Microfluidic lab-on-a-foil for nucleic acid analysis based on isothermal recombinase polymerase amplification (RPA)

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For the first time we demonstrate a self-sufficient lab-on-a-foil system for the fully automated analysis of nucleic acids which is based on the recently available isothermal recombinase polymerase amplification (RPA). The system consists of a novel, foil-based centrifugal microfluidic cartridge including prestored liquid and dry reagents, and a commercially available centrifugal analyzer for incubation at 37 °C and real-time fluorescence detection. The system was characterized with an assay for the detection of the antibiotic resistance gene *mecA* of *Staphylococcus aureus*. The limit of detection was <10 copies and time-to-result was <20 min. Microfluidic unit operations comprise storage and release of liquid reagents, reconstitution of lyophilized reagents, aliquoting the sample into ≤30 independent reaction cavities, and mixing of reagents with the DNA samples. The foil-based cartridge was produced by blow-molding and sealed with a self-adhesive tape. The demonstrated system excels existing PCR based lab-on-a-chip platforms in terms of energy efficiency and time-to-result. Applications are suggested in the field of mobile point-of-care analysis, B-detection, or in combination with continuous monitoring systems.

Introduction

Nucleic acid amplification of DNA or RNA is a commonly used method in all fields of life sciences and of great importance in sectors like clinical medicine[1,2] or genotyping. The most widespread method for amplification is the polymerase chain reaction (PCR) used in nearly all biological or clinical laboratories.[3,4] Many lab-on-a-chip systems have therefore already proven compatibility to PCR or reverse transcription PCR (rtPCR).[5,6] Nevertheless, the requirements for PCR are technically demanding. The utmost demand is rapid thermocycling between temperatures of 95 °C and ~50 °C, together with a precise temperature control. As a consequence, compared to a standard thermoblock, PCR devices are complex, expensive and energy consuming, rendering the adaption of PCR to mobile point-of-care (POC) applications difficult. Nevertheless the development of such POC devices for nucleic acid analysis is an important task, especially for third world countries. A POC analysis system would allow epidemiological surveillance of diseases in developing countries with poor infrastructure, without the need to transport patients samples to centralized laboratories.[7] A development of portable POC-based nucleic acid analysis devices would therefore contribute to an improvement of the general health situation.[8]

As a matter of fact when developing PCR based lab-on-a-chip systems for POC testing, rapid thermocycling and temperatures of up to 120 °C during the assay are absolutely required. Furthermore, the materials and sealing processes used to manufacture a microfluidic lab-on-a-chip cartridge have to be compatible to the biochemistry of the PCR assay. This limits the range of applicable materials and sealing processes.[9] This restricted availability of suitable materials and processes complicates the development of lab-on-a-chip systems for PCR, regardless if they are focused on the demands of the first or the needs of the third world.

Despite the problems emerging from the demanding thermal requirements, the microfluidic integration of nucleic acid amplification protocols into lab-on-a-chip platforms has several opportunities when compared to state of the art laboratory techniques: a shortened time-to-result, reduced consumption of reagents, better defined reaction conditions leading to a higher reproducibility, good cost-efficiency, and in particular a significant reduction of handling steps.[10,11]

In order to circumvent the demanding thermal requirements of state of the art PCR based DNA analysis new types of DNA amplification reactions have been developed that allow exponential amplification at both constant and low temperature, thus rendering the use of microfluidic platforms significantly easier. Some examples of this development are the nucleic acid sequence based amplification (NASBA) reaction for DNA and RNA

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amplification,[12] the single strand displacement amplification method (SDA),[13,14] the loop-mediated isothermal amplification (LAMP),[15] the self-sustained sequence replication (3SR)[16] the rolling circle amplification (RCA)[17] and helicase-dependent isothermal amplification.[18]

In addition to some of the above mentioned reactions NASBA has already been integrated in lab-on-a-chip platforms. The integration of a NASBA reaction in a lab-on-a-chip was demonstrated in 2004 and 2008.[19,20] The integration of LAMP assays into microchambers was demonstrated by Zhang *et al.* and Noji *et al.*[21,22] Despite the attractiveness of these techniques some drawbacks have to be mentioned. A problem for the integration of LAMP assays in point-of-care devices is still the temperature of 65 °C needed during the reaction. Even without the need of thermocycling, keeping this temperature makes devices for this assay quite energy consuming.

