A NOVEL MICROFLUIDIC PLATFORM FOR CONTINUOUS DNA EXTRACTION AND PURIFICATION USING LAMINAR FLOW MAGNETOPHORESIS

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ABSTRACT

We present a novel microfluidic platform using laminar-flow magnetophoresis for combined continuous extraction and purification of DNA. All essential unit operations (DNA binding, sample washing and DNA elution) are integrated on one single chip. The key function is the motion of magnetic beads given by the interplay of laminar flow and time-varying magnetic field. The time for extraction was 1 minute. The device is a central part of a complete biochemical system for continuous monitoring of cell-growth in bioreactors. The novel platform allows continuous purification of DNA, but is also applicable to purification of RNA, proteins or cells, including their subsequent real-time analysis in general.

INTRODUCTION

Monitoring of biological agents, including the pathogenic microorganisms, protein and free nucleic acids, is highly relevant in the field of security (B-detection), blood monitoring and process control in pharmaceutical fermentations. Key requirement to perform these monitoring tasks is the continuous processing of biochemical assays. Such continuously working and automated monitoring systems are currently not available.

Continuous on-chip PCR systems for amplification and detection of DNA have already been developed [1] including a transcription into cDNA for RNA detection [2]. A concept for continuous molecular enrichment using segmented flow has also been proposed [3]. However, a method for continuous DNA or RNA extraction from any sample such as whole blood or cell cultures was not achieved. Therefore we develop a novel microfluidic platform in order to realize a fully integrated microfluidic continuous extraction and analysis system for on-line monitoring of cell growth in bioreactors.

Among various techniques for DNA purification, we use DNA adsorption onto superparamagnetic beads. Different microfluidic approaches using this method in a batch-wise manner have already been reported [4-6]. In contrast our device allows the continuous operation in a flow through manner. Until now the only existing continuous magnetophoretic devices are for single-step cell separation [7;8], while we now report well-controlled manipulation of magnetic beads through at least three subsequent assay steps necessary for nucleic acid purification.

WORKING PRINCIPLE

The working principle is depicted in Fig. 1. The first step of DNA extraction and purification after sampling and lysis of the cells is binding of the DNA onto the superparamagnetic beads in order to separate the DNA from impurities such as enzymes and/or cell debris. After the separation a washing step is included to remove the high salt buffers required for DNA binding as well as any residual impurities. As a last step the beads are transferred into the elution buffer where the DNA dissociates from the beads to allow real-time analysis.

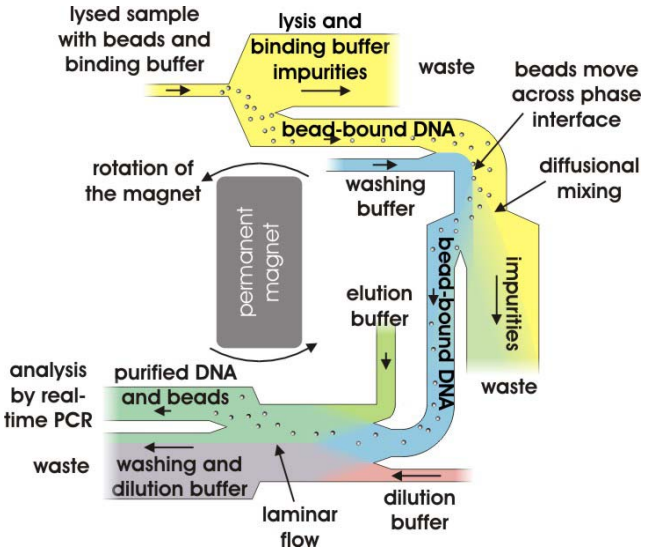

Figure 1: Schematic view of the microfluidic chip for continuous DNA extraction. The rotation of the permanent magnet is opposite to the direction of the buffer flow.

Chip design

Our microfluidic chip applies magnetophoresis to control superparamagnetic beads in different buffer solutions under continuous laminar flow (Fig. 1).

Bead-bound DNA samples flow through three chambers for impurity separation, sample washing, and DNA elution from the beads. The circular arrangement of the microfluidic structure (Fig. 2), together with a single rotating permanent magnet positioned below the chip center, allows the transfer of magnetic beads from one reagent to the other in each separation chamber. Subsequently, the magnetic beads transport the DNA through three separation chambers for the separation of impurities, sample washing and the final elution of DNA elution.

The separation chambers consist each of one or more inlets and two outlets which are different in diameter. This is a key design feature for the division of the buffer flow into two fractions of different flow rates; a fraction of small volume containing a high concentration of magnetic beads and thus high concentration of sample, and a large fraction without any magnetic beads but most of the buffer solution.

