SAMPLE METERING AND PARALLEL LIQUID PLUG ACTUATION FOR MULTIPLE BIOCHEMICAL ASSAYS

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ABSTRACT

We demonstrate a passive microfluidic system for simple and efficient metering, handling and control of parallel nanoliter samples on-chip. The system consist of two chips; one for sample loading, splitting and metering (based on capillary forces only), and another for sample plug movement in parallel reaction channels. The latter is based on a set of capillary valves and one single external pressure source only. The strength of the capillary valves determines the logic movement of the parallel sample droplets on-chip, permitting robust system control. In general, the presented system is applicable to a variety of multi-step reaction protocols with a large number of parallel channels.

Keywords: microfluidics, capillary valve, lab-on-a-chip, sample control

1. INTRODUCTION

Passive fluidic manipulation is a promising approach in Lab-on-a-Chip (LOC) systems due to the inherent mechanical simplicity of the resulting device, rendering it well suited for mass production with a minimum of (costly) assembly needs. Such manipulation has been previously explored by e.g. [1], [2], and [3].

Here, we present a passive system designed for on-chip amplification of mRNA and ssDNA by Nucleic Acid Sequence-Based Amplification (NASBA, see [4]), in our case requiring two separate isothermal steps and mixing with two different dried reagents stored on-chip. We demonstrate a passive system providing efficient multi-step control of liquid samples in several separate, parallel microchannels. Specifically, simultaneous fluidic control of seven parallel channel systems is demonstrated, permitting the analysis of a single sample for e.g. seven different markers.

3. EXPERIMENTAL

The experiments were performed using two types of test chips, one for metering (fig.1a) and another for control of sample plug movement in parallel channels (fig.1b). The chips were manufactured in Cyclic Olefin Copolymer (COC) by micromilling (features >100µm) and laser ablation (features of 100µm and below). Because native COC is hydrophobic, the chips were coated with 0.5% polyethylene glycol (PEG) in methanol (Sigma Aldrich). The contact angle of DI water on PEG surface was approx. 30º. The valve structures consisted of a tapered part and a narrow restriction channel, see fig. 2. Teflon fluorpolymer (AF1600, DuPont) was spotted using PipeJet spotting system (BioFluidix) onto the tapered part of the valve, filling the narrow valve structures by capillary action. The contact angle of DI water on Teflon surface was measured to be approx. 110º. After coating and spotting, the chips were sealed with adhesive tape.

Both DI water and pre-mixed NASBA reagents with sample were used as working liquids to verify the chip functionality.

The parallel sample plug movement chip (fig.1b.) was mounted in an aluminium frame with fluidic interconnects, and coupled to a syringe pump (PHD2000, Harvard Apparatus) which was used to apply suction to the common chip outlet, withdrawing air from the system at a rate of 10 µl/min. A pressure sensor (TP3100 001A 0P from MEMSCAP) was used for monitoring the pressure in the common tube connection to the parallel channels (see fig. 1b).

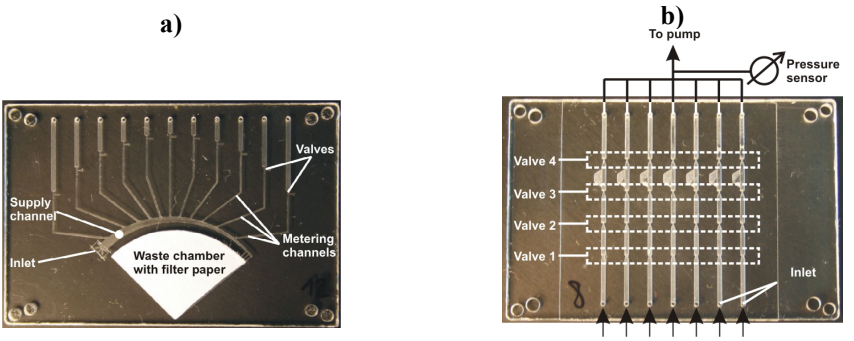

Figure 1: **a)** Sample metering chip. The sample is introduced at the inlet, filling up the supply channel (depth 200µm) and the eleven individual metering channels (75µm×300µm, volume 335nl) up to the hydrophobic capillary valves. Excess sample is drawn into the waste chamber, which contains an absorbing filter paper. **b)** Parallel reaction/actuation chip. The capillary valves increase in strength in the downstream direction, with valve widths of 380µm, 150µm, 75µm and 33µm. The depth of all channels is 200µm. The width of the parallel channels is 800µm.