NASBA assays are performed at a temperature of 42 °C only. But with NASBA it is not possible to amplify double-stranded DNA without an initial temperature step of 95 °C.

In this paper we, for the first time, present a lab-on-a-chip cartridge designed for the automated amplification of double-stranded DNA based on recombinase polymerase amplification (RPA). The reaction was first published in 2006 by Piepenburg and colleagues.[23] Characteristic of the reaction is a constant temperature, the typical optimum being 37 °C. Compared to other isothermal amplification approaches, temperatures above 37 °C are not required. Sensitivity of RPA is similar compared to PCR, and amplification and detection of less then 10 copies of DNA have been demonstrated. The amplification of DNA is based on the binding of oligonucleotide primers to template DNA and a polymerase-based primer extension like in PCR. But instead of the temperature-driven melting of the double-stranded DNA like in PCR, RPA uses the properties of a nucleoprotein complex formed by the recombinase enzyme and the oligonucleotide primer, to facilitate the strand exchange of non-template strand and primer at the primer binding sites.[24–26] Primer elongation is performed by a strand displacing DNA polymerase, the DNA polymerase I homologue from *Staphylococcus aureus*. Single-stranded DNA molecules, such as amplification reaction intermediates are stabilized by single-stranded DNA binding proteins (gp32). The proteins exploited for this application are important components of the DNA recombination and repair systems of bacteriophages.[27]

Due to the robustness of its biochemistry and the possibility to present the reagents in a lyophilized form, RPA can be rendered into a simple-to-use format that lends itself to POC applications by untrained personnel, especially if the number of samples tested at the same time is low. However, for certain applications there are distinct advantages in using a more sophisticated detection platform such as the presented lab-on-a-chip system. These advantages are: (a) an overall reduction in liquid handling time for medium-throughput tests, (b) the option to split a single test sample into individual reaction chambers and thus increase the number of samples analyzed in parallel (spatial multiplexing) while drastically reducing the costs per assay and (c) the possibility to control the synchronous start of all reactions in one experimental setup, thereby allowing an estimation of starting amounts of template. The lab-on-a-chip format described here delivers these advantages by reducing the liquid handling performed by the user to the initial addition of the sample, while automating all subsequent liquid transfer and mixing operations. To allow compatibility of our system to the exigencies of mass production the fluidic cartridge is based on a foil structured by a thermoforming process. The foil cartridge includes fluidic cavities for the processing of up to 30 samples in parallel. Under rotation the liquid is transferred to the fluidic chamber with the lyophilized RPA mix. A unidirectional shake mode mixing protocol guarantees a homogeneous distribution of the molecules that are necessary for the reaction, in the buffer. After mixing the total volume of 50 μL reaction buffer is split into five 10 μL aliquots. These aliquots enter the reaction chambers containing the sample fluid *via* a spinning frequency controlled centrifugo-pneumatic valve. As proof of principle, the RPA-lyophilisate was prepared for the detection of the antibiotic resistance gene *mecA* from *S. aureus* and stored on disc.

Experimental

Fabrication and preparation

The fabrication of the foil substrates comprises three steps. First, a CAD-constructed positive master structure is micromilled in a cyclic olefin copolymer (COC) substrate. The structured COC master is washed with isopropanol followed by a wash with DI-water and then dried under nitrogen-flow.

Second, the positive master is cast with PDMS Elastosil RT 607,[28] forming a negative master containing all cavities of the milled original.[29] Directly after the two components of Elastosil are mixed, the PDMS prepolymer is degassed in a laboratory exsiccator at 100 mbar for 5 min. Then the PDMS is cast onto the positive master. Reproducible curing is achieved in a modified hot embossing machine (MB series, Schmidt-Maschinentechnik, Bretten, Germany),[30] where another degassing process is implemented for 3 min at 3 mbar, after which the PDMS is cured at a temperature of 95 °C for 30 min. After demoulding a final curing step is performed for 3 h at a temperature of 120 °C.

