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Lab on a Chip

Cite this: *Lab Chip*, 2012, **12**, 1384

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PAPER

Rapid microarray processing using a disposable hybridization chamber with an integrated micropump†

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Received 15th November 2011, Accepted 25th January 2012
DOI: 10.1039/c2lc21110f

Published on 27 January 2012 on http://pubs.rsc.org | doi:10.1039/C2LC21110F
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We present a disposable microarray hybridization chamber with an integrated micropump to speed up diffusion based reaction kinetics by generating convective flow. The time-to-result for the hybridization reaction was reduced from 60 min (standard protocol) down to 15 min for a commercially available microarray. The integrated displacement micropump is pneumatically actuated. It includes two active microvalves and is designed for low-cost, high volume manufacturing. The setup is made out of two microstructured polymer parts realized in *polycarbonate* (PC) separated by a 25 µm *thermoplastic elastomer* (TPE) membrane. Pump rate can be controlled between 0.3 µl s^{-1} and 5.7 µl s^{-1} at actuation frequencies between 0.2 Hz and 8.0 Hz, respectively.

Introduction

The trend towards miniaturization and wearable systems such as portable diagnostic and therapeutic medical devices imposes strong requirements in terms of size, weight, accuracy and system integration capability. This leads to a growing demand for integrated microflow management components such as sensors, pumps and valves.[1] Platforms based on silicon or glass show the highest geometrical accuracy but the material itself and the microstructuring technology are expensive if chip sizes cannot be scaled down. Polymer materials enable cost-efficient disposable devices even for larger chips.[2] In particular low-cost manufactured Lab-on-a-Chip solutions are expected to have a major impact in biotech industries, pharmacology, medical diagnostics, forensics, environmental monitoring and basic research.[3,4] So far most polymer platforms are based on *polydimethylsiloxane* (PDMS) which combines accurate manufacturing at low cost and the ability to integrate active components for fluid management.[5,6] However, this approach lacks a high throughput production technology. In contrast, the fabrication technology presented here combines cost-efficient mass production capability by injection molding and laser welding with the ability to realize various microfluidic functions like valving, pumping, mixing, *etc.*

Microarrays can be regarded as an analytical method that combines small size with the capability to detect several hundreds of different biomolecules simultaneously. Microarray based analytics is expected to have a broad range of applications such as *Deoxyribonucleic acid* (DNA), protein and antibody detection.[7] The time-to-the-result of such a heterogeneous assay is limited by the time a target molecule needs to diffuse from the bulk liquid to the probe immobilized at the bottom of a chamber. For large molecules like DNA fragments, it takes several minutes to hours to bring feasible results.[8] As described in several modeling works,[9,10] this time can be dramatically reduced by superimposing this diffusion limited process with a convective flow. Several stand-alone instruments are commercially available for that purpose. They generate the convective fluid movement by surface acoustic waves,[11] electrochemically generated gas bubbles[12] or cyclic mechanical displacement of the sample.[13] In addition, fully integrated microfluidic chips have been proved to enhance the quality and time-to-result of microarray based analytics. They employ convective flow by integrated peristaltic micropumps or inertial forces during centrifugation.[14–18] These mostly *PDMS* based microfluidic devices are mainly used for research and lack the possibility of a high throughput fabrication. In contrast, in this work we present a hybridization chamber with an integrated micropump made with a technology suitable for mass production. To prove its performance we used this technology with two different microarrays, *A* and *B*. Microarray *A* is a commercially available DNA microarray[19] for molecular genetic determinations of *HLA-B27*, a marker for *Bechterew's disease*; microarray *B* is a custom made microarray based on a genotyping assay which discriminates the resistance of

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† Electronic supplementary information (ESI) available. See DOI: 10.1039/c2lc21110f

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Escherichia coli (*E. coli*) against fluoroquinolone based antibiotics.

