TWO-DIMENSIONAL PROTEIN SEPARATION ENABLED BY MICROVALVE ARRAYS

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ABSTRACT

Two-dimensional (2D) protein separation was demonstrated in a plastic microfluidic device by integrating isoelectric focusing (IEF) in 1 channel and polyacrylamide gel electrophoresis (PAGE) in 29 parallel channels that are orthogonal to the IEF channel. An array of microfluidic valves was developed for introducing different separation media into two dimensions and for transferring proteins from the first to the second dimension. Two types of microvalve arrays have been evaluated. One is gel-based pseudo-valve array that is passive whereas the other is true microvalves that are thermally or pneumatically actuated. Both valves have been realized in the device with the total separation time less than 30 minutes, which is favorable when compared to 1-2 days for conventional 2D slab gel electrophoresis.

KEYWORDS

Two-dimensional separation, microvalves, electrophoresis, proteins.

INTRODUCTION

Among the approaches being developed for proteomics, two-dimensional gel electrophoresis (2DGE) is an essential tool [1]. 2DGE consists of isoelectric focusing (IEF) as the first dimension and polyacrylamide gel electrophoresis (PAGE) as the second dimension. One major advantage of 2DGE is its enormous separation resolution, whereas its key limitations include poor reproducibility and time-consuming procedure. This work is carried out to address the limitations.

Our device can be distinguished from those using two orthogonal separation channels, in which capillary electrophoresis (CE) is coupled with either micellar electrokinetic chromatography [2, 3] or IEF [4]. The key difference is the use of multiple channels, rather than a single channel, for the second dimension to match the separation capacity of the slab in 2DGE. Our method is also different from the existing work on miniaturized 2DGE [5, 6]. The key advances are twofold: (1) an array of microfluidic valves allows the seamless introduction of two different separation media; and (2) a fluidic network with densely-packed channels enables the transfer of proteins from the first to the second dimension.

EXPERIMENTAL

Cyclic olefin copolymer was used to fabricate 2D separation devices using compression molding. **Figure 1** shows the device design.

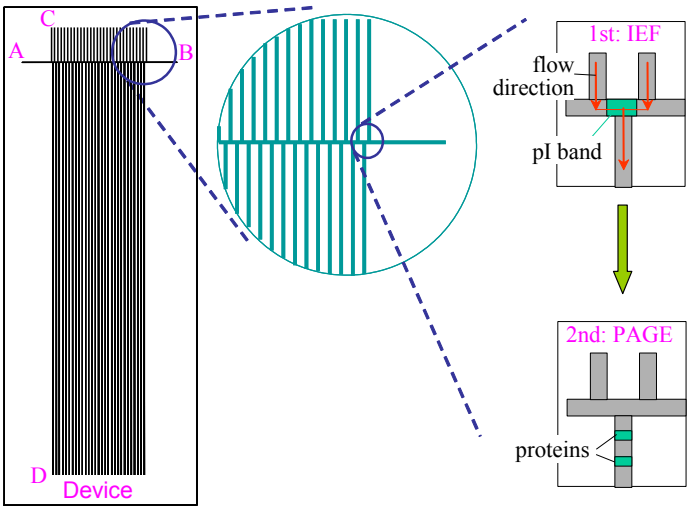

Figure 1. *Design of a microfluidic device for 2DGE.* ***(left)*** *The device consists of one horizontal channel (AB) for IEF and vertical channels (CD) for PAGE. The size of the device is 1" x 3", about the size of a microscope slide. Cyclic olefin copolymer was exploited to fabricate devices using compression molding. All channels are 110 µm wide and 40 µm deep. The distance between channels (center-to-center) is 360 µm.* ***(middle)*** *An exploded view of a part of the device.* ***(right)*** *The interface between IEF and PAGE illustrates proteins in each pI band being transferred from the first to the second dimension and then separated. The arrows indicate the direction of protein migration.*