The third fabrication step, the thermoforming of the foil, is executed in the hot embossing machine. A cyclic olefin polymer (COP) foil (Zeonex ZF14 from Zeon Chemicals L.P.) with a thickness of 188 μm[31] is fixed above the PDMS master by a blow-molding tool. Foil structuring is realized by heating to 180 °C, evacuation of the process chamber, followed by blow-molding at a nitrogen pressure of 2.5 bar.

After thermoforming, the foil is cleaned with isopropanol and water. Global hydrophilization to alleviate capillary priming of microfluidic channels and to avoid unspecific adsorption of molecules onto the foil is realized with a BSA blocking buffer containing 0.1% (w/v) of BSA fraction V (Carl Roth) in d-PBS (GIBCO) with 0.1% (w/v) Tween 20 (Riedel de Häen). Coating is performed by incubating the cartridge in a petridish overnight at a temperature of 4 °C. Afterwards the foil is washed three times in PBS containing 0.3% (w/v) Tween 20. The cartridge is dried under a constant nitrogen-flow.

RPA rehydration buffer is prepared for prestorage in glass ampoules (Hilgenberg, Malsfeld, Germany). The capillaries are filled with 55 μL of rehydration buffer and sealed using a double nozzle Bunsen burner (Hilgenberg, Malsfeld, Germany). The ampoules are placed inside the storage chamber which is located

adjacent to the center of rotation of the centrifugal cartridge. Then, the lyophilized reagents are placed in the cartridge. Finally the lab-on-a-chip system is sealed with a self-adhesive sealing foil (order no. 90.1994, Sarstedt, Nümbrecht, Germany).

Instrumentation

The readout is performed in a thermocycler for real-time PCR, a modified Rotor-Gene 2000 from Corbett Life Sciences (now part of Qiagen), Australia. Modification of the device allows the rotor to run with two spinning frequencies of 6.6 Hz and 27 Hz. After the glass capillaries are crushed the foil cartridge is inserted into a custom-made rotor inside the device. The rotor in combination with our fluidic disc ensures that all reaction cavities are located in an identical position as PCR tubes used in the "standard" Rotor-Gene, thus enabling "standard real-time readout" and analysis by the Rotor-Gene 2000 software.

Recombinase polymerase amplification

All materials for the RPA reaction including buffers and lyophilisates are supplied by the company TwistDX, UK.[32]

The DNA sample is a double-stranded PCR product of a 420 base pair long fragment of the *mecA* gene of *S. aureus*. For the RPA reaction the DNA stock solution is dissolved in dilution buffer consisting of TE-buffer (10 mM Tris–HCl/pH 8, 0.1 mM EDTA) and 1 ng μL^{-1} herring sperm DNA. The rehydration buffer contains potassium acetate, magnesium acetate, dithiothreitol and tris(hydroxymethyl)-aminomethane and PEG35000, as supplied by TwistDX. The lyophilisate contains the polymerase system, including dNTPs, adenosine-5′-triphosphate, phospho-creatine, the *mecA* specific primers and detection probe, creatine kinase, *S. aureus* DNA polymerase I and components of a bacteriophage derived recombination system consisting of the proteins gp32 (single-stranded DNA binding protein), uvsX (recombinase) and the uvsY (recombinase load factor). The probe molecule contains three functional groups: the fluorophore fluorescein, the quencher BHQ1 and an abasic site mimic (tetrahydrofuran). The latter is a substrate for the enzyme exonuclease III, which is present in the reaction. Detection is enabled by cleavage of the probe in a double-stranded DNA after recognition of the amplification material by the probe sequence.

