Diagnostic Polymer Disc for Process Speed-Up in Microarray-based Bacterial Classification

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We present an injection molded microfluidic polymer disc enabling DNA amplification and fluorescent labeling within one step. The device is ready for large-lot production and biocompatible. The fluorescently labeled product generated in our flow-through reaction device can be directly hybridized onto microarrays to classify bacterial species. Combining DNA amplification with labeling and the application of a continuous-flow polymerase chain reaction reduced the overall microarray processing time by a factor of four. DNA amounts equivalent to 100 cells per reaction could be detected, which validates this polymer disc as a sensitive and rapid assay for bacterial classification.

Introduction

Infectious diseases are a major health threat including diseases like sepsis, pulmonary tract infections and meningitis. The most effective therapy is still early treatment, which requires early and fast diagnosis. In addition the causative bacterial species are rarely identified prior to initiation of therapy, which decreases treatment efficiency and promotes a further increase in antibiotic resistances. Therefore a rapid assay for species-specific identification is of great importance for a targeted therapy, a most effective patient convalescence and for confining drug resistances of pathogens.

While culturing of the specimen is still the gold standard, it is time-consuming and can last up to days for slowly growing bacterial species. More rapid assays are often implemented with molecular methods like quantitative polymerase chain reaction (qPCR) or DNA microarray technology. They have a clear time advantage and reduce analysis time from days to hours. For example DNA microarray analysis of 65 different pathogens can be performed within 6 hours [1].

A typical microarray workflow consists of DNA amplification by PCR, fluorescence DNA labeling by linear PCR (forward primer only), microarray hybridization and signal read-out, including statistical analysis (Fig. 1). In the workflow, PCR and labeling account for over 80% of the overall processing time and are therefore the processes which are accelerated with the presented microfluidic device. Applying our newly developed polymer disc, we can reduce processing and hands-on time and increase process automation. Rapid diagnosis of bacterial pathogens is therefore possible, which can very much improve therapy success.

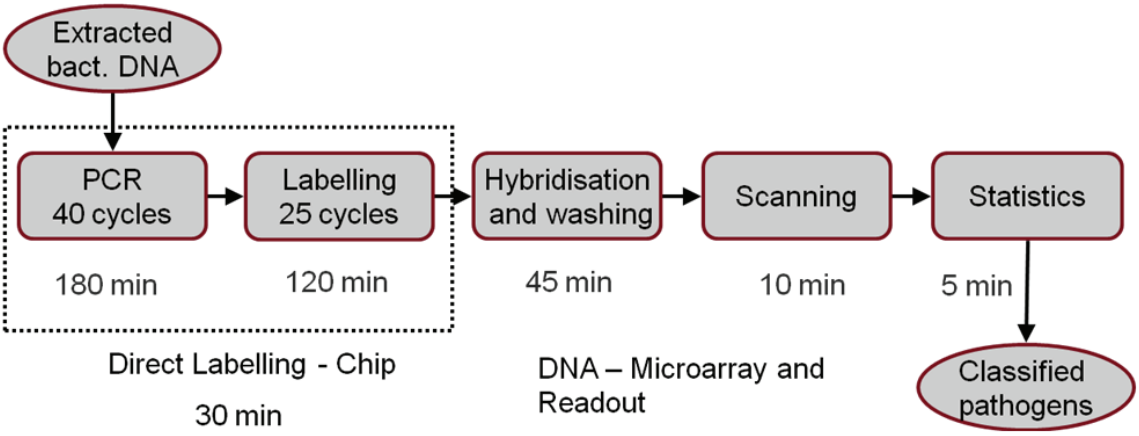
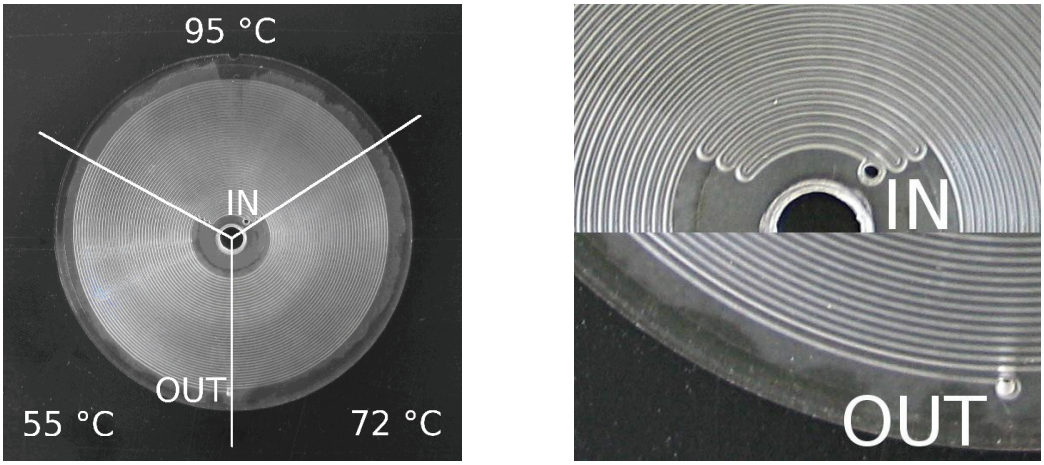


