FREE-STANDING HYDROGEL MICROARRAYS: OPEN-CHANNEL MICROFLUIDICS FOR MASSIVELY PARELLEL PROTEIN ELECTROPHORESIS

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ABSTRACT

We report here for the first time a new open-microchannel architecture for rapid protein electrophoresis. Owing to its open architecture, this technology is optimized for interfacing with automated robotic controllers and downstream processing (e.g., sample spotters, immunological probing, mass spectroscopy). We show the design, fabrication and initial feasibility testing of an open-microchannel array with favorable attributes for up-scaling into high-throughput electrophoretic separations. This multiplexed open-channel platform is uniquely well-suited for massively parallelized proteomics, a major unrealized goal from bioanalytical technology.

KEYWORDS: open-channel microfluidics, polyacrylamide gel, protein electrophoresis, moving boundary electrophoresis, photo-polymerization, free-standing microchannel

INTRODUCTION

Microfluidic technologies have largely been realized within enclosed microchannels. While powerful, a principle limitation of closed-channel microfluidics is the difficulty for sample extraction and downstream processing. While digital microfluidics (e.g., EWOD) has remedied this for discrete droplet reactors [1], such advances have been attempted [2] but have had limited success in separation science. Departing from enclosed microchannels [3], we demonstrate moving boundary electrophoresis (MBE) in a free-standing polyacrylamide gel microchannel-array as a powerful protein separation medium. We introduce a mask-based photolithographic method for parallel device fabrication atop a glass microscope (Figure 1). This microfluidic platform is realized simply, avoiding cumbersome micro-fabrication steps (drilling, etching, or bonding), and from materials that are already abundant in many labs. In addition, semi-dry polyacrylamide gel electrophoresis is a procedure that is already commonly implemented in life science labs. All together these factors improve the potential for free-standing polyacrylamide gel microchannels to be adopted by life science labs conducting proteomics research.

EXPERIMENTAL

Free-standing polyacrylamide gel microchannels are fabricated as follows. A gel precursor solution confined between the slide and a support wafer is polymerized with UV light through a photomask (Figure 1A, B). After a 200 second exposure, the desired micropattern polymerizes, excess precursor is removed and the devices are soaked in 10% glycerol. Next, the microarray is dried in ambient conditions and can be stored indefinitely in its dehydrated state [4]. Microdevices are rehydrated in any aqueous buffer of interest and ready for experimentation in minutes.

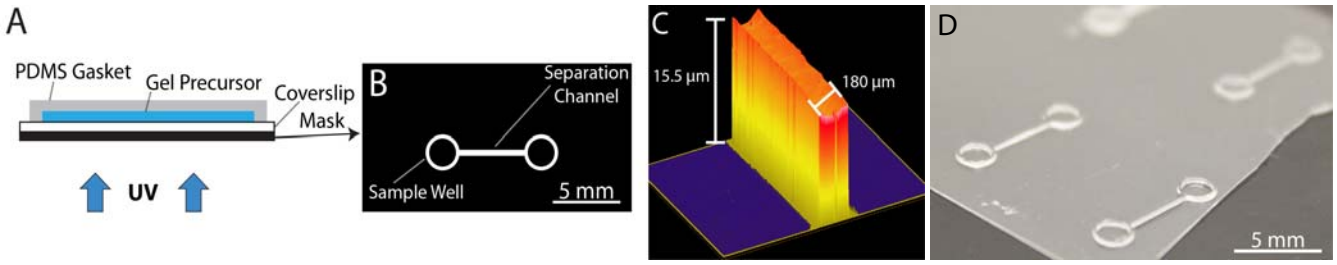

Figure 1: *Free-standing polyacrylamide gel microchannels are fabricated using a (A) mask based photolithography technique. A reservoir of gel precursor is contained above a methacrylate functionalized coverslip. The desired micropattern is defined by a photo-mask, which determines the portions of the reservoir that are polymerized by a UV light source. Our design (B) consists of a single channel connecting two fluid reservoirs. (C) Optical profilometry shows a well-defined free-standing hydrogel with dimensions and uniformity appropriate for supporting electrokinetic protein separations. (D) Using this technique, free-standing polyacrylamide microchannel arrays are fabricated in parallel atop a glass surface.*

RESULTS AND DISCUSSION

Application of protein sample and a driving electric field results in the MBE separation of fluorescently labeled proteins along the open separation-channel (Figure 2A). In MBE the moving boundaries of analytes are analyzed as opposed to discrete zones [3]. Elimination of an injection channel allows for greater device density and multiplexing. Figure 2B shows a baseline separated protein ladder in the first 250µm of the free-standing separation channel (false color) at 130s after the potential was applied; a 2.7x reduction in separation length in comparison to the closed-microchannel analog [3]. Figure 2C displays the corresponding intensity profile and the spatial derivative of the intensity, dI/dx, which yields clear identification of analyte fronts.

