STATIONARY FLUIDICS: MOVING TARGET MOLECULES ON BEADS THROUGH NON-MOVING LIQUIDS FOR MOLECULAR DIAGNOSTIC ASSAYS

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ABSTRACT

Commonly, microfluidic devices are constructed to move fluidics. For molecular diagnostics assays which include steps like PCR, this practically always involves a more or less complicated set of external pumps, valves and liquid controls. In the presented experiment, the fluid after sample introduction remains stationary and the main bioactive sample molecules are moved through a chain of reaction compartments which contain different reagents. The big advantage of this concept is the lack of any external fluid actuation/control. Results on sample carry-over experiments and complete assays will be given.

KEYWORDS

Stationary fluidics, molecular diagnostics, beads, PCR.

INTRODUCTION

Molecular diagnostics is one of the fastest growing segments in the diagnostics field and well suited for the use of microfluidics [1]. As molecular diagnostic assays contain a significant amount of process steps such as lysis, DNA extraction and amplification (e.g. by PCR), the conventional approach is to develop more or less complex microfluidic devices through which various liquids are moved. This approach practically always involves a more or less complicated set of external pumps, valves and liquid controls. In the presented experiment however, the fluid after sample introduction remains stationary and the main bioactive sample molecules are moved through a chain of reaction compartments which contain different reagents (buffers, PCR reagents). These reagents are either simple buffers or PCR reagents which have been stored in lyophilized form on chip and are resolubilized by the buffer. This allows to realize a system which exhibits a high degree of simplicity, both in the microfluidic cartridge as well as the accompanying instrument.

EXPERIMENTAL SET-UP

In our approach, the molecules of interest are attached to functionalized paramagnetic polymer beads. The beads are moved from chamber to chamber using an electromagnet on a linear stage. The big advantage of this concept is the lack of any external fluid actuation/control. A single fluid (buffer) as well as the sample is introduced into the cartridge using a Luer-syringe. A linear stage with an electromagnet is the only other mechanically active component of the whole set-up which allows for a very simple instrument which is an important issue for real-world diagnostic applications.

The main challenge of this stationary-fluid concept is to realize the inter-cavity transfer of the sample-laden beads without carry-over of reagents from one cavity to the next, especially in the case of PCR-inhibiting reagents and without significant sample loss. This was realized by the geometrical design of the various reaction compartments. Figure 1a and b show the complete cartridge (dimensions 97 mm × 48 mm excluding the PCB) with 3 tracks of 9 compartments each plus a final sensor chamber where a giant-magnetoresistivity (GMR) sensor detects the PCR products. The overall volume of each fluidic track is about 140 µl.

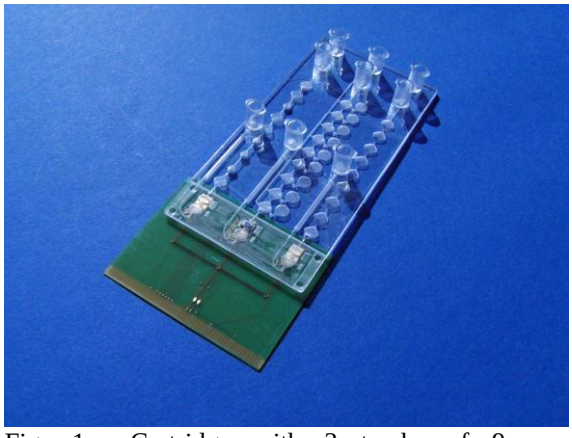

Fig. 1a: Cartridge with 3 tracks of 9 reaction compartments. Buffer and sample are introduced through the top Luer connectors. The lower connector is a simple venting outlet.

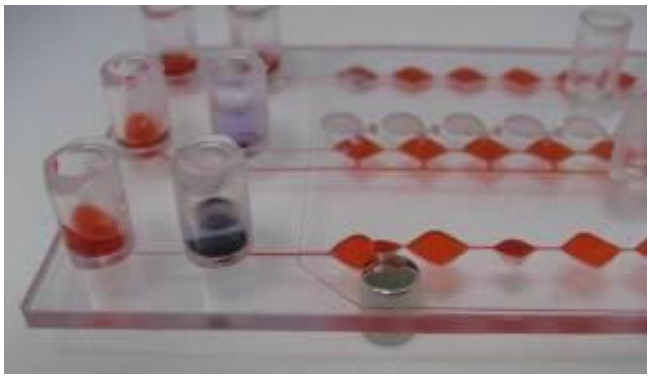

Fig. 1b: Details of buffer (left Luer) and sample (right Luer) inlets.

