Integrated Microfluidic Device for Cell Lysis in a Continuous Flow Mode

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***Abstract*—When working with cells, the first step is often to lyse them in order to get the cellular content before analysis. Some bacteria, especially gram-positive, may be difficult to lyse with conventional lysis buffer. If the cells are not properly lysed, the quality of the analysis results might suffer. In this paper, a planar micromixer design based on a splitting and recombination (SAR) concept with ellipse-like micropillars is proposed for cell lysis. With a splitting and recombination concept, the diffusion distance of the fluids in a micromixer is decreased. Thus, space usage for micromixer of an automatic sample collection system is also minimized. The efficiency of the proposed micromixer is comparable to other conventional lysis methods.**

***Index Terms*—micromixer, passive mixing, splitting and recombination, microfabrication, lysis**

I. Introduction

With a few exceptions, all bacteria are enclosed by a protective cell wall, comprised of peptidoglycan. It is common to distinguish bacteria according to the composition of their cell walls; Gram negative bacteria have a thin layer of peptidoglycan covered with a second, outer membrane. Gram positive bacteria cell walls have a simpler structure without the outer membrane, but with a thicker layer of peptidoglycan. This cell wall structure makes Gram positive generally more rigid and better fit to withstand external forces than Gram negative bacteria.

To conduct isolation of nucleic acids for various molecular biological methods it is crucial that cells are properly lysed. Especially Gram positive bacteria may be challenging to break. Therefore, conventional isolation methods have resulted in poor recovery of nucleic acids from Gram positive bacteria [1], [2].

Various procedures have been used to break cell walls; chemical lysis, enzymatic lysis and physical disruption [2]-[5].

Enzymatic break down of cell walls works well on gram positive bacteria [5]. But enzymes are often expensive and lysis by enzymatic methods may demand long incubation periods. Niwa et al. were able to decrease the incubation time of lysis with the lytic enzyme labiase by the addition of SDS (sodium dodecyl sulphate), to 5 minutes [3].

De Boer et al. found that beating bacteria with beads prior to isolation could increase the DNA outcome [2]. The quality of the outcome depended both on the bead size and isolation method. For bacteria 0.1 mm beads had a positive correlation, whereas 1.4 mm had a negative correlation with DNA outcome. A rapid protocol of breaking Gram positive cells with glass beads prior to RNA isolation was also reported to be successful [4].

In this paper, a simple and low cost splitting and recombination micromixer with ellipse-like micropillars is proposed for cell lysis in a continuous mode. The effect of the lysis treatment was verified by Nucleic Acid Sequence Based Amplification (NASBA).

II. Design of Micromixer

A. Micromixer Unit

The term ellipse-like micropillar is an element having the shape of an ellipse (Fig. 1) [6]. There are three steps in a splitting and recombination process: flow splitting; flow recombination; and flow rearrangement. When the main flow reaches the ellipse-like micropillar, the flow is split into two separated flows in the smaller channels. For an incompressible fluid, the equation of continuity (mass conservation of fluid) is defined as [7]

$$A_1 . u_1 = A_2 u_2 \quad (1)$$

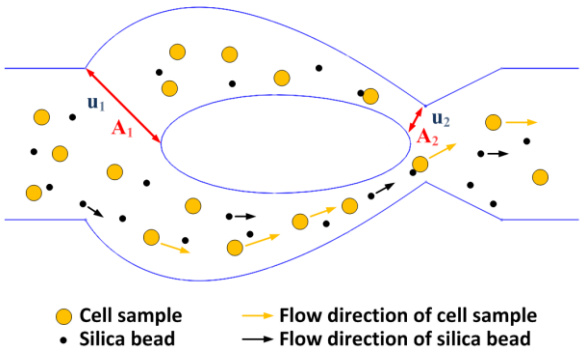

Figure 1. Mixing unit of SAR micromixer

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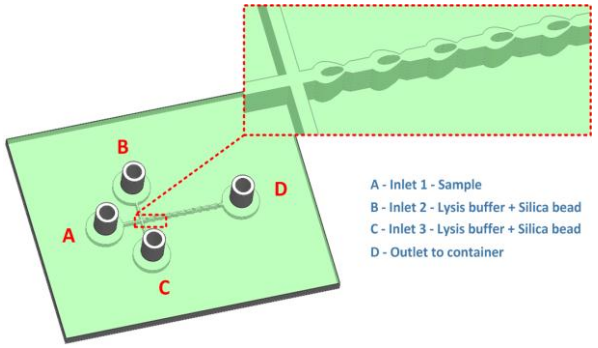


