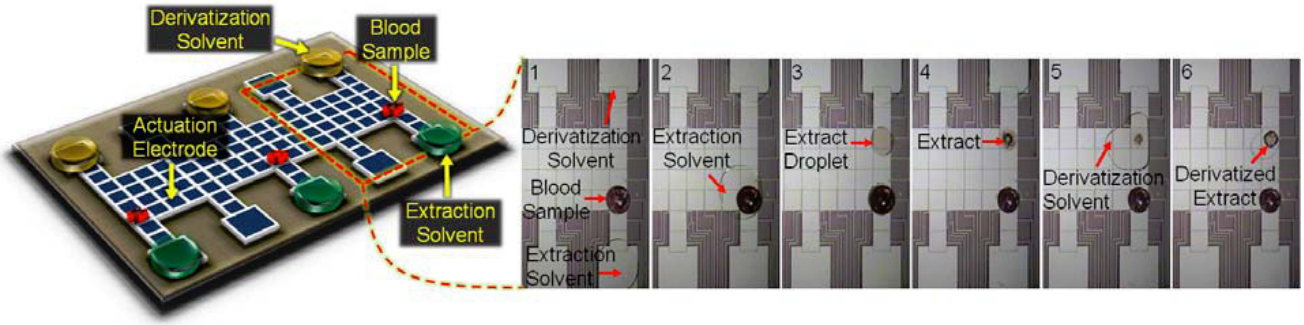

Figure 1: Sample processing by digital microfluidics (DMF). The left image is a schematic of a DMF device which allows for the processing of 3 blood samples simultaneously. The right image is a sequence of frames from a movie (left-to-right) depicting several stages in sample processing by DMF including: (1) a dried blood sample; (2) mixing and incubating an extractant droplet with the sample; (3) a droplet containing sample extractate after translation away from the dried sample; (4) a dried extract; (5) mixing and incubating a derivatization reagent droplet with the dried extract; and (6) the dried, derivatized product.

Nanoelectrospray ionization tandem MS (nESI-MS/MS) was used to quantify AAs in samples of blood processed by the DMF method. Calibration curves with R^2 greater than 0.996 (Fig. 2) were generated by analyzing standards processed by DMF at known concentrations from the abundance ratio of each AA to its deuterated standard peak in the secondary (MS2) spectra.

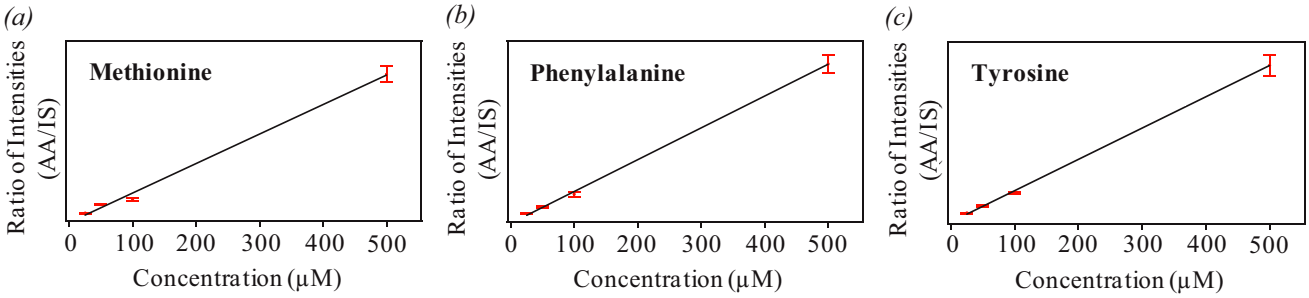

Figure 2: Calibration curves generated by DMF for quantification of (a) methionine (Met), (b) phenylalanine (Phe), and (c) tyrosine (Tyr) in blood. Data were generated by plotting the intensity ratios of the daughter ions of each amino acid (AA) relative to their deuterated internal standard (IS) (i.e., d3-Met, d5-Phe, d4-Tyr, respectively) as a function of AA concentration. Each data point represents at least four replicate measurements, and error bars represent ±1 S.D.

The calibration curves facilitated measurement of AA concentrations in blood samples from a healthy male volunteer. As listed in Table 1, values obtained were in the expected physiological range and the precision in the method was high with coefficients of variation (CVs) ranging from 5 to 11%. Fluorescence and MS/MS were used as an orthogonal tests to evaluate the extraction efficiency of the new DMF technique. As listed in Table 2, the new technique was very efficient (recovery ≥80%) with high precision (CVs 1 to 10%). To validate the new DMF method as a platform for analyzing AA disorders in blood, spiked blood samples (mimicking diseased states) and non-spiked blood samples (mimicking healthy state) were analyzed by mass spectrometry.

Table 1: Measured and normal adult concentrations of amino acids in blood.

Amino Acid	Measured Blood Concentration (μM)	Normal Blood Concentration (μM)
Methionine	25 ± 2	16-33
Phenylalanine	38 ± 2	41-68
Tyrosine	46 ± 5	45-74

Table 2: % Recovery of the DMF method measured by fluorescence and MS/MS.

