Centrifugal blood sample preparation for metabolite derivatization and analysis by solid matrix laser desorption/ionization mass spectrometry (SMALDI-MS)

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ABSTRACT

A microfluidic approach is developed to automate and simplify multiplexed metabolite assays. Solid matrix assisted laser desorption ionization mass spectrometry (SMALDI-MS) on nanoporous thin films is suitable for analyzing small molecules, particularly metabolites, with a simple spot test. We present a system for blood sample preparation for metabolite analysis using centrifugal fluidics, coupled with derivatization and SMALDI-MS. This centrifugal microfluidic device enables semi-automated on-chip cleanup of blood samples, delivering a clean matrix for MS detection. A derivatization reagent is applied in our work to label the target molecules with fluorous affinity tags, so that higher S/N is observed by SMALDI-MS.

KEYWORDS: Centrifugal Microfluidics, Mass spectrometry, metabolite, nanoporous, whole blood

INTRODUCTION

In the analysis of the human metabolome, microfluidics and related technologies can be introduced to automate and miniaturize assays. The biological sample matrix is extremely complex, making extraction and purification of relatively hydrophilic, ionic, small target molecules extremely challenging for analysis [1], in a background of salt and proteins. We previously reported a polyester-toner centrifugal microchip to prepare small volumes of human serum samples for metabolites analysis by solid matrix laser desorption/ionization mass spectrometry (SMALDI-MS) [2]. That work involved off-chip mixing steps and serum preparation; here we present a semi-automated multichannel centrifugal microfluidic device for multi-step preparation of whole blood samples. The devices are assembled by laser cutting poly(methylmethacrylate) (PMMA) and pressure sensitive adhesive (PSA) to avoid wet chemical bonding procedures [3]. To facilitate liquid control and precise metering, dissolvable film(DF) based centrifugal-pneumatic valves [4,5] were introduced to automate on-chip decanting. The fluidic device removes proteins and lipids from blood, so that metabolites can be detected by directly spot test of SMALDI-MS in a clean matrix with high response and stable intensity. The SMALDI surface provides a necessary desalting step required for MS, eliminating the need for chromatographic preparation. To validate the on-chip cleanup efficiency and broad target molecule coverage, the metabolites are detected in native form by SMALDI-MS and also by SMALDI-MS after derivatization. Derivatizing low mass analytes by using fluorous affinity tag shifts them to a higher mass, resulting in better S/N. Given the fluorous coating on the SMALDI surface, the fluorous tags also provide removal of salts by simple washing steps. The system reported here allows multiplexed, simultaneous analysis of many ionic, hydrophilic metabolites in blood.

EXPERIMENTAL

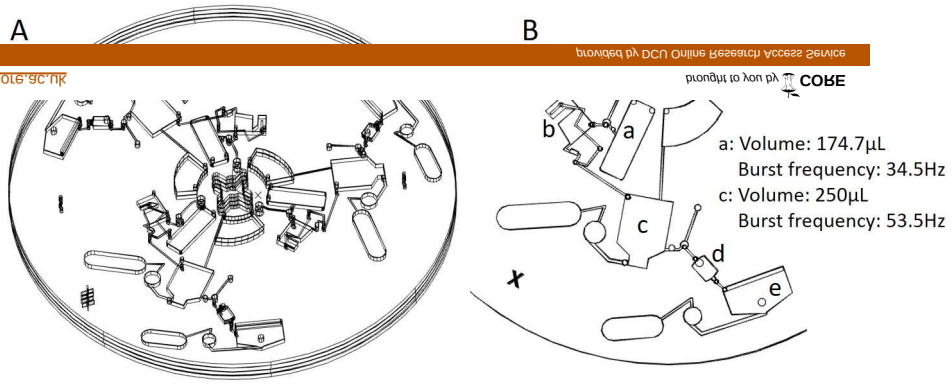

Figure 1: 3D view (A) and design for each unit (B) of centrifugal disc for blood sample preparation.

