ON THE WAY TO A FULLY INTEGRATED DNA-PURIFICATION SYSTEM ON A STANDARD LABORATORY CENTRIFUGE

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ABSTRACT

For the first time, we combine liquid reagent storage in glass capillaries [1], time-controlled reagent release [2], and a novel solution for unidirectional centrifugal routing, paving the way to a fully integrated DNA extraction on a standard laboratory centrifuge. The average yield of the extraction is 192 ± 30 ng DNA out of 32 µl blood, corresponding to 53 ± 8 % of a reference extraction. The overall processing time of ~66 minutes can be reduced to ~8 minutes after optimization of the burst-valves. This novel approach demonstrates a convenient way for fully automated DNA-extraction on a standard laboratory centrifuge.

KEYWORDS: Microfluidics, centrifugal routing, DNA extraction, time-controlled release, liquid reagent storage

INTRODUCTION

DNA purification from blood is an essential step in sample preparation for many molecular diagnostic tests. DNA extraction is very time consuming due to numerous manual steps. Therefore, microfluidic integration of DNA extraction receives increasing attention. Manual working steps were reduced heavily but up to now there is no integrated system, which includes liquid reagent storage and a time-controlled release of the reagents. We propose an approach for integrated DNA purification in a standard laboratory device with pre-stored liquid reagents, rendering the development of a special centrifugal processing device obsolete.

WORKING PRINCIPLE

The presented structure integrates the following steps: Binding of DNA from a lysed blood sample to a silica membrane, two consecutive washing steps, and elution of the purified DNA from the silica membrane. Unbound sample residues and washing buffers are routed into a waste chamber, whereas the eluate into a separate collection chamber. The extraction disk harbors two glass capillaries for pre-storage of 100 µl washing buffer 1 (AW1) and washing buffer 2 (AW2), three burst valves for time-controlled release of reagents, one silica extraction matrix and one microfluidic router that enables collection of waste and eluate in two different chambers. The storage of elution buffer and a lysis step have not yet been implemented on disk. Glass capillaries are crushed simultaneously at the beginning of the DNA extraction to release the liquid reagents into the microfluidic system. The buffer solutions are then controlled by the burst valves., which are realized by delaminated lids during fast rotation [2]. Elution buffer and lysed blood have to be filled in before starting the rotational protocol. As an alternative to a previous routing approach [3], a novel routing mechanism for separation of the eluate from waste liquids is presented: By blocking the air vent of the waste chamber with a liquid plug during the elution step *(Figure 1)* the purified DNA is guided into a different liquid path.

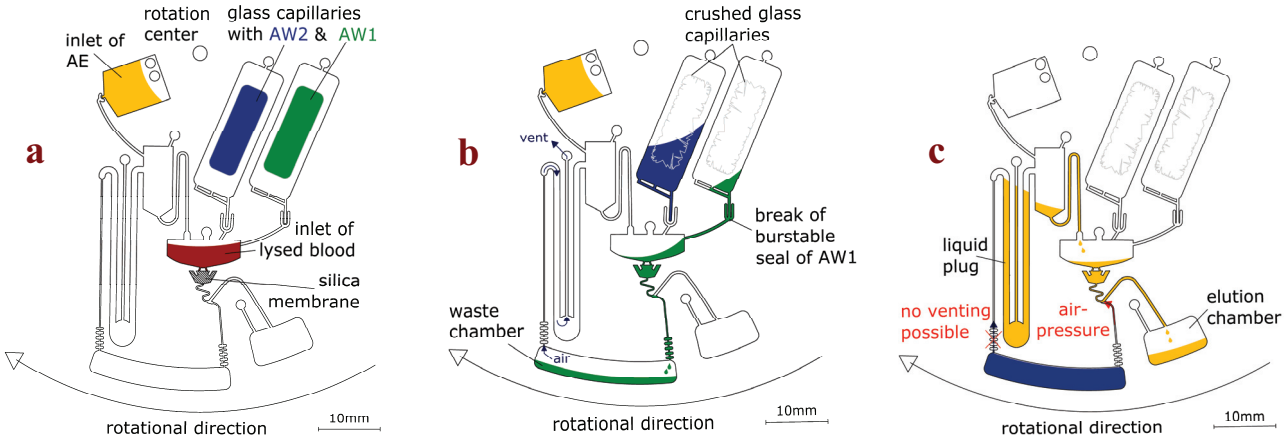

Figure 1: Microfluidic principle. a) Filling levels before rotation and uncrushed glass capillaries containing washing buffers AW1 and AW2; b) break of burstable seal of AW1 and centrifugation of AW1 into the waste chamber; subsequent release of AW2; c) elution step after breakthrough of burstable seal of AE and blocking the switch channel by a liquid plug. Thereby the elution buffer AE is guided into the elution chamber.