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The sequence of cavities follows the primary functions of typical molecular diagnostics assay. In the first cavity, the magnetic beads are collected after introduction. The second cavity is used for a first wash step. Cavity 3 contains lypophilized lysis buffer for cell lysis and DNA extraction. DNA is now captured on the functionalized beads. Cavities 4 and 5 are used for sequential washing in order to remove unbound molecules from the beads. PCR takes place in cavities no. 6-8. Figure 2 shows this part of the cartridge sitting on top of the temperature controller having three temperature blocks for the respective PCR temperatures. Alternatively, a conventional thermocycling can take place in one of these cavities. Cavity 9 again is for washing, while the actual sensor is mounted in cavity 10. The operation principle can be exemplified as shown in Fig. 3, presenting a detail of the compartments. The compartments are sequentially filled with a single buffer through a side-channel (top of picture) which is connected to all reaction chambers. Air leaves through a venting channel (bottom); and the compartments are separated by a 1.15mm long, 200 µm wide channel. The length of the channel has been chosen as to minimize diffusion from one chamber to the others as well as to minimize reagent carry-over during bead transport. The trapezoidal shape of the compartments proved to be important in order to remove all air as well as allow for a smooth bead transport from one compartment to the next. The magnetic beads can be seen as a black cluster in the second cavity.

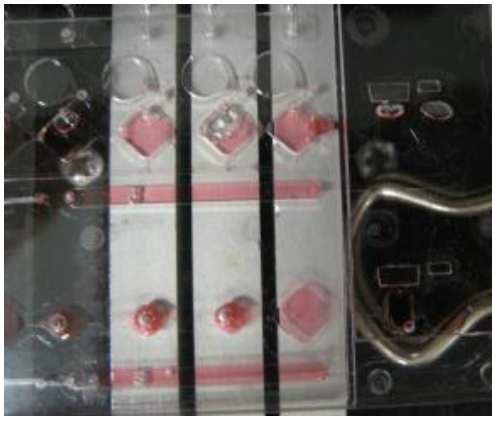

Fig. 2: Cartridge mounted on temperature controller.

Fig. 3: Detail of reaction compartments. The filling is performed through the common filling channel (top). The black cluster in the compartment consists out of the magnetic beads. Air leaves the chamber through a venting port (bottom).

EXPERIMENTAL RESULTS

One of the first questions to be answered was with respect to the transport properties of the magnetic beads. In the first experiment, 10 µl of bead suspension (bead diameter of 1 µm) with different concentrations were pipetted into the cards and the efficiency of bead transport depending on the absolute amount of beads was determined by extracting the beads again from the last chamber. Up to 10 µg beads could be recovered with an efficiency >90% as can be seen from the data in Fig. 4.

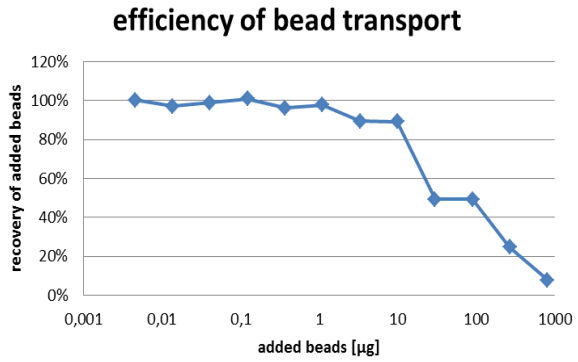

Fig. 4: Efficiency of bead transport through the cartridge. For amounts <10 µg, more than 90% of the beads are transported through the card.

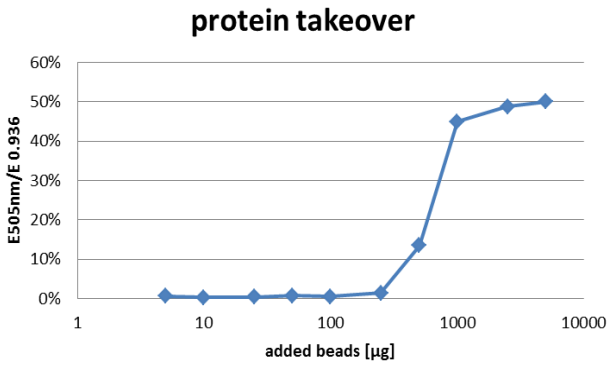

Fig. 5: Extinction measurements to measure the amount of protein carried over with the magnetic beads.

A second parameter which is important for the overall functionality of the concept is the carryover of proteins which can eventually lead to an inhibition of the PCR or a reduced sensitivity of the sensor due to the development of a protein layer on the sensor surface. As shown in Fig. 5, for the relevant amounts of beads, the amount of protein carry-over is very limited as measured using optical density (extinction) measurements.

