Optimization of Microfluidic “Lab-on-a-Chip” Devices for Capillary Electrophoresis Separations

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Abstract

Microfluidic devices (MDs) are powerful tools for performing an array of applications. However, the volume of parallel processing are advantages of MDs that make them ideal for biochemical analysis, high-throughput screening, and other limited reagent scenarios. A major challenge associated with the desired features of an MD is to simultaneously reduce the number of prototyping steps needed to test the devices while maintaining the sample volume over several reactions or separation steps. Capillary electrophoresis is a technique that has shown great promise with coupled to microfluidic devices. Although CE has gained widespread use because of its versatility there is still the need to prepare samples at variable concentrations which inherently slows down the analysis rate. Using multilayer soft lithography (MSL), fluid and control channels are fabricated to allow for manipulation of material on the device without the need of time-consuming prototyping steps. Subsequent electrophoresis using CE affords separation of material. Herein, we describe our work on coupling affinity CE (ACE) to “lab-on-a-chip” devices using as a model system the interaction of aryl sulfonamides to carbonic anhydrase B (CAB, EC 4.2.1.21).

Introduction

The development of new molecular biological techniques has provided for a re-task of biological interactions. One technique that has shown great promise in quantifying receptor-ligand interactions is the assay developed by capillary electrophoresis (CE). In a typical form of CE a sample of receptor and a non-interacting standard(s) are loaded into the capillary column and are electrophoresed in an increasing concentration of ligand in a running buffer generating an electropherogram readout. A change in migration time of the receptor relative to the standard(s) is indicative of the formation of the receptor-ligand complex. This change in migration time is then used for Scatchard analysis. Based on the Scatchard analysis, an equation is used to calculate a binding constant value (Kd) on a relative scale using the non-interacting standard(s).

In these studies, when quantities of materials were limited, CE was the ideal technique to be utilized to measure receptor-ligand interactions. Multilayer MDs have been fabricated and shown to be capable of running comparable CE experiments to those previously seen on large electrophoresis machines, on a smaller scale and at a fraction of the cost. Microfluidic devices and the analytical workflow that have made the fabrication and experimentation of MDs desirable.

Discussion

MD’s created during the course of this research were made using Multilayer Soft Lithography (MSL, utilizing poly(dimethylsiloxane) (PDMS), a silicone rubber using 2-component elastomer materials (GE RTV or Sylgard)). An AutoCAD design was developed onto a mold by incorporating photolithography SU-8 (neg) photoresist. There were two major components necessary for microfluidic devices: two separate layers created in a 4:1 (thick layer: thin layer) ratio, which are representative of the “flow layers” where reagents are deposited and manipulated and the “control layer” where air is utilized to control the flow direction of R15 AI055515-01 (R15 AI065468-01). Programs, and the National Institutes of Health (R15 AI055515-01)

Conclusion

We have demonstrated that affinity capillary electrophoresis (ACE) coupled to a microfluidic format can be utilized in the estimation of binding constants between a receptor and a ligand. Using the model system carbonic anhydrase B (CAB) and its binding to 4-carboxybenzenesulfonamide (Ligand 1), in theses studies, a plug of increasing concentrations of 1 was injected into the microfluidic device at 10 sec, followed by buffer for 10 sec, then by a sample containing DMF and CAB. DMF is a non-interacting standard which does not interact with CAB. The samples are then pushed past the anode and into the capillary. Upon electrophoresis, the sample flows into the zone of separation. A major challenge associated with the desired features of an MD is to simultaneously reduce the number of prototyping steps needed to test the devices while maintaining the sample volume over several reactions or separation steps. Capillary electrophoresis is a technique that has shown great promise with coupled to microfluidic devices. Although CE has gained widespread use because of its versatility there is still the need to prepare samples at variable concentrations which inherently slows down the analysis rate.