Separation resolution between proteins improves with time and location (Figure 3), as expected, and crosses the critical 'baseline resolved' resolution metric (SR, mean difference between peaks normalized by peak width, a separation is complete when SR > 1) in less than one minute as compared to hours for a conventional benchtop slab-gel separation.

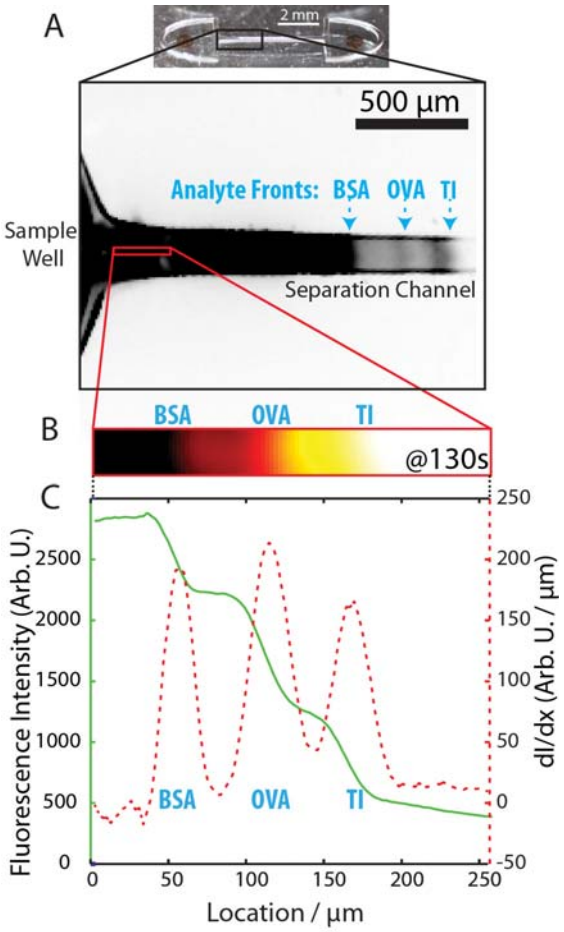

Figure 2: *A free-standing hydrogel acts as both the microchannel and sieving matrix for a native MBE protein separation (A). A 250 nM fluorescently labeled protein ladder consisting of Trypsin Inhibitor (TI), Ovalbumin (OVA) and Bovine Serum Albumin (BSA) is captured in an epi-fluorescence image. (B) A false color image of the first 250µm of the separation channel at 130s clearly shows well separated proteins. (C) The corresponding intensity profile and derivative (dI/dx) are overlaid.*

CONCLUSION

We report on a new platform for open-channel microfluidics. Free standing polyacrylamide gel microchannels enable rapid protein separations in a small device footprint and a format compatible with upstream robotic handling, post-separation processing, all of which are important factors for up-scaling into a massively multiplexed proteomics system. Our new separation paradigm advances proteomics towards the performance demanded by biomarker studies, as well as systems and synthetic biology.

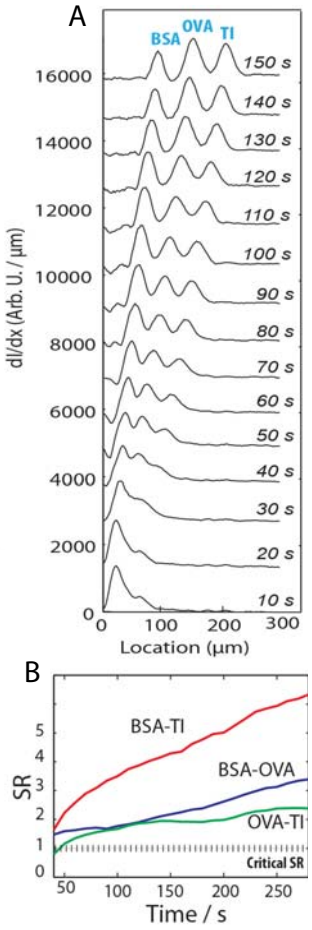

Figure 3: *The time evolution of the native protein separation from Figure 2 is displayed (B) by vertically staggering the derivative (dI/dx) from 10 s to 150 s. (B) SR is plotted for each protein. The separation is completed in 50 s and 150 µm yielding an ultra-fast, short-separation distance assay.*

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