Fig. 1: DNA microarray workflow: The polymer device replaces the conventional and time-consuming PCR- and labeling step in the protocol, resulting in an overall 4-fold process speed-up

Design and Fabrication

The newly developed polycarbonate chip is designed to transform low amounts of bacterial DNA into fluorescently labeled amplicons, ready for microarray hybridization. This is performed in a spiral microfluidic channel with 40 turns and two meanders for predenaturation and post-annealing respectively (Fig. 2).

The chip was fabricated by injection molding, which makes it suitable for mass production, essential for a disposable device. The channels are 290 µm wide and the rims between are 150 µm. To achieve a constant volume per turn the channel depth decreases with increasing radius (from 597 µm to 175 µm). To seal the channels a polycarbonate film (125 µm) was thermally bonded to the injection molded substrate.



(a) (b)

Fig. 2: Chip design: (a) top view with the three temperature zones, (b) detail of predenaturation meander with inlet (top) and post-extension meander with outlet (bottom)

The whole chip is tempered with three symmetric external heating sectors for denaturation (95 °C), annealing (55 °C) and extension (72 °C) (Fig. 2). When the samples are

pumped through the spiral, they undergo a polymerase chain reaction (PCR) protocol. Cy3-conjugated primers are used for direct labeling the DNA within the PCR. All processed samples are interspaced with silicone oil as transport buffer. Before the reaction the sample sequence is aspirated in a loading column as described before [2].

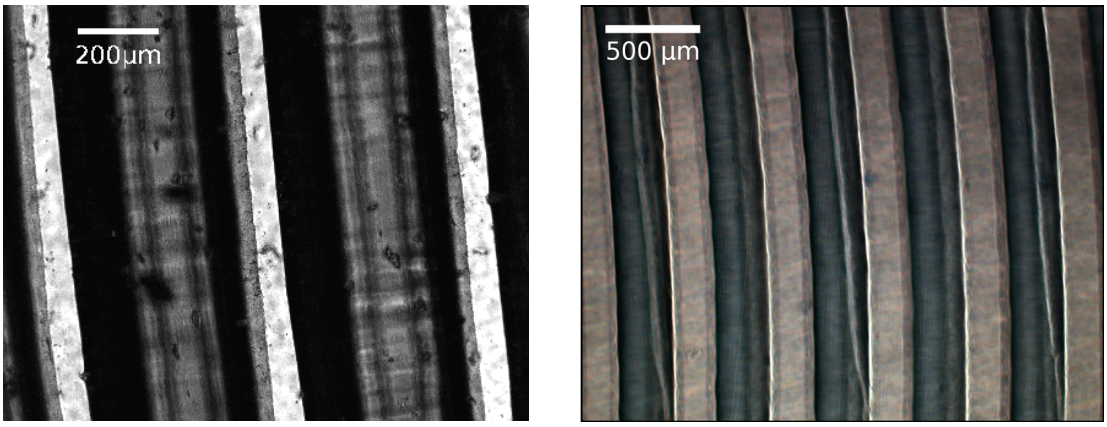
Results

The fabricated polycarbonate disc was compatible to all biological samples and reagents. No adhesion of polymerases or nucleotides was observed, which would have resulted in significant efficiency loss. However it was crucial, that bovine serum albumin was included in the used PCR mastermix, as presented before [2]. In addition the transport buffer did not affect the polycarbonate material or the bonding interface.

