Detection of Biological Threats with Fully Automated Lab-on-a-Chip Systems in the project SONDE

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Abstract
Incidents and terrorist attacks with biological agents bear the risk of causing widespread damage and panic. Rapid detection of such threats is not possible with state of the art technologies, since laboratory methods are time-consuming and often not available in the vicinity. However, to enable a fast assessment of patient symptoms and allow an appropriate quarantine and decontamination response, a robust point-of-care diagnostic system is required. To address this problem, novel lab-on-a-chip platforms can be used. One solution is the centrifugal microfluidic platform. It allows automated liquid handling on a rotating structured polymer disk enabling the integration and automation of complex biochemical analysis, such as nucleic acid tests and immunoassays for B-detection.

Project goal SONDE
The consortium of the BMBF funded SONDE project unifies leading competences in microsystems technology and molecular diagnostics. Partners from industry and academia are:

- Robert Koch Institute, Centre for Biological Security
- ESE Embedded System Engineering GmbH
- Institut für Mikrotechnik und Informationstechnik (HSG-IMIT)
- University of Freiburg, Department of Microsystems Engineering, (IMTEK), Laboratory for Sensors
- University Medical Center Goettingen, Institute of Virology
- University Medical Center Freiburg, Institute for Molecular Medicine and Cell Research
- University of Freiburg, Zentrum fuer Angewandte Biowissenschaften

The scenario of a bioterrorist attack requires fast, automated test systems for rapid diagnoses in a potentially large area. This need is not met by state-of-the-art diagnostic procedures which rely on labour-intensive and slow laboratory tests or highly integrated but large and immobile pipetting robots. The SONDE-consortium will implement a microfluidically integrated, automated and mobile lab-on-a-chip system. Target pathogens include *Bacillus anthracis* and *Yersinia pestis*. The final goal of the project is a simulated test with the target pathogens under field conditions.

Preliminary work of HSG-IMIT and IMTEK includes centrifugal microfluidic structures for nucleic acid testing, including DNA extraction from whole blood and real time PCR. Proof-of-principle experiments for these operations on the centrifugal microfluidic platform have been successfully carried out and are presented here.

Centrifugal microfluidics has the charm of relying only on one rotor and a structured polymer disk for liquid control [1;2]. Since no connections to pumps are required, this platform promises robust, easy to use and contamination free integration of analytical protocols. There are numerous unit operations for the centrifugal microfluidic platform, including structures for valving, metering, mixing, switching and aliquoting of liquids as well as protocols for binding, washing, sedimentation and separation of bio-molecules and cells.

Preliminary Results
We present the set of unit operations for our centrifugal microfluidic platform [3] which is required for the implementation of nucleic acid based testing. These unit operations provide solutions for solid-phase extraction of nucleic acids, aliquoting of extracted nucleic acids, and parallel analysis of nucleic acids of up to 16 aliquots based on real-time PCR. A DNA extraction structure with a yield of 290 ng ± 80 ng DNA per 100 µL of eluate from a 32 µL lysed blood sample (Fig. 1) was realized. The extracted DNA showed no real-time PCR inhibition compared to a reference extraction (QIAamp DNA Blood Mini Kit) [4].

