CONTINUOUS MICROFLUIDIC DNA PURIFICATION FOR ONLINE MONITORING AND PROCESS CONTROL

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ABSTRACT

We demonstrate the first continuous microfluidic platform for flow-through DNA purification from cell lysate. Using superparamagnetic beads, DNA is continuously transported across interfaces between co-flowing laminar streams of extraction reagents. In on-chip experiments DNA was continuously purified over a time period of 110 min. After the on-chip purification, DNA content and purity of the eluate sampled at different stages of the continuous extraction experiment was analyzed off-chip via qPCR. Results show successful flow-through purification with constant output over almost the complete duration of the experiment. Possible applications are seen in biological safety and environmental monitoring or bioprocess control.

KEY WORDS: Nucleic Acids, Extraction, Continuous, Flow-Through

INTRODUCTION

Optical and electrical sensors are applied to continuously monitor various parameters like concentrations of airborne pathogens or cell count in bioreactors. If continuous monitoring could be extended to nucleic acids based approaches, the measurement results would provide additional valuable information — such as the presence of pathogenic or spoilage bacteria, viruses, or genetically modified organisms. To keep reagent consumption low, a microfluidic approach is advisable. Lab-on-a-Chip modules for flow-through lysis [1,2] and flow-through PCR are already well-established [3,4]. However, corresponding modules for continuous DNA purification have not been available so far, rendering integrated monitoring systems impossible. Therefore, we developed a microfluidic platform for continuous DNA purification from cell lysate based on "Phase-Transfer-Magnetophoresis" (PTM) which showed an on-chip purification efficiency better than conventional off-chip systems [5]. Here, we focus on characterizing the performance over time of the system.

WORKING PRINCIPLE

Cell lysate of an *E. coli* culture is mixed with superparamagnetic beads and binding buffer of a commercially available DNA extraction kit. After binding of the DNA to the beads, the mixture is continuously injected into the microfluidic chip. Washing buffer and elution buffer are introduced through a second and third inlet, respectively. On-chip, the reaction reagents are brought into contact forming a laminar interface. Circularly arranged microchannels guide different buffer flows around a central rotating permanent magnet (Fig. 1). The magnet generates a time-varying magnetic field and attracts the beads towards the inner channels walls, thus transferring the beads together with the bound DNA across the laminar interface.

RESULTS

Precision syringe pumps controlled the flow of the sample and the DNA purification reagents within the microfluidic chip. The extraction chip was fabricated by micromilling into polycarbonate (Fig. 2). An inlet flow velocity of 11.9 $mm \cdot s^{-1}$ (0.75 $\mu l \cdot s^{-1}$) led to an average bead velocity of 0.7 $mm \cdot s^{-1}$ and a sample transition time of approximately 2 minutes. To confirm the continuous working principle, DNA was purified from an *E. coli* lysate over the course of 110 min with 10 min sampling intervals. Sampling was started as the first magnetic beads left the chip defining time stamp t = 0 s. The syringe containing the lysate and magnetic beads was stirred every 30 min to circumvent bead sedimentation. After the on-chip purification the DNA content of the eluate samples was determined off-chip via qPCR (Fig. 3). After a short period of initial ramping, the extraction efficiency was constant within the error bars at a DNA concentration value of 24800 $copies \cdot \mu l^{-1}$. Stirring of the feed syringe could not fully prevent bead sedimentation. Therefore, the bead concentration increased towards the end of the experiment resulting in increasing DNA concentration in the eluate.

CONCLUSION

With the novel platform for Phase-Transfer-Magnetophoresis we successfully demonstrated continuous DNA purification from bacterial lysate with (almost) constant performance over a period of 110 min. The module bridges the missing path between continuous cell lysis and continuous DNA amplification to complete powerful microsystems for online monitoring and process control. Using magnetic beads with appropriate surface modification, the fields of application can be extended to purification of other biomolecules like RNA, proteins or even cells.

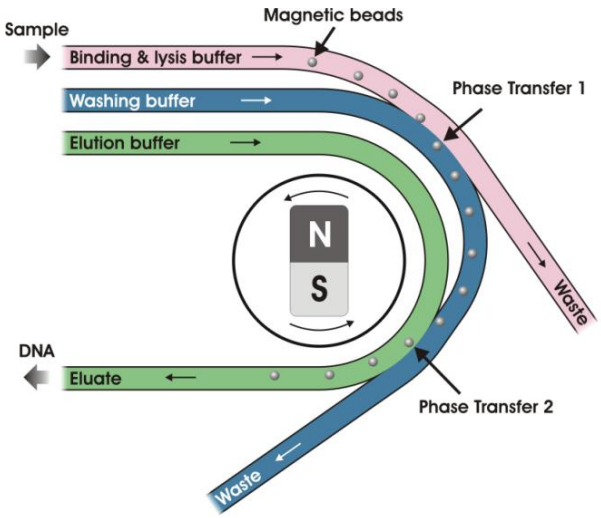

Figure 1: Schematic view of the microfluidic structure for continuous DNA extraction. The direction of rotation of the central permanent magnet is opposite to the direction of the buffer flow.

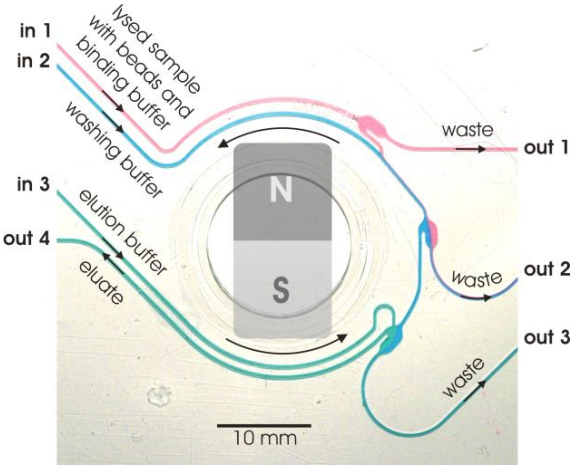

Figure 2: Photograph of the microfluidic chip. To illustrate the purification process, DI water dyed with ink has been injected into the chip. The different colours denote the different buffer solutions. Red: lysis and binding buffer including the cell sample and the magnetic beads, blue: washing solution, green: elution buffer.

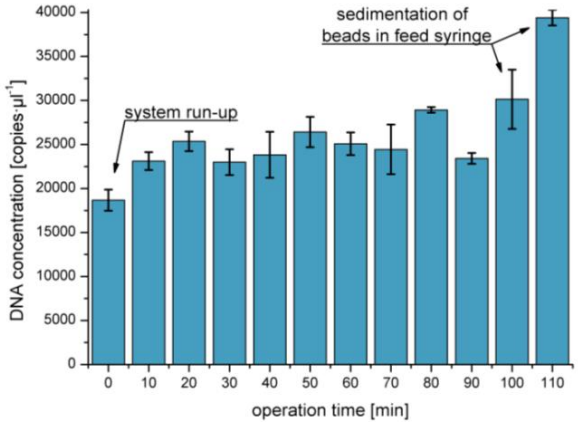

Figure 3: Continuous long-term on-chip DNA extraction. Lysate with binding buffer and magnetic beads was stored in a syringe. The sample and purification reagents were continuously injected into the microfluidic chip. The error bars indicate the standard deviation of three qPCR analyses.

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