The 15 cm diameter centrifugal microfluidic device consists of four layers of 1.5 mm-thick PMMA (Acrylite FF, Johnston plastics) and four layers of 127 µm-thick PSA. Features are cut by CO_2 laser ablation (Epilog Zing Laser Series) and aligned layers are laminated (Cheminstruments HL-100 rolling laminator) with 100 psi. PSA layers serve as bonding layers between PMMA layers. The assembled disc is fixed on a centrifuge (Eppendorf 5415C centrifuge) by using an adaptor. Figure 1 represents the 3D view of the microfluidic design of one disc with three processing units for sample preparation.

Blood was collected from a healthy female volunteer and loaded to plasma separation chamber (a). Then 50 µL of separated plasma is metered by chamber (b) after on-chip blood cell separation in chamber (a). The metered plasma is diluted 3:1(v/v) with methanol in reservoir (c) for protein precipitation. C18 beads are preloaded in chamber (d) for lipid removal. Sample drying, re-dissolution and mixing with silica nanoparticles for further cleanup are performed in reservoir (e). An oscillating spinning speed, below the DF valve burst frequency, mixes the suspensions to accelerate precipitation and adsorption on the nanoparticles. Then precipitates and nanoparticles are settled to the chamber bottom by a stable spinning speed. The cleaned sample is pipetted out and spotted onto a SMALDI-chip for analysis, as our work flow in Figure 2 demonstrates. The SMALDI film which is a nanoporous silicon structure deposited by glancing angle in vacuum, is modified with a perfluoralkane monolayer [6].

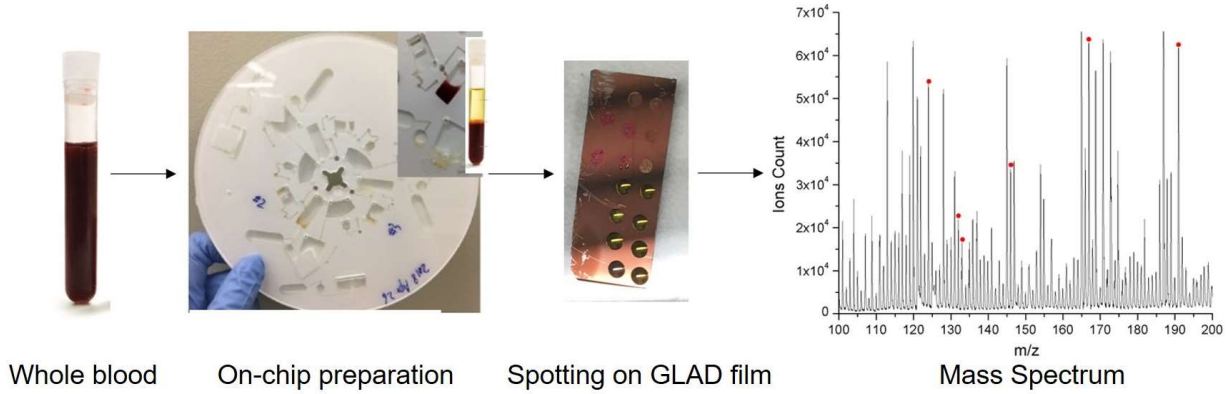

Figure 2: Work flow of on-chip sample preparation, followed by SMALDI-MS

An 40 µL aliquot of the processed sample is vacuum dried and recovered by methanol for derivatization. The target molecules in the recovered supernatant were labeled with 3-(Perfluorohexyl)propan-1-ol in conc HCl, incubating 1 h at 65°C. Sample was then spotted on per-fluoro$SiCl_3$ coated GLAD Si-films for SMALDI-MS.