The practical performance in the above described microfluidic cartridge was demonstrated using a

Streptococcus-specific PCR producing 153 bp DNA fragment. As shown in Fig. 6, both two- and three-step PCR protocols could be successfully performed on the cartridge. Furthermore, the absence of possible PCR inhibiting influences from either the materials or the manufacturing methods of the cartridge could be proven. Figure 6 shows the following data: expected 153BP amplification products were not obtained by GBS-specific PCR with water (Ø, negative control) but with extracts from *Streptococcus sp.* (+). PCR was performed in a desktop cycler (lanes with 1) or in a cavity of the cartridge (lanes with 2-4). To monitor possible PCR inhibition from materials or manufacturing methods of the cartridge, PCR performed in the desktop cycler (lanes with 1) was spike with additionally 25%vol/vol mastermix (a), water (b) or water (c) that had been incubated within the respective cavity of the cartridge by placing them onto heating plates (a, b) or incubation at room temperature (c) for 30 minutes. PCR in the cartridge was performed by filling in water first, and then 10µl of mastermix in a way that the mastermix containing fluid is placed in the cavity located on top of the selected heating zone. A three-step procedure was used for heating in 2, a two-step protocol was used for 3. In 4, the neighboring cavity has been heated, indicating a good thermal isolation of the respective cavities as no amplification was observed. M_{low} indicates a size marker (10/20/50/100/200 bp).

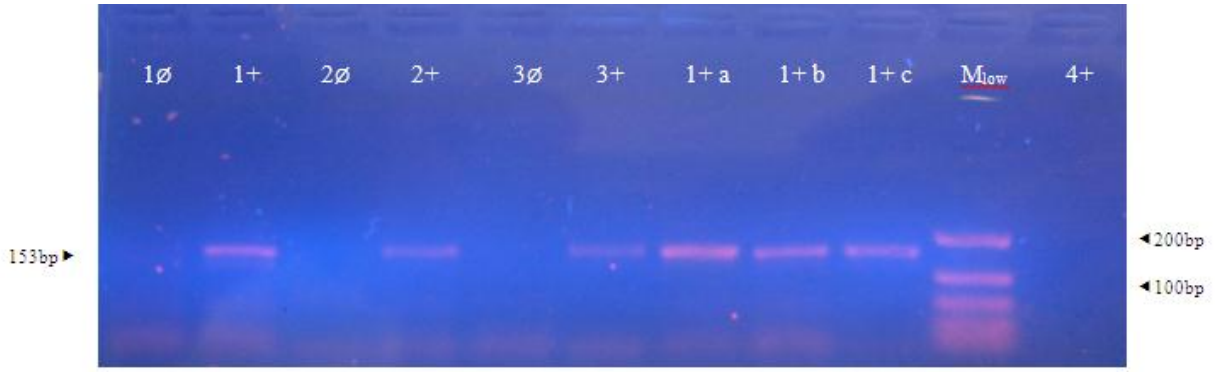

Fig. 6: Amplification of a 153 bp DNA fragment from *Streptococcus sp.* For details, see text.

CONCLUSIONS

In this paper, we present a microfluidic platform for molecular diagnostics using stationary fluidics and magnetic beads. While the concept of stationary fluidics has been published using immiscible liquids [2], our approach uses a single buffer liquid and protocol specific reagents which are stored in lyophilized form in the respective cavities of the cartridge. The big advantage of this approach is the simplicity of both the cartridge and the instrument as, besides a mechanism for moving the magnet, no mechanically active components such as pumps or valves are needed. The viability of the concept has been verified by experiments about the transport properties of the magnetic beads which indicate an efficiency of >90% of the initially dispensed beads for amounts ≤ 10 µg while at the same time the protein carry-over which could be detrimental for the assay proved to be sufficiently low. Further experiments showed that the resolubilization of the lyophilized reagents is possible with the buffer amounts available. Finally, DNA amplification using PCR was successfully carried out, indicating the feasibility of the process in the presented microfluidic cartridge.

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REFERENCES

[1] D. Eicher and C. A Merten, "Microfluidic devices for diagnostic applications", Exp. Rev. Mol. Diag., 11, 505-519, (2011)

[2] S. M. Berry, E. T. Alarid and D. J. Beebe, "One-step purification of nucleic acid for gene expression analysis via Immiscible Filtration Assisted by Surface Tension (IFAST)", *Lab Chip*, 11, 1747-1753, (2011).

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