ACTIVE MICROFLUIDIC MIXER USING VIRTUAL SOURCE-SINK PAIRS FOR DNA PURIFICATION

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ABSTRACT

We present an active mixer based on the creation of virtual source-sink pairs in a polydimethylsiloxane (PDMS) chip. Perfect mixing is achieved in only a sub-second time interval for a compact 5 μL mixing volume. This excellent performance is achieved thanks to the implementation of co-rotating vortices inside the mixing chamber by using microplumbing technology. A DNA isolation and purification protocol using magnetic beads in the mixing chamber is performed on-chip, showing the enhanced DNA binding performance due to active mixing.

KEYWORDS: Active mixing, source-sink flow, DNA purification

INTRODUCTION

Mixing is a challenging issue in microfluidic devices due to the low Reynolds number characteristics of the flow inside microfluidic channels. A rotary mixer can present a general solution for active mixing in affordable polydimethylsiloxane (PDMS) chips realized using microplumbing technology, but this mixing concept requires long mixing times and channels to get efficient mixing, even for nanoliter volumes [1, 2]. This situation can pose a bottleneck for high-volume (microliter) applications, such as in clinical diagnosis [3]. Here, we present an active microfluidic mixing in a compact 5 μL volume for DNA purification. Perfect mixing is achieved in only a sub-second time interval (i.e. 3300 fold faster than pure diffusion-based mixing) with a virtual source-sink pair (VSSP) flow concept realized using PDMS-based microplumbing technology. This extremely fast mixing performance is also of great benefit to DNA purification.

THEORY

The mixer with a 5 μL mixing chamber, shown in Figure 1a, is fabricated from PDMS by using a multilayer soft lithography technique [4]. Four integrated microfluidic valves are used for fluid filling and isolation in our device [4]. After pressurizing the former, a flow is generated by pressurizing one mixing control chamber and at the same time depressurizing the neighboring one, as illustrated in Figure 1b. This creates an unidirectional fluidic flow from the squeezed region (source) to the released region (sink). Pulsed source-sink flows assure a fast mixing process with chaotic advection, characterized by exponential stretching and folding of microfluidic streams [5]. As described in Figure 1c, optimum mixing is performed with 4-VSSPs by periodically pressurizing four PDMS membranes between control and mixing chambers to form pulsed source-sink flows. During operation, the PDMS membranes preserve their previous status until a fresh stroke pressurizes or releases them.

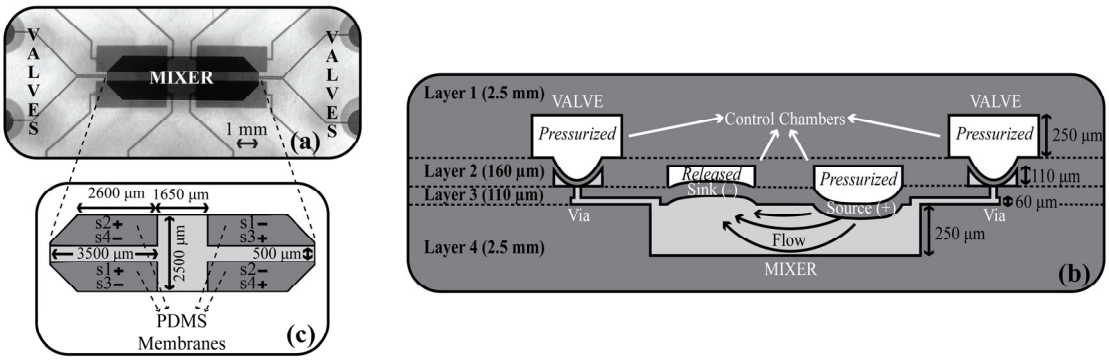

Figure 1: (a) Photograph of the PDMS chip with four valves, and a 5 μL mixing volume. (b) Cross-sectional schematic view of the device composed of four bonded PDMS layers with an illustration of a virtual source-sink pair (VSSP) flow generation. (c) 4-VSSP mixing configuration studied in the microfluidic mixer chamber and the actuation sequence of the four PDMS membranes. The character 's' on top of a pair of PDMS membranes indicates the source (+) or sink (-), respectively, at a particular sequence of the mixing cycle (expressed by the number 1, 2, 3 or 4).

EXPERIMENTAL

MHP1 normally-closed 3/2 way solenoid valves (Festo SA, Yverdon-les-Bains, Switzerland) are utilized to control compressed air for pressurizing the control chambers. All fluidics are driven with a Nemesys dosing system (Cetoni GmbH, Korbußen, Germany) equipped with two 1 mL borosilicate syringes (ILS Innovative Labor Systeme GmbH, Lausanne, Switzerland). The flow rates of the syringes are set with the Nemesys software.