RESULTS AND DISCUSSION

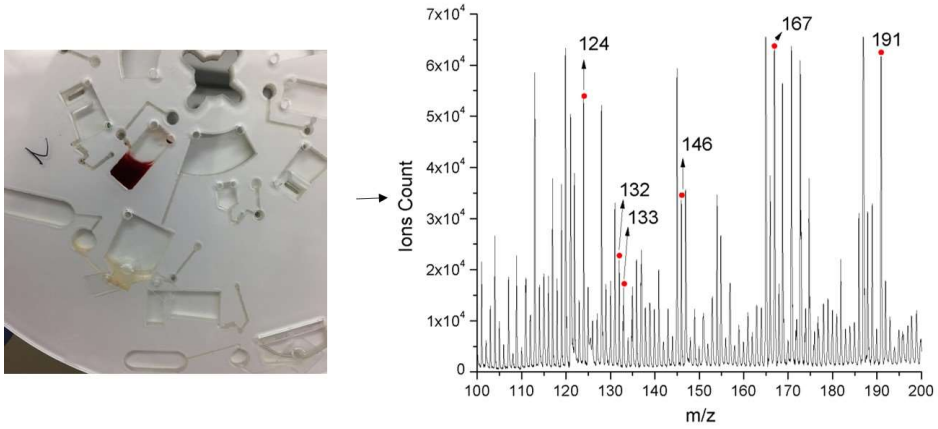

Figure 3: Blood sample on-chip preparation photo and corresponding mass spectrum with metabolite peaks. (taurine: 124; aspartic acid: 132; glutamic acid: 146; uric acid: 167; citric acid: 191).

After the on-chip processing, clear sample was obtained in the last chamber, shown in Figure 3 and the mass spectrum after cleanup shows tens of low mass metabolite peaks. We selected 6 analytes including amino acids

and acids related to major diseases such as Parkinson's disease, stroke and cancer according to the Human Metabolome Database (HMDB), identified by their m/z values.

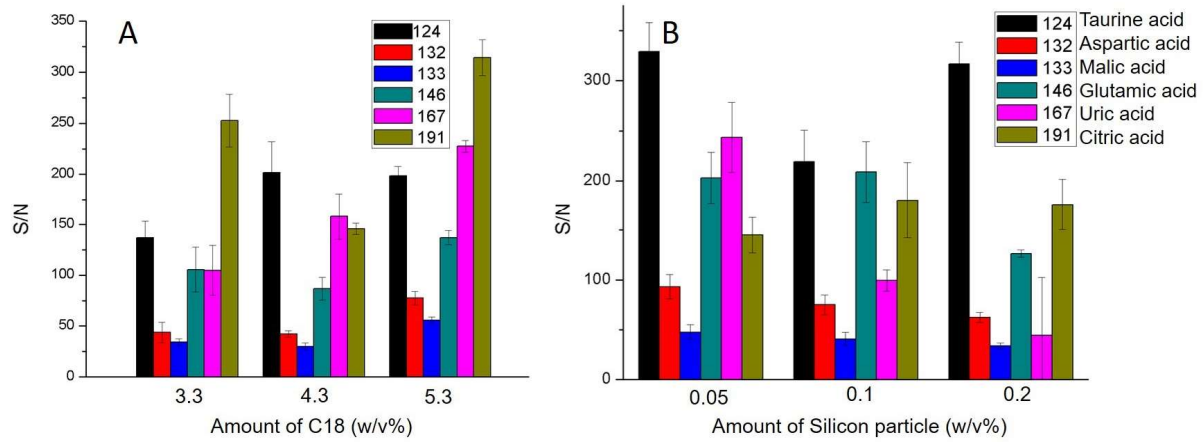

Figure 4 : Signal to noise ratio of different metabolites in serum samples prepared on-chip with different densities of C18 beads (A) and silica nanoparticles (B).

