MICROFLUIDIC UNIDIRECTIONAL PNEUMATIC SWITCH FOR AUTOMATED DNA-EXTRACTION ON STANDARD LABORATORY CENTRIFUGES

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ABSTRACT

For the first time, we present a new, unidirectional liquid switching concept for the centrifugal microfluidic platform. The concept relies on a controlled liquid-air interface instability. It enables automated DNA-extraction and potentially other microfluidic protocols on standard laboratory centrifuges, superseding the need for expensive base instruments, which are common to all state of the art Lab-on-a-Chip platforms. As a proof of concept we fabricated a Lab-on-a-Chip cartridge for automated DNA extraction from 32 µL whole blood on a standard laboratory centrifuge. The obtained DNA yield was 88 ± 44 ng (42 % of the optimized reference extraction [1]).

KEYWORDS: Centrifugal, Microfluidics, Switch, DNA extraction, Lab automation

INTRODUCTION

Centrifugal microfluidics is an attractive platform for µTAS-implementations because no connections to pumps are required. However, many applications require specific instruments with fine control over spinning frequency and acceleration of the Lab-on-a-Chip disposable. Such application-specific instruments are significant investments for the potential customer. The use of a standard laboratory centrifuge could lower the entry barrier for the customers significantly. Here, a protocol for automated DNA extraction is demonstrated on such a standard laboratory centrifuge.

THEORY

The microfluidic structure consists of an inlet chamber connected to a silica membrane [1] for reversible DNA binding which in turn is connected to two chambers: a waste chamber for gathering washing liquids and an elution chamber for collecting the purified DNA (Figure 1). The waste chamber is unvented and the elution chamber is only reachable over an “energy barrier”. This is realized by an arching channel which leads a short distance radially inwards before continuing into the elution chamber. At high centrifugal forces the air in the waste chamber is displaced alongside intruding water due to an interface instability [2]. At low centrifugal forces, when the interface is stable, the liquid is forced over the “energy barrier” into the elution chamber (Figure 2). Thus, an unidirectional switch is realized.

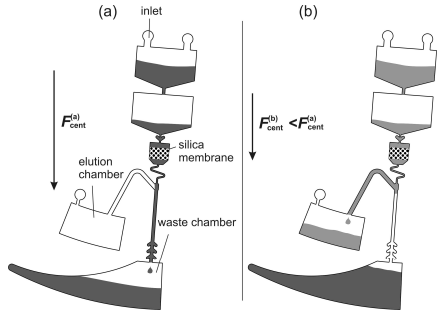

Figure 1: Principle of the unidirectional switch: (a) At high centrifugal forces, liquid enters the unvented waste chamber displacing the air. (b) At low centrifugal forces, a stable liquid-air interface forms and forces liquid into the elution chamber.

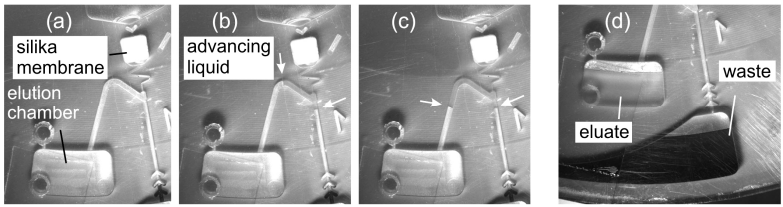

Figure 2: Switching in elution chamber at low centrifugal forces. (a) Start of rotation. (b) Liquid enters the switch and (c) is stopped by the air pressure towards the unvented waste, propagating into the elution chamber. (d) Result of a DNA extraction with washing liquids in the waste and purified DNA in the elution chamber.

Figure 3: Experimental Setup. Microfluidic DNA-extraction disk on standard laboratory centrifuge.

EXPERIMENTAL

Experiments were performed on a standard lab centrifuge (Sigma 1-15, Sigma GmbH, Germany). The axle of a standard haematocrite rotor was used to fix the mi-

crofluidic substrate in place (Figure 3). A lysed blood sample, two washing buffers, and an elution buffer were consecutively pipetted into the inlet hole and processed (Table 1). The elution buffer was then collected and the DNA content measured.

Table 1. Process steps on laboratory centrifuge for DNA-extraction

Liquid (100 µL each)	Frequency /rpm	Time /s
Lysed sample	500	10
	8000	60
Washing liquid 1	8000	60
Washing liquid 2	8000	180
Elution liquid	700	10
incubation	0	60
elution	600 (1000, 2000, 8000)	90 (10 each)

RESULTS AND DISCUSSION

In 7 experiments, an average of 88 ± 44 ng DNA was extracted. The variation is probably due to fluidic by-passes at the silica membrane. Purity of the extracted DNA was determined by real-time PCR of the extracted DNA with 3.3 µL of the extracted DNA in an overall 33 µL PCR mix. The average threshold cycle (C_T) of the reference extraction was 24.3 compared to 27.4 for the microfluidic extraction. At 42 % DNA mass compared to the reference extraction, this shows an inhibition of less then 2 cycles. Thus, the extraction protocol (buffer volumes, centrifuge frequencies) and switch design shows satisfactory results but has to be optimised further.

CONCLUSIONS

We designed and experimentally validated a structure enabling automated DNA-extraction on a standard laboratory centrifuge. The next steps will be to integrate pre-stored liquid reagents [3] and to program the computer interface of the centrifuge to proof a fully automated extraction sequence without the need for any user interaction except applying the sample. This will enable a truly automated DNA-extraction on a standard centrifuge without the need for manual steps like changing tubes or pipetting of buffers, and also without any investment for a new instrument.

REFERENCES

[1] Extracted from: QIAamp DNA Blood Mini Kit (Qiagen GmbH, Deutschland).

[2] D. Mark, S. Haeberle, T. Metz, S. Lutz, J. Ducrée, R. Zengerle, and F. von Stetten, Aliquoting structure for centrifugal microfluidics based on a new pneumatic valve, Proc. MEMS 2008, Tucson, USA, pp. 611-614 (2008).