Figure 2. SAR micromixer for cell lysis

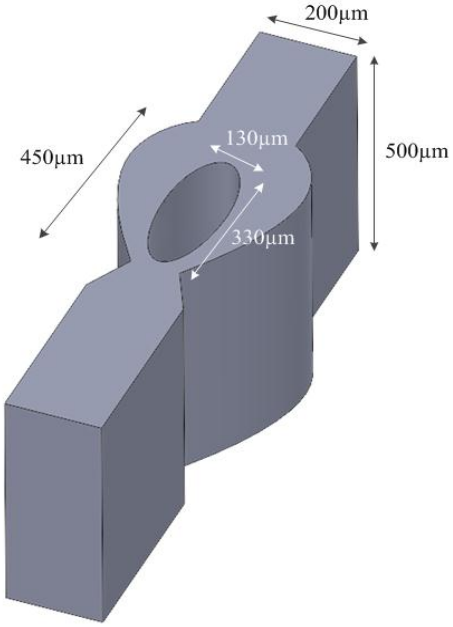


Figure 3. Configuration of micromixer unit

Since cross-section area A_2 is less than cross-section area A_1, the local velocity u_2 will be larger than velocity u_1 (Fig. 1). This phenomenon together with the high velocity region of the ellipse-like micropillar will create high velocity at the right end of the micropillar's contour. At the outlet end of the micropillar, the two separated flows from the small channels are recombined with high velocity. The contact interface of fluids is increased throughout each mixing unit so that the mixing effect is enhanced.

B. Device Design

Our SAR micromixer with ellipse-like micropillars for cell lysis includes 3 inlet channels (cell sample, lysis buffer and silica beads), one outlet channel, and ten mixing units. The geometry of SAR micromixer with ellipse-like micropillars is shown in Fig. 2.

With the special geometry of the SAR micromixer, lysis buffer carrying silica beads flow through the small channels of the micromixer units, and meet at the end of each micropillar with high velocity. When beads and bacteria collide at high speed it causes the cell walls to rip and release the cellular content.

The configuration of each micromixer's unit is shown in Fig. 3. It consists one ellipse-like micropillar with the major axis of 330 µm, minor axis of 130 µm. The width and the depth of the main channel is 200 µm and 500 µm, respectively.

III. Microfabrication of Micromixer

A bare silicon wafer was coated with negative photoresist SU-8 2150 (MicroChem Corp., MA, USA). The thickness is approximately 500 µm. The wafer with SU-8 thin film was contacted with a photomask in hard contact mode and the resist was exposed to UV light (Fig. 4a). The dose of UV light was 600 mJ/cm^2. The unexposed SU-8 was removed in SU-8 developer (MicroChem Corp., MA, USA).

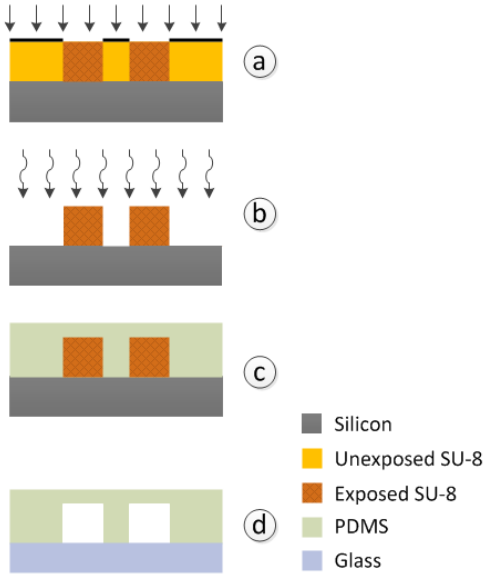
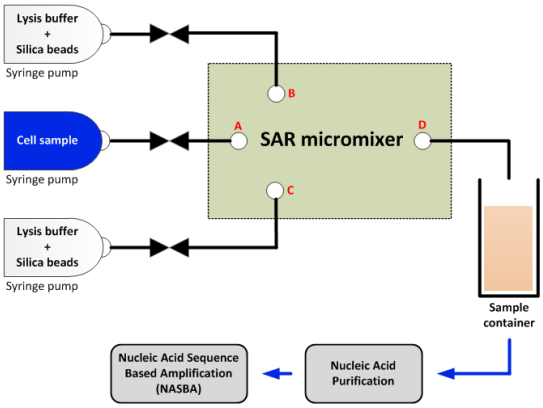


