Membrane based sample preparation chip

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Abstract

Microfluidics plays an important role for the miniaturization of chemical and bio-chemical processes used for the detection of pathological states. Aside from the detection process itself sample preparation is a crucial but often neglected step in the process chain. While traditional methods use many manual handling steps in tubes or wells we present the integration of nucleic acid extraction in a small polymer chip by using proven extraction in a silica membrane. The results are compared to a conventional in tube protocol with the same volume of lysed sample.

1 Introduction

The miniaturization of molecular diagnostics for decentralized and point-of-care testing requires the integration of all necessary molecular biological processes in one, self-contained and autonomous system. User intervention, such as manual handling of liquids, pumping or switching of valves, has to be reduced to a minimum in order to minimize possible errors and misreadings of the system.

The advances in microfluidic research[1, 2] have created a set of microfluidic technologies like integrated pumps and valves or miniaturized optical readouts that enable even the integration of highly complex processes such as sample preparation.

Since pre-analytics for molecular diagnostics in laboratory scale commonly require a lot of manual liquid handling steps we developed a disposable sample preparation chip with integrated pumps and valves for the silica membrane based extraction of nucleic acids from liquid samples.

1.1 Nucleic acid extraction methods

The extraction of nucleic acids from different sample materials is the key technology for highly sensitive molecular biological analytics. Main goal of the extraction process is the isolation of specific nucleic acids for further processing(PCR) and the removal of interfering or inhibiting substances like proteins or alcohols. Various methods exist utilizing the different solubility of nucleic acids and contaminant (phenol/chloroform), the different molecular weight (gel filtration) or the temporary selective binding of nucleic acids to solid matrices(ion exchange chromatography, absorption chromatography)[3,4].

For the fast extraction from small sample volumes absorption chromatography with silica matrices is the dominating technology. The silica matrix is usually provided a silica surface of magnetic beads or as a solid membrane (composite).

1.1.1 Magnetic beads

Polymer capped magnetic particles are widely used for the affinity based separation since the technology was invented in 1976 by John Ugelstad[5]. Silica coated magnetic beads combine the principle of absorption chromatography with the easy and fast handling of magnetic bead technology. Usually the beads are given to the lysed sample under high-salt conditions binding the nucleic acids to the beads. For the removal of supernatant the beads are temporarily immobilized by an external permanent magnet.

1.1.2 Silica membranes

In contrast to magnetic bead based extraction methods here the silica surface is provided as a solid membrane. For high purity and reduction of filtration time the membranes are commonly provided as spin columns for the use in laboratory centrifuges. In principle the membrane based extraction consists of four steps: lyses, binding, washing and elution (see figure 1).

![Figure 1 Schematic of silica membrane based extraction (clockwise): 1. Lysis of cells, 2. Binding of nucleic acids to membrane, 3. Washing off of debris, 4. Elution](image-url)
1.2 Integrated nucleic acid extraction

In literature various publications describe the integration of silica based nucleic acid extraction for sample preparation on chip. Karle et al. [6] describe a microfluidic system for the continuous DNA extraction by phase-change magnetophoresis. The sample is mixed with super-paramagnetic beads which are manipulated by a rotating permanent magnet. Resulting from the continuous flow regime the principle is not suited for an extraction in an all-contained microfluidic cartridge. The integration of a silica membrane in a CD-shaped rotational microfluidic platform was reported by Focke et al.[7]. In the system fluid transport is realized by inertial forces in a laboratory centrifuge. Although the system is highly integrated and suited for small sample volumes its main drawback is seen in the missing possibility of combining the system with cheap electrochemical sensors.

2 Methods

Resulting from the need for an integrated disposable cartridge for nucleic acid extraction a polymer based system was developed. The system was tested with yeast cells (saccharomyces cerevisiae) and the obtained results were compared to in parallel extractions with commercial spin columns.

2.1 Cartridge configuration and working principle

The cartridge consists of the following parts: The disposable polymer chip with four integrated reservoirs (40 µl) for the necessary buffers, the PCB with the integrated low cost pumps and valves and the silica membrane for the temporary binding of nucleic acids. The microfluidic pumps and valves were realized by using low cost electrolysis generators published before [8]. The polymer chip consisted of Makrolon® (Bayer) and was fabricated by injection molding. The PCB was obtained by an ordinary PCB job shop. The silica membrane was integrated in the chip using medical grade adhesive tape. The adhesive tapes and flexible membrane for the electrolysis pumps and valves were structured using laser micromachining.