For mixing characterization, the mixing chamber is filled with E122 red-colored solution (Coop Co., Lausanne, Switzerland) and deionized water (CMI, EPFL, Lausanne, Switzerland), side-by-side introduced at 100 nL/s flow rates to the chamber. During the mixing operation, the mixing index (*MI*), i.e. the normalized deviation from the average color

intensity, is monitored at the center region of mixing chamber, which is uncovered with control chambers, to quantify the mixing performance [6].

The DNA purification is conducted in our device by using Roche MagNA Pure LC DNA isolation kit I reagents (Roche Diagnostics AG, Rotkreuz, Switzerland) composed of paramagnetic glass particles in isopropanol (99-100%), lysis/binding buffer (guanidinium thiocyanate (1–5%) and Triton X-100 (1–5%)), wash buffer I (ethanol (30–40%) and guanidinium chloride (1–5%)), wash buffer II (ethanol (40- 50%)) and elution buffer (TE buffer at pH 8.0 : Tris-HCl (10 mM) and EDTA (1 mM)). Paramagnetic particles are rinsed twice in PBS and they are diluted 10 times in a lysis/binding buffer. λ-DNA (Invitrogen AG, Basel, Switzerland) solution is also prepared in the lysis/binding buffer. All the solutions are introduced in the mixing chamber at a 100 nL/s flow rate using the syringe pump after the following chip treatment steps. First, the microfluidic channels are cleaned with ethanol 95 % and washed with phosphate buffered saline (PBS) thoroughly. To eliminate unspecific absorption DNA on the channel walls, 1 % bovine serum albumin (BSA) in PBS is incubated for 10 min and subsequently the channels are rinsed with PBS, which was purchased from Sigma-Aldrich Chemie GmbH (Buchs, Switzerland) like the ethanol 95 % and BSA.

A PicoGreen ds-DNA quantification kit (Invitrogen AG, Basel, Switzerland) is used for DNA quantification. The recovered DNA solution from the chip (5 µL) is diluted in TE buffer (75 µL) and mixed with a PicoGreen solution (80 µL). The prepared sample is transferred to a 96-well plate and it is distributed into 3 wells that each contains equal amounts (50 µL). Then the fluorescent intensities of each well is read in a microplate fluorometer (Cytofluor series 4000, Applied Biosystems, Rotkreuz, Switzerland) with 485 nm excitation and 530 nm emission wavelengths. The mean value of the measured results is calculated and consequently the eluted DNA concentration is quantified using standard curves.

RESULTS AND DISCUSSION

Figure 2a shows sequential photographs of the mixing operation of deionized water (DIW) and a E122 red-colored solution using a 70 kPa actuation of the control chambers at 10 Hz. Within the closed mixing chamber, color homogeneity is obtained in a matter of a few tens of mixing cycles due to the induced co-rotating vortices, shown in Figure 2b, resembling a chaotic mixing process [7]. From the *MI* values of Figure 2c, we deduce that the fastest mixing performance is achieved for the highest actuation pressures and frequencies, which evidently give rise to the highest flow velocities resulting in a maximum number of mixing cycles per unit time. The best mixing performance is achieved with 200 kPa actuation at 20 Hz and is characterized by a mixing time, required to reach a *MI* value of 0.1, of only ~ 750 ms, which is ~ 3300-fold faster than a pure diffusion-based (passive) mixing process.

