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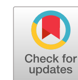
Research paper

A lab-in-a-foil microfluidic reactor based on phaseguiding



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ABSTRACT

We demonstrate a microfluidic reaction chamber that mimics a microcentrifuge tube where reagents can be mixed sequentially at a known stoichiometry. The device has no moving parts or valves and is made by hot embossing in a polymer foil. Sample and reagents are filled in the reaction chamber by controlled guiding of the air/liquid interface in a rectangular array of pillars. The operation of the device is demonstrated by performing isothermal DNA amplification in nL volumes. In our device, 28 pg of DNA from λ-phage, a virus with a 48 kilo base genome, is amplified 500 times thus the amplification product is suitable for library preparation for second generation sequencing. We show that fabrication by hot embossing does not introduce significant contamination and that our device is performing comparably well to test tube amplification and current PDMS-based chip technology.

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1. Introduction

Microfluidics enables the miniaturisation of fluidic systems for performing chemical reactions. It allows integrating several functionalities in a single lab-on-a-chip device where multiple samples can be analysed in parallel. Miniaturisation and parallelisation reduce the cost per analysis by reducing the sample and reagent consumption as well as the device fabrication cost by economy of scale.

Single cell genome analysis has received increasing attention in the recent years. Nucleic acids of a single cell can be amplified in μL volumes in a microcentrifuge tube. However, the quality of the amplification product is improved when amplification is performed in nL volumes [1,2]. Microfluidics and lab on a chip devices thus have a crucial advantage beyond parallelisation and become a central technology for single cell genomics.

So far pressure-driven microfluidics using the pneumatic valve [3] has been the most successful for whole genome amplification of RNA from single cells [4]. The pneumatic valve is based on a multilayer device, that consists of a pneumatic and a microfluidic network separated by a flexible polydimethylsiloxane (PDMS) layer. Ideally, to realise a true economy of scale, a lab-on-a-chip device should be mass produced by industrial processes well-established in automobile and pharmaceutical industry like foil blowing [5] and injection moulding [6–8]. As an intermediate step, hot embossing is a prototyping technique for devices that can be fabricated by injection moulding [9]. However, the device design must be based on passive microfluidics only without moving parts for those techniques to be used. This is feasible for centrifugal and droplet microfluidics. Centrifugal microfluidics allow arraying of single cells [10,11] and integration with lysis and nucleic acid amplification for multiple cell samples [5,12]. Droplet microfluidics enables single cell analysis and sorting at high throughput, so that it is a powerful platform for high throughput detection of PCR amplification products from single cells [13]. Still, pressure driven microfluidics platforms may have the advantage that it can more easily include single cell and single molecule techniques that require high NA optics such as manipulation with optical tweezers [14,15] and visualisation in nanofluidics [16]. Passive pressure driven devices allow the parallel capture of single cell and the sequential DNA and RNA extraction for whole genome sequencing [17]. In this context, a pressure driven amplification unit based on passive microfluidics is needed.

Here we focus on designing a device capable of sequential mixing of reagents that can be integrated with a pressure driven platform and can be fabricated by hot embossing. We report on a microfluidic device that uses only passive components such as capillary burst valves [18] and a phaseguiding structure [19,20] made of a single material. We then demonstrate on-chip amplification of 28 pg λ-phage DNA, a 48 kb viral genome, by Multiple Displacement Amplification (MDA)[21].

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2. Material and methods

2.1. Fabrication

A Silicon stamp is fabricated on a 4” wafer by UV photolithography and deep reactive ion etching at depth of h=500 nm and H=10 μm (Fig. 2C). The stamp is completed by cleaning and deposition of a perfluorodecyltrichlorosilane anti-stiction coating by chemical vapour deposition. The Silicon stamp is embossed in a 250 μm thick COC foil (grade 5013, T_g = 134 °C, TOPAS Advanced Polymers Inc.) at 6 bar and 190 °C for 20 min using a nanoimprint lithography imprinter (CNI, NIL Technology ApS). A second foil, 100 μm-thick, is flattened between two clean anti-stiction 4” coated Silicon wafers using nanoimprint lithography at 6 bar and 190 °C for 10 min. This reduces the peak-to-valley roughness of the foil from 831 nm measured on a 120 μm distance to 16 nm. Eight access holes are drilled in the chip before the lid is bonded to the embossed part by thermal bonding at 121 °C for 20 min. The foil assembly is then cut to 2×2 cm devices (Fig. 2A).

2.2. Experimental setup

The device is mounted on a chuck and o-rings are placed in-between the device reservoirs and the chuck. Air hoses are connecting the chuck to an eight-channel pressure control device (MFCS-FLEX, Fluigent). The pressure of the three inlets, waste and four outlets can be adjusted independently with a resolution of 0.3 mbar. That equals to a flow-resolution of 0.01 pL.s^{-1} through the nano-slit and into the reaction chamber. The Device is then imaged on an inverted microscope (Nikon TE2000-U) equipped with an EMCCD camera (Photometrics), and a FITC filter cube (excitation: 465–495 nm, dichroic mirror: 505 nm, emission: 515–555 nm, Nikon) for fluorescence microscopy.

