MAGNETIC ON-CHIP DNA EXTRACTION IN A DROPLET BASED MICROSYSTEM

U. Lehmann[1], C. Vandevyver[2], V.K. Parashar[1], M.A.M. Gijs[1]
1) Institute of Microelectronics and Microsystems
2) Research Commission EPFL-SNF
Ecole Polytechnique Fédérale de Lausanne (EPFL), Switzerland

Abstract
Magnetic droplet manipulation is a promising new approach towards the miniaturization of bioanalytical procedures. We present a system that combines droplet microfluidics and magnetic microparticles for the extraction and purification of DNA from µl-sized lysed cell samples. The DNA is detected on-chip via fluorescent microscopy or via an off-chip amplification step. We are able to extract and detect the DNA from as little as 10 cells in our system.

Keywords: droplet manipulation, magnetic particles, DNA extraction, cell lysis

1. Introduction
The miniaturization of processes for bio-analysis is an area of vast potential for droplet based microfluidic systems. In this respect the extraction of DNA from crude cell samples is gaining interest since most detection procedures rely on an amplification step that requires DNA of high purity. Here, we present a system (figure 1) that combines the magnetic manipulation of freely suspended droplets with bead-based DNA purification protocols. The use of small droplets as sample containers helps to reduce reagent consumption and processing time and in addition decreases the contact interfaces with the manipulation platform, thus minimizing problems of bio-molecule adsorption to sidewalls. The droplets are actuated via magnetic microparticles contained within, which act as force mediators and allow us to profit from the comparatively large force and long range of magnetic actuation [1]. These particles additionally serve as mobile substrates for the bio-molecules [2].

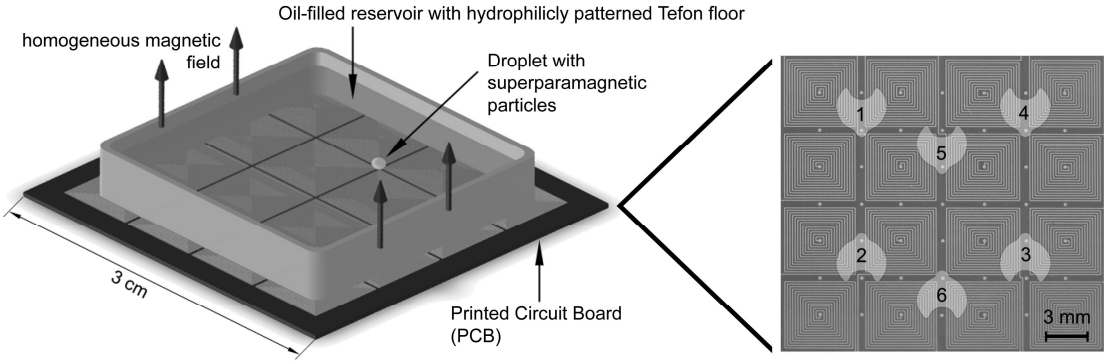

Figure 1. Schematics of the droplet manipulation system. The figure on the right-hand side demonstrates the placement of the 10 µl reservoirs that contain the different reagents for the DNA extraction (1: Lysis buffer, 2-4: washing reagents (ethanol), 5-6: elution buffer).

2. Purification Procedure and Setup

The stages of the on-chip DNA purification are schematically shown in figure 2. Starting from a set of six immobilized droplets of 10 µl, we are able to miniaturize a protocol for the extraction and purification of DNA from crude cell samples. The cells are lysed in a solution containing Guanidine thiocyanate, which also help to selectively attach the DNA to the magnetic silica particles. After extracting a small droplet containing the magnetic particles and the attached DNA from the immobilized lysis buffer droplet, the particle-and-DNA compound is passed through three stages of washing. Thus we are able to remove transferred cell debris or proteins, since these would inhibit subsequent steps of amplification. As a last step of the on-chip DNA extraction protocol, the purified DNA is eluted from the particles in a buffer of low ionic strength. Subsequently the eluted DNA can be either detected using fluorescent microscopy or it can be transferred to a step of amplification via Polymerase Chain Reaction (PCR).

