Comparison of the Efficiency of Different Methods for the Lysis of Cells in Lab-on-Chip Systems

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ABSTRACT

Quantitative or qualitative examination of DNA has enormous impact on medical, forensic, or genealogical, analysis. The macro as well as micro world knows a panel of different methods for cell lysis that has to happen prior to the purification of the DNA. To our knowledge the efficiency of those protocols as well as the purity/amplifiability of extracted DNA has not been compared for a Lab-on-a-Chip application so far. To address this issue, human HeLa cells were lysed on-chip by chemical, enzymatic, thermal or mechanical treatment, or by application of strong pH gradients. These tests are currently complemented by approaches using exposure to strong electrical fields, ultrasonic treatment or radio frequencies. In the first instance quantity and purity of DNA was inspected by agarose gel electrophoresis. Up to now, the chemical lysis by applying Guanidinium SCN and the electrochemical lysis represent to be the best DNA isolation protocols in our chip-based test system.

Keywords: cell lysis, DNA/RNA extraction, Lab-on-a-Chip

1 INTRODUCTION

The analysis of DNA or RNA has become a routine process in medical, veterinary or forensic laboratories, in basic, applied or industrial research and development, in food-chemical monitoring etc. The Lab-on-a-Chip approach is predestinated for the analysis of nucleic acids because (1) it allows high content screening, (2) being automized it is an ideal tool for a none scientific user, (3) it can handle biosample with low volumes or concentrations of analytes, and (4) it facilitates use of small volumes of otherwise expensive reactants or enzymes to save costs.

An all-in-one Lab-on-a-Chip DNA analysis system in its true sense has to start with the earliest possible stage being the lysis or disintegration of cultured cells, whole blood, smears, or compact tissues. Different standard techniques for the release of intact nucleic acids from such specimens such as chemical lysis with Guanidinium SCN, (bio)-chemical lysis with the help of proteinase K and lysis by boiling are commonly used in macrosystems. These methods were transferred to a microsystem and the lysis efficiency on-chip was compared. For further comparison alternative lysing techniques that have partly been reported for on-chip systems, such as exposure to a strong pH gradient induced by localised electrolysis or strong electrical fields parallel to the flow direction were subject of investigation. Besides the application of strong shear forces achieved by sharp turns of the sample flow in narrow channels for mechanical lysis, the effect of radio frequencies is analyzed at present.

According to the technical demands the cells were either immobilized on a polyethersulfone (PES) filter membrane (pore size: 1.2 µm) or lysed in special chambers. Yield and quality of extracted DNA was semi-quantitatively checked by agarose gel electrophoresis. Visual inspection of the electrophoretic pattern of the DNA allowed determining the degree of degradation and the extent of undesired complexation of DNA and protein.

2 RESULTS

The design of the basic cell lysis functionality on-chip is given in Fig. 1. Each of the five schematically drawn grooves in the center of the chip represents a support for a PES membrane for cell retention welded into this groove prior to the experiments. To confirm permeability of this membrane for genomic DNA, a defined amount of bacteriophage lambda DNA was pumped through the lysis chips. Photometric measurement of the genomic DNA content of the filtrate revealed an up to 100 % permeability of the membrane for genomic DNA sizes (10-100 kb) that were typically obtained after applying standard DNA isolation protocols.

At first, the question whether heating of intact cells is sufficient for extracting genomic DNA of amplifiable quality, was addressed. For this, 100000 HeLa cells were resuspended in 100 µl PBS buffer (pH 7.4) and pumped into the chip with a syringe pump (0.03 ml/min). The cells in the retentate were incubated in PBS at 95°C for 10 minutes and the resulting suspension was sucked out of the chip with a syringe pump again (0.1 ml/min).

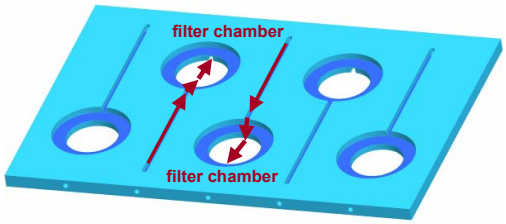

Fig. 1: Basic lysis test chip. Red arrows indicate the flow direction of the cell suspension.

Aliquots of the samples were analyzed using agarose gel-electrophoresis followed by ethidium bromide staining. Comparison of the intensity of chip-extracted versus conventionally isolated DNA gave a very rough estimation of the recovery. Additionally, the electrophoretic mobility of the DNA mirrored the grade of complexation between DNA and protein. As shown in Fig. 2, lanes ´B´ no DNA could be visualized after recovery of the nucleic acid from the chip.