For a conventional RPA reaction in a standard reaction tube 1 μL of the diluted *mecA* sample is mixed with 48 μL of rehydration buffer in a 200 μL reaction tube. The tube is vortexed 3 times for 5 s and spun down in a centrifuge at 96*g*. A volume of 49 μL of the mixture of DNA sample and rehydration buffer is pipetted into a 200 μL polypropylene tube containing the lyophilisate for the RPA reaction. After rehydration the total tube volume is 50 μL. The amplification reaction starts immediately when the DNA sample is added into the tube containing the lyophilisate. The tube has to be mixed thoroughly (*e.g.* by vortexing) before it is mounted into the processing and readout device. The Rotor-Gene 2000 establishes a constant temperature of 37 °C for the RPA reaction. According to the protocol after 3 min the tubes are removed from the device and an additional vortexing step is performed to get a proper mixing and better defined reaction. Then the tube is placed in the Rotor-Gene 2000 again and the amplification is monitored in real-time.

Fluidic design and protocol

The presented microfluidic foil cartridge (Fig. 1) for isothermal DNA amplification by RPA features 6 identical fluidic structures. Each of these structures is capable of testing up to 5 different samples in the presence of a specific target, allowing testing of up to 30 different samples per run. All fluidic operations are controlled by the spinning frequencies which are applicable on the processing device, a modified Rotor-Gene 2000, and no active valves or pumps are needed.

As the presented disc exploits centrifugal microfluidics also high viscous liquids such as concentrated PEG solutions which are required for RPA can be transported and problems related to the formation of gas bubbles are excluded.[33]

As shown in Fig. 2A the radial inner part of the fluidic structure comprises the liquid reagent storage chamber that contains 55 μL of prestored reaction buffer, which is sealed into a glass ampoule. Before the analysis starts the rehydration buffer is released from the ampoule by crushing the glass by mechanical compression of the elastic storage cavity. The liquid reagent storage chamber is interconnected *via* a comb structure that holds back glass splinters, to a mixing chamber, where the dry reagents are prestored. By spinning the cartridge at a frequency of 27 Hz for 1 min the complete buffer volume is transferred into the mixing chamber, without glass splinters. In the mixing chamber fast dissolving of the lyophilisate is achieved by a unidirectional shake mode protocol.[34,35] In this protocol an alternating acceleration and deceleration of the foil cartridge enhance mixing due to the Euler force and thus generate a homogeneous distribution of the reagents in the buffer within 2 min (Fig. 2Bii).

For the unidirectional shake mode the spinning frequency varies between 6.6 Hz and 27 Hz with an acceleration of 5 Hz s^{-1} periodically. By stopping the rotation of the cartridge a siphon connecting the mixing chamber with an aliquoting structure primes due to capillary forces (Fig. 2Biii). By spinning the cartridge again at 6.6 Hz the buffer will be released through the primed siphon from the mixing chamber into the aliquoting structure (Fig. 2Biv).

Aliquoting is implemented as follows: a supply channel fills 5 metering channels with a volume of 10 μL each, while excess fluid is transported into a waste reservoir. During aliquoting at a rotation frequency of 6.6 Hz, centrifugo-pneumatic valves keep

Fig. 1 Photograph of a foil disc assembled with liquid reagent containers and lyophilisate reagents featuring 6 fluidic structures, each capable of processing 5 assays in parallel. For demonstration purposes the buffer is replaced by red ink.

