INJECTION AND ULTRAFAST MIXING OF ATTOMOLE SAMPLES VIA MICRO-NANOFLUIDIC GATES FOR ON-CHIP BIOCHEMICAL ANALYSIS

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ABSTRACT

A three-dimensional microfluidic circuit with nanofluidic interconnecting gates has been developed for the injection and extremely rapid mixing of analytes for chip-based biochemical assays. Polycarbonate nanoporous membranes containing 15- to 200-nm diameter cylindrical pores are employed as electrically-controlled molecular gates to inject bands of ultrasmall analyte samples (~10 attomoles) from one microchannel channel to another, and to completely mix and react analytes within microns of the injection point. The molecular gating, injection, and rapid mixing functionalities depend on the electrokinetic flow through the nanopores, which separates the microchannels.

Keywords: biochemical analysis, laminar flow injection, micro-mixing, nanofluidic

INTRODUCTION

Injecting, mixing, and reacting analytes are important functions for the many chip-based biochemical assays being developed(1-3). We report on the fabrication and testing of a three-dimensional microfluidic circuit with nanofluidic interconnecting gates that

Figure 1. (a) Top view of a micro-nanofluidic bioassay chip where analytes are first separated in a channel and bands are then injected into another channel via nanofluidic membrane sandwiched in between. (b) A close-up of the injection point. (c) A side view of the stack, with the interconnect layers bonded between two glass layers, with a PDMS top layer to facilitate attaching inlet and outlet tubes.

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allow injection and extremely rapid mixing of ultralow attomole \((10^{-18})\) quantities of analytes. Polycarbonate nanoporous membranes containing 15 to 200 nm diameter cylindrical pores are employed as electrically-controlled non-moving gates to establish fluidic communication between microfluidic channels in vertically separated planes. The nanofluidic pores in the regions of crossing microfluidic channels are electrokinetically addressed, enabling these nanofluidic gates to determine flow based upon chemical composition, molecular size, or electrophoretic mobility\(^{[4-6]}\). This hybrid microfluidic/nanofluidic architecture allows ultrasmall analyte samples (thus far down to tens of attomoles) to be separated in one channel, single bands to be injected and collected into another channel with near 100% mass efficiency\(^{[7]}\), and then completely mixed and reacted within \emph{microns} of the injection point.

**RESULTS AND DISCUSSION**

Figure 1 shows a glass-based separation and injection chip consisting of a stack of patterned dissimilar materials: glass, polycarbonate membranes, PDMS, and other polymer layers. The bottom piece serves as the assembly platform and comprises a glass substrate with standard electrophoresis channels etched into it. To this substrate are bonded alternating layers of polycarbonate membranes with addressable nanopores and

![Figure 2](image-url)

Figure 2. (a) Schematic representation of injection channel connected to separation channel via nanofluidic membrane. (b) Electrical bias configurations for active electrokinetic injection control were repeated for serial separations with either manual or automated timed computer control. (c) Fluorescence image (captured from video) demonstrating the dependence of injection (membrane gate) bias on amount injected.
polymer spacer layers with microfluidic channels etched in them. The crossed channels visible in Fig. 1b have polycarbonate nuclear-tracked nanopore membranes (~6-10 μm thick) in between glass wafers, as shown in Fig. 1c. The crossed channels are conceptually shown in Fig. 2a to show the separation and injection channels. By controlling the voltage between the channels, electrokinetic flows can induced within each channel, and then across the nanopore member, as shown in Fig. 2b. As illustrated in Fig. 2c, analyte can be selectively removed from the separation channel and injected into the collection channel. For the nanopore membranes used, no measurable cross over of analyte is observed without a bias applied across the membrane. As discussed in [7], the electrokinetic injection across the nanofluidic gate occurs with near 100% mass efficiency. Plus, extremely narrow bands of analyte can be removed from the separation channel and injected into the collection channel[1]. Control of the analyte injection for 15 nm pore membranes is on the order of 0.1 attomoles (10^{-18}) per volt, with a nearly linear response until saturation occurs at about 200 V drop.

In addition to exquisite control in the quantity of injected analyte, another notable feature of the micro-nanofluidic gate is ultrafast mixing of the analyte in the collection channel. Figure 3 illustrates this ultrafast mixing by monitoring the Ca^{2+}-CGD binding reaction that can only occur when Ca^{2+} is injected into the collection channel. As the fluorescence image in (a) and the intensity plot in (b) shows, the Ca binds completely within the gating time, and well before fluid has traversed the width of the injection channel, or about 30 μm. Note that the flow velocity in both channels, as well as through

Figure 3. (a) Fluorescence images of Ca^{2+}-CGD binding reaction. Left, membrane reverse bias. Right, membrane forward bias, Ca^{2+} is injected into the horizontal CGD channel. (b) Fluorescence intensity (left ordinate) and applied bias state, (right ordinate, ...) as a function of time showing transport of Ca^{2+} across 200 nm pore diameter PCTE membranes into the 2-μM CGD-containing channel. (c) Fluorescence intensity of CGD-Ca^{2+} in the receiving channel as a function of [Ca^{2+}].
the nanopore itself, is creeping, with \( \text{Re} \ll 1 \). The flow is highly laminar and under such conditions, complete mixing between the two microchannels would be expected to occur by diffusion over many millimeters.

The mechanisms for the ultrafast mixing are still under investigation. However, mixing in the lateral direction due to the close spacing of the nanopores in the nuclear-tracked membranes is conceptually shown in Fig. 4. Diffusion between the fluid leaving the pores and the surrounding fluid occurs on the order of microseconds, thereby laterally mixing the fluid streams within microns of fluid travel in the lateral direction, depicted by the dimension \( d \). The average distance \( d \) is determined by the pore density and ranges from \( \sim 0.1 \) to \( 10 \mu m \). Interestingly, the distance \( x \) that the mixing occurs over appears to be surprisingly large, on the order of many microns, for electrokinetic injection using the nanopore membranes. At this time, we believe this transverse mixing may be due to the high electric fields present at the nanopore exit, creating electrophoretic velocities high enough to effectively increase mixing in the \( x \) direction. The result of both interactions is that the two streams completely mix within microns of the intersection between the two crossed channels.

REFERENCES