Next, the efficiency of biochemical lysis on chip was investigated. After retention and subsequent concentration of the cells on the filter, the filter chamber was filled with 100 µl of proteinase K solution (3.6 mg/ml PBS, pH 7.4) and incubated at 55°C for 10 minutes. The lysate was sucked out and analyzed for isolated genomic DNA. As already observed in the thermal lysis approach no DNA signal could be detected on the agarose gel (Fig. 2, lanes ´A´).

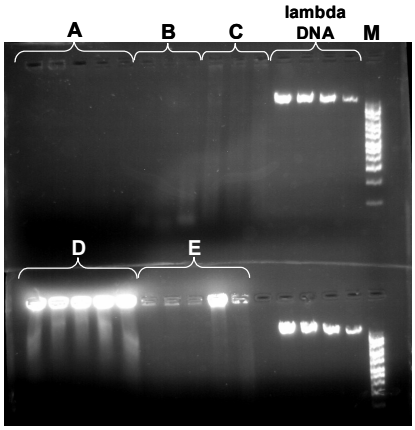

Fig. 2: Gel electrophoresis after lysis on-chip with proteinase K (A), thermal lysis (B), a combination of thermal lysis supported by proteinase K (C), lysis with Guanidinium SCN (D), and the amount of DNA that could be washed off from the filter membrane via back flush in the last case (E). For reference lambda DNA (120, 60, 30, and 15 µg/ml) and 1 kbp markers are shown at the 5 right lanes.

Subsequently, a combination of thermal and biochemical lysis was investigated. Therefore, 100000 HeLa cells were heated to 95°C as described above. The lysate was removed and replaced by 100 µl of proteinase K solution. After a 10 minutes incubation step at 95°C, the solution was sucked off and analyzed as described above. The outcome was, however, as negative as observed for both stand-alone experiments (Fig. 2 lanes ´C´).

For further comparison of on-chip lysis approaches, 3M guanidinium SCN was used for lysis in the basic microchip. The technical procedure was the same as for the biochemical lysis tests. With this approach the lysate gave a strong signal on the agarose gel (Fig. 2, lanes ´D´). Back flush experiments showed that in the majority of experiments DNA had been sucked off from the filter almost completely (Fig. 2, lanes ´E´). However, the DNA did not leave the slot where the sample was loaded. This phenomenon indicated a complexation of DNA to proteins. The high salt concentration of samples may not explain the absence of electrophoretic mobility because addition of 3M Guanidin SCN to marker DNA only decelerated its electrophoretic mobility in the gel (data not shown).

Alternative to those experiments where cells were retained by a filter membrane system a simple, reagentless mechanical lysis has been investigated as well. Efficient mechanical lysis shall be obtained by flowing of the sample through sharp turns in narrow channels that give rise to shear forces. The corresponding channel structure as well as a simulation of shear forces within the channel is presented in Fig. 3. After passing the mechanical lysis structure the lysate was pumped over the membrane to retain intact cells. This ensures comparable conditions to the above experiments. Going from narrow to wider parts of the mechanical lysis structure induces only strong forces behind the obstacle like mathematical simulations have shown (Fig. 3b) and which was already successfully tested for the fragmentation of DNA [1]. In the opposite flow direction additionally forces occur when the fluid is accelerated again. Therefore, the mechanical lysis structure has been tested in both flow directions to compare the applied shear forces and resulting lysing properties. Experiments done so far resulted in lysis efficiencies up to 50% as observed by microscopic analysis. Up to now no difference for the forward and backward mode was found. Slow flow rates turned out to be more effective. These observations still have to be confirmed on DNA level by gel electrophoresis. Besides the investigated structure with a channel width of 150 µm and a maximum width of 500 µm of the active structure two additional channel and structure sizes will be studied.

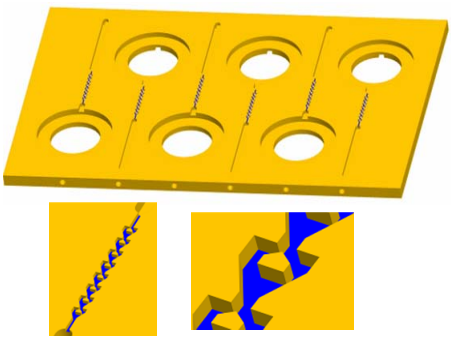

Fig. 3a Drawing of the mechanical lysis chip. Active lysis structure is shown in detail.