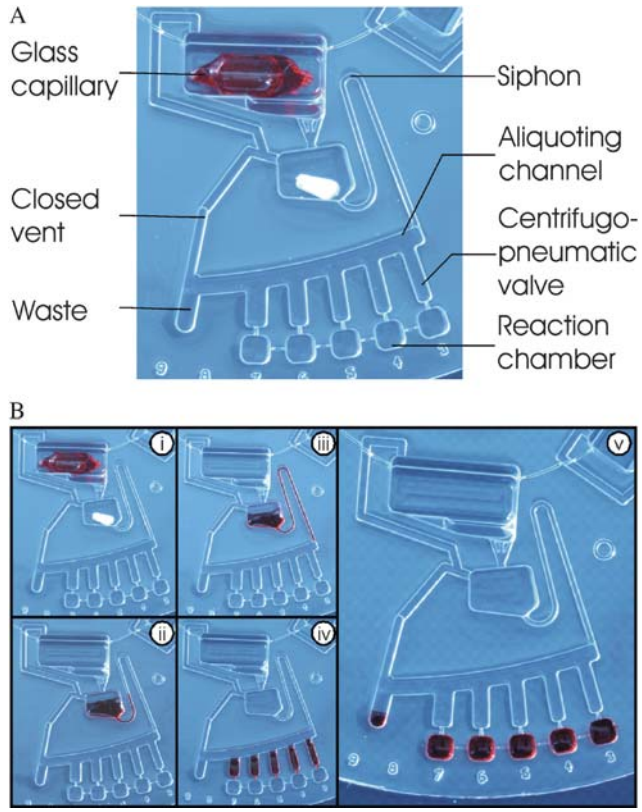

Fig. 2 (A) Photograph of the thermoformed lab-on-a-chip cartridge. The foil disc features a chamber with a glass capillary containing 50 μL buffer for RPA and a chamber with a lyophilisate. A capillary siphon and a centrifugo-pneumatic valve are integrated for fluid control. An aliquoting structure splits the 50 μL buffer into 5 × 10 μL. (B) (i) The disc is ready to be processed. (ii) After the glass capillary is crushed the liquid is spun into the lyophilisate chamber. (iii) A capillary siphon allows valving between lyophilisate chamber and aliquoting structure. (iv) The 50 μL buffer volume is split into 5 × 10 μL aliquots. (v) The fluid fills the reaction chambers *via* a centrifugo-pneumatic valve. To achieve a higher quality of the photographs the buffer is colored with red ink.

the fluid in the metering chambers. After metering, by switching the spinning frequency between 6.6 Hz and 27 Hz the buffer gradually enters the reaction chambers (Fig. 2Bv), and after 150 s the entire buffer volume is transferred.[36] The DNA sample is added directly into the reaction chambers to minimize the adsorption of DNA molecules along microfluidic channels. Therefore the presented design minimizes the effect of surface adsorption that is a typical problem of microfluidic systems due to high surface to volume ratios. To ensure proper mixing of sample DNA and the reagents in rehydration buffer the spinning frequency alternates from 6.6 Hz to 27 Hz for another minute. The reaction starts immediately as soon as the first droplets of buffer enter the reaction chamber. The reaction chamber is separated from the upstream metering chamber by the centrifugo-pneumatic valve to prevent any well-to-well cross-contamination.

Results and discussion

Analogous RPA tests were performed with both, the novel lab-on-a-foil system and, as a reference, in a standard laboratory tube based process. Results were compared with respect to sensitivity and time-to-result. For all of the tests DNA samples containing concentrations of 200, 20, and ~2 copies per μL (*i.e.* per reaction) have been prepared from a stock solution by dilution with dilution buffer.

The performance of the fluidic design was tested using 32 structures. All 32 fluidic structures showed full functionality, allowing execution of all fluidic unit operations exploiting the previously described frequency protocol.

Tube based reference reactions with 50 μL volumes

Reference RPA reactions were performed in standard 200 μL polypropylene tubes. The reactions were setup at room temperature by manually pipetting. Each of the reactions had a volume of 50 μL, including 1 μL of sample DNA. No-template control (NTC) reactions were setup with all assay reagents and pure water instead of sample DNA. The tubes were placed in a 36 tube standard rotor of a Rotor-Gene 2000 instrument which was already pre-heated to a temperature of 37 °C. Readout was performed at a rotational frequency of 6.6 Hz enabling real-time fluorescence measurement of all reactions in parallel.

The results are depicted in Fig. 3. After a lag-phase, the fluorescence intensity increased exponentially for all tested DNA concentrations, surpassing the threshold signal (4 fold NTC signal) in a corresponding threshold time of less than 8 min. For an accurate overall time-to-result for the RPA performed in a tube the time taken for all manual sample processing steps preceding the start of the reaction (time 0 min in Fig. 3) has to be added. A final saturation occurs after 25 to 30 min at a signal intensity in the range of 75 to 90 Relative fluorescence units (RFUs). Certain effects on fluorescence signal intensity after reaching the plateau are probably due to changed reaction conditions towards the end of the incubation and a resulting change in protein binding equilibrium of the DNA binding proteins in the mixture.

