Development of a Fully Automated Centrifugal Lab-on-a-Chip System for Rapid Field Testing of Biological Threats

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Abstract— The world's growing mobility, mass tourism, and the threat of terrorism increase the risk of the fast spread of infectious microorganisms and toxins. Today's procedures for pathogen detection involve complex stationary devices, and are often too time consuming for a rapid and effective response. Therefore a robust and mobile diagnostic system is required. We present a microstructured LabDisk which performs complex biochemical analyses together with a mobile centrifugal microfluidic device which processes the LabDisk. This portable system will allow fully automated and rapid detection of biological threats at the point of need.

The project S.O.N.D.E.
The project "scenario-based emergency diagnostics system for field use" (Szenario-orientierte Notfall-Diagnostik für den Feldeinsatz, S.O.N.D.E.) is funded by the Federal Ministry of Education and Research (BMBF) under the research programme for Civil Security of the German Federal Government as part of the high-tech strategy for Germany.
A mobile and fully integrated diagnostic system for the detection of bacterial pathogens (such as *B. anthracis* and *Y. pestis*) and toxins (such as ricin and botulinum toxin) is being developed and field-tested as part of the project.
The S.O.N.D.E. consortium unifies leading experts in molecular diagnostics, microsystems technology and hardware development. Partners from industry and academia are:

- Robert Koch Institute, Centre for Biological Security
- QIAGEN Lake Constance 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 für Angewandte Biowissenschaften

The diagnostic system
The diagnostic system consists of a microstructured LabDisk which in which the biochemical analysis is performed, and a mobile centrifugal processing device which controls fully automated liquid handling of the LabDisk (Figure 1). The system is designed for point-of-care applications: the ready-to-use disposable LabDisk is equipped with pre-stored reagents and the portable centrifugal processing device allows processing at the site of patient care.

Figure 1: Portable diagnostic system for point-of-care applications comprising a disposable LabDisk for the nucleic acid based detection of pathogenic microorganisms and the immunoassay based detection of toxins, as well as a centrifugal processing device.

LabDisk

The LabDisks are microstructured polymer disks which include all of the required liquid handling operations to perform biochemical analysis. The foil based production approach by micro-thermoforming of the LabDisks offers unique features such as low thermal resistance for efficient thermocycling and low material consumption, which is attractive for a cost-efficient and large-scale production of disposables[1]. Microthermoforming of polymer films of typically 180 µm thickness is performed in a modified hot embossing machine, using a defined pressure and vacuum protocol. The use of this novel process has already been reported for the manufacturing of LabDisks used for sensitive subtyping of pathogenic bacteria by real-time PCR[2] and isothermal amplification of an antibiotic resistant gene of methicillin resistant *Stapphylococcus aureus* (MRSA)[3].
Objective of the project S.O.N.D.E is to implement an immunoassay and a nucleic acid analysis into the disposable LabDisk. The immunoassay allows the detection of ricin from blood plasma samples; the nucleic acid analysis includes a DNA/RNA extraction and an isothermal amplification for the detection of several microbial pathogens such as *Y. pestis* and *B. anthracis.* For both assays, the complete microfluidic structures have been integrated into the foil-based disks and all the required microfluidic unit operations required to perform the assays have been verified. This includes in particular (i) the storage of liquids and lyophilised reagents on the LabDisk and their time-controlled release, (ii) the transfer of sample material by the use of antibody-coated microbeads and (iii) the aliquoting of sample material for simultaneous analysis on one LabDisk.

(i) On the disposable LabDisk the required buffer solutions are stored in aluminium pouches. Applying a well-defined ultrasonic welding protocol the liquid filled pouches are equipped with a frangible seal. Due to the hydrostatic pressure of the liquids during centrifugation of the LabDisk the frangible seal bursts at a defined rotational threshold frequency of the disk and the reagents are released. Figure 2 shows a LabDisk with the microfluidic design for the immunoassay with integrated aluminium pouches for reagent pre-storage. The pouches are filled with the required washing buffers as well as with skimmed milk powder that, after rehydration, is used to block unspecific binding.

Figure 2: Disposable test carrier for immunoassay: reagents are stored in aluminium pouches. If centrifugal force is applied, the pouches burst due to the increased hydrostatic pressureof the liquid.

(ii) Both assays, the immunoassay and the nucleic acid extraction, are based on magnetic beads as mobile solid phase. Centrifugal acceleration forces mix the sample material with the magnetic microbeads, and magnetic forces allow the transfer of the microbeads between reaction chambers. Figure 3 indicates the transfer-procedure of microbeads from the sample chamber to the detection via the washing chambers. The transfer is performed automatically rotating the LabDisk over stationary magnets that are integrated into the processing device.

