

PROCESSING PROTEINS IN SERUM BY DIGITAL MICROFLUIDICS

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ABSTRACT

We present the first digital microfluidic (DMF)-driven method capable of processing proteins from heterogeneous fluids. In this study, proteins were extracted from serum and other heterogeneous mixtures from nanoliter-size droplets via precipitation. This method is an important step towards the construction of a fully automated system for clinical proteomics.

KEYWORDS: Digital Microfluidics, Lab-on-a-Chip, Proteins, Proteomics, Protein Precipitation

INTRODUCTION

Clinical proteomics has emerged as an important new discipline, promising the discovery of new biomarkers that will be useful for early diagnosis and prognosis of disease [1]. While clinical proteomic methods vary widely, a common characteristic is the need for chemical processing of extremely heterogeneous fluids (e.g., serum, whole blood, etc.) prior to analysis. Here, we report a new method for automated proteomic sample processing, making use of digital microfluidics (DMF). In DMF, droplets are actuated (i.e., dispensed, merged, mixed, split, translated) on an open array of electrodes [2]. DMF has recently become popular for biochemical applications such as profiling proteomics [3], enzyme assays [4], and cell-based assays [5], because of the benefits of reduced sample size and analysis time and the potential for multiplexed analysis.

Here, we introduce the first DMF-based method for chemical processing of proteins in serum. Methods were developed to extract proteins (via precipitation) from droplets containing serum, followed by sequential biochemical processing (reduction, alkylation and digestion) of the extracted analytes. This work is an important first step in our efforts to develop fully automated methods for biomarker discovery (i.e., clinical proteomics).

EXPERIMENTAL

1,2-Dibutyroyl-*sn*-glycero-3-phosphocholine (PC)/bovine serum albumin (BSA) (80:1 mol) mixtures were prepared in 10 mM Tris-HCl (pH 7.8) buffer with Pluronic F127 (0.08% w/v). BSA and proteins from fetal bovine serum (FBS) were precipitated using droplets containing trichloroacetic acid (TCA).

DMF devices were fabricated in the University of Toronto Emerging Communications Technology Institute (ECTI) cleanroom facility as described in detail elsewhere [5]. Briefly, gold electrodes were patterned by photolithography and wet etching, and the electrodes were coated with parylene-C (2 μ m, chemical vapor deposition), and Teflon-AF (50 nm, spin-coated at 2000 rpm for 1 min). In addition to patterned devices, unpatterned indium-tin oxide (ITO) coated glass substrates

were coated with Teflon-AF (50 nm, as above). Devices were assembled with an ITO-glass top plate and a patterned bottom plate separated by a spacer formed from two pieces of double-sided tape (~150 μm thick). Droplets were sandwiched between the two plates and actuated by applying driving potentials (70–100 V_{RMS} , 18 kHz) between the top electrode and sequential electrodes on the bottom plate.

Matrix assisted laser desorption/ionization mass spectrometry (MALDI-MS) spots were formed by dissolving extracted protein samples in a 1.5 μL aliquot of MALDI matrix solution (10 mg mL^{-1} sinapinic acid in 80% (v/v) acetonitrile/water) and allowing the droplet to dry on a target plate. One hundred shots were collected per spectrum, with laser power tuned to optimize the signal to noise ratio.

RESULTS AND DISCUSSION

Figure 1 illustrates our novel DMF-based method for extracting proteins from serum. In Fig. 1a,b, a droplet containing a precipitant, TCA, was dispensed from a reservoir and merged with a droplet containing FBS. The droplet was then mixed by oscillating between adjacent electrodes and allowed to incubate (Fig. 1c,b) such that proteins precipitated from solution. The unwanted supernatant was then driven away, leaving the precipitated proteins on the surface (Fig. 1e,f).