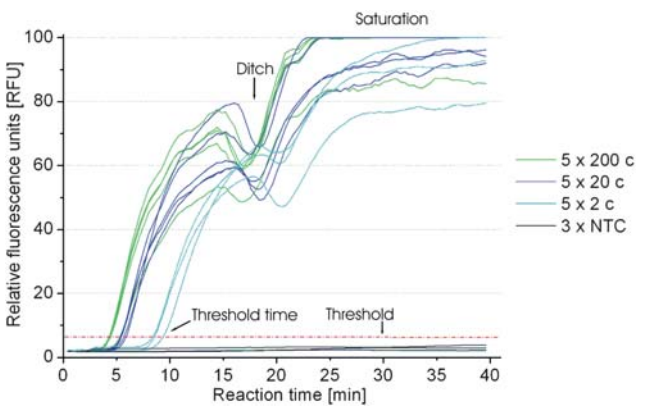

Fig. 3 Amplification plot of tube based reference reactions with 50 μL volumes. The plot shows the amplification of a sample dilution series. The graph demonstrates the typical progress of the fluorescence signal for RPA. All threshold times (Ct) are below 8 min. The test was performed with samples containing a dilution series of the PCR product of the gene *mecA*.

Lab-on-a-foil based reactions with 10 μL volumes

In the following, the performance of the lab-on-a-foil cartridge based RPA is compared to the performance of the tube based reference reactions. In the presented cartridge the reagents volume per tested sample is reduced from 50 μL to 10 μL. Blow-molded cartridges were manufactured and loaded with reagents: each mixing chamber with one 50 μL piece of lyophilisate, and each buffer reservoir with one glass capillary containing rehydration buffer. Afterwards the cartridges were sealed with adhesive tape.

For setup of the assay, DNA samples of 1 μL were pipetted into the inlets, which were subsequently sealed with adhesive tape. The cartridge was placed into the Rotor-Gene 2000 instrument, which was pre-heated to 37 °C. The glass capillaries were crushed to release the reaction buffer and the processing of the assay was started: the reaction buffer was transferred to the mixing chamber to dissolve the lyophilisate by an automated frequency protocol. The resulting mixture was aliquoted into 10 μL portions and transferred into the reaction chambers containing the DNA samples. Real-time fluorescent detection of the RPA reaction was performed at a rotational frequency of 6.6 Hz.

Results of the lab-on-a-foil based RPA with samples containing 200 copies of the gene *mecA* are depicted in Fig. 4. The diagram demonstrates a successful RPA reaction in five out of five DNA containing samples within 12 min. The amplification of DNA samples containing 20 copies of the *mecA* gene showed a similar performance (Fig. 5). All DNA containing samples reach the threshold level within 15 min. Samples containing statistically two copies of *mecA* have been amplified in the RPA cartridge in two out of seven samples within 12 min (Fig. 6). The reason why samples containing two copies of the gene *mecA* did not yield a signal could be due to adsorption of the DNA onto the surface and as such decrease of sensitivity of the assay. In all cases the NTCs stayed below the threshold value.

The results show that the RPA reaction is successfully implemented into the presented lab-on-a-chip cartridge. All samples containing 200 and 20 copies have been amplified within 15 min, whilst 2 copies are on the edge of the limit of detection. Therefore a RPA in the presented lab-on-a-chip cartridge can be processed with samples containing low copy numbers of 20 DNA molecules in total.

