Integration of Isothermal Polymerase Amplification into a Centrifugal Microfluidic Cartridge

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ABSTRACT

Recombinase polymerase amplification (RPA) is a new isothermal DNA amplification method that runs at 37°C, amplifies single copies in less than 15 minutes, and allows real-time fluorescence detection. For the first time we automated this method by microfluidic integration into a centrifugal lab-on-a-chip system, comprising unit operations for reconstitution of reagents, mixing with the sample, and aliquoting to test cavities. As the cartridge contains all of the required liquid and dry reagents only the addition of sample DNA is required. The system was demonstrated by the qualitative detection of < 10 copies of the antibiotic resistance gene *mecA* in less than 15 minutes.

KEYWORDS: Isothermal amplification, centrifugal microfluidics, reagents prestorage

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 or genotyping. To avoid the technically demanding requirements for PCR, like high temperatures and fast thermocycling new types of reactions have been developed that allow an exponential DNA amplification at constant temperature. This also leads to a reduced complexity regarding the implementation of nucleic acid amplification assays in microfluidic platforms. For the first time we present a lab-on-a-chip cartridge designed for the automated amplification of doublestranded DNA based on recombinase polymerase amplification (RPA). Our fluidic cartridge consists of a polymer foil. To demonstrate the potential of the presented system a qualitative test for the antibiotic resistance gene *mecA* was implemented and we could demonstrate successful amplification out of samples containing less than 10 copies of DNA .

WORKING PRINCIPLE

The presented disk-shaped Lab-on-a-Chip cartridge consists of a COP-foil structured by blow-molding [1]. The surface is globally coated with BSA for blocking and hydrophilization. Glass ampoules with liquid reagents, and lyophilized dry re-

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agents are inserted into reservoirs and the disk is sealed by an adhesive foil, AB-0558 from Thermo Scientific (fig 1A). Before the cartridge is used for analysis the sample is added, the ampoules are manually disrupted to release the reaction buffer, and the cartridge is placed into a centrifugal analyzer with integrated fluorescence detection (modified RotorGene 2000, Corbett Research, Australia). At a spinning frequency of 27 Hz 50 µl of reaction buffer is transferred from the ampoule to the lyophilized RPA reagents. After dissolving the lyophilisate the solution is divided into 5 x 10 µL aliquots and transferred into the test cavities via a centrifugo-pneumatic valve (fig 1B). Each of the test cavities may contain a different primer and probe system to allow a multiplexed screening for genetic markers out of one aliquoted sample, or different samples are analzed for one target. The RPA reaction is based on a special recombinase-primer-complex that allows local strand displacement and amplification at 37 °C [2]. Similar to real-time PCR, the detection mechanism is based on the cleavage of a fluorescently labeled oligonucleotide probe.

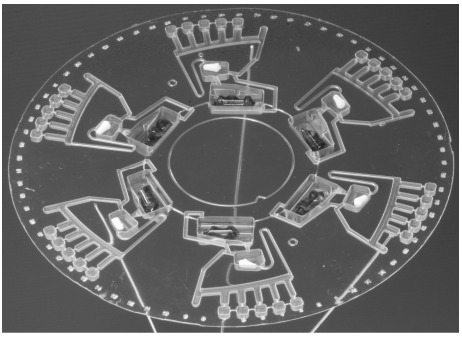

Figure 1. Photograph of a foil disk assembled with liquid reagent containers and lyophilisate reagents featuring 6 fluidic structures; each capable of processing 5 assays in parallel. For demonstration purposes the buffer is replaced by red ink. To achieve a higher quality of the photographs the buffer is colored with red ink.

