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# Development of a fast miRNA extraction system for tumor analysis based on a simple lab on chip approach

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**Abstract**

MiRNAs are small (20 to 23 nucleotides in length) noncoding RNAs regulating numerous essential cell functions. They operate by targeting messenger RNAs for cleavage or translational repression, influencing cell development and cell differentiation. MiRNAs are identified to play an important role in human cancers. In gene expression studies for tumor diagnostics, an extraction system with high extraction efficiency from low sample amounts is mandatory for any biomarker identification. A fast on chip RNA extraction module formerly used in pathogen detection was modified for the extraction of miRNAs from human cell cultures. This fast method (~8 min) yields purified and amplifiable miRNAs for subsequent expression analysis. Compared to commercial extraction kits, the on chip miRNA extraction system shows 100fold higher extraction efficiencies for cell cultures.


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*Keywords:* miRNA; Lab on Chip; tumor diagnotics; gene expression analyses; extraction system

## 1. Introduction

MicroRNAs are small (20 to 23 nucleotides in length) non-coding RNAs regulating numerous essential cell functions[1]. They operate by targeting messenger RNAs for cleavage or translational repression[2] and it is known that their expression profiles classify human cancers[3, 4]. In breast cancer (BC), emerging evidence suggests a potential role for deregulated miRNAs as modulators of carcinogenesis, proliferation, apoptosis and drug-

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resistance[5]. Most data exists for tumor tissue or breast cancer cell line-based miRNA expression profiles[6,7]. However, there are numerous hypotheses for a pivotal role of miRNAs in intercellular communication[8,9] partially based on the leakage of miRNAs into circulation[10] as well as by active and passive transport mechanisms, respectively[11]. Recent studies documented the feasibility to detect stable miRNAs in serum and plasma. This opened the field for these circulating miRNAs as potential novel biomarkers in breast cancer for early detection and outcome prediction[12-14]. Furthermore, urinary miRNAs show a potential role as a non-invasive innovative biomarkers in the detection of breast cancer[15]. MicroRNA analysis for rapid tumor classification lately became an immediate demand in surgery and diagnostics. Standard laboratory procedures in hospitals usually take hours and involve many manual steps. By accelerating this procedure the physician can decide much earlier on the type of treatment for the now classified type of cancer. By applying the sample preparation chip, microRNAs are extracted from eukaryotic tumor cells in less than 10 minutes. This chip based extraction method is very promising, because a first comparison to a commercially available method revealed benefits regarding time and efficiency.

## 2. Material and Methods

Thermoelectric cell lysis is conducted by applying an alternating current (AC) to the lysis chamber (a). Under a microscope the disintegration of the target cells by joule heating and electroporation can be directly observed (Figure 2). At this step, the nucleic acids are released from the interior of the cell. The miRNA can be extracted subsequently without any further preparation steps. By applying a direct current (DC) to perform a gel electrophoresis through the integrated hydrogel the miRNA is purified immediately after cell lysis and collected in the elution chamber (c). The method yields purified and amplifiable miRNAs for subsequent expression analysis. The extraction module consists of three chambers, namely lysis- (a), separation- (b) and elution chamber (c) as shown in Figure 1.

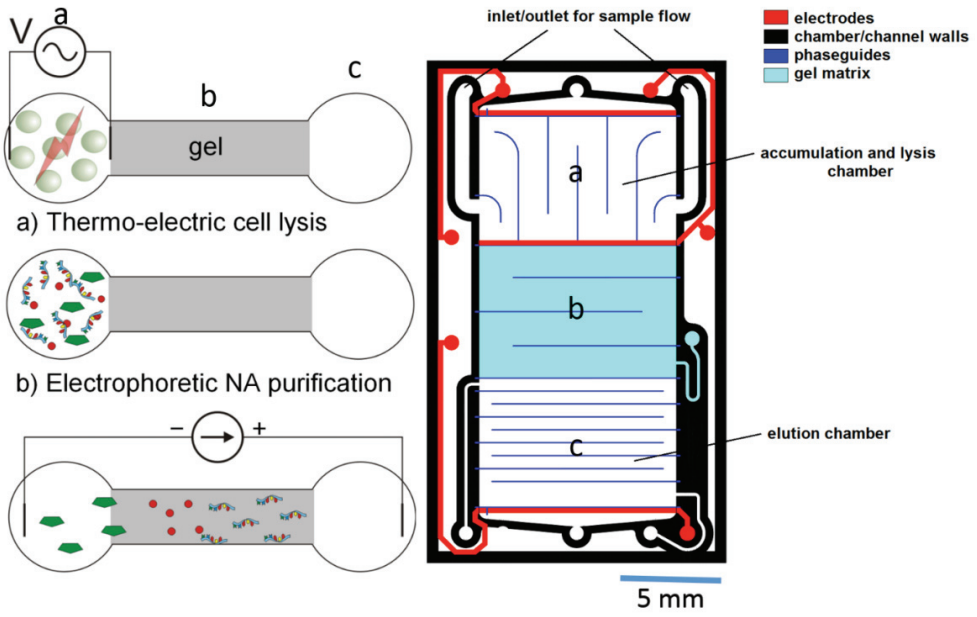


Figure 1: (Left) Principle of on-chip cell lysis and electrophoretic purification of nucleic acids. (Right) Microfluidic electrophoresis chip with labeled microstructures with lysis- (a), separation- (b) and elution chamber (c) [16].