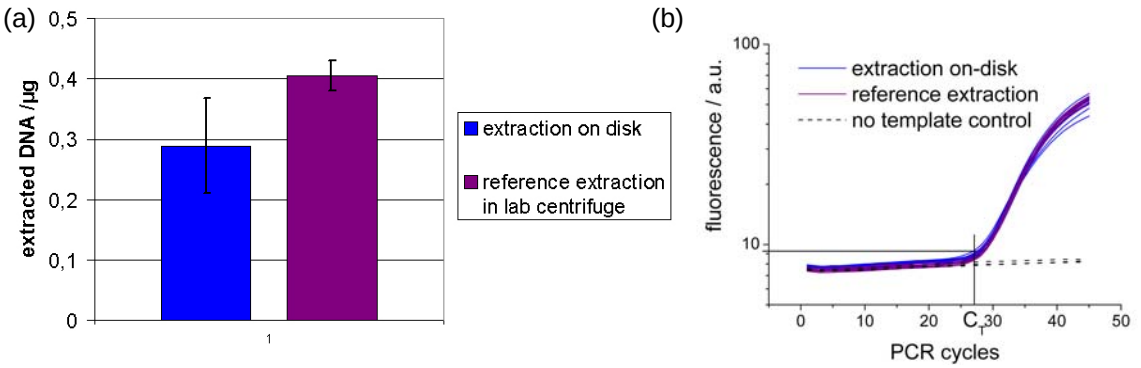

Figure 1: Performance of DNA extraction from whole blood on a centrifugal microfluidic disk versus a standard reference extraction with spin columns in a lab centrifuge. (a) DNA quantity: extracted DNA mass. (b) DNA quality: Amplification plot of ~ 600 starting copies of DNA. No inhibition was observed at the DNA extracted on-disk compared to the reference [4].

For aliquoting the extracted DNA, a novel aliquoting structure was designed and tested. A 105 µL volume was split into 16 aliquots with a volume CV of 3.0 % [5]. The aliquoting structure does not require local surface modifications and works for a large range of liquids (including ethanol, liquids containing detergents and aqueous solutions).

Rapid thermocycling based real-time PCR analysis requires a good thermal conductivity and low thermal mass of the microfluidic substrate. This was achieved by introducing a novel foil-based approach as test carrier (Fig. 2 a). The compatibility with PCR protocols was demonstrated by real-time PCR with DNA samples containing 3 to 300,000 copies of the *exfoliatin A* gene of *Staphylococcus aureus* in foil cavities. The resulting standard curve was consistent with standard polypropylene tubes at an R^2 of 0.97, proving the excellent suitability of the approach [6] (Fig. 2 b). A commercial centrifugal thermocycler was used for thermocycling.

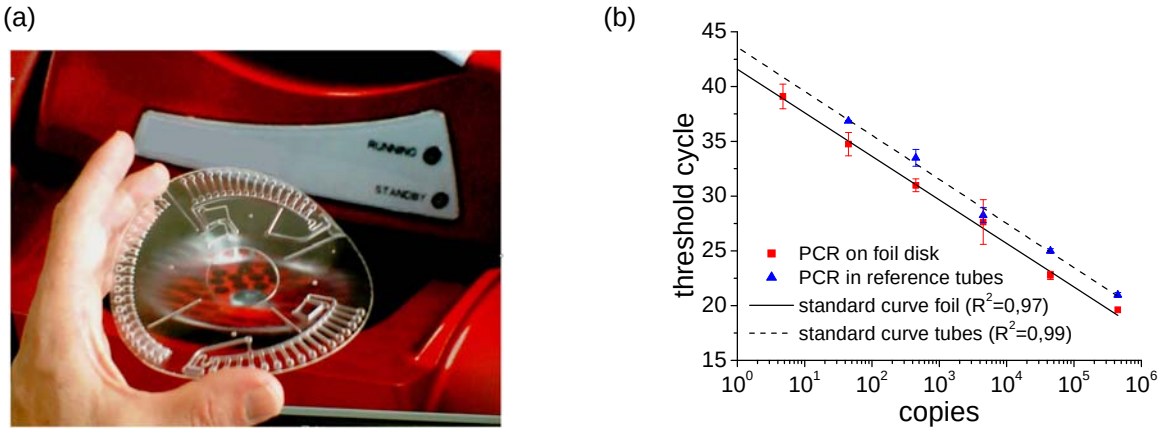

Figure 2: Real-time PCR on a microfluidic disk. (a) Foil disk with aliquoting structure after blow molding and thermocycler. (b) Standard curve of a real-time PCR dilution series of the *Exfoliative A* gene performed on disk and, as a reference, in standard PCR tubes.

Conclusions and Outlook

Microsystems technology is a promsing solution for fast and sensitive detection of biological threats. In prelimary work, the excellent suitability of the centrifugal microfluidic foil-disk approach has been demonstrated.

The SONDE consortium will combine its competences in molecular microbiology and microsystems technology to fully integrate diagnostic protocols for B-threats. The modular design of the platform will ease fast implementation of new protocols for emerging threats.

Since mobile diagnostic systems need to be extremely energy efficient, future lab-on-a-chip solutions will favour isothermal protocols rather than thermocyling based approaches. We already demonstrated the full integration of the isothermal recombinase polymerase amplification (RPA) in a centrifugal

microfluidic system and its use for the detection of bacterial pathogens such as methicillin resistant *Staphylococcus aureus* [7].

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