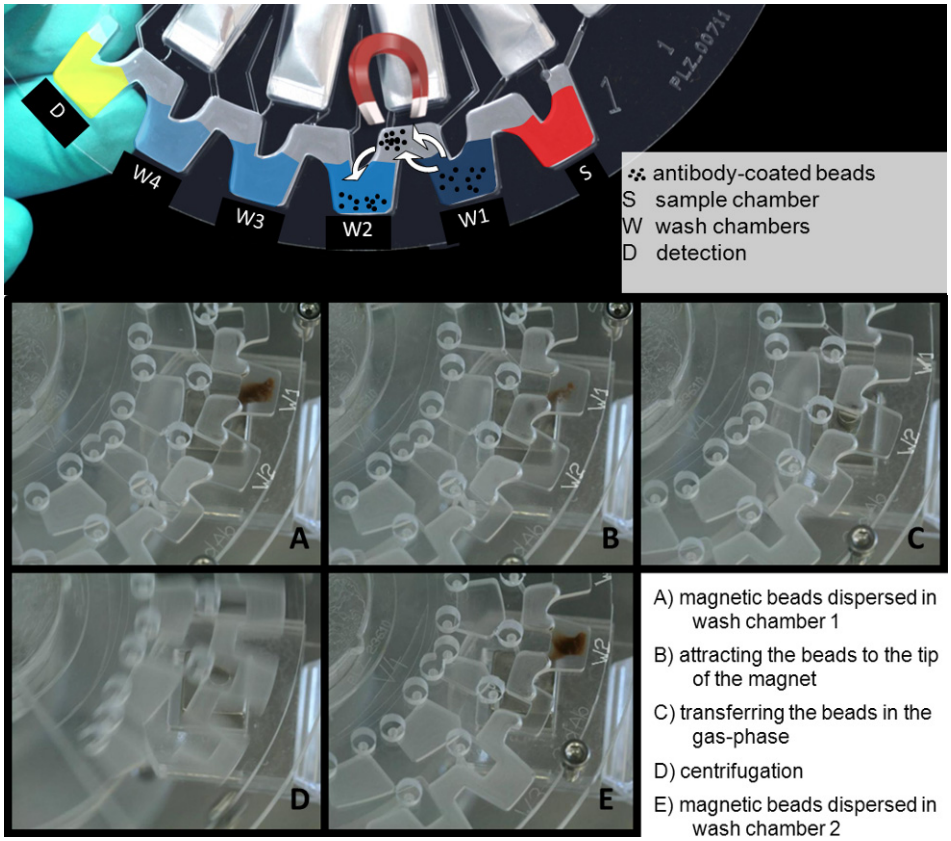

Figure 3: Transportation of magnetic microbeads in the LabDisk. The transfer of the microbeads is fully automated by rotating the LabDisk over a magnet that is integrated into the processing device.

(iii) Aliquoting of liquid sample material enables the simultaneous detection of multiple biological threats. Figure 4 shows the aliquoting structure of the nucleic acid analysis. After the extraction of DNA/RNA from blood plasma the the sample is divided into multiple chambers with identical volumes for isothermal amplification of individual target sequences used for specific identification of pathogens.

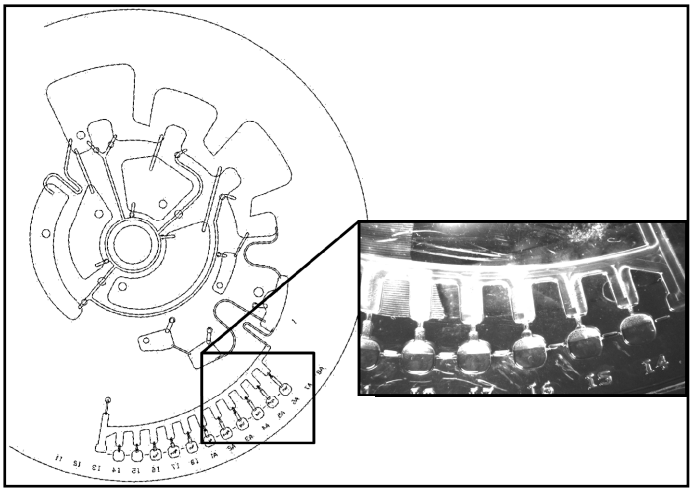

Figure 4: Aliquoting structure of the nucleic acid analysis. Dividing the sample enables the simultaneous detection of multiple biological threats. After extraction and amplification the sample is divided into multiple chambers with identical volumes of 10 µl.

Processing Device:
In addition to the LabDisk, a centrifugal device for processing of the LabDisk is being developed within the project. After being loaded with the sample the test carrier is inserted into this device which is able to run a fully automated and defined rotation- and temperature protocol to control the complete analysis. Integrated into the processing device are a heater for isothermal incubation and detection units for a fluorescence and chemiluminescence readout. No additional manual handling steps are needed to perform the required unit operations of the analysis such as valving, mixing and aliquoting. The required frequencies of rotation for processing the test carrier are in the range of 0 – 50 Hz allowing mixing and valving;.magnets that are integrated into the device allow the handling of the LabDisk-integrated microbeads. The heater allows isothermal heating up to 70 °C, however the required temperatures for the nucleic acid analysis is only in the range of 25 °C – 55 °C. The portable processing device has a weight of 2 kg and allows user-friendly handling and therefore fulfils the requirements for point-of-care applications.

Conclusion and outlook
The project S.O.N.D.E. aims at developing a mobile and fully integrated system that provides rapid detection of pathogenic microorganisms and toxins. The production of the microstructured disposable LabDisks and the development of a portable centrifugal platform have already been successfully demonstrated. All the basic handling procedures to perform the assay have been developed and integrated. Currently the performance of the complete assays is under validation. Future steps are to fabricate a pilot series of test carriers in a prototyping line and to perform field tests with the integrated S.O.N.D.E. system.

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