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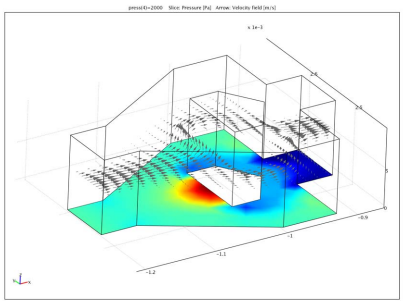

Fig. 3b Simulation of shear forces in the mechanical lysis chip.

Motivated by the publication of Di Carlo et al. 2005 [2] testchips for electrochemical lysis have been realized. In contrast to the flow through design of Di Carlo et al. where cathode and anode were separated by a filter structure to build a retention structure for intact cells the system here was established as a batch arrangement. Gold-electrodes were manufactured on the side walls of the channel and cell lysis shall occur while passing the electrodes. The experiments revealed that various applied voltages and frequencies did not affect cell integrity. Therefore, channel structures were studied and the two most effective designs are shown in Fig. 4. Parameters investigated were electrical current, flow rate, and channel geometry. In contrast to the basic chip design, the lysate was not pumped over a membrane to enable simple detection of remaining intact cells. Using this set-up alternating current gave better results than direct current. A flow rate of up to 1 ml/h showed suitable for efficient cell lysis. The efficiency of lysis was up to 97-99% using different channel geometries (different width, structure). In addition efficiency was related to the number of used cells. Nevertheless, time needed to perform lysis (Fig. 5) with this efficiency took about 15 sec compared to about 10-20 minutes with thermal, biochemical and chemical methods.

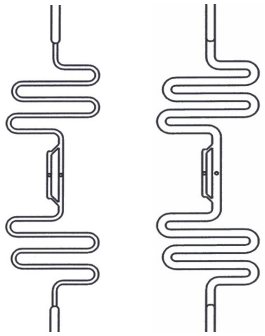

Fig 4: Channel structures for most successful electrochemical cell lysis. Left (D1): channel cross section 0.5 x 0.2 mm^2, right (D3): 1 x 0.2 mm^2.

Besides the electrochemical lysis depending on generation of a strong pH-gradient, lysis of cells by exposition to strong electrical fields parallel to the flow direction is currently investigated (electroporation) which might be also the main effect in the AC case of the prior design.

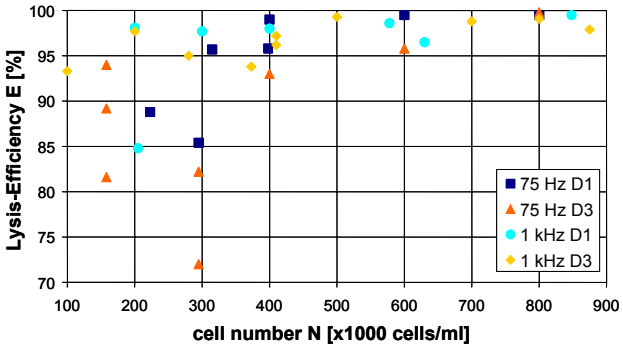

Fig 5: Lysis efficiency dependent on the number of used cells for different chip designs and applied voltage.

The design of the test-chip for electroporation is based on the design presented by Wang and Lu 2006 [3]. The local electrical field is amplified by a geometric variation of the microfluidic channel. This geometric structure of the channel including a dramatic restriction of the channel width (300 µm to 30 µm) has been realized with slight modifications concerning the electrode position (Fig. 6). The electrodes are not in contact with the buffer solution within the microfluidic channel, in order to ensure the absence of electrochemical reactions at the electrodes.

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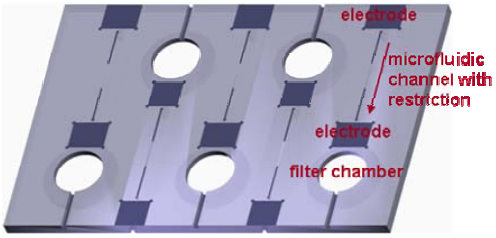

Fig. 6 Drawing of the high voltage chip for electroporation.