![Figure 2](image.png)  
**Figure 2** Extraction chip with four reservoirs, membrane and integrated pumps and valves

The working principle of the chip can be described as follows: After the lysed sample is loaded in the chip it is automatically pumped over the silica membrane. One after another two washing buffers are flushed over the sample. After the valves have closed the waste reservoir the eluate buffer is used to rinse the extracted nucleic acids to the chip outlet.

2.2 Testing the cartridge system with yeast cells

2.2.1 Sample lysis

For the experiments on chip and in tube saccharomyces cerevisiae was suspended in TRIS-based lysis buffer with a concentration of 8,6x10^7 cells/µl. Proteinase K was added to brake down cell proteins in an incubation step at 55°C form 5 hours. Afterwards the samples were centrifuged 3 min with 8000 g. The resulting pellet of cell remains was discarded and the nucleic acid in the supernatant was fall out with ethanol.

2.2.2 Reference extraction in spin columns

QuickGene DNA tissue SP kit from Fujifilm was used as a reference extraction system. Differing from the standard assay conditions the extraction was carried out with reduced volumes for sample, washing and elution buffers by analogy with the volumes in the cartridge. Therefore 40 µl lysed sample was pipetted in the spin column with the silica membrane and centrifuge with 6000 g for 1 min. Then the membrane was washed with 40 µl washing buffer twice. Subsequent the waste tube was changed and the nucleic acid was washed out with the elution buffer in the last spin step.

2.2.3 Extraction on chip

For on chip extraction 40µl of lysed sample was pipetted into the microfluidic chip. After the sample inlet was sealed the chip was placed in an electrical connector for pumping. Pumping by electrolysis was done with 10/20mA for 40s for each reservoir. Subsequently the eluation valve was closed, the sample was pumped through the membrane and the membrane was washed twice with 40µl of washing buffer. After the waste valve was closed nucleic acids were eluted with 40µl of elution buffer. The eluate was collected with a pipette from the outlet and analyzed in a spectrophotometer. For the optimized extraction an additional flushing step with air was introduced after each emptying of a reservoir.

2.2.4 Evaluation the nucleic acid concentration and purity

The samples were evaluated with the spectrophotometer UvLine 9400 from SCHOTT instruments. Optical density was measured at 260nm and 280 nm. Since nucleic acids
absorb specifically at 260 nm and proteins at 280 nm the proportion OD260/OD280nm can be used to determine the purity of the sample [4]. Because the yeast genome consists of 98 to 99% of RNA [9] the yield of the extractions was calculated using the optical density at 260nm the multiplications factory 40µg/ml for RNA [4].

3 Results

3.1 On-chip extraction

It was possible to extract nucleic acids on chip. In first experiments 0,7 µg nucleic acid has been extracted with a purity of 1,4. The extraction with spin method provides 8,5 µg nucleic acid with an purity of 2,3. The results of spin method are superior to the first results on chip. This is caused in the wet-on-wet method on the chip. In the spin method the silica membrane dehumidify during the centrifugation and the individual washing steps are separated. In the chip system it was not possible the separate the washing steps and so the membrane could not dry between the separate steps.

3.2 Optimized results

To optimize the extraction on chip an intermediate dehydration step was included. Therefore the micro channels have been flushed with air after each extraction step. With this air flushing the purity could increase up to 2 (Figure 3). Also the crop of nucleic acid could increase up to 2,56 µg (figure 4).

Figure 3 Purity (OD260/OD280) of extracted samples: in tube (spin column), on chip and on-chip after optimization

Figure 4 Nucleic acid yield of extracted samples: in tube (spin column), on chip and on-chip after optimization

4 Conclusion

We presented a microfluidic cartridge for the extraction of nucleic acids from liquid samples. Compared to in parallel extraction in commercial spin columns the purity of extracted samples reached the reference after optimization of the extraction process on chip. Regarding the amount of the extracted nucleic acids the chip reached only one third of the performance of the chip. The time for an extraction was reduced from ~30min in tube to ~6min on chip mainly by reduction of manual handling steps. Further optimizations will include drying of the membrane and an improved flow regime through the silica matrix.

5 References