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REMOVAL OF PCR INHIBITORS USING DIELECTROPHORESIS FOR SAMPLE PREPARATION IN A MICROFABRICATED SYSTEM.

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Abstract

In this abstract we present the results of a novel method for sample preparation to remove inhibitory substances from samples that would prevent DNA amplification. The method is based on dielectrophoresis and is suitable for integration in μTAS.

Keywords: Sample preparation, Dielectrophoresis, PCR inhibitors, DEP, Cells

1. Introduction

Polymerase chain reaction (PCR) has been used to amplify specific DNA sequences and to detect microorganisms in clinical diagnostic and food industry. However, when conducting PCR with biological materials (blood, milk, feces etc.), residual materials in the sample may significantly or totally inhibit the PCR process [1, 2]. Conventional methods for sample preparation prior to PCR can involve intensive manual operations that may be very difficult or impossible to integrate in μTAS. We present a novel method for sample preparation based on dielectrophoresis (DEP). DEP is the movement of dielectric particles e.g. cells by the forces imposed upon them by an inhomogeneous electric field and DEP has been used for manipulating cells in microsystems [3].

2. Experimental

The microsystem was a DEP-chip fabricated on a silicon substrate. A 2 μm LPCVD (Low Pressure Chemical Vapour Deposition) nitride layer was deposited on the silicon substrate to insulate the electrodes from the substrate. On top of that a 180 μm LPCVD polysilicon layer was deposited. An 80 nm layer of titanium was deposited using E-beam evaporation and structured using a lift of process. To remove the natural oxide a 30 second HF (hydrofluoric acid) dip was applied just before titanium deposition. The silicide was formed using rapid thermal annealing for 1 minute at above 700°C in an argon atmosphere. The electrode design was an interdigitated-finger structure [4] with a characteristic dimension of 10 μm and a length of 2 mm (figure 1). The walls of the channel were made of SU-8 [5] and the channel was height width = 70 400 μm. The total channel length in the microstructure was 11 mm.

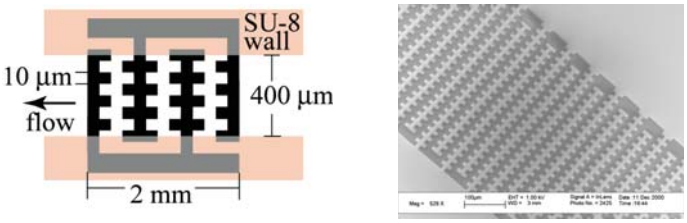

Figure 1. Left: diagram of microfluidic structure with integrated silicide electrodes. Right: SEM picture of the electrodes

PCR was performed using the primers of a yeast housekeeping gene (ribosomal protein S3). PCR program: 10 minutes at 94°C, 35 cycles of 94°C for 30 seconds, 59°C for 30 seconds and 72°C for 1 minute. The final step was 72°C for 10 minutes.

3. Results and Discussions

In this study, we investigated the inhibitory effect of hemoglobin and heparin on PCR. Both hemoglobin and heparin are natural part of blood and heparin is furthermore often added to blood samples to prevent coagulation. Hemoglobin or heparin was added to PCR mixture. The target DNA for amplification was in the form of whole yeast cells (*Saccharomyces cerevisiae*) and the PCR reactions were performed in tubes using a conventional thermocycler (table 1). These initial experiments show that the PCR reactions were completely inhibited by a hemoglobin concentration of 0.1 mg/ml and by a heparin concentration of 1.3×10^{-3} mg/ml.

Hemogl.	10 mg/ml	1 mg/ml	100 µg/ml	10 µg/ml	1 µg/ml	100 ng/ml	pos	neg
PCR result	–	–	+	+	+	+	+	–
Heparin	1.3 mg/ml	130 µg/ml	13 µg/ml	1.3 µg/ml	130 ng/ml	13 ng/ml	pos	neg
PCR result	–	–	–	+	+	+	+	–

Table 1. PCR inhibition effects of hemoglobin and heparin, respectively. (+): PCR positive (-): no PCR product, (n.t.): not tested. Pos/neg: positive and negative controls.

