MICROVALVE-ADDRESSABLE PICOLITER CHAMBERS FOR SINGLE-MOLECULE ENZYMOLOGY

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ABSTRACT

This paper reports a novel microfluidic device for characterizing low-copy enzymatic reactions via parallel mixing of picoliter-scale fluid volumes. Integrated polydimethylsiloxane (PDMS) microvalve arrays are used to control filling and mixing of a nanoliter-sized chamber with 1-9 picoliter-sized chambers. A proof-of-concept enzymatic reaction was performed in which the device was loaded with the enzyme alkaline phosphatase (ALP) and the fluorogenic substrate fluorescein diphosphate (FDP). Fluorescence microscopy was used to observe the rate of fluorescence increase once enzyme and substrate solutions were allowed to diffusively mix.

KEYWORDS: Microvalves, Enzymology, Tunable, PDMS

INTRODUCTION

Miniaturization of benchtop biochemical assays is revolutionizing our ability to precisely measure and understand a variety of biological functions. Microfluidic systems, when combined with soft lithography techniques, provide simple, low-cost, and high-throughput platforms for analysis [1]. Portable microfluidic systems with precise fluidic handling and volume dispensing control functions are of great interest in many biomedical assays. Various methods have been established for controlling enzymatic reactions in confined volumes using photoresist-reflow [2] microvalves or capping of PDMS wells with a glass slide [3]. While these designs have shown a significant improvement over traditional methods for biochemical analysis, they suffer from particular drawbacks. Photoresist-reflow microvalves have rounded cross-sections microchannels where the channel width is a function of the channel height. This means that a wide range of channel or chamber design heights is not possible on a single microfluidic chip (a crucial advantage for certain microfluidic cellular studies [4]) and the curvature of the channel acts as a lens (an impediment for phase-contrast microscopy). The design presented here offers enhanced portable storage of sub-nanoliter fluid volumes and allows for characterization of enzymatic reactions, potentially down to single-molecule concentrations.

THEORY

A schematic of the "picoliter device" is presented in Fig. 1. Standard soft lithography techniques were used to create the channels and chambers. Two independent groups of chambers, one set of "nano-chambers" (100 μm height, ~9 nL volume), and another set of smaller "pico-chambers" (~5 μm height, ~3.5 pL volume), are separated by automated control valves. Upon opening of the microvalves, the nano and pico-chambers are put in communication and fluid is allowed to mix.

978-0-9798064-1-4/μTAS2008/$20©2008CBMS

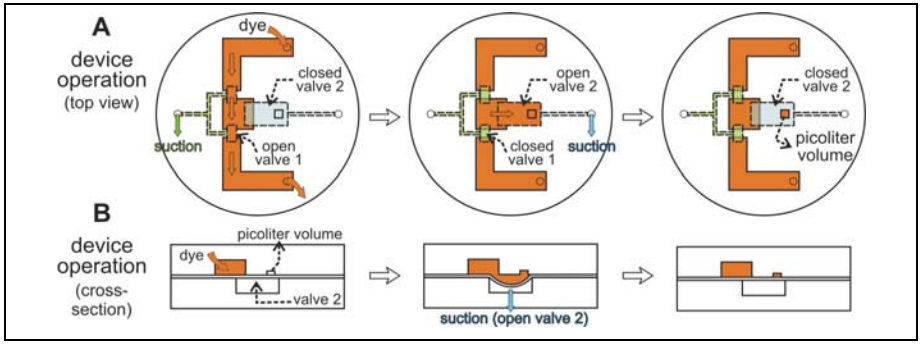

Figure 1. Device operation from a top view (A) and side view (B).

EXPERIMENTAL

To perform a proof-of-concept enzymatic reaction, the picoliter chambers were (first) filled with the enzyme ALP and (second) the nanoliter chambers were filled with the fluorogenic substrate FDP. Cleavage of the phosphate groups from FDP (a low-fluorescence compound) releases fluoresecein (a high-fluorescence compound); hence the increase in fluorescence as a function of time can be used to measure enzymatic reaction rates. With the microvalves closed (i.e. no fluid communication between the picoliter and nanoliter chambers), hydrolysis of FDP was negligible, indicating that background adsorption of enzyme onto the device surface can be neglected. Upon opening of the microvalves, the fluorescence signal increased as a function of time, at a rate that was a function of the number of picoliter chambers that were communicating with any given nanoliter chamber. Fig. 2 shows a typical fluorescence microscopy image in which fluid has been trapped in the pico-chambers.

