MICROFLUIDIC SOLUTIONS FOR MINIATURIZATION, INTEGRATION, AUTOMATION AND PARALLELIZATION OF TESTS ON COMMERCIALLY AVAILABLE INSTRUMENTS

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ABSTRACT

We demonstrate that Lab-on-a-Chip implementations can be designed for operation in common commercialized instruments like laboratory centrifuges, DVD drives, or real-time PCR cyclers. On the one hand this approach could render the expensive development of tailor-made instruments superfluous and could significantly reduce market entry barriers for developing Lab-on-a-Chip solutions. On the other hand this provides instrument companies with the opportunity to upgrade their devices e.g. by integrating complex sample preparation protocols prior to the detection for which the instruments are designed. We demonstrate this approach for the following applications:

- automated DNA-purification operated on a standard laboratory centrifuges with 50% yield compared to gold standards,
- DNA pre-amplification, aliquoting, and real-time PCR operated on a slightly modified real-time thermocycler (Rotor-Gene) with < 10 copy sensitivity,
- isothermal DNA amplification by recombinase polymerase amplification with < 20 copy sensitivity and a time-to-result of less than 15 minutes, also operated on the Rotor-Gene platform,
- hematocrit measurement of less than 10 µL of whole blood in a DVD drive.

KEYWORDS

Lab-on-a-chip, standard instruments, microfluidics, microfluidic platforms, PCR, RPA, DNA purification, hematocrit

INTRODUCTION

Microfluidics is an enabling technology for miniaturization, integration, automation and/or parallelization of tests in diagnostics, drug development or biology research. During the last decade several products were realized by microfluidics and introduced to the market, but in general the current market penetration and user acceptance, especially in the field of diagnostics, is less than expected.

Assay miniaturization requires the sequential combination of many different microfluidic unit operations such as reagent storage and release, extraction of certain molecular species from complex samples, metering, mixing, incubation, enrichment and/or amplification and detection. Those microfluidic operations have to be combined ideally in a seamless monolithic manner on a low-cost substrate. Although all of those unit operations have been demonstrated in the past, until now the development of fully integrated tests in a sample-in/result-out manner is seldom realized and bears high development risks. In addition, suitable instrumentation for the processing and read-out of the microfluidic chips has to be developed. The end-user will benefit significantly more from microfluidics research, if the processing instruments for operating the microfluidic chips are already available on the market or used in his own lab.

This does not only reduce the investments for the end user but also for the developer of the microfluidic chips. He usually has to invest large sums in developing and distributing the instruments. This can significantly impede a fast market penetration.

This analysis stimulated us to focus on centrifugal microfluidics as the microfluidic platform. On the one hand it enables an easy way to implement a wide range of different assays, on the other hand the lab-on-a-chip cartridges can be processed on commercially available instruments, like laboratory centrifuges, real-time-PCR cyclers, or DVD drives. The microfluidic cartridges are either solid plastic discs and can be produced by injection molding, or they are made of thin polymer films in a micro-blister technology derived from the low-cost technology for the packaging of pills.

MICROFLUIDICS FOR COMMERCIALIZED INSTRUMENTS

We demonstrate the implementation of a wide range of different applications ranging from DNA purification to genotyping of antibiotic resistant bacteria as an upgrade for existing or minimally modified lab equipment. The approach of tailoring microfluidic disposables to existing processing instruments and not vice-versa enables the use of existing instrumentation and the expansion of their utility into automated assay processing [1].

DNA purification on a standard laboratory centrifuge

DNA purification is a common task in analytical laboratories and molecular diagnostics. Usually, DNA purification is either performed manually in a labor intensive process or by large and expensive automated pipetting work stations. The following example

demonstrates that also comparably cheap and simple laboratory instruments such as lab centrifuges can perform automated DNA purification, if a suitable microfluidic cartridge is employed. To demonstrate this, we implemented a solid phase DNA purification protocol into a microfluidic cartridge to be operated in a standard laboratory centrifuge [2]. The microfluidic cartridge contains pre-stored washing buffers in glass ampoules and an integrated silica membrane as a solid phase for DNA extraction (Figure 1). After the disposable is inserted into the centrifuge, a lysed blood sample and the elution buffer can be dispensed into the cartridge, all glass ampoules are crushed and the centrifugation starts. In 3 preliminary experiments, 192±30 ng DNA was extracted from 32 µL lysed whole blood. This corresponds to ~50% yield when compared to a reference extraction based on the QIAamp DNA blood mini kit. The only manual steps are to pipette the sample and elution buffer into the disk, crush the ampoules and to close the lid before starting the centrifugation.

