AUTOMATION OF NUCLEIC ACID EXTRACTION BY A CORIOLIS-FORCE ACTUATED DROPLET ROUTER

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ABSTRACT

We present an automated nucleic acid extraction protocol by a simple frequency and dispensing protocol of a microfluidic rotor. The key enabling steps are the centrifugally induced generation of droplets which are routed by the Coriolis pseudo force either to the waste or to the eluate reservoir. At centrifugally driven flow rates below 2 µL/s through a packed column of 11 mg silica particles, up to 0.7 µg of purified DNA (recovery ratio of 16%) is collected in an onboard reservoir or – to smoothly interface with common lab environments – in a standard Eppendorf tube ("Eppi") attached to a "flying bucket" rotor.

Keywords: centrifugal microfluidics, droplet formation, routing, DNA extraction

1. INTRODUCTION

Nucleic acid analysis has gained increasing importance for in vitro diagnostics within the last years. The integrated extraction of DNA from e.g. whole blood samples is an essential step towards the implementation of an automated nucleic acid analysis. The process is usually implemented by the reversible adsorption of the DNA molecules to a silica solid phase (the so called "Boom chemistry" [1]). Within this work we use silica microparticles of approximately 110 µm diameter as solid phase and restrain the flow rates to meet the time frame of an optimal physicochemical binding. The applied droplet switch [2] enables the routing of small liquid flows by means of the Coriolis pseudo force.

2. FUNCTIONAL PRINCIPLE

Figure 1 shows the microfluidic channel network comprising an extraction chamber where silica particles are retained by a geometric aggregation step. The extraction buffers

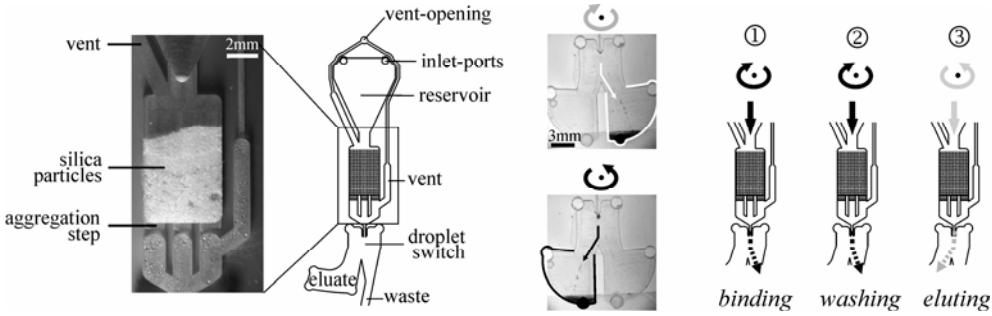

***Figure 1**. Design of the microfluidic structure for centrifugal DNA-extraction. Silica particles are aggregated in front of a geometrical aggregation step at the radial end of the extraction chamber. The droplet switch after the extraction chamber enables the switching between two downstream flow paths as depicted in the middle. The complete process sequence for DNA extraction of binding, washing and eluting is shown on the right.*

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are filled via the inlet-ports into the reservoir. After the centrifugal flow has passed the stationary phase, the liquid disintegrates and proceeds to the hydrophobic orifice [2]. The issued droplets are deflected against the direction of motion into the waste (clockwise) or eluate reservoir (counter-clockwise), depending on the sense of rotation imposed by the spinning protocol. The silica particles can efficiently be dried by centrifugation prior the elution step, thus ensuring a high purity of the extracted DNA.

The automated protocol consists of three basic steps: the binding of the DNA to the silica particles ①, the subsequent washing in order to remove any unwanted impurity ② and the final eluting to gain the purified DNA ③ (Fig. 1, right). The clockwise sense of rotation for the first two steps routes the binding and washing solutions into the waste reservoir. Within the last flow-through, the sense of rotation is altered and the purified DNA ends up in the eluate reservoir while preventing cross-contamination.

3. EXPERIMENTAL RESULTS

The experimental performance is qualified by the extraction of nucleic acid solutions from calf thymus [3] on silica particles taken from a commercial buffer kit [4]. The particles are filled into the extraction chamber via the inlet reservoir and subsequently compacted by centrifugation. The flow rate through the silica particle aggregation has been measured and is displayed in Fig. 2. The flow rate scales with the square of the rotational frequency as expected from the centrifugal hydrodynamic theory. Flow rates remain below 2 µL/s for frequencies of rotation not exceeding 15 Hz. This is the working range for the flow-rate critical steps during the extraction protocol, namely the binding and eluting step.