The signal to noise ratio (S/N) of the selected targets was employed to evaluate the sample preparation performance with different suspension densities of C18 beads and silica particles. Figure 4A shows S/N from the sample preparation with 5% (m/v) C18 is larger than the sample prepared with lower C18 density, since the hydrophobic molecules in serum such as lipids are sufficiently removed. Figure 4B shows S/N of the sample prepared with 0.05% (m/v) silica particles is higher than sample prepared with higher density of silica particles. According to previous study [7], this arises because silica nanoparticles can also adsorb some metabolites when protein is absent. In conclusion, densities of 5% (m/v) C18 and 0.05% (m/v) silica particles were selected to achieve optimized sample cleanup efficiency.

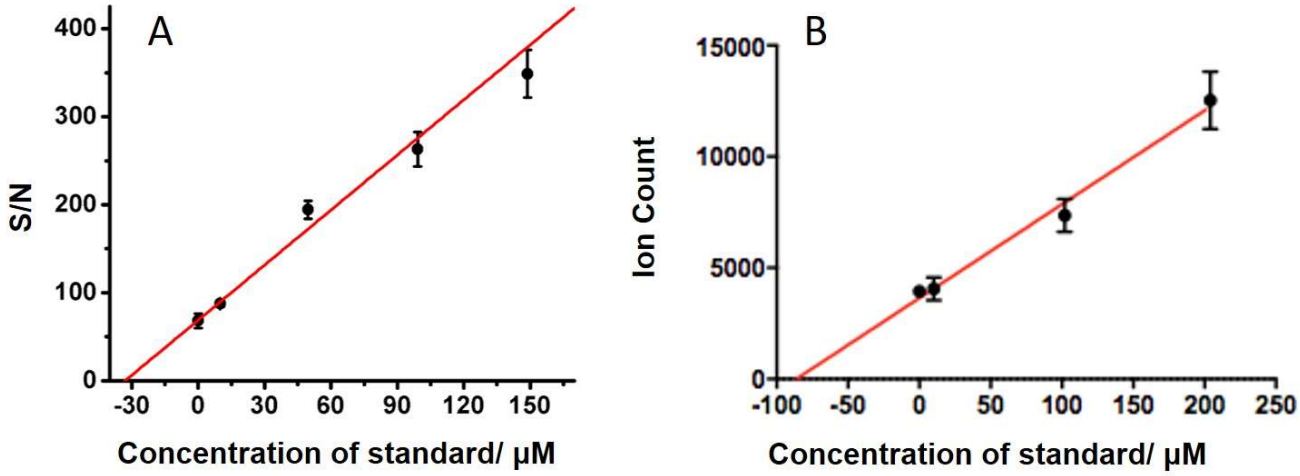

Figure 5: Calibration curves of aspartic acid after on-chip cleanup (A) and glutamic acid after on-chip cleanup and derivatization (B).

The quantification of analytes in blood sample prepared on-chip was carried out by the standard addition method. As shown in Figure 5, linear regression was observed before and after derivatization. For glutamic acid in blood, the ion count was 3300 before labeling. After derivatization, higher ion counts were achieved due to the raised mass of the target molecules and better response of standard addition was observed because of the enhanced retention on the per-fluoro coated GLAD films during rinsing. The concentration of aspartic acid found in blood was 33.4 µM, with 85.8 µM for glutamic acid. These are very close to the concentration obtained for serum samples. The results agree with NMR measurements, and the concentration range in human blood (Human

Metabolome Database, HMDB, www.hmdb.ca). Many reported studies for serum show higher concentration of aspartic acid than the database, because the concentration of aspartic acid in blood will increase with storage time[8]. Our result is more consistent with the database (HMDB: 20 ± 5 μM) because of the efficient automatic on-chip preparation of fresh blood. In addition, results from derivatization prove that the on-chip processed sample is clean enough for further metabolite labeling and improved detection.

CONCLUSION

The centrifugal fluid device works effectively for sample cleanup, allowing metabolite quantification from human whole blood. The device, when coupled with the nanoporous GLAD thin films and per-fluoro labeling, provides sensitive, quantitative analysis. The method is competitive with chromatographic techniques, and is well suited to batch, clinical analysis of samples.

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