Figure 1: Microfluidic disposable disk for DNA purification in a standard laboratory centrifuge. The required washing buffers are pre-stored.

Geometrically multiplexed real-time PCR in the Rotor-Gene 2000 instrument

DNA analysis is a very attractive, yet challenging field for µTAS. Especially when a fast amplification of DNA is needed, a good thermal heat transfer from the instrument into the Lab-on-a-Chip cartridge is desirable. This enables quick heating and cooling cycles and leads to fast results. Applying injection molding for the mass fabrication of microfluidic cartridges does not enable to fabricate reservoirs with thin walls and thus reasonable thermal conductivity. To solve this problem, we developed a technology for microstructuring and replication of thin polymer films of approximately 188 µm thickness [3].

We designed these polymer film cartridges to be processed on the real-time PCR thermocycler "Rotor-Gene" from Corbett Life Science (recently acquired by Qiagen) (Figure 2). The instrument is designed to perform real-time PCR of DNA in up to 72 individual samples which are manually pipetted into 72 vertically oriented reaction wells. We replaced this rotor by our microfluidic cartridge and additionally we slightly modified the Rotor-Gene's electronics to allow programming higher centrifugation frequencies of ~27 Hz.

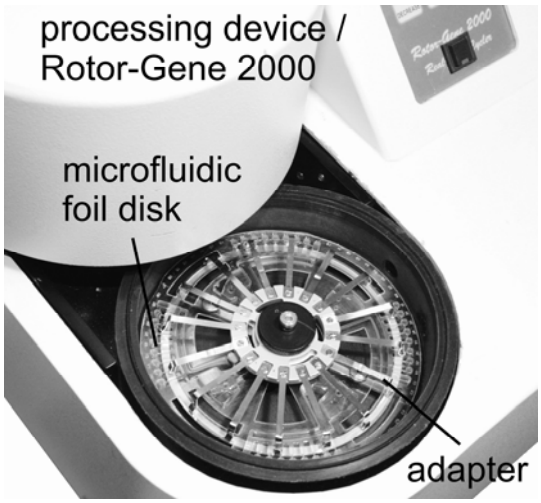

Figure 2: Microfluidic foil disk in a modified version of a commercially available centrifugal real-time thermocycler. A simple and inexpensive mechanical adapter and a slight adaptation of the device's integrated electronics are the only modifications to the original instrument [4].

To demonstrate the capabilities of this modified Rotor-Gene platform, we developed a microfluidic cartridge for genotyping assays from a 90 µL sample. The implemented processing steps allow automatic splitting of the sample into 8 independent cavities and mixing with pre-stored primers and probes. In this way the manual handling steps are significantly reduced. Then, individual real-time PCR is performed in each cavity according to the standard use of the instrument [4]. Less than 10 DNA copies per cavity could be detected (Figure 3), allowing the successful distinction between seven different genotypes of methicilin-resistant *Staphylococcus aureus* (MRSA) plus positive and negative controls.

In a second stage we integrated additional microfluidic functions. We implemented a generic pre-amplification of DNA by roughly a factor of 1000 by a primary PCR, followed by subsequent mixing with a secondary PCR buffer, aliquoting of the solution into 8 independent cavities and finally the secondary PCR to identify if a certain genotype of a pathogen is present or not. This increased the sensitivity of the assay to < 10 DNA copies per sample (which is about 8 times more sensitive than 10 copies per cavity, since each sample is split into 8 cavities) [5].

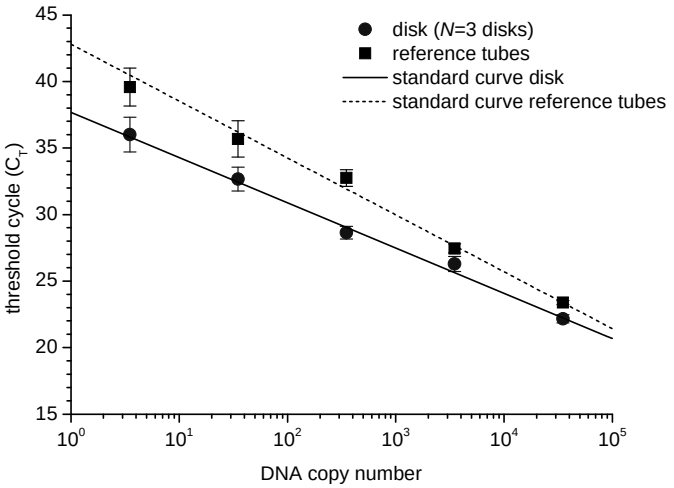

Figure 3: Comparison of the measurements performed in the reference polypropylene tubes and the microfluidic foil disk. Shown are threshold cycles of the qPCR according to the applied DNA copy number in the sample. The PCR protocol was the same for the reference and the disk (data according to [4]).