To test whether DEP can be used to remove the PCR inhibitors, we used a microfluidic structure with integrated silicide electrodes (figure 1). The yeast cells were mixed with hemoglobin (10 mg/ml) or heparin (1.3 mg/ml) and these inhibitor/yeast samples were prepared for PCR in 3 steps (figure 2 and 3): First the sample is sucked into the microstructure. During this step the yeast cells are captured on the electrodes by the electric field using positive dielectrophoresis (DEP) (figure 4). Secondly, the inhibitor is wash away while the cells remain captured on the electrodes. Finally, the electric field is turned off, and cells are released and collected. The PCR were performed directly on

these yeast cells. The initial hemoglobin or heparin concentration was 3 orders of magnitudes higher than the PCR inhibition level (table 1). A washing for 5 and 10 minutes for hemoglobin and heparin could remove the inhibitory effect, respectively (table 2).

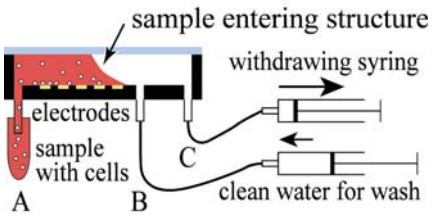

Figure 2. Schematic overview shows the first step in which the sample is sucked into the microstructure and cells captured on the electrodes

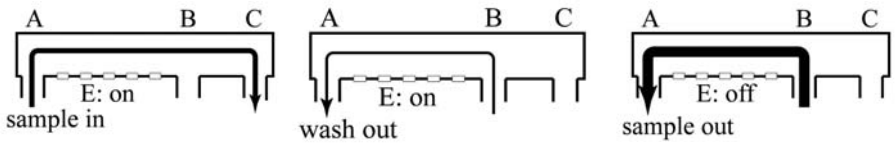

Figure 3. The 3 steps in the sample preparation: collection of cells, washing, and sample release

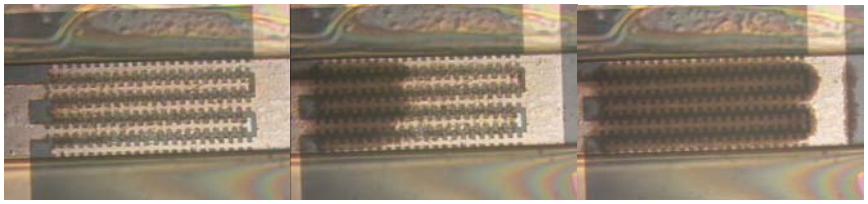

Figure 4. A series of 3 images showing the build up of cells as they collect on the electrodes under the influence of positive DEP.

4. Conclusions

In conclusion, in the present study we showed that yeast could be selectively withheld while PCR inhibitors are removed by using dielectrophoresis in a microsystem. The required volumes (sample and wash) are small, and in the order of 30 µl. The dielectrophoretic filter can be used to collect any cell type, without altering the set up much. In contrast to conventional methods the sample preparation method presented here

is suited for integration with microstructures for PCR reaction and DNA analysis. This work is an important contribution towards sample preparation, PCR reaction and DNA analysis in a micro total analysis system for molecular diagnostics.

min.	hemogl. 1	hemogl. 2	heparin 1	heparin 2
0	–	–	–	–
2.5	–	–	–	–
5	+	+	–	–
7.5	n.t.	+	n.t.	–
10	+	+	+	+
30	+	n.t.	+	n.t.
pos	+	+	+	+
neg	–	–	–	–

Table 2. Sample preparation in the microstructure. Result of the PCR reaction after washing with a flow rate of 0.2 ml/h for various time. (+): PCR positive (-): no PCR product, (n.t.): not tested. Pos/neg: positive and negative controls.

Acknowledgements

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