## 3. Results and discussion

For a successful application the on chip thermoelectric lysis has to work also for eukaryotic cells, but it has been verified only for bacteria so far. For verification the same procedure which has proven of being suitable for bacteria is applied on the breast cancer cell line MCF7. The accumulation step is omitted. The MCF7 cells are directly observed during the lysis process which is shown in figure 2. After 10 seconds the cells start to expand and after 20 seconds they begin to burst. Sometimes it can even be observed how single cells release their contents. After 30 seconds nearly all cells are disintegrated and only residuals of the cell membrane and other components are visible. This is regarded as visual proof that the integrated on chip lysis is applicable also for the eukaryotic MCF7 cells.

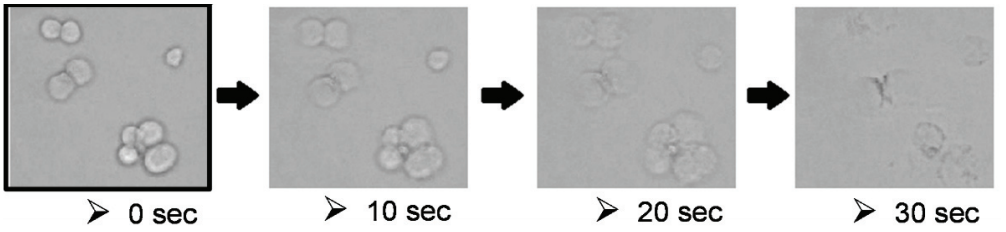


Figure 2: Thermoelectric lysis can be performed in less than one minute. Fast cell Lysis of MCF 7 tumor cells can be observed visually during the lysis process.

For an optimized microRNA extraction procedure, the electrophoretic migration time through the hydrogel needs to be determined. For that purpose, 1X TBE buffer is spiked with synthetic miRNA 34a and introduced into the lysis chamber. The extraction protocol as described in [17] is followed without the accumulation or lysis steps. Every two minutes the eluate is collected and the elution chamber is refilled with fresh buffer. The collected eluates are analyzed with the *Bioanalyzer 2100* (Agilent) capillary electrophoresis system. The result is shown in figure 3. "NTC" indicates the no template control where just the marker peak "M" is visible. "REF" indicates the initial material before chip actuation where a concentration of 6840 pg/µl is measured at the expected length of slightly above 20 nucleotides. When looking at the same position of the eluates which are collected after 2 min and 2-4 min actuation time, no peak is visible. It can be concluded that the microRNA is still within the gel matrix after 4 minutes and hasn't yet reached the elution chamber.

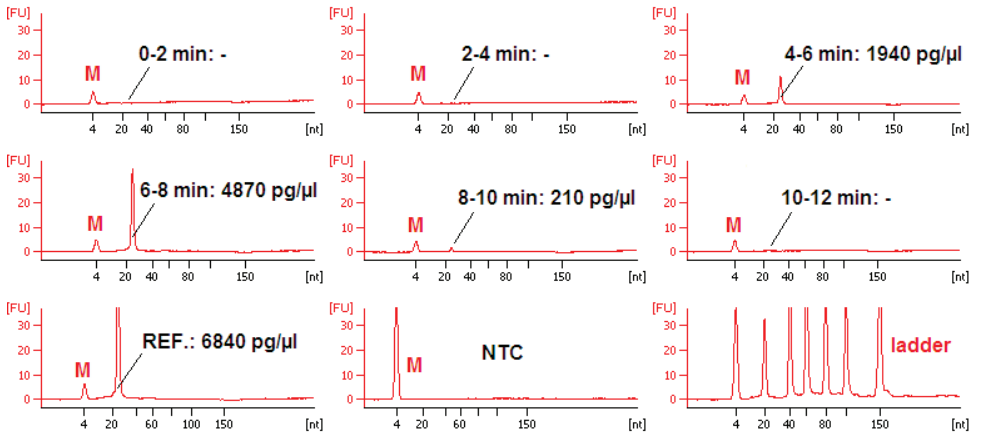


Figure 3: Bioanalyzer measurements show the yield in relation to the chip actuation time. The starting reference material (REF) is synthetic micro RNA 34a at a concentration of 6840 pg/µl.