The complete DNA extraction protocol comprises the initial binding, three washing and the final elution step. The amount of DNA within the sample and the eluate is measured by a fluorescence labeling kit (*Invitrogen*, *Quant-iT*). Figure 3 identifies a working range between 0.5 µg and 4 µg wherein the mass of DNA in the eluate increases with the amount of DNA in the input sample. The working range corresponds to a volume of whole blood between 16.7 and 133.3 µL (amount of DNA in whole blood: ~ 30 µg/mL).

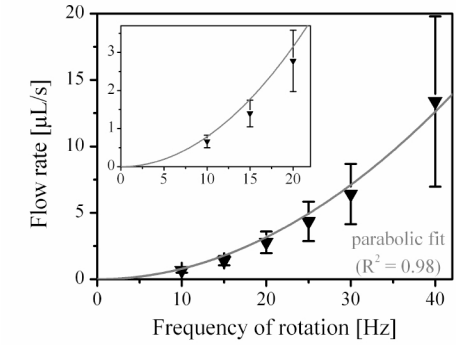

Figure 2. *Defined flow-conditions through the solid-phase (silica particle aggregation). The ratio of diffusion time and transit time can easily be adjusted by the frequency of rotation. The binding and eluting step of the DNA extraction are done below 15Hz.*

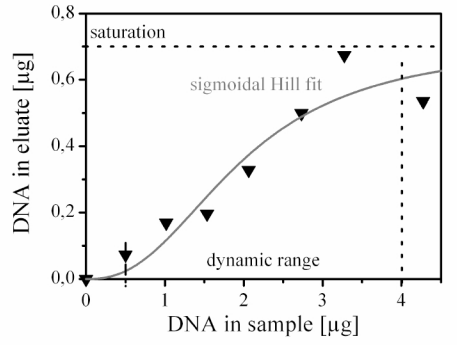

Figure 3. *Results of the integrated DNA extraction on the rotating disk. The amount of DNA in the eluate increases with the amount of DNA in the sample. The maximum amount of extracted DNA within the first eluate is 0.7 µg, corresponding to a recovery ratio of 16 % in the dynamic range.*

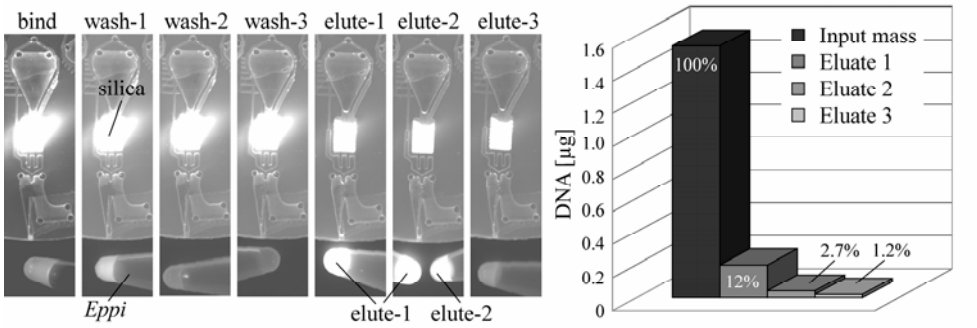

Figure 4. *Left: To investigate the extraction performance, the process is visualized by fluorescent labelling of the DNA molecules in the sample. A fluorescent image is captured after each extraction step: the binding, the three washing and the three elution steps. Besides the microfluidic extraction structure, also the flow of elute into a standard Eppendorf tube ("Eppi") is included in the picture. Right: measured total mass of DNA in the sample and recovery of DNA in each of the three elution steps.*

The experimental data represents a fit to a sigmoidal curve to account for the saturation of the extraction system at 0.7 µg. The recovery ratio, i.e. ratio between input and output DNA, respectively, amounts to 16 % within the dynamic range.

In Figure 4, the fluorescently labeled DNA is tracked at different stages of the extraction procedure in order to qualify the extraction process. Therefore, all liquids are switched into the waste outlet and transferred into a standard laboratory tube ("*Eppi*") for inspection. The small traces of DNA which are initially bound to the channel walls are removed by the first washing step and no measurable amount of DNA is present in the second and third washing solution (liquid within the *Eppi* tubes placed below the microfluidic structure in the fluorescent images). The overwhelming fraction of DNA is eluted by the first and second elution step. Even after the third elution, there is still some DNA bound to the stationary phase. Thus, for a further improvement of the recovery ratio, the elution step should be optimized (see measured amount of DNA in the three eluates in Fig. 4, right).

4. CONCLUSION AND OUTLOOK

The here presented, centrifugal microfluidic technology bears great potential to replace costly, state-of-the art equipment for the integration and automation of nucleic acid extraction procedures. Future work will focus on improvements of the extraction recovery ratio as well as the extension of the process chain by upstream cell lysis and downstream amplification (PCR).

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