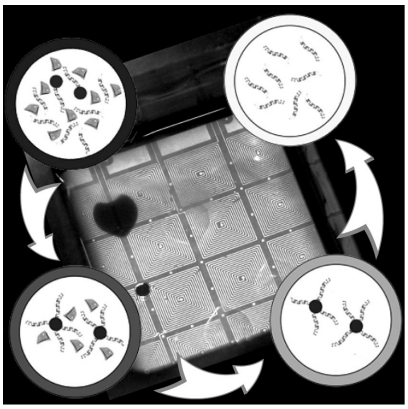

Figure 2: Schematic principle of the DNA extraction and purification process. First, the cells are lysed and the DNA is bound to the magnetic particles. The magnetic particles are separated from the initial droplet and washed in three stages. Finally the particles are mixed with an elution buffer where the DNA is removed

3. Experiments and Discussion

We tested the functionality of the proposed procedure using different concentrations of mammalian cells (Jurkat, ACC-TIB 152)[3]. The eluted DNA was detected via fluorescent microscopy using PicoGreen as quantification agent. Figure 3 shows the results of the on-chip detection, where we can clearly distinguish between different initial cell contents. Due to the low DNA concentration, which ranges from 60 pg for 10 cells to 6000 pg for 1000 cells in a 10 µl droplet, the fluorescent signal is close to the lower limit of detection. We additionally transferred the extracted and eluted DNA to a subsequent amplification step. This PCR-step also verifies if the extracted DNA is intact and sufficiently pure, because cells debris, proteins or lysis reagents generally inhibit the amplification. Figure 4 shows an agarose gel electropherogram of different samples, where the successful amplification of human β-actin proves the purity of the eluate. In the electropherogram, the difference in cell amounts is also visible, especially in the band of the unused primer. The intensity of the primer for 10 cells is significantly higher

than for 100 or 100 cells which indicates differences in the concentration of the initial DNA material subjected to the amplification.

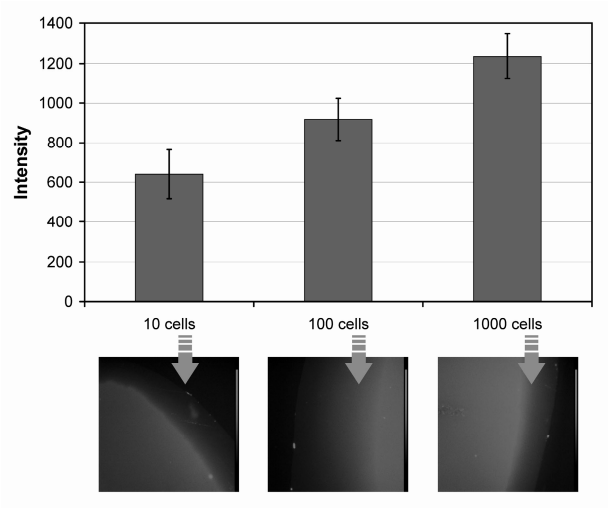

Figure 3. Fluorescent on-chip detection of the eluted DNA in a 10 µl droplet. The upper diagram shows the intensity of the fluorescence as determined from the fluorescent microscopy photographs below.

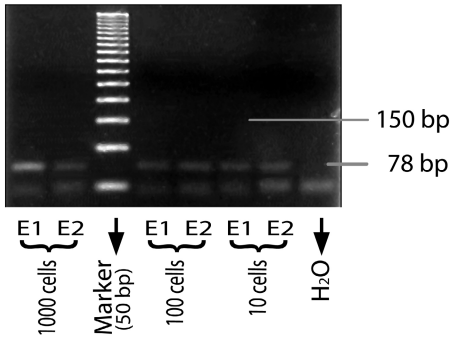

Figure 4. Agarose gel electropherogram of human β-actin after 35 cycles of PCR starting from 3 µl of eluate, which contains the DNA extracted from different concentration of Jurkat cells. The lowest band shows the primers whose consumption varies with initial cell number. (E1, E2: first and second elution; M: 50 bp ladder marker)

4. Conclusion

Our results demonstrate the potential of the presented 2D magnetic droplet manipulation system as a promising platform for bioanalytical applications. We were able to extract, detect and amplify the DNA of as little as 10 cells using sample volumes of 10 µl. The use of droplets allows an easy conversion of titerplate-based procedures onto a microchip, which will result in lowered reagent consumption, higher system sensitivity and less contamination.

References:

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