Acrylamide/bisacrylamide (19:1), 1-hydroxycyclohexylphenylketone (HCPK), proteins were purchased from Sigma-Aldrich (St. Louis, MO) while carrier ampholytes were from Bio-Rad Laboratories (Hercules, CA). Alexa-488 conjugated proteins were purchased from Invitrogen (Grand Island, NY). The detection was carried out using a laser-induced fluorescence, whole-channel imaging system or a fluorescence microscope.[7]

RESULTS AND DISCUSSION

We have developed two types of microvalve arrays. The first one is gel-based pseudo-valve array as shown in **Figure 2**, and the concept has been reported previously.[8] The valve array was created by *in situ* gel polymerization. To make gel valves at a precise location, acrylamide monomer solution containing HCPK was first filled in all channels. HCPK is a photosensitive agent to initiate gel polymerization, replacing chemical initiators. After the AB channel of the device in Figure 1 was covered by a mask, photopolymerization was conducted by exposure to UV light. The solution in the exposed region (CD channels) polymerized to form gel, whereas the solution in the AB channel was blocked by the mask and did not polymerize. The non-polymerized solution was then replaced with the IEF medium. The gels in CD channels functioned as "closed" valves when an IEF separation medium was introduced into channel AB, without flowing into CD channels. After IEF, they functioned as "open" valves when an electric field was applied to the CD channels, allowing the focused proteins to be electrokinetically transferred form the first to the second dimension. This transfer took place because proteins can be electrokinetically injected through gels.

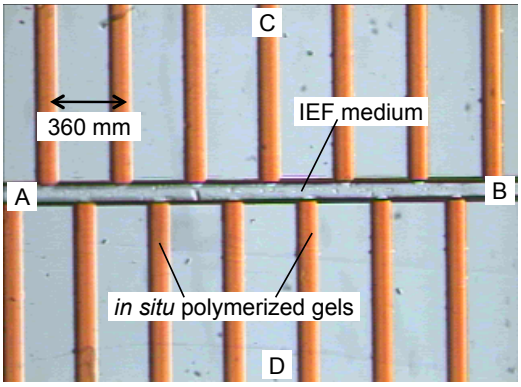

Figure 2. *Micrograph of the pseudo-valve array formed by in situ gel polymerization. Gel was formed in the CD channels whereas the IEF medium was in the AB channel. Polymerized gels were dyed for easy visualization.*

An alternative valve array is comprised of true microvalves; the operation principle of a single valve is shown in **Figure 3a**. The valve is made of four plastic layers as reported previously.[9] The top layer contains microfluidic channels; the second layer is an elastomer. The third layer is a valve layer that houses a thermal-sensitive liquid. The bottom heater layer contains micro-patterned resistors created on a cover film. When a temperature-sensitive fluid is heated, its volumetric expansion deflects the elastomeric film into the microchannel, closing the valve. In addition to thermal actuation, the valves might be actuated pneumatically as demonstrated by Quake's research group.[10]

The locations of the thermally or pneumatically actuated microvalves are indicated by the rectangular blocks on the both sides of the IEF channel in **Figure 3b**. The operation of such a microvalve has been demonstrated by the observation of synchronization between heat supply and valve actuation in the device.

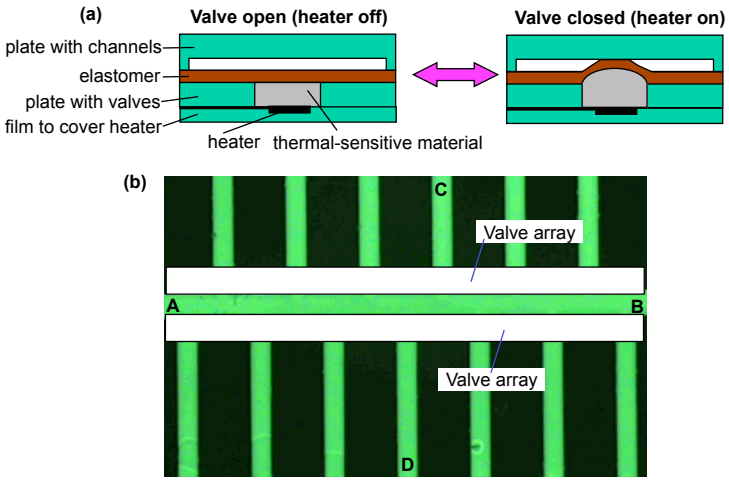

Figure 3. (a) *Schematic of a thermally actuated microvalve. On the left is when the valve is open and the heater is off. On the right is when valve is closed and the heater is turned on. The drawing is not to scale.* ***(b)*** *Picture of the interface of 2DGE device (filled with a fluorescent solution), in which two valve arrays are indicated by two rectangular blocks.*