Figure 4. Microfabrication of the SAR micromixer

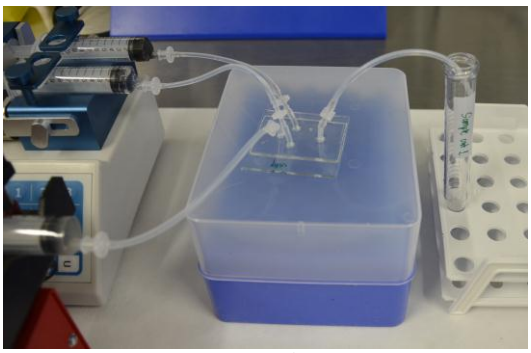
Before casting Polydimethylsiloxane (PDMS, Sylgard 184, Dow Corning, USA) onto the SU-8 resist mold, the patterned wafer was passivated with tridecafluoro-1,1,2,2-Tetrahydrooctyl trichlorosilane to prevent PDMS from sticking (Fig. 4b). PDMS was mixed at a 10:1 ratio of elastomer base to curing agent. The polymer mixture was stirred until the mixture was "milky" due to air bubbles. The PDMS was degassed to remove air bubbles, and poured over the SU-8 mold to achieve a 1 mm thickness (Fig. 4c). The PDMS was degassed again and cured at 65°C for 3 hours in a leveled oven. The PDMS was cut and peeled off from the SU-8 mold with tweezers. Both PDMS and glass substrate were treated with O_2 plasma, and placed on a hotplate at 65°C for 2 hours to complete the bonding process (Fig. 4d).

IV. Experiments

A test loop for the SAR micromixer was set up with all the components connected by flexible fine tubing, as shown in Fig. 5. The sample fluid was driven by a digital syringe pump, in which the flow rate could be precisely controlled.



(a) Schematic drawing



(b) Photo of the test loop

Figure 5. Experimental setup for characterizing micromixer performance

The variable for the mixing studies is the flow rate corresponding to the characteristic dimensionless number *Re* (Reynolds number). The Reynolds number is defined as

$$Re = \frac{uL_c}{\nu} \qquad (2)$$

where *Re* represent the ratio between momentum and viscous friction, u is velocity, L_c indicate the characteristic length of the flow, and ν is the kinematic viscosity of the fluid.

The gram-positive bacterium *Geobacillus toebii* WCH70 (BGSC, Columbus, OH, USA) was used in this lysis experiment. The specific flow rate and the amount of lysis buffer and silica beads of each inlet is given in Table I.

TABLE I. EXPERIMENTAL PARAMETERS FOR CELL LYSIS TESTING

Case	Silica bead (μl)	Lysis buffer (μl)	Flow rate (ml/min)
I	50	1450	0.5
II	100	1400	0.5
III	100	1400	1.0
IV	100	1400	1.5
V	100	1400	2.0

The samples were collected at the outlet channel. As a positive control, *G. toebii* WCH70 was lysed for 10 minutes in an ultrasonic bath. Nucleic acids were extracted from the control and from each sample using the NucliSENS easyMAG kit (bioMerieux, France). Nucleic Acid Sequence Based Amplification (NASBA, PreTect HPV-Proofer kit, NorChip, Norway), using primers and probe targeting 16S rRNA, was used to determine the effect of the lysis treatments. RNase and DNase free water was used as a negative control in the NASBA reaction.

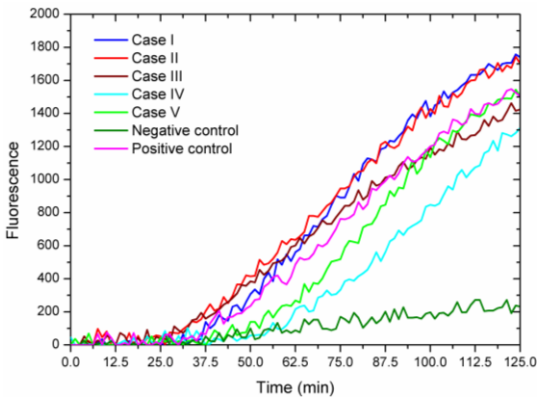


Figure 6. NASBA results

V. RESULTS AND DISCUSSION

According to our results (see Fig. 6) all cases of lysis treatment in the SAR micromixer were successful in lysing the cells prior to analysis of nucleic acids. From our NASBA data it is difficult to state whether some treatments, (flow rate and bead concentration) are better than other. In case I and case II, the amount of silica beads was stirred in lysis buffer before testing are 50 μl and 100 μl, respectively. There is no observable difference between case I and case II. A slight tendency in case IV and case V could indicate that a higher flow rate in the mixer does not improve the lysis efficiency.

However, the treatment in the micromixer is comparable lysis by long ultrasound exposure. Ultrasound lysis has been very successful in our previous work with *G. toebii* (data not shown). In all, our SAR micromixer proved to be a good alternative method for cell lysis. The micromixer has the advantage that it can easily be integrated into an automatic system for lysis and sample treatment.

VI. CONCLUSION

In this paper, micromixer with ellipse-like micropillars was proposed for cell lysis. With a splitting and recombination concept, homogeneous mixing can be obtained in short distance. Hence, the quality of the sample after lysis for further process (Nucleic Acid Purification, Nucleic Acid Sequence Based Amplification) is also improved. Moreover, this kind of micromixers may be ideal for a user friendly, rapid and optimal collection and mixing related sample preparation of whole blood or any other complex human or biological fluidics used for point-of-care or point-of-need diagnostic applications and technologies.

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