Micropump concept and assembly

The micropump consists of three layers. The upper and lower layers are microstructured *polycarbonate* (PC) parts while the middle layer is an elastic membrane made of a weldable *thermoplastic elastomer* (TPE) with a thickness of 25 μm. The upper layer contains channels, which can be pressurized with a control pressure P_c for deflecting the elastic membrane layer in the middle. In the lower hydraulic layer, all channels that are in contact with liquids are realized. When the pneumatic control pressure P_c is larger than the hydraulic pressure P_h inside the liquid channels the membrane is deflected towards the hydraulic layer. Based on this, a microvalve or a micropump can be implemented easily as shown in Fig. 1 and 2.

The upper and lower bulk layers can be structured using high throughput fabrication processes such as injection molding.[20] All three layers are joined together in one laser welding step (Fig. 3) creating a mechanically stable assembly which shows no leakage even at a hydraulic pressure of 0.90 MPa, significantly higher than the control and hydraulic pressures used for operation (10–50 kPa). The used materials offer high chemical resistance against a broad range of commonly used liquids;[21,22] they are transparent even to UV light with wavelength down to 300 nm and show reasonable low fluorescence, which qualify the materials to be used for *micro total analysis systems* (μTAS).

Valving is realized by applying a control pressure $P_c > P_h$ inside a control chamber. This deflects the *TPE* membrane towards the hydraulic layer containing two separated liquid channels, which are this way sealed (Fig. 1). The principle is similar to *PDMS* based setups[5,6] except that the materials used here enable mass fabrication. The diameter of the control chamber is 1.0 mm, the liquid channel is 400 μm in width and 300 μm in depth. The two liquid channels are separated by a 200 μm support. The design parameters were chosen to combine a low fluidic resistance with a full sealing capability. The fabricated microvalves offer switching times in the order of 100 ms and can be operated at very low control pressures in the range of 10 kPa. With this setup, a pneumatically actuated displacement micropump is easily built by combining two active microvalves with a displacement chamber.[23,24] The working principle of the displacement micropump is described and illustrated in Fig. 4. The micropump itself is arranged on top of a hybridisation

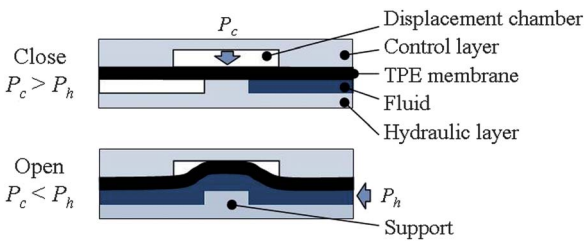

Fig. 1 Schematic cross-section of the setup and working principle of the pneumatically actuated microvalve. The functional unit is made of a three layer stack consisting of an upper control layer and a lower hydraulic layer both made of microstructured polycarbonate separated by an elastic *thermoplastic elastomer membrane* (TPE) in the middle.

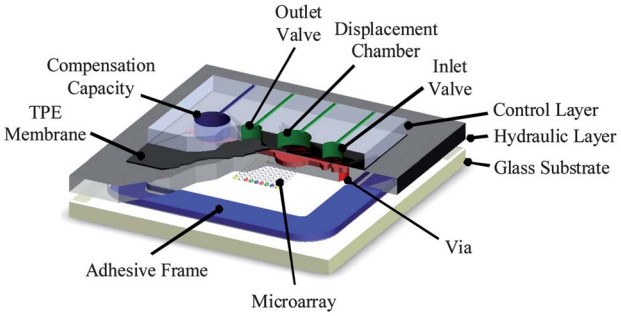

Fig. 2 Schematic of a membrane micropump assembled on top of a substrate with a microarray.

chamber (Fig. 2) and is used to circulate the sample liquid within the hybridization chamber (Fig. 3).