The peak appears in the eluate taken after 4-6 min actuation time with 28 % of the reference material's concentration. In the eluate taken after 6-8 min actuation time the peak reaches 71% of the initial concentration. After 8-10 min actuation time just a small peak with 3% of the initial concentration is visible. No peak is detected in the eluate taken between 10 and 12 minutes chip actuation time. This is most likely due to all microRNAs having already passed the gel matrix. Summing up 99% of the input material is detected after an overall chip actuation time of 8 minutes. The fact that altogether 102% of the initial concentration is detected is due to measurement errors. From this result, 8 minutes is determined as an optimized electrophoretic chip actuation time for obtaining a maximum yield of microRNAs.

The electrophoretic on-chip extraction method is compared to a commercial extraction kit regarding time and efficiency. The chosen commercial kit is the *mirVana* kit (life Technologies), which is optimized for small and micro RNAs. From a fresh MCF7 cell culture two microRNA extracts are prepared according to the mirVana kit's protocol. Two different initial cell concentrations are used. From the same cell culture samples also two chip extractions are performed according to the method described in [16]. Those four eluates are compared using the RT & TaqMan PCR assay hsa-mir-195 (life Technologies).

The result is shown in figure 4, which reveals different $C_t$-values for both extraction methods. The chip eluates are marked with "chip extract 1,2,3" and while the commercial kit extracts are marked with "Kit extract1,2". The "-RT" indicates the negative controls. With a higher sample to eluate volume ratio, also a higher eluate concentration has been expected for the commercial kit method. So taking into account the sample to eluate volume ratio and similar $C_t$ values, this suggests higher extraction efficiency for the electrophoretic chip extraction method. Observed yield indicates 100fold higher extraction efficiencies for the electrophoretic chip extraction method compared to the commercial miRNA extraction kit (Figure ). The commercial method is a 30-45 min procedure while the on-chip method on chip is performed within 10 minutes. The analysis of the extracted samples is performed using RT-qPCR without any preamplification.

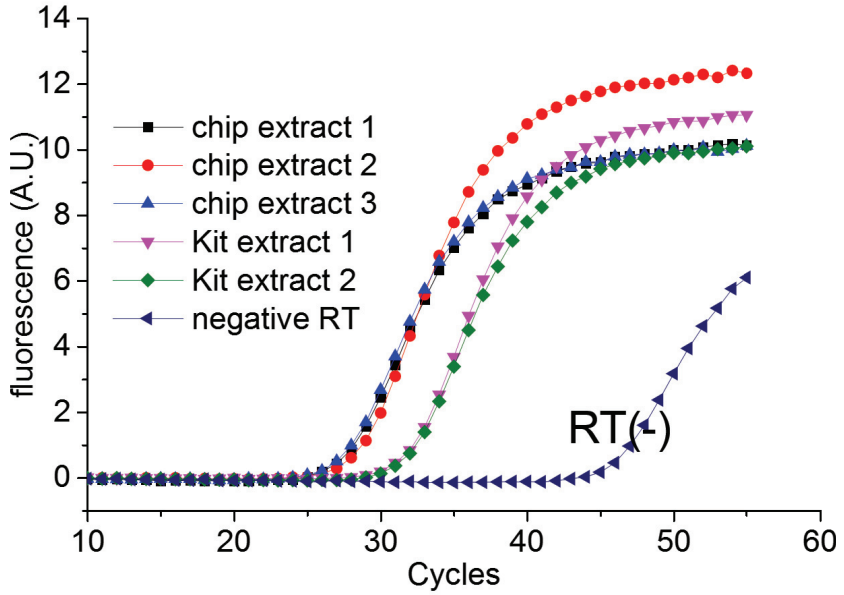


Figure **4**: RT-qPCR comparing the yield of on-chip extracted miRNA (e.g. miRNA 195) to a commercially available kit (mirVana, Ambion). The analysis of the extracted samples is performed using RT-qPCR without any preamplification.

## 4. Conclusions

The extraction time is adjusted for an optimized microRNA yield. A miRNA extraction through the implemented hydrogel via gel electrophoresis is possible in less than eight minutes (verified with Bioanalyzer 2100). The

comparison with a commercial kit reveals better extraction efficiencies (commercial extraction process ~1h). Ribosomal RNA (rRNA), messenger RNA (mRNA) or genomic DNA are excluded due to the hydrogel separation. The analysis of the extracted samples is performed using RT-qPCR without any preamplification. Compared to current extraction procedures in clinical laboratories, the benefits would be faster extraction times and less manual steps.

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