The disc itself showed high quality, where channels were molded correctly and there was no major deviation of the desired geometry. The sealing with the polycarbonate film was successful and no cross-talk between channels was observed (Fig. 3). In the experiments the bonding withstood the used flow rates of 111 $nl.s^{-1}$. Therefore the fabrication technologies are appropriate for large-scale production, which is important for the application as a disposable device. The thermal distribution across the polycarbonate disc showed deviations of maximal 1.5 °C in the outer regions, which was appropriate for fast transitions between the temperature stages.

The chip was tested with *Staphylococcus aureus* and lead to a sensitivity of 100 cells per reaction, which qualifies the system for clinical applications. The direct comparison of microarray results using our new disc chip and the standard method showed lower signal intensities for PCR amplicons synthesized by the chip. Although the absolute signals were lower, the correct bacterial species could be classified (Fig. 4).

Performing the reaction in the microfluidic disc showed a 10-fold acceleration of PCR and labelling (Fig. 1), because of absent ramping times between temperature stages, which limit the speed in conventional thermocyclers. This resulted in a decrease of the total processing time for microarray analysis from 6 h to 1.5 h.



(a) (b)

Fig. 3: Sealing of channels: (a) empty channels, bright parts represent the bonding interface between rims and film; (b) channels filled with methylene blue stained water for testing the tightness of the chip

Conclusion

The combination of DNA amplification and fluorescence labeling in a one-step approach reduced hands-on time and increased process automation. We have overcome the limit of analysis time for microarrays by accelerating the overall process 4-fold, resulting now in a processing time of only 1.5 h. The fabricated disc is biocompatible and the production technique is capable for mass production. Therefore our diagnostic disc device enables very rapid identification of pathogen species in a disposable assay format.

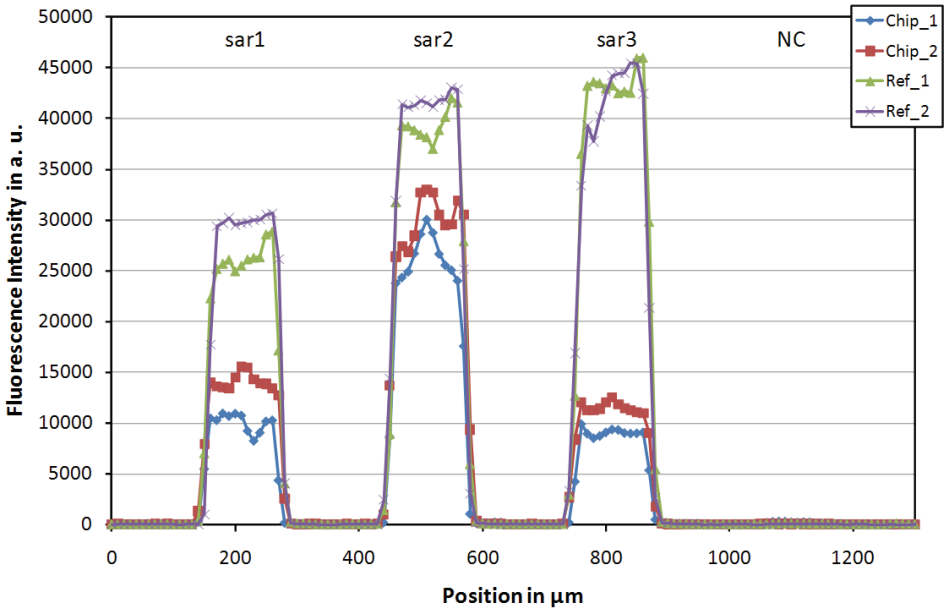


Fig. 4: Line-plots of scanned microarray slides: The output of the polymer disc (Chip_1, Chip_2) is compared to the standard method (Ref_1, Ref_2). Although the absolute signals from the chip were lower, the bacterial species could be classified correctly; sar1-3: species specific DNA probes for *Staphylococcus aureus*.

Acknowledgements

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References

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