Pump characteristics

The frequency characteristic of a micropump containing a displacement chamber of 2.0 mm in diameter is depicted in Fig. 5. The control pressure P_c is switched between 50 kPa and vacuum. It is applied periodically to the active microvalves and the displacement chamber as well. The resulting average flow rate is measured using a precision flow meter.[25] Pump rate can be controlled between 0.3 μl s^{-1} and 5.7 μl s^{-1} at actuation frequencies between 0.2 Hz and 8.0 Hz, respectively, with a highly linear characteristic. Higher actuation frequencies could not be tested due to surrounding equipment restrictions. To be able to circulate liquid in a closed loop (Fig. 2), a nonactuated capacity chamber has been put in series to the micropump to compensate the displaced volume during the displacement stroke.

Reaction kinetics

The molecules in the sample react with the immobilized probe molecules at the bottom of the channel causing a depletion of potential binding partners near the microarray spot. The distance a new binding partner needs to diffuse to get to the reactive surface enlarges over time at a rate of $\sim(Dt)^{1/2}$ (Fig. 6B) Therefore the concentration gradient ∇c decreases causing the flux j_D of new molecules to the reactive surface to decrease over time (eqn (1)). With an overlaying convective flux Q on the other hand, the depletion zone over the array is steady and comparably thin (Fig. 6B) because new molecules are constantly transported near the reactive spot causing the concentration gradient to be constant. This leads to a large decrease in time for the reaction to be in an equilibrium. Here a 25 μl hybridization chamber is

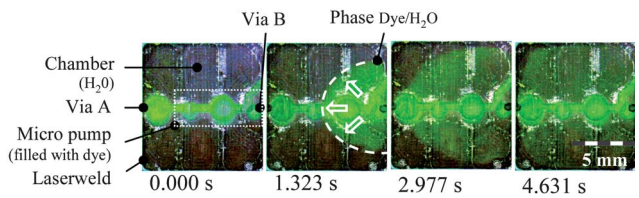

Fig. 3 Top view picture of the micropump assembled by laser welding. For visualization of the transporting flux, a fluorescent dye is pumped in a circle ($f_P = 8.0$ Hz) *via* A to B.

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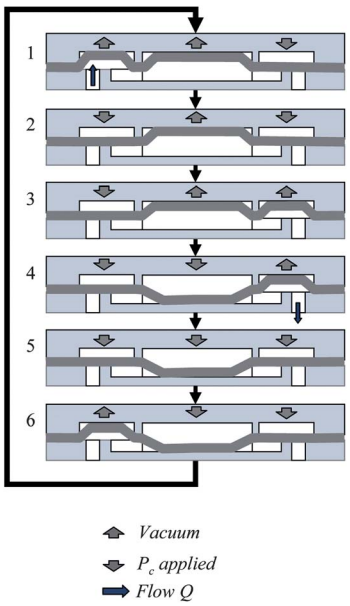

Fig. 4 Schematic working principle of the displacement micropump with vacuum and a control pressure as actuation levels. Step 1: Suction of liquid through the open inlet valve. In step 2 the inlet valve is closed. The outlet valve is opened in step 3 before the displacement chamber is actuated in step 4 pushing the liquid towards the outlet. Step 5 illustrates the closing of the outlet valve to block any backflow. In step 6 the inlet valve is opened again to initiate the suction step 1. This cycle is repeated periodically at a frequency f_P.

created using an adhesive frame[26] surrounding the microarray. The dimensions are 10 mm × 10 mm × 0.25 mm ($L \times W_c \times H$). The flow rate is $Q = 5.7$ µl s^{-1} (Fig. 6). The diffusion constant of a 200 base pairs DNA sequence is assumed to be $D = 2.19 \times 10^{-11}$ m^2 s^{-1}.[27]

$$j_D = -D\nabla c \quad (1)$$

Experimental

The fluorescent signals from two microarrays are used to investigate the influence of a convective flux. For the dynamically