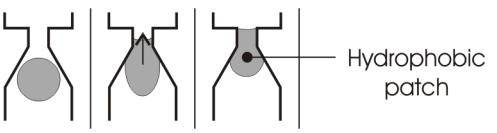

Figure 2: Capillary valve geometry. Hydrophobization was achieved by spotting a droplet of Teflon in the tapered part of the valve, filling the narrow restriction by capillary forces.

4. RESULTS AND DISCUSSION

The metering chip (fig.1a) was tested using different sample volumes at the inlet. It was found that the smallest sample volume permitting proper chip operation was 17µl. For this and larger volumes, the sample was drawn into the supply channel, filled all metering channels up to the position of the capillary valves, while excess sample was absorbed into the waste chamber. During the accompanying drainage of the supply channel, the liquid contained in the parallel metering channels was pinched off at the inlets, leaving a precisely metered sample plug of 335nl in each metering channel. The whole process required approximately 2 minutes from sample insertion till completed absorption of waste. Smaller sample volumes resulted in incomplete filling of metering channels, or incomplete drainage of the supply channel to waste. When the waste chamber was ventilated to the atmosphere, a smaller sample volume (15µl) could be applied at the chip inlet.

The parallel plug control chips (fig.1b) consisted of 7 parallel channels, each containing 4 hydrophobic valves of increasing strength. During operation, sample plugs introduced at the inlets all passed valve 1 before any plug passed valve 2, etc., such that all plugs resided at the same downstream position in the channels. In this manner, parallel, multi-step motion of the sample plugs was achieved.

A typical pressure trace of the movement of seven parallel plugs through the system is shown in fig.3a. For each valve, seven pressure minima can be observed, indicating the passing of a liquid plug through its respective valve. Figure 3b shows average burst pressure data collected from 5 chips. The solid line is the plot of the Young-Laplace equation representing the burst pressure required to overcome a capillary burst valve, see [5]. As can be seen from the figure, the valve performance is well predicted by the theoretical approximation.

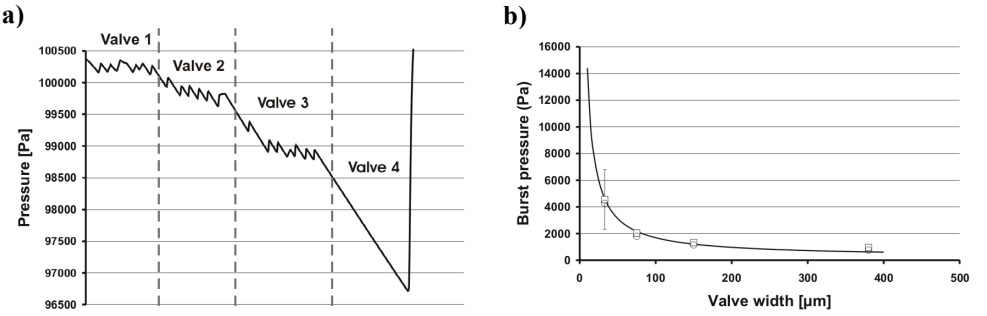

Figure 5: **a)** Pressure trace of seven parallel plugs moving through the parallel actuation chip. At the fourth valve, the first plug to break the valve effectively ventilates the system, and hence the pressure in the system returns to atmospheric conditions. **b)** Pressure characteristics of the capillary valves. Symbols: □ DI water; ○ reagents; solid line represents the analytical values for water.

5. CONCLUSIONS

A passive system capable of robust, parallel sample control on-chip using a single external pressure source has been demonstrated. The system relies on capillary valves of varying strength. The presented method is applicable to a variety of multi-step reaction protocols and can be extended to a large number of parallel channels.

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