In the presence of gel-based microvalves, 2D separation of proteins was implemented in the device as shown in **Figure 4**. The pH gradient was established using carrier ampholytes with a pH gradient of 3-10. It should be noted

that the IEF analysis time was typically in minutes, much shorter than hours needed in the traditional slab gel format. Seven fluorescently-labeled proteins were used in this experiments. All proteins were separated by their difference in either pI value or size except for trypsin inhibitor and S-100b (since they have essentially same pI and molecular weight). The double peaks for some proteins are likely due to heterogeneity in labeling and/or and splitting of protein bands into neighboring PAGE channels during the transfer stage. The result demonstrates the feasibility of 2D separation in a device enabled by microvalve arrays. The total analysis time for IEF and PAGE is <30 minutes. Compared to typical analysis time of 1–2 days for obtaining a map using an IEF strip and slab gel electrophoresis, the device shows a significant improvement in analysis time.

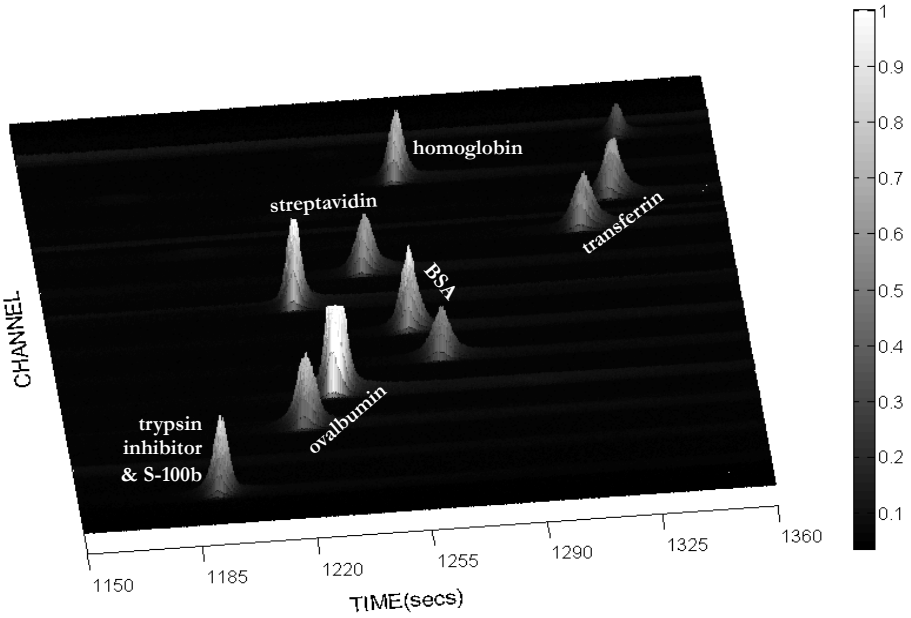

Figure 4. *2D separation of proteins including S-100b, transferrin, bovine serum albumin (BSA), ovalbumin, trypsin inhibitor, hemoglobin and streptavidin. The channel number in the Y-axis is related to pI (isoelectric point) of proteins and the migration time in the X-axis is related to the molecular weights of proteins; and the signal in the Z-axis indicated the amount of the protein. The bar indicates the relative abundance of the protein, represented by the signal intensity.*

CONCLUSION

We demonstrated the integration of IEF and multi-channel PAGE in a plastic microfluidic device. The key element that enables 2D electrophoresis in the device is an array of microfluidic valves, which was either passively controlled as in the gel valves or actively controlled as in the true valves. The valve arrays allowed the introduction of different separation media into two dimensions and the transfer of proteins from the first to the second dimension.

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