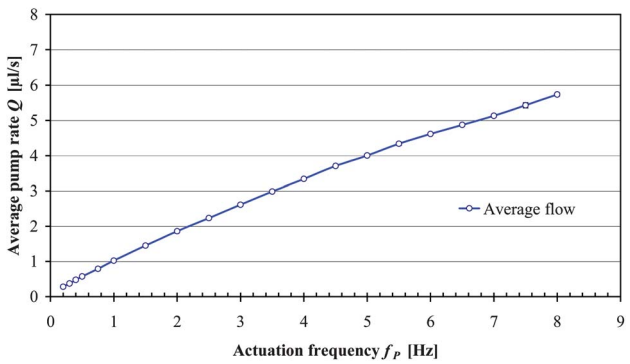

Fig. 5 Average pump rate Q *vs.* actuation frequency f_p. The data shown are from 20 pump cycles per frequency. Ramping is repeated five times. Average standard deviation for the measured flow is <2%.

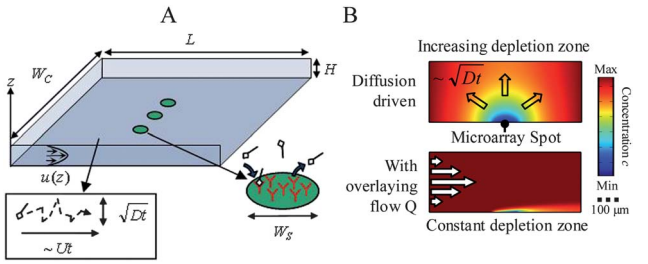

Fig. 6 Schematic model system. A: Sample solution flows with velocity u and volumetric flow rate Q through a channel of height H and width W_C over a microarray spot of diameter W_s. The diffusivity of the target molecules is D.[9] B: Sample concentration over a microarray spot after 900 s. The depletion zone increases over time for pure diffusion. Small steady depletion zone when molecules are actively transported to the spot.

driven reaction each sample is pumped in a circle with $Q = 5.7$ µl s^{-1}. To fill the micropump volume, an additional volume of 4 µl is needed. Since the reaction kinetics strongly depends on the reactant concentration c_0 in spite of the total volume, this additional volume is considered negligible.

Microarray A is a component of a commercially available DNA microarray test system[19] for molecular genetic determinations of *HLA-B27*, a marker for *Bechterew's disease*. After hybridization of a reference PCR product[28] at 45 °C, the microarray is immediately washed using three washing buffers (wb) included in the standard kit: 1 min (wb 1), 2 min (wb 2) and 5 s (wb 3), followed by a drying step with N_2. Data are obtained by acquisition of fluorescence signals from a microarray scanner.[29]

Microarray B contains spots for detection of the resistance of *Escherichia coli* (*E. coli*) against fluoroquinolone based antibiotics. It is spotted on a surface modified glass substrate[30] using an automated contact spotting robot[31] and contains spots with the investigatory probe for a point mutation (*single nucleotide polymorphism, SNP*) significant for the fluoroquinolone resistance of the *E. coli* bacteria in bases 260 of the *gyrA* gene.[32] For sample preparation genomic DNA from susceptible *E. coli* strain was extracted followed by a labeling polymerase chain reaction

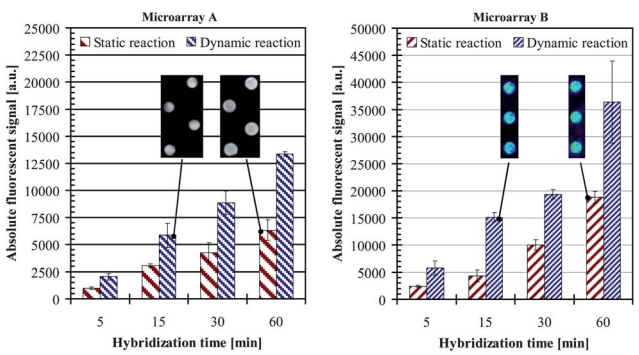

Fig. 7 Absolute signal intensity in arbitrary units of the fluorescence signal. Reference signal is after 60 min for both microarrays. Microarray A: probes for *HLA B27 EX 3* polymorphism. Microarray B: probes with a point mutation in bases 260 of the *gyrA* gene. Active pumped (dynamic) hybridization is compared to diffusion driven (static). One data point contains the average of four (A)/three (B) redundant spots.