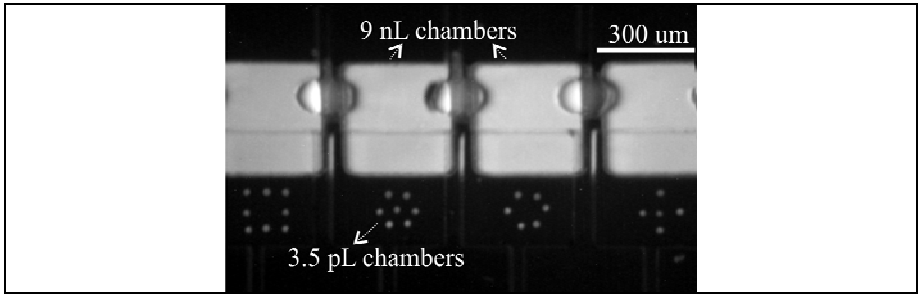

Figure 2. Fluorescence micrograph showing fluid separation in both nano and pico chambers.

RESULTS AND DISCUSSION

Fig. 3 (A) shows a typical experimental result; fluorescence intensity is highest in the nano-chambers that are in communication with the highest number of pico chambers, as shown in (B). Simple calculations demonstrate that it is possible to

control the entrance of a single molecule of enzyme into the nanoliter chambers. This could be accomplished by diluting the enzyme substrate before experimentation, or by altering the device to have smaller pico-chamber volumes, thus trapping smaller volumes of fluid.

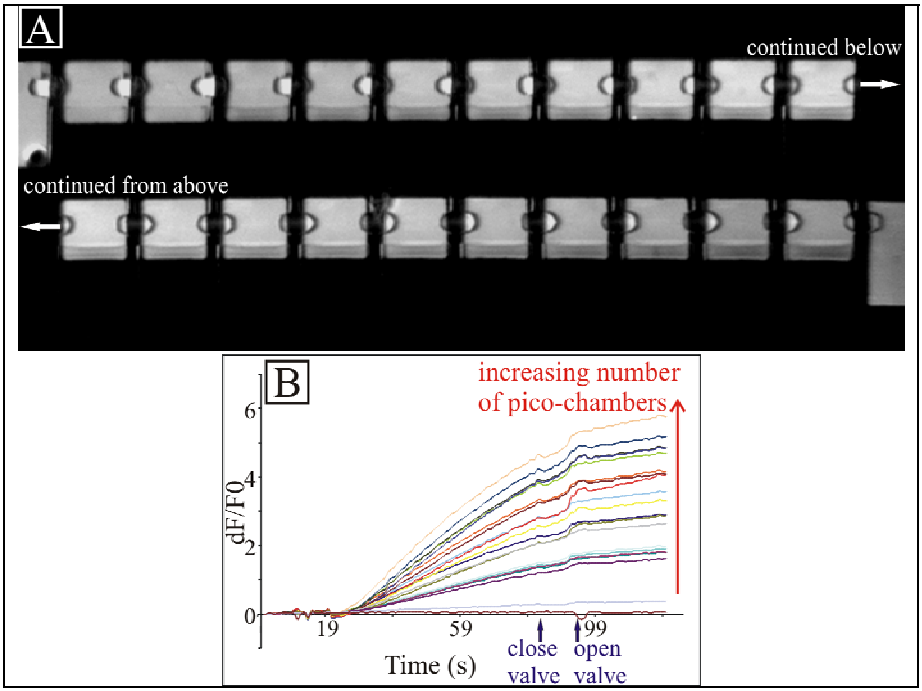

Figure 3. Visualization of results (A) and analysis of fluorescence increase (B).

CONCLUSIONS

In comparison to existing microfluidic mixing devices, this picoliter-scale device offers enhanced portable storage of subnanoliter fluid volumes and allows for easy characterization of single-molecule enzymatic reactions.

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