2.3. Sample and reagents

0.5xTBE with 0.01% Triton X-100 is used as wetting solution and as buffer for the DNA sample. λ-phage DNA (Wako, Japan) is stained at a 5:1 ratio with YOYO-1 (Invitrogen) for the purpose of visualising the flow conditions. The DNA is used at a concentration of 1 μg/mL in 0.5xTBE to which 3% β-mercaptoethanol (BME) is added to prevent photo-nicking. MDA is performed using reagents from a commercial kit (REPLI-g Midi Kit, Quiagen) to which 0.01% of Triton X-100 is added to prevent DNA from sticking to the polymer surfaces.

2.4. Device operation

The bus is primed with 5 μL of the wetting solution from the waste outlet at P=200 mbar (Fig. 1B) while a pressure at the outlet of the reaction chambers is maintained. The DNA sample is loaded in inlet 1 and introduced to the bus at P=100 mbar, then to the reaction chamber, first at ΔP=80 mbar (thus the capillary burst valve at the nano-slit is burst) then at ΔP=50 mbar (Fig. 1B). During this step, the solution is moved slowly through the bus at δP=2 mbar. Before adding a new reagent, we apply a pressure of P=100 mbar for 30 s to ensure complete exchange of solution in the bus. The denaturation solution, the neutralising solution are introduced following the same procedure from inlet 2, and so is the reaction mix from inlet 3.

2.5. DNA quantification and library preparation

DNA was collected from the reaction chamber outlet by adding 5 μL of milli-Q water, then collecting the solution. The polymerase was inactivated by incubation at 65 °C. DNA was cleaned by ethanol precipitation. Briefly, 2.5 μL of 5 M ammonium acetate and 5 μL of 99.9 % ethanol was added. The solution was kept at −20 °C overnight and centrifuged at 4 °C and 14 000 rpm. The supernatant was discarded and the DNA resuspended in 2.5 μL milli-Q before it was

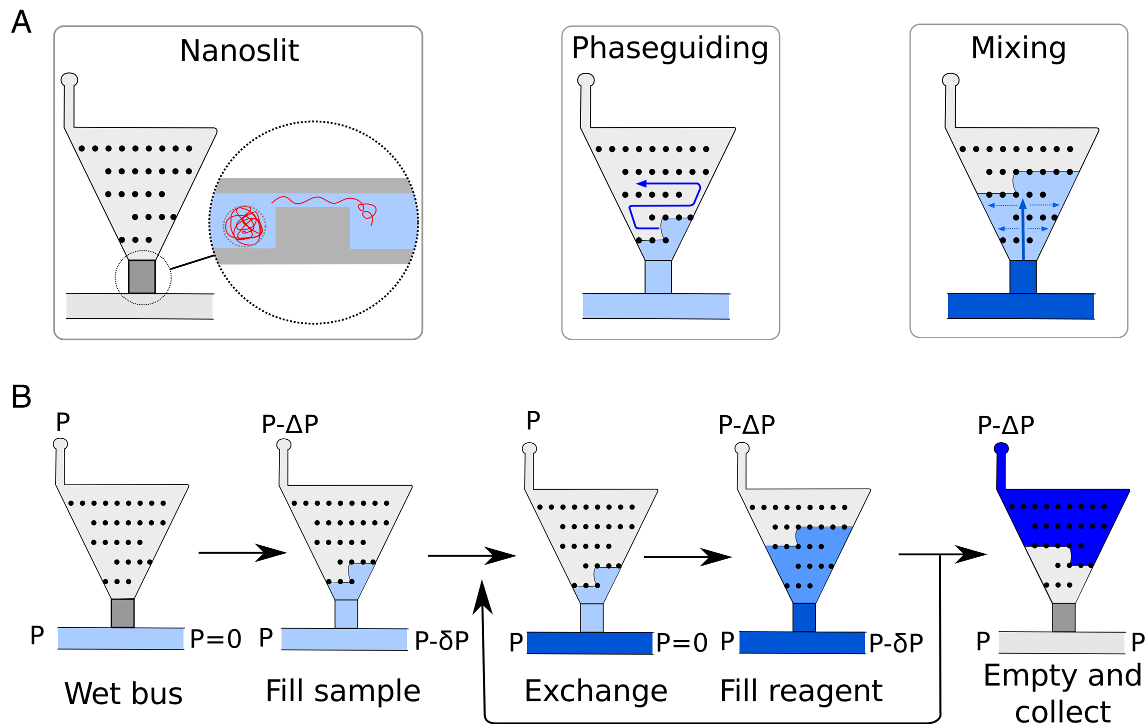

Fig. 1. (A) Design elements: the nano-slit works as entropic barrier preventing diffusion of macromolecules, a pillar array works as a phaseguiding structure, mixing is improved by the phasequiding. (B) Sketch of the on-chip reaction protocol: sample is introduced to the bus then to the reaction chamber by lowering the pressure at the outlet by ΔP while a pressure drop δP is applied to the bus. New reagents are introduced sequentially to the bus then to the chamber. The reaction product is pushed to the outlet by air and collected.

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