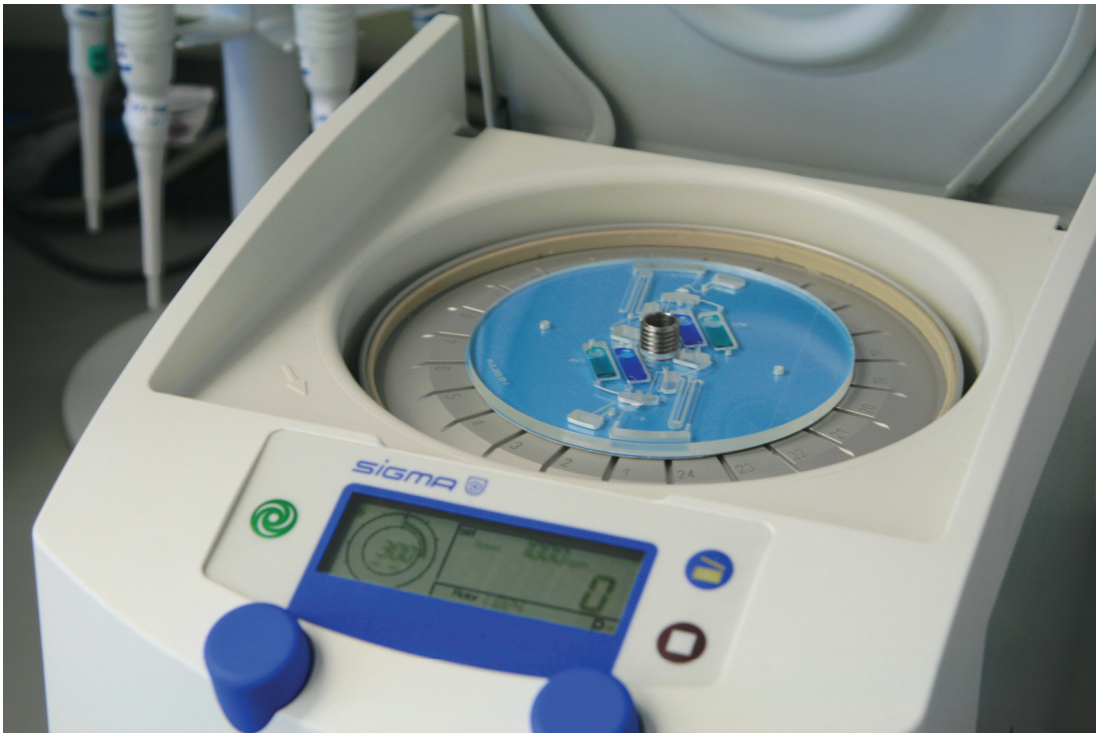

Figure 2: DNA-Extraction disk in a standard laboratory centrifuge using a haematocrite rotor to fix the substrate in place. The green glass capillary contains washing buffer 1 (AW 1), the blue capillary contains washing buffer 2 (AW 2). (buffers are colored for visualization)

EXPERIMENTAL

The extraction disks were fabricated by a milling machine (Minitech Machinery Corporation GA, USA) after designing the fluidic layout with a CAD software. The substrate is a Cyclic Olefin Copolymer (COC) disk with a diameter of 115 mm and a thickness of 4 mm. Each substrate accommodates two DNA extraction structures depicted in Figure 2. After milling, the disks were cleaned with DI-water and isopropanol. The glass capillaries, which were fabricated with a double-nozzle burner, have to be inserted into the substrate before sealing with pressure sensitive adhesive PCR foil (900320, Polyolefin-foil, HJ-Bioanalytic GmbH, Mönchengladbach, Germany).

All experiments were implemented on a standard laboratory centrifuge *(Figure 2)* (Sigma 1-15, Sigma GmbH, Osterode am Harz, Germany) that was provided by the manufacturer with an automated control option via an RS232 interface. For fixation of the disk a haematocrite rotor is used. DNA extraction reagents and the silica extraction matrix were taken from a QIAamp DNA Blood Mini Kit (Qiagen GmbH, Hilden, Germany). Prior to rotation, elution buffer (AE) and the lysed blood are pipetted into respective inlets. Thereafter the glass capillaries containing both washing buffers are crushed. Then the frequency which is shown in Table 1 is started.

For a higher DNA yield it is possible to add an extra step at the beginning of rotation: For complete wetting of the silica membrane the spinning frequency has to be reduced to 7 Hz for a few seconds. Also an incubation step for 1 minute can be added during the elution step after the elution buffer has reached the membrane.

Table 1: Frequency protocol of the automated DNA purification on disk after pipetting of blood and crushing of the glass capillaries containing both washing buffers

DNA extraction step	**Frequency /rpm**	**Time /min**
Centrifugation of lysed blood, AW 1 and AW 2 (100 µl each) into the waste chamber	6000	5
Breakthrough of burstable seal of AE (190 µl)	6000	57
Capillary filling of the siphon	0	1
Incubation step (optional for increased elution efficiency)	300 0	5 60
Elution step: centrifugation of the elution buffer in the elution chamber	900	3

RESULTS AND DISCUSSION

In three independent experiments an average of 192 ± 30 ng DNA has been extracted from 32 µl of blood, which is a yield of 53 ± 8 %, compared to a reference extraction with the QIAamp Kit *(Table 2)*. Currently, the processing time for the complete extraction is about 66 minutes, since the opening time of the burst valves has not been optimized yet. Burst times of washing buffer AW1, AW2, and elution buffer are 50 ± 20 s, 105 ± 40 s, and 62 minutes (!) respectively, whereby the latter is to be reduced to ~ 6 minutes with an adapted layout. In total, three out of four experiments with the completely integrated system were successful. Due to sealing failure in the fourth experiment the air vent of the waste chamber was not blocked so that the elution buffer flows directly in the waste chamber and the measurement of DNA-yield was not possible.

Table 2: DNA yield in 3 experiments with the lab centrifuge compared to the reference extraction with standard spin columns of the QIAamp kit

Number of test	**DNA [ng]** ***(on disk)***	**DNA yield [%] compare to reference** ***(test tube)***
1	201.4	55.7
2	216.6	59.9
3	158.08	43.7
average	**192.03**	**53.1**

CONCLUSION

We present an integrated microfluidic liquid control system for DNA extraction which has the potential of reducing DNA purification to a one-step automated protocol on existing laboratory centrifuges. The next step will be the addition of a lysis structure on the same substrate to completely integrate the whole process. Additionally, the processing time will be reduced to ~ 8 min by optimizing the burstable seals. The automation process will be completed by programming the frequency protocol with the control option via an RS232 interface of the standard laboratory centrifuge.

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