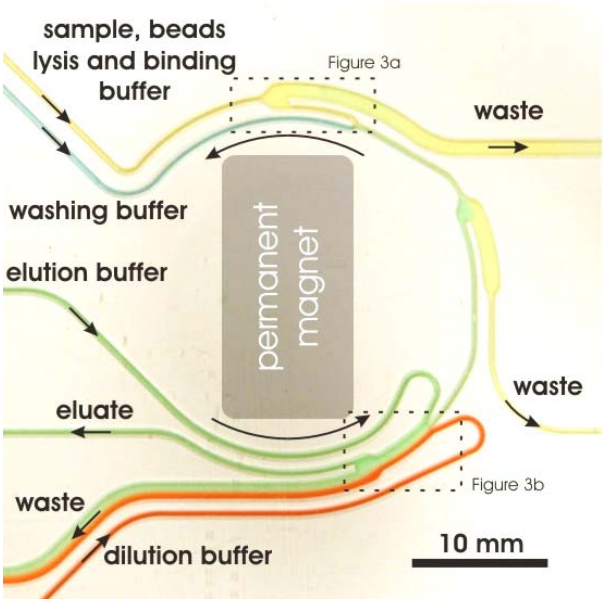

Figure 2: Polycarbonate chip with micro-milled microchannels. This chip design was used for the DNA extraction experiment. To illustrate the extraction process dyed buffer solutions were injected into the chip. Yellow: Lysis and binding buffer, blue: washing solution, green: elution buffer, red: dilution buffer.

The magnetic beads exit the separation chambers through the small outlet and are transferred to the next section whereas the large fraction of the buffer solution leaves through the wider outlet that directly leads to the waste.

Time-varying magnetic field

The circular arranged microchannels guide different buffer flows from different inlets of the chip around a single external permanent magnet located beneath the center of the chip. The permanent magnet is rotated slowly using a stepping motor to provide the required periodically changing magnetic field to attract the magnetic beads in the radially inward direction. According to the oscillating strength of the magnetic field, the beads are attracted and stick to the side-wall during the strong field phase but are released during the weak field phase. With the circular microfluidic design shown in Fig. 2, the DNA-bound beads are attracted by sufficiently strong magnetic force during the strong field phase at each of the separation chambers. The average magnetophoretic velocity of beads is determined by the balance between the flow rate and the strength of magnetic field, the latter of which can be controlled by altering the magnet rotation speed. We have also observed that by rotating the magnet rotation in the direction opposite to the flow, the streamwise bead velocity is larger. Thus, we realize efficient separation of the magnetic beads without immobilizing them on the walls of the microchannels.

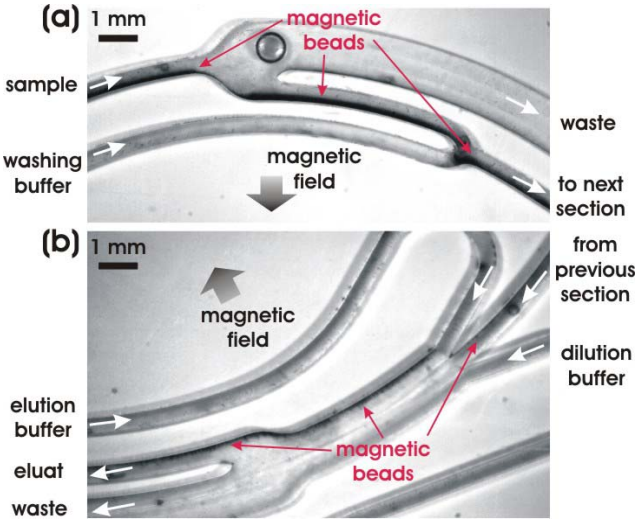

Figure 3: Instantaneous image of (a) the first separation chamber and (b) the third separation chamber. The beads are strongly attracted by the magnetic field and flow in the vicinity of the inner side wall throughout the device. The inlet flow velocity of each stream is 12.5 mm·s^{-1} and the average velocity of beads estimated from consecutive images is approximately 1.6 mm·s^{-1}.

Buffer flow protocol

A mixture of cell lysate, binding buffer and superparamagnetic beads, which are already bound to the DNA, enters the chip through the 1st inlet (Fig. 2, top left). In the first separation chamber the beads are concentrated by the central permanent magnet and separated from most of the buffer solution containing impurities (Fig. 3a).

During the transfer to the next section, washing buffer is added forming a laminar flow with the remaining binding buffer. The permanent magnet attracts the beads to the inner part of the channel system and ensures the transfer of the magnetic beads across the phase interface.

In the second separation chamber the magnetic beads are again concentrated and most of the buffer is transferred to the waste, while the magnetic beads leave through the small outlet towards the third separation chamber (Fig. 3b).

Here the beads are transferred to the elution buffer, in which the DNA is released from the bead surface. The eluate is collected for further analysis. A key feature in the third separation chamber is the introduction of a dilution buffer.