Isothermal DNA amplification and detection in a Rotor-Gene

Novel biochemical protocols also allow isothermal amplification and detection of DNA which can speed up nucleic acid testing significantly. Similar to the geometrically multiplexed real-time PCR disc, we established an integrated microfluidic assay based on the recombinase polymerase amplification (RPA) technology [6]. It enables the detection of the antibiotic resistance gene mecA of *Staphylococcus aureus* from less than 20 starting copies of DNA in less than 15 minutes at a constant temperature of 37 °C [7]. All required dry and liquid reagents were pre-stored in the microfluidic cartridge (Figure 4).

Hematocrit measurement in a DVD drive

The incentive of using "microfluidic DVDs" is quite obvious: Due to the high number of drives and DVDs produced, the instrument and replication costs are minimal and therefore attractive for laboratory automation or even home diagnostics. One application which can easily be realized in a microfluidic DVD is a fully automated hematocrit test (Figure 5). This application was realized in a microstructured DVD operated in a DVD drive with modified firmware [8,9]. After insertion of 8-10 µL blood, the sedimentation and readout are fully automated by the device.

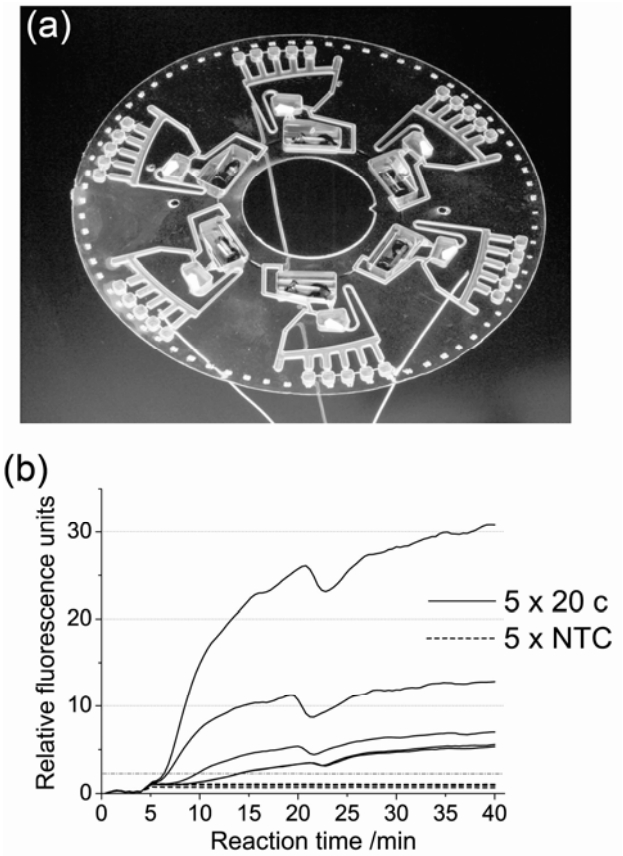

Figure 4: Microfluidic polymer foil cartridge for RPA amplification and detection of DNA in the Rotor-Gene. (a) The assay buffer and the dried master-mix are pre-stored in the disposable. (b) Assay curves for 20 starting copies and no template controls (NTC) (figures according to [7]).

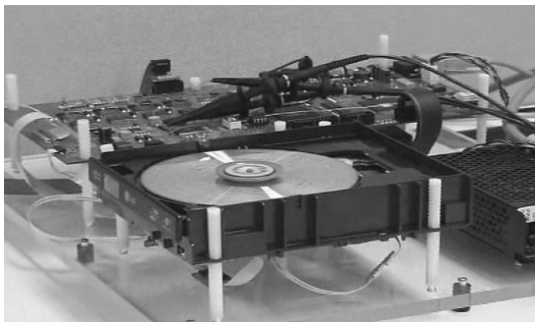

Figure 5: DVD with microfluidically integrated hematocrit testing structures in a DVD drive with modified firmware (figure according to [8]).

Conclusions and outlook

We demonstrated that complex laboratory protocols can be implemented in low-cost microfluidic cartridges which can be processed in existing standard lab-instruments. DNA purification, multiplex DNA amplification as needed e.g. for genotyping of pathogens, and hematocrit measurement were presented. By using common laboratory instruments as processing devices for microfluidic automation, instrument costs can be reduced and distribution can be enhanced.

Consequently, a large target group could benefit from microfluidic automated laboratory protocols without significant investments. In the future this could significantly reduce the market entry barriers for lab-on-a-chip based assay implementations and might support the breakthrough of lab-chips in everyday lab situations.

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