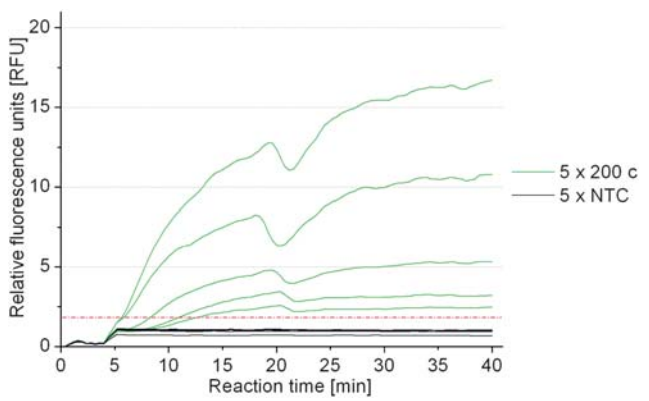

Fig. 4 Amplification plot of a sample containing 5 × 200 copies of the *mecA* gene and 5 NTCs processed in the RPA cartridge. The graph shows an exponential rise in the fluorescence signal intensity between 5 and 12 min for all DNA containing samples.

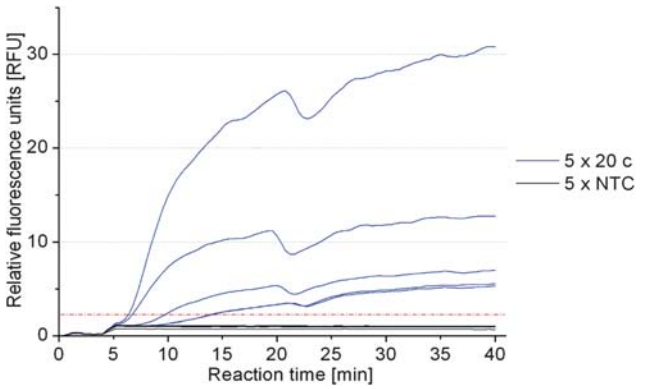

Fig. 5 The graph shows the results of an amplification plot of samples containing 20 copies of the *mecA* gene processed in the RPA cartridge. All samples have been successfully amplified reaching the threshold value within 15 min. No rise in the fluorescence intensity of the NTCs can be detected.

Taking into account the time for preparation steps like the insertion of the samples into the cartridge, sealing the inlets and putting the disc into the Rotor-Gene 2000, the total time-to-result is below 20 min.

Lab-on-a-foil based cross-contamination tests

To evaluate the risk of cross-contaminations between adjacent reaction chambers on the foil cartridge another experiment was performed. Out of the five reaction chambers the central one was filled with 1 μL of sample containing 2000 copies of DNA. All surrounding reaction chambers contained NTCs. Then the lab-on-a-chip cartridge was processed as described before. The results of this experiment are depicted in Fig. 6. Due to the NTC signal of 2 RFU at the reaction start the threshold value was set to 8 RFU. The blue line of the 2000 DNA copies shows a RPA-typical progression. 7 min after the buffer entered the reaction chamber, this sample crosses the threshold value showing an exponential fluorescence signal increase. The fluorescence intensities of the neighboring reaction chambers that contain the NTCs show a constant signal height of 2.5 RFU. No signal in the NTC containing chambers, as shown in Fig. 7, was observed.

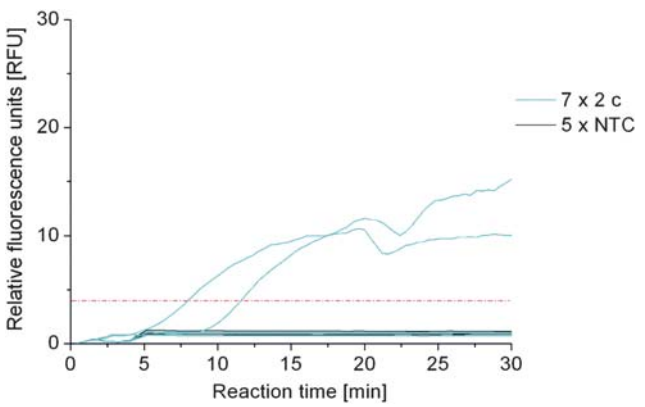

Fig. 6 This graph depicts results of a RPA reaction processed in the cartridge. Two out of seven samples containing statistically 2 copies of the *mecA* gene have been amplified and detected within 12 min. All other samples including the NTCs show no amplification.