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Table 1 Ratio of perfect match to the highest mismatch (microarray B)

	5 min	15 min	30 min	60 min
Static	4	14	14	33
Dynamic	12	26	36	52

(PCR) and a purification step. A thermomixer[33] is used to gain the reaction temperature of 55 °C. The hybridization buffer used is a 6× SSPE buffer and a 5× Denhardt's solution with a DNA concentration of 7.58×10^{-4} μM. After hybridization the microarray is immediately washed in three steps, each 10 min, and dried with N_2 afterwards. Data are obtained by acquisition of fluorescence signals from a microarray laser scanner with a constant amplification factor from a photomultiplier tube (PMT) of 70. The signal for a perfect match is used to benchmark the reaction kinetics together with its ratio to the signal obtained from the highest mismatch.

Results and discussion

The *absolute fluorescence signals* shown in Fig. 7 prove that the supporting flow approximately doubles the signal intensity for all investigated durations of hybridization for both investigated microarrays *A* and *B*. For microarray *A*, it is possible to reach a signal intensity after 15 min with pumping similar to the signal appearing after 60 min without pumping. For microarray *B*, a signal intensity appearing after 30 min with pumping is similar to that appearing after 60 min without pumping.

Microarray *B* contains spots for the *discrimination* of non-specific binding probes (mismatches). Comparing the signals of these spots to the specific perfect matching spot indicates the level of sensitivity of the array. Table 1 lists this ratio by comparing the signals of the perfect match and the highest mismatch for static and dynamic reaction.

The higher ratio for the dynamic experiment shows that the active flow shifts the reaction kinetics to a more specific binding between probe and target. For a static hybridization reaction, a discrimination ratio of 33 appears after 60 min. With pumping the perfect match signal is already 36 times higher than the signal from the highest mismatch after 30 min.

Conclusions

We presented a new Lab-on-a-Chip concept made of polymers suitable for mass production. The microfluidic device consists of two structured thermoplastic polycarbonate bulk layers with an elastic thermoplastic elastomer in between. With this setup, a membrane micropump with two active microvalves at the inlet and outlet of a displacement chamber has been realized and characterized showing a broad range of tunable flow rates from 0.3 to 5.7 μl s^{-1} needing only very low actuation pressures of 10–50 kPa. The reproducibility of the tested device is outstanding with an average deviation in flow rate of <2%. The micropump was used to accelerate a diffusion limited microarray hybridization experiment, and the improvement was demonstrated with two different microarray experiments. For this, a device was used consisting of a 25 μl reaction chamber with a micropump as a cap pumping the sample in a circle at a pump rate of 5.7 μl s^{-1}. We compared the resulting fluorescent signals to those of a pure diffusion driven hybridization experiment. The ratio of signal intensities increased in the dynamic experiment by a factor of two. In addition, it was demonstrated that not only is the reaction kinetics accelerated by the convective flow but also a more specific binding can be achieved at the same time. The discrimination ratio between a perfect matching and a mismatching probe was increased at the same time. This way we could accelerate an experiment, which usually takes 60 min down to 30 min (microarray *B*) or even 15 min (microarray *A*). The lower detection limits together with the acceleration of the reaction show the potential and reliability of the concept presented here. This flexible setup with passive and active microfluidic components addresses a broad range of analytical tasks that need complex fluid networks and a robust fluid management. The active components promise a fast on-chip assay implementation, and the concept and material enable cost-efficient production technologies for the mass fabrication of disposable devices.

Acknowledgements

Parts of the present study were supported by the German Federal Ministry of Education and Research (01EZ0709).

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