MOVING THE SOLID PHASE: A STATIONARY MICROFLUIDICS PLATFORM TECHNOLOGY FOR CARTRIDGE BASED SANDWICH IMMUNOASSAYS

Raiah Gottheil¹, Nadja Baur¹, Holger Becker², Andreas Geiger¹, Verena Hummel³, Andrea Normann³, Angelika Haage³, Gorden Link¹, Dimitri Maier¹, Nicole Schneiderhan-Marra¹, and Martin Stelzle¹

¹ NMI Natural and Medical Sciences Institute, GERMANY
² microfluidic ChipShop GmbH, GERMANY
³ Mediagnost GmbH, GERMANY

ABSTRACT

We report on a stationary liquid phase microfluidic based platform which is applicable to various immunoassay formats. It employs magnetic beads which constitute both the solid phase for immobilizing capture molecules and in addition are used for magnetic field activated assay processing steps. Externally applied magnetic fields enable a comprehensive set of assay procedures such as active incubation, accumulation, dispersion, and transport between different assay solutions. This thus, allows for flexible adaptation of individual assay steps. The target molecules are detected through a fluorescence sandwich immunoassay.

Assays for a protein biomarker (interleukin 8) and a viral pathogen (Hepatitis A Virus) were established and yielded a limit of detection of 1.49 pg/mL (0.186pM) and $10^4$ virus particles/ml, respectively.

KEYWORDS: microfluidic cartridge, immunoassay, magnetic particle, Point-of-Care Testing (PoCT)

INTRODUCTION

Microfluidic cartridge based assays hold great promise for application in Point-of-Care Testing (POCT) as they enable complex multi-step biochemical reactions with minimal user interference. Therefore, they may also be employed by untrained users. Ideally, assay components, sample and waste are contained in the cartridge and disposed of together with the cartridge once the assay is completed thus avoiding any risk of contamination. On the other hand such a scheme requires a maximum of automation to be integrated in the system operating the cartridge based assay. Exchange of fluids between system and cartridge would require pumps, valves, and reservoirs to be included in the peripheral system, thus increasing the risk of malfunction and also involve more complex regulatory requirements to be met. In contrast, moving the solid phase by using magnetic beads and applying suitable magnetic fields enables performance of complex assays yet completely avoids fluid handling between system and cartridge. In magnetic actuation schemes previously investigated by other research groups, magnetic beads were used for separation [1], [2] or mixing [3] but were not exploited for the integration of a complete assay procedure which is at the core of the system discussed in this report.

EXPERIMENTAL

Figure 1: Cartridge comprising reservoirs for sample, wash buffers, detection antibody, and waste. Phase guides provide for robust and reproducible filling. Capillary stop valves help metering of fluid and enable transfer of particle aggregates by external magnetic actuation.
The cartridge was injection molded from cyclic olefin polymer (COP) and comprises several chambers separated by capillary valves (Figure 1). Chambers contain the assay reagents, through which the beads are manipulated via externally applied magnetic fields. Active incubation is made possible by magnetic field induced self-assembly of the beads into microstirrers [4] and systematically scanning them through a chamber (Fig.2a). The beads are transported by focusing them to form an aggregate which subsequently is dragged through the valves (Figure 2b). Notably, these valves avoid the requirement of an oil phase to separate chambers and prevent intermixing of fluids, in contrast to previous reports by other groups [1], [5]. Once the aggregate enters a chamber, it is re-dispersed and magnetic actuation is used to reassemble the beads into microstirrers. The assay protocol involves an incubation of sample with antibody-coated magnetic beads, followed by steps for washing or separation, labeling with fluorescent detection antibody and finally fluorescence detection.

**RESULTS AND DISCUSSION**

An interleukin-8 assay served as a model for evaluating the system and a concentration as low as 1.49 pg/ml (0.186 pM) was successfully detected (Figure 3a). Furthermore, hepatitis A virus as a model pathogen was detected with an LOD of $2 \times 10^4$ virus particles / ml (Figure 3b).

**CONCLUSION**

The platform presented shows potential to be developed into a diagnostic tool to be used in a PoCT setting. “Moving the solid phase” is a paradigm that enables comprehensive and robust assay performance in a cartridge, without resorting to external peripheral devices for fluidic control to run the assay. This substantially simplifies the assay procedure and enhances its robustness.
The cartridge was used as fabricated without any further preparation and achieved sensitive detection, as shown by the results obtained. Apparently, blocking or pretreatment steps of the cartridge are not necessary for the concentration ranges tested. An important feature of this platform is its compatibility with commercially available buffers, even including those containing detergents. The actuation parameters can also be adapted to a given assay by means of facile changes to a software protocol. These features can be exploited to further simplify the transfer of additional assay parameters to this system.

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REFERENCES

CONTACT
Martin Stelzle, tel: +49-7171-5153075; martin.stelzle@nmi.de