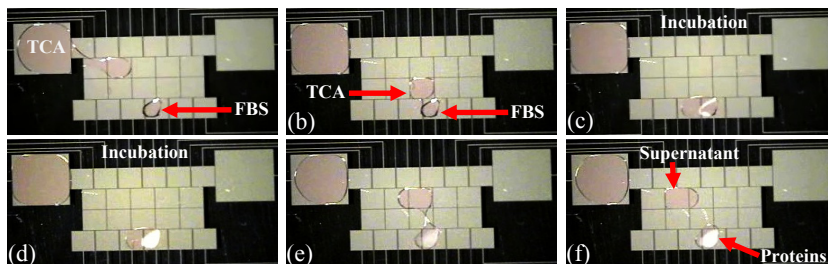


Figure 1. In (a,b), a droplet (~70 nL) containing a precipitant (TCA) was dispensed and merged with a droplet (~50 nL) containing FBS. In (c,d), the merged droplet was incubated (~10 min, 4 °C), and in (e,f), the unwanted supernatant was driven away from precipitated proteins.

Figure 2 depicts an experiment demonstrating the full protocol required for extracting and purifying proteins from a heterogeneous mixture. As shown, a droplet containing a mixture of model protein (BSA) and model contaminant (PC) was merged with a droplet containing TCA to precipitate the protein. The supernatant (containing PC) was then driven away, and the precipitate was washed in a droplet of acetone and allowed to dry. Finally, the purified protein was resuspended in a droplet of Tris-HCl buffer for subsequent reduction of disulfides, alkylation of thiols, and enzymatic digestion (not shown). Figure 2c shows representative mass spectra generated from control and extracted solutions. As shown, both spectra include peaks in the 22 000 – 66 000 m/z region corresponding to BSA. In contrast, a peak at m/z 397 (graph inset), corresponding to PC, is seen only in the control sample (not in the extracted sample). In on-going work, we are evaluating the efficiency of extraction quantitatively using fluorescence (not shown).

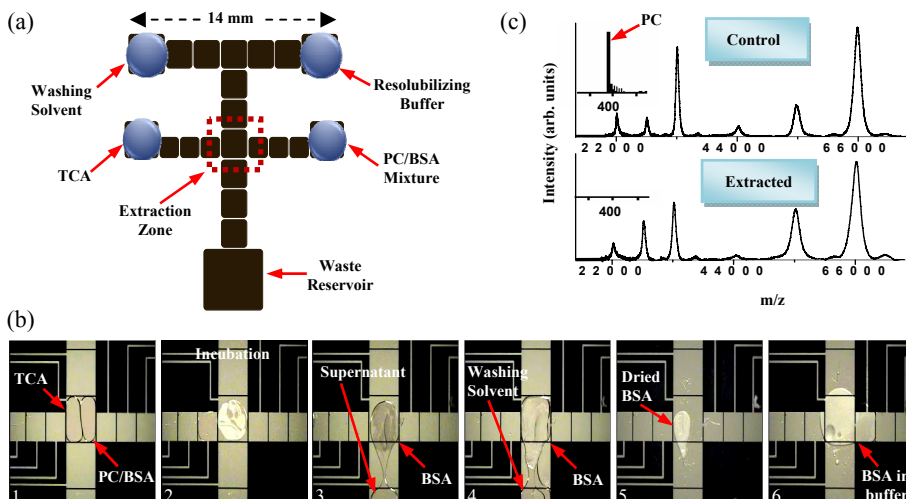


Figure 2. (a) Schematic and (b) video sequence depicting the purification of protein from a droplet (~ 70 nL) containing BSA and PC. The droplet was merged with precipitant, and then incubated (~ 10 min, 4°C) until BSA precipitated from solution. The precipitate was then washed in solvent, dried ($\sim 95^\circ\text{C}$), and resuspended in buffer. (c) MALDI-MS spectra of control and extracted samples.

CONCLUSIONS

We present the first DMF-driven method for processing protein(s) in heterogeneous mixtures. These results suggest great potential for digital microfluidics for automated clinical proteomic biomarker discovery.

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