The washing buffer contains a high concentration of ethanol, which is a PCR inhibitor. However, the hygroscopic nature of ethanol leads to rapid dispersion throughout the sample flow, and it is thus not possible to establish a clear laminar interface between the washing and elution buffers. Therefore we introduce a dilution buffer that is injected from the opposite side. The purpose of the dilution buffer is to assimilate the ethanol from the washing buffer and therefore reduce the ethanol concentration in the eluate or even exclude ethanol from the eluate.

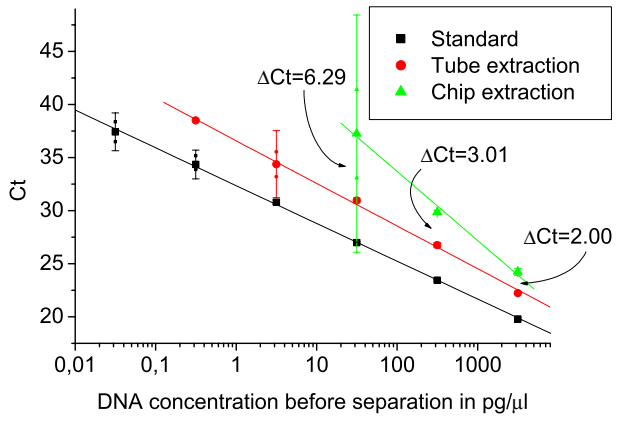

Figure 4: Real-time PCR of extracted genomic E. coli DNA. Shown in the graph is the result of a DNA extraction experiment performed both in a test tube and on the microfluidic chip. The genomic E. coli DNA was diluted and spiked with herring sperm DNA (therefore the gap between the standard curve and the tube extraction) . At E. coli DNA concentrations of 3.15 ng·µl^{-1} the on-chip purification achieves 25% of the reference in the test tube.

On the other hand, DNA molecules and magnetic beads are large enough that they do not diffuse in the sample solutions so rapidly. Thus, after the mixing of elution and washing buffer on one side of the third separation chamber and the mixing of washing and dilution buffer on the opposite side, the purified DNA remains in the elution buffer stream to be collected at the outlet without any loss.

CHIP PRODUCTION

The 2D-layout of the microfluidic channels was designed with CAD software and converted to G-code compatible to a precision milling machine. For chip production polycarbonate was used. The mircochannels were milled directly into the chip using a 400 µm mill, with a standard width and depth of 400 µm. After the milling process the channels were sealed with adhesive foil.

RESULTS

The flow of sample and buffers of a commercially available DNA purification kit was controlled by syringe pumps (neMESYS, cetoni GmbH). The permanent magnet was mounted below the chip system close to the surface and rotated by a stepping motor with a rotational frequency of 1 Hz.

We extracted and purified a sample of genomic DNA from *E. coli* DH5αZ1 [9] bacteria on the chip in a flow-through manner. An inlet flow of 12.5 mm·s^{-1} lead to an average bead velocity of 1.6 mm·s^{-1} and a sample transition time of approximately 1 minute.

The extracted DNA was successfully amplified off-chip via real-time PCR to demonstrate that PCR inhibitors included in the buffers are sufficiently diluted and/or excluded during the on-chip purification. At a starting concentration of 3.15 ng·µl^{-1} the fluorescence signal of the chip-extracted total DNA crossed the threshold value only 2 cycles later than the reference extraction performed in a test tube (Fig. 4).

CONCLUSION

We established a microfluidic platform for continuous DNA purification by magnetophoretic manipulation of magnetic beads in a time-varying magnetic field. The platform closes the gap between continuous cell lysis and continuous DNA amplification to set up powerful microsystems for the monitoring of biological agents, pathogenic microorganisms, protein, free nucleic acids and cell growth in bioreactors.

The magnetic beads enable the transport of the DNA across the interfaces between co-flowing laminar streams in a circular channel arrangement around a central rotating permanent magnet inducing time-varying magnetic field, which prevents the beads from sticking to the channel walls and enables controlled transfer of beads between different extraction reagents. An inlet flow velocity of 12.5 mm·s^{-1} lead to an average bead velocity of 1.6 mm·s^{-1}. The sample transition time is approximately 1 minute. Compared to a reference extraction in a test tube, 25% of DNA could be detected by Real-Time PCR. However, we expect significant improvements in the recovery rate and purity by optimizing the magnet motion and fluid resistance at each section of the microfluidic structure.

Applications of this concept for the extraction and purification of biomolecules are the monitoring of cell growth in fermenters or continuous safety monitoring of drinking water against biological contaminations. Other potential applications are in the field of continuous protein purification, immuno- and cell based assays or other biological assays that are compatible with target-binding to magnetic beads.

ACKNOWLEDGEMENT

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