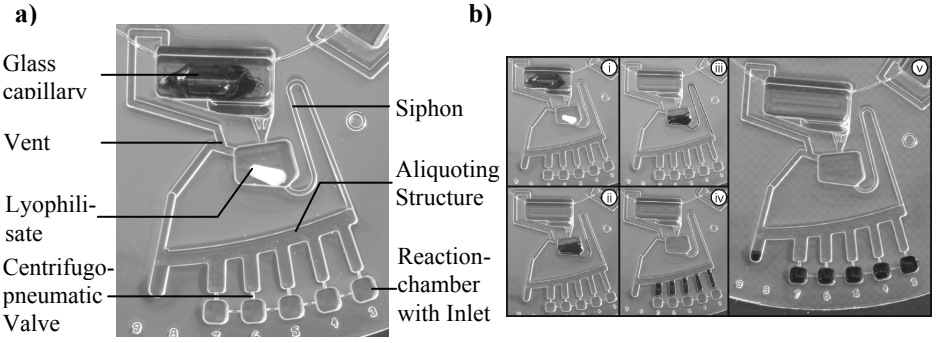

Figure 2.a) Fluidic structure of the foil cartridge. b) i) The disk is ready to be processed. ii) After the glass capillary is crushed the liquid is spun into the lyophilisate chamber. iii) A capillary siphon allows valving between lyophilisate chamber and aliquoting structure. iv) The 50 µL buffer volume is splitted into 5 x 10 µL aliquots. v) The fluid fills the reaction chambers via a centrifugo-pneumatic valve.

EXPERIMENTAL RESULTS AND DISCUSSION

In first experiments the sensitivity of our approach was evaluated. Samples containing < 10, 20 and 200 copies of the *mecA* gene are pipetted into the reaction chambers and analyzed. The graph in fig. 2B shows the real-time amplification plot of the samples. The *ct*-values for < 10, 20, and 200 copies are already reached after 12, 10, and 5 minutes, respectively. All curves show a "ditch" following the exponential rise of the signal intensity, a typical characteristics of the RPA reaction. In another experiment the reaction chambers are tested with respect to cross contamination. Therefore a sample containing 2000 copies is added in one reaction chamber while the adjacent reaction chambers contain no template controls (NTC). The graph in fig. 3 demonstrates that during the assay no DNA propagates to the adjacent NTCs chambers, demonstrating a cross-contamination free aliqoting structure.

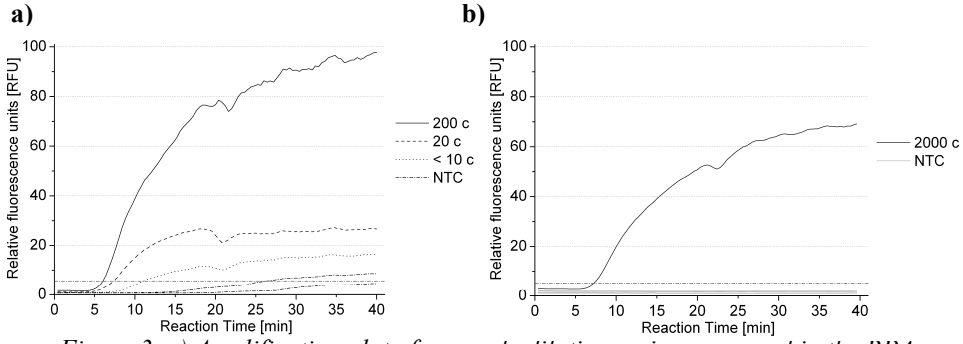

Figure 3. a) Amplification plot of a sample dilution series processed in the RPA-cartridge. The graph shows an exponential rise in the fluorescence signal intensity between 5 and 10 minutes for all tested copy numbers. The signal rise in the NTC is determined due to an unspecific detector drift. b) Results of a cross-contamination test of a foil disk. A sample containing 2000 copies of the mecA gene is transferred into the central reaction chamber, while all surrounding chambers contain NTCs.

CONCLUSIONS

A fully integrated centrifugal Lab-on-a-Chip system is presented for isothermal amplification of DNA using the RPA reaction. Our microfluidic cartridge features prestorage of all reagents required for the assay. The successful detection and amplification of less than 10 copies of the mecA gene out of a sample is shown without cross-contamination between adjacent reaction chambers.

REFERENCES

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[2] O. Piepenburg *et al.*, "DNA detection using recombinant protein", PLoS Biology, **4**, Issue 7, 1115-1121, (2006).