Amino Acid	% Recovery by Fluorescence in Standards	% Recovery by MS/MS in Blood
Methionine	98 ± 10	100 ± 1
Phenylalanine	86 ± 9	85 ± 5
Tyrosine	82 ± 10	84 ± 7

To validate the new DMF method as a platform for analyzing AA disorders in blood, spiked blood samples (mimicking diseased states) and non-spiked blood samples (mimicking healthy state) were analyzed by mass spectrometry. Figure 3 shows a comparison of measured concentration of AAs in normal and spiked blood samples. The dashed line indicates typical threshold value for diagnosis of homocysteinuria (100 μM Met), phenylketonuria (120 μM Phe), and tyrosinemia (150 μM Tyr). As shown, the method is useful for distinguishing between these states. Note that for Type 1 Tyrosinemia, other markers such as succinyl acetone are often measured as a primary screen.

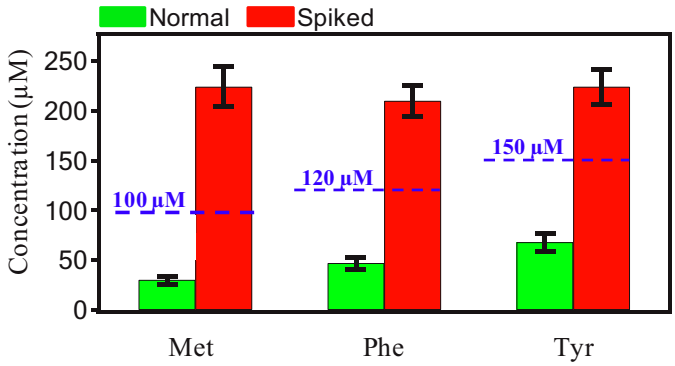

Figure 3: A comparison of Met, Phe, and Tyr concentrations in normal (green) and spiked (red) blood samples as biomarkers for homocystinuria, phenylketonuria, and tyrosinemia, respectively. The dashed lines indicate the upper levels for normal concentrations in newborn blood samples. Each data point represents at least four replicate measurements, and error bars represent ±1 S.D.

As a further step towards system integration, the digital microfluidic platform was coupled directly to a nanoelctrospray mass spectrometer emitter for in-line analysis. As shown in Figure 4a, the central feature of this design (building on our recent work with hybrid microfludics [5]) is an intersection of a DMF electrode and a microchannel through a vertical hole. In this device, droplets are manipulated on the top surface, and are subsequently transferred to microchannels on the bottom of the device through the hole. The principle of operating the hybrid device for on-chip sample analysis is shown in Figure 4b. Representative mass spectra generated from samples processed and analyzed on-chip are shown in Figure 4d. The entire process requires ~1 h from sampling to analysis, and requires only the hybrid DMF device and a mass spectrometer (i.e., no complex nanoflow pumps, robots, samplers, or control software).

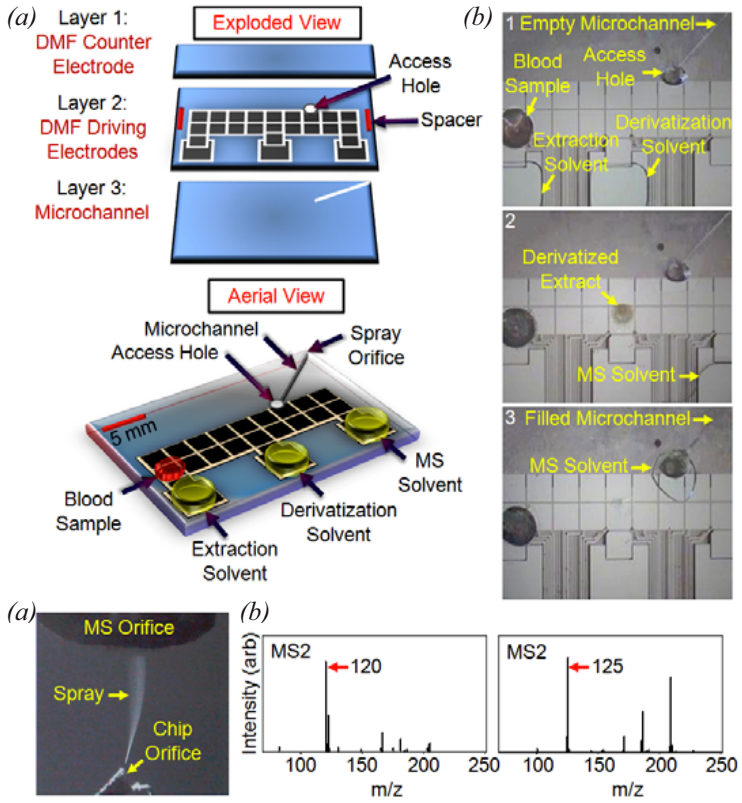

Figure 4: (a) Schematics showing the hybrid DMF-microchannel device used for in-line mass spectrometry analysis. (b) Series of frames from a movie (top-to-bottom) demonstrating derivatization and extraction of AA, resolubilization in solvent, and analyte solution in spray microchannel. (c) Image of sample spraying from the fabricated emitter. (d) MS2 spectra of Phe and d5-Phe generated from blood samples.

CONCLUSION

We report a new method for rapid processing and analysis of inborn AA diseases using an integrated microfluidic device. The new method facilitates reduction in reagent consumption and analysis time. Most importantly, the new method has built-in plumbing for direct interfacing with mass spectrometry, which has the potential to contribute to a new generation of fast, inexpensive screening for congenital diseases.

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