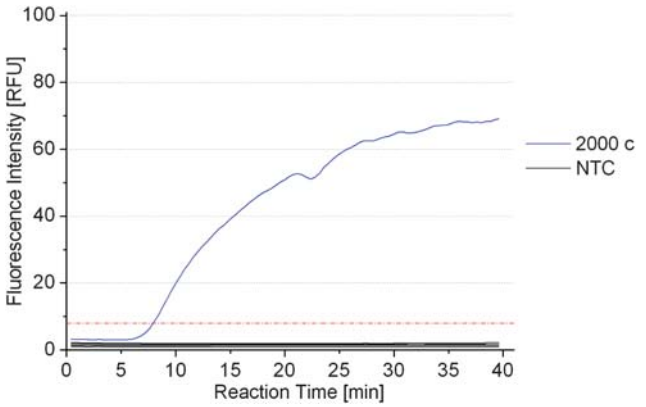

Fig. 7 Results from a cross-contamination test in the lab-on-a-chip. The central chamber out of five reaction chambers is filled with a sample containing 2000 copies of the *mecA* gene whereas all adjacent cavities contain NTCs. The graph shows an increase of the fluorescence signal intensity only for the chamber containing the DNA sample. All other chambers exhibit a constant signal proofing that no amplification appeared.

This experiment clearly demonstrates that no DNA from the central chamber entered the neighboring reaction chambers and cross-contamination did not occur.

Conclusion and outlook

In this work a fully integrated centrifugal lab-on-a-chip system is presented for isothermal amplification of DNA using the RPA reaction. The polymer foil cartridge features prestorage of all necessary liquids and dry reagents for the RPA reaction. The lab-on-a-chip cartridge allows the parallel processing of up to 30 samples while reducing the number of manual handling steps from approximately 240, excluding the uptake and disposal of pipette tips required in the manual setup of a tube based reaction (30× open tubes with lyophilisate, 30× add rehydration buffer, 30× close tubes, 30× vortexing, add sample, vortex, wait, vortex again, and put into readout device), to 68 handling steps in the cartridge for 30 samples (30× pipetting sample in, 30× seal sample chamber, 6× crush liquid storage (can be automated), 1× put disc in instrument, 1× start instrument). Therefore a drastic reduction of manual working steps is realized compared to the standard protocol for RPA.

We have demonstrated that a downscaling of the volume of reagents by a factor of five from 50 μL to 10 μL per reaction is possible, leading to a drastic cost reduction per assay run. The successful detection and amplification of 20 copies of the *mecA* gene out of a sample were shown and it was proven that no cross-contamination between reaction chambers occurred. All DNA detections were made within 20 min. The short time-to-result in combination with easy handling steps and high sensitivity makes our system attractive for point-of-care applications like the screening of methicillin resistant bacteria in hospitals.

In a clinical environment many samples include genomic DNA featuring a high tendency to be adsorbed to the walls of the reaction chambers. Clinical samples also include a very complex matrix of DNA, proteins and other molecules that may affect the assay performance as well as coat the surfaces of the microstructures. This potentially results in a decrease of the sensitivity of the presented lab-on-a-chip system. Nevertheless, the demands regarding sensitivity of most clinical applications are well met by the sensitivity of the presented lab-on-a-chip system, making our approach suitable for most clinical exigencies.

For our demonstration, a commercially available real-time PCR cycler was used for processing of the cartridge, incubation and readout. A significant cost reduction for the device could be achieved by replacing the thermocycling capability to a much less complex feature of incubating the cartridge at a constant temperature like 37 °C. It is conceivable that such a device could use batteries as energy supply and could therefore also be applicable for diagnostic approaches in third world countries and field studies.

For further developments, multiplexing of the targets is an interesting task that can be realized by exploiting the fluidic design presented in this paper with a little adaption: the sample inlet has to be inside the mixing chamber, while the lyophilisates containing target specific primers are stored inside the reaction chamber. Such a cartridge could be used for genotyping or the analysis of a panel of targets out of one patient sample, like it is necessary for the detection of viral or bacterial subspecies.

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