Because of the separation of the electrodes from the liquid and the conductivity of the sample fluid, the electric field inside the channel will be negligible for DC voltages. However, due to the finite conductivity of the fluid and the capacitive coupling between the electrodes and the conductive fluid channel, this will be different for AC voltages. The electrical behaviour of the electroporation chip has been analyzed theoretically taking into account the molar conductivity of the sample fluid and electrical properties of the chip polymer. The main outcome of these investigations was that the chip behaves like a 1st order high pass filter. The cut-off frequency was measured experimentally to be 12 kHz. Therefore, the chip will be further tested with frequencies higher than 12 kHz. In addition the mechanical stress caused by the channel restriction on the cells will be studied.

Apart from the described methods for lysis alternative approaches using ultrasound or radio frequencies are currently realised on chip and their efficiency will be compared to the alternative lysis methods.

Heat lysis of bacteria (10^3/100 µl Bacillus thuringiensis) at 95°C in a lysis buffer with detergent from Molzym GmbH (Germany) was tested on-chip as well. Subsequent to lysis the DNA has been purified and the efficiency of lysis was compared by amplification of equal aliquots in a PCR reaction. The results for different heating intervals are shown in Fig. 7. No conclusion is possible since even without heating significant amounts of lysed cells were obtained.

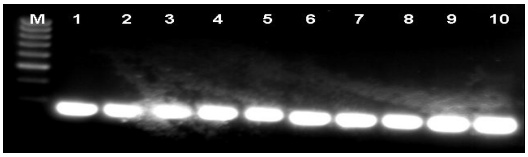

Fig. 7: Lysis at 95°C after 2.5 min (lane 2+7), 5 min (lane 3+8), 7.5 min (lane 4+9), and 10 min (lane 5+10) heating. Blank values without heating are lane 1+6. M=Marker.

Heating up to 95°C resulted in a significant loss of sample volume (up to 30%) if lysis chamber was structured as a reservoir. The evaporation was reduced to ~10% by using a meandering channel as lysis chamber.

3 DISCUSSION

The first objective of the presented work was to test different lysis methods that allow extracting amplifiable DNA from intact cells. The focus of this report was to present suitable lysis protocols for a fluidic chip environment. Such a protocol could be quite different to those known in the macro world. With other words, the distinctive feature of the approach was to avoid pre-selection and subsequent optimisation of a promising technique for this new platform.

Each specific lysis technique requires its distinct chip structure. Nonetheless, it was tried to standardize the design of the chips as much as possible. In addition, it was planned to analyze semi-quantitatively the yield of recovered DNA after agarose gel electrophoresis, check the degree of purification by taking the electrophoretic running of the isolated DNA into account, and, finally, to quantify the exact recovery by quantitative PCR. Unfortunately, the inspection of the DNA pattern on agarose indicated already that in no case the quality of the extracted DNA is sufficiently good to try the subsequent quantification via PCR without further purification. Nonetheless, quantitative PCR to determine the yield of extraction methods as precise as possible is an option that is under process.

Thermal lysis alone was tried because it is a rather simple approach and, accordingly, an ideal application for microsystems. Boiling of the sample, however, did not allow recovering any genomic DNA as indicated by agarose gel electrophoresis. This is most probably due to changes of the structure of the DNA as well as changes in the interaction of the DNA with molecules of the cellular environment. The performance in the tested chip is considerably increased when chemical or biochemical reagents are added.

These reagents might interfere with subsequent sample processing or detection methods and the removal might be complicated. Since lysis methods using 3M guanidinium SCN are commonly used in lab scale protocols, many assays for DNA detection can handle this by applying further purification steps. The fact that a heating element is required for thermal supported lysis methods is of less relevance since heating is always needed in every amplification step.

The lysis of cells only by mechanical shear stress is very attractive since no elements have to be integrated into the chip and the structure for the lysis can be fabricated in the same injection moulding step as the fabrication of the chip. But the lysis of cells requires very high shear forces which can be realised mainly by adequate flow rates and very small channel structures. Besides the difficulties arising for the fabrication of these small structures the risk is considerably high that clogging of the channels occurs mainly if samples with a high number of cells are investigated.

Methods that induce a locally high OH^--concentration by the application of electrical fields show very high lysis efficiency. Another advantage is that there are no disturbing reagents required. The major draw back especially for industrial applications that need cost effective disposable chips is the need to implement electrode structures into the chip. The second electrical method that generates an electrical field parallel to the flow direction has the additional draw back that a significant constriction of the channel is essential which increases the danger of clogging.

The application of ultra sound might be a very attractive method. No internal components and reagents are required and cheap actuators can be implemented into the instrument. Thus, future experiments will focus on this method as a good candidate for the lysis method of choice.

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