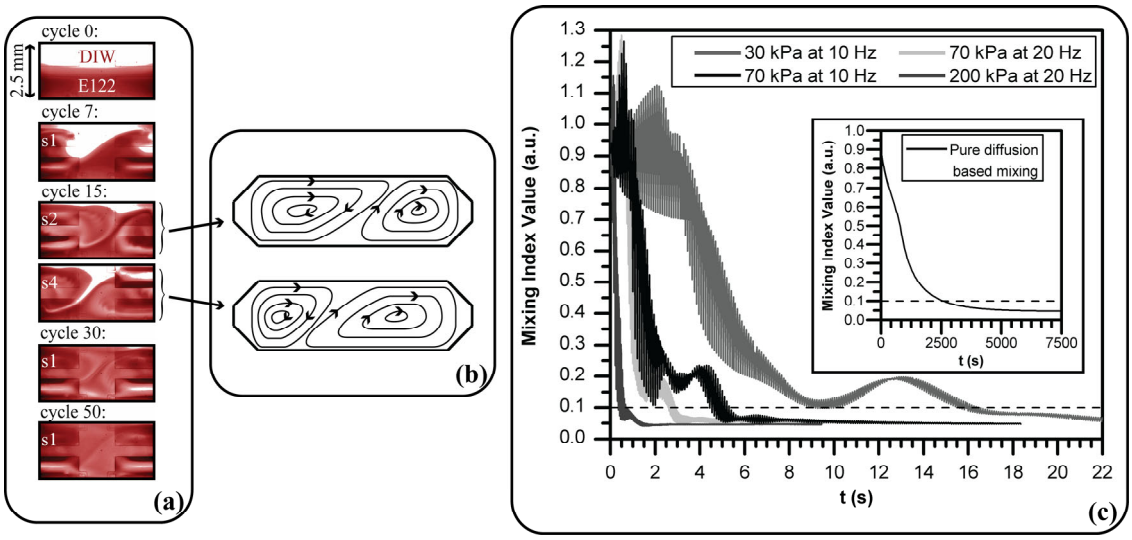

Figure 2: (a) Sequential optical micrographs of the mixing of a colored (E122) solution and color-less deionized water (DIW) using active mixing with a 70 kPa actuation pressure at 10 Hz. (b) Schematic flow patterns of the mixing configuration during the second (s2) and fourth (s4) sequences of the fifteenth mixing cycle. (c) MI values at different times of the mixing process using different actuation pressures and frequencies, compared with the MI values of pure diffusion-based (passive) mixing.

The mixer performance is also characterized by conducting on-chip the DNA purification protocol shown in Figure 3a. 1000 ng/mL initial concentration of λ-DNA is purified with various mixing conditions during the DNA binding step (third step in Figure 3a), where DNA strands are captured on the silica surface of the magnetic beads due to electrostatic interactions in the presence of chaotropic salts [8]. As shown in Figure 3b, the time needed for DNA elution is strongly improved when using active mixing. The process is essentially achieved when the magnetic particles' DNA binding capacity is reached, that is only after 10 s for 200 kPa actuation at 20 Hz.

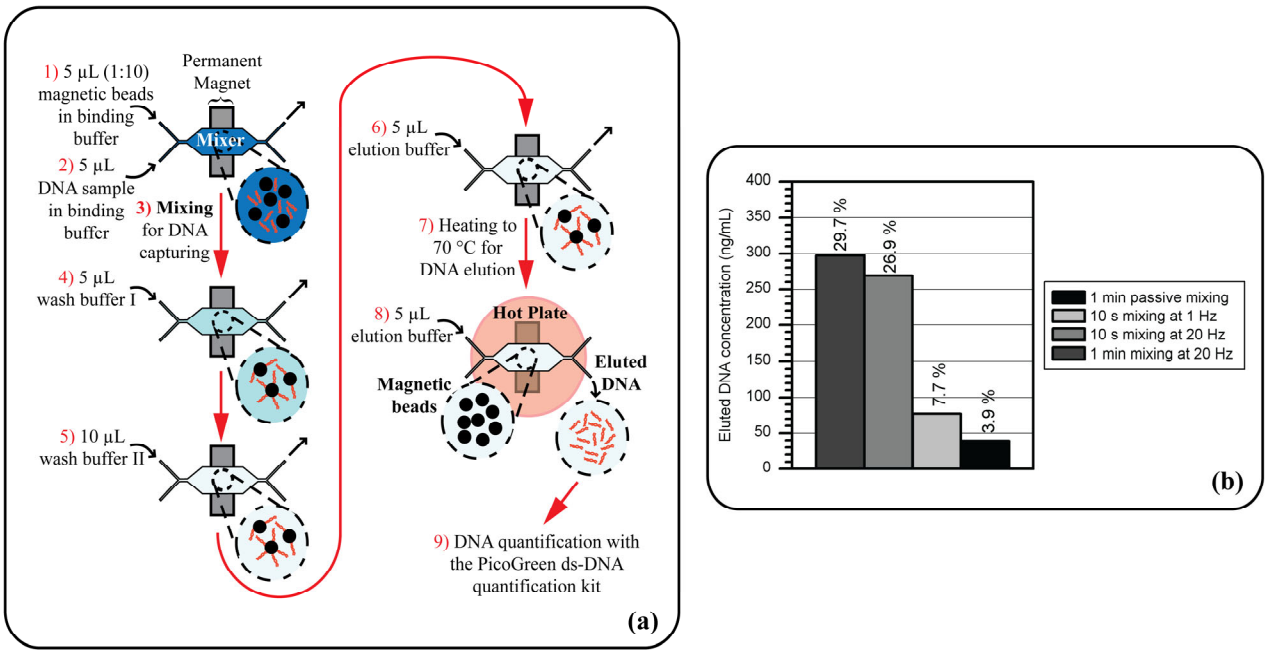

Figure 3: (a) Protocol of the DNA purification and quantification process using the Roche MagNA Pure LC DNA isolation kit and the PicoGreen ds-DNA quantification kit, respectively. (b) The eluted DNA concentration obtained for different mixing conditions in the binding step of the protocol presented in Figure 3a for 1000 ng/mL initial concentration of λ-DNA. Mixer control chambers are actuated with 200 kPa pressure at different frequencies for active mixing.

CONCLUSION

Mixing with VSSPs assures an extremely fast mixing performance for microliter volumes. Successful on-chip DNA isolation is also demonstrated and it is shown that DNA binding performance is improved when using active mixing. We therefore think that the proposed mixer will offer new opportunities for integrated, low-cost, fast and high-volume biological analysis applications.

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