PHASEGUIDE ASSISTED LIQUID LAMINATION FOR MAGNETIC BEAD-BASED ASSAYS

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ABSTRACT

We demonstrate a simple, pump-free platform for performing rapid magnetic bead-based processes via their transfer through sequentially laminated liquid streams, made possible by the use of phaseguide technology. We have applied this strategy to two on-chip assays: (i) a streptavidin-biotin binding assay, and (ii) a sandwich immunoassay for the detection of C-reactive protein (CRP). Here, functionalized magnetic beads were pulled through alternating lanes of reagents and buffer solution, allowing multiple binding and washing processes to be reduced into a single step, significantly shortening procedural times compared to conventional multi-step bead-based assays.

KEYWORDS: Magnetic beads, Phaseguides, Liquid lamination, Immunoassays, C-reactive protein

INTRODUCTION

Conventional bead-based assays require multiple reaction, incubation, and washing steps, thus yielding laborious and time-consuming procedures. However, by containing the different reagent and washing buffers within the different lanes of the microfluidic chip, functionalized magnetic beads could be pulled consecutively through each lane, allowing sequential reaction and washing steps to be performed in a single process. Therefore, multi-lane fluid systems are elegant platforms for magnetic bead-assisted assays. The transfer of beads from one fluid to the next enables a range of on-bead operations including functionalization, washing and binding steps to be performed within rapid timeframes. The adjacent patterning of multiple liquids, however, is not straightforward and normally requires either laminar flow [1], a multi-pipette [2], or the use of immiscible phases, such as oil-water [3].

Here we present a magnetic bead-based assay platform in which beads are transferred between five aqueous liquids to carry out immunoassays (Fig. 1). The five liquids are sequentially pipetted and patterned in adjacent lanes within a rectangular chamber, without the need for an additional external pump. Lamination of liquids in reproducible lanes is facilitated by the use of phaseguides [4]. Although gel-based stratification of square chambers has been reported before [5,6], it is much more complex to laminate various liquids without a gelling step. Using this phaseguide assisted liquid lamination device, a one-step streptavidin-biotin binding assay was first performed, followed by a two-step sandwich immunoassay for C-reactive protein (CRP), an inflammatory biomarker found in blood (usually below 10 μg mL^{-1}) whose concentration increases following inflammation or infection.

LAMINATION PRINCIPLE

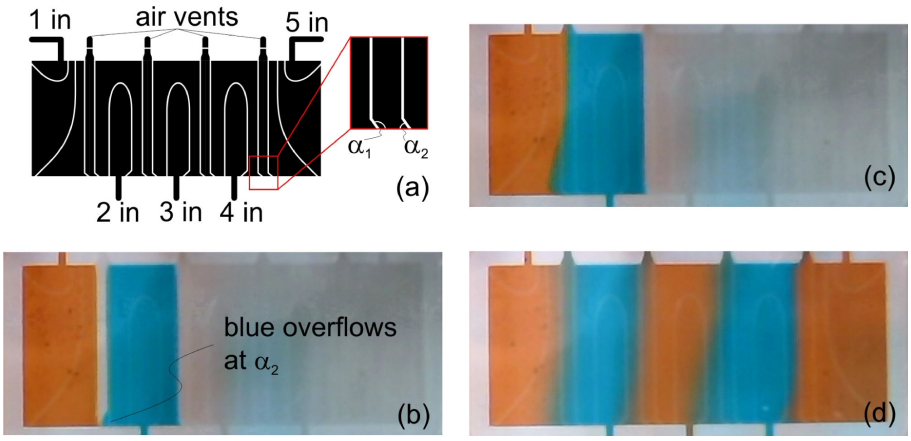

Figure 1: Principle of liquid lamination. (a) The chamber is subdivided into five lanes by parallel phaseguides. (b-c) The parallel phaseguides are designed such that the first liquid is kept stable in its lane, while the second liquid over-flows at a position distal with respect to the air vent. (d) Five lanes of aqueous liquids.

The design of the liquid lamination device is shown in Fig. 1a. The chip featured a large rectangular chamber that was subdivided into five "lanes", each having a width of 2 mm and a length of 4 mm. Each lane was fed by a single inlet, while air vents were situated at the top of the chamber, between the adjacent lanes. The device was fabricated on glass substrate using Ordyl SY-330 (Elga Europe) as the structural material. Fabricated structures were sealed by a glass substrate with access holes drilled. The chamber contained phaseguides that were patterned as thin lines on the bottom substrate. When a first liquid is introduced into lane 1 via a pipette, the advancing liquid-air interface is manipulated by the phaseguides such that the liquid is contained within this first lane. A second liquid is injected adjacent to the first, with a minimal gap maintained between the two, until the second liquid has filled the lane (Fig. 1b). Once filled, the gap between the two liquids is bridged by phaseguide overflow (Fig. 1c). The position of the overflow is controlled by a small angle (α) between the phaseguide and the microfluidic chamber wall, at a location distal to the air vent in order to prevent air entrapment. Additional liquid lanes are patterned in the same manner to ultimately achieve a five-lane configuration of aqueous solutions that contain various reagents and buffers (Fig. 1d).

EXPERIMENTAL

Stock magnetic bead suspensions were prepared by dispersing Dynabeads® M-270 Streptavidin beads (2.8 μm, diameter, Invitrogen, Norway) in phosphate buffered saline (PBS, pH 7.4, Sigma-Aldrich, UK) containing 0.05% Tween20 (Sigma Aldrich, UK). Reagents, buffers, and magnetic bead suspensions were injected into the microfluidic chamber by pipetting 1.5 μL of solution into the appropriate lanes of the microfluidic device.

In order to perform a streptavidin-biotin binding assay, streptavidin coated magnetic beads (2 x 10^6 beads in total) were introduced into lane 1 of the lamination device, while lane 3 contained biotin-4-fluorescein (Molecular Probes, UK), and lanes 2, 4 and 5 consisted of PBS buffer solution (Fig. 2a). Once all five lines had been filled, a 20 x 10 x 5 mm^3 rectangular NdFeB magnet (Magnet Sales, UK) was placed directly on top of the glass chamber, over lane 1, before being moved over the chamber from lane 1 to lane 5 (Fig. 2b). The fluorescence intensity of the magnetic beads was measured before and after magnetic actuation using an inverted fluorescence microscope (TE 2000-U, Nikon, UK). Images and videos were recorded via a CCD camera (WAT-221S, Watec, Japan), and fluorescence intensities of the beads were analyzed using ImageJ software (http://rsbweb.nih.gov/ij/).

For performing a CRP sandwich immunoassay, primary antibody coated magnetic beads were first prepared by incubating streptavidin functionalized magnetic beads with 10 μg mL^{-1} biotinylated anti-CRP antibody (R&D systems, UK). The primary antibody coated beads were introduced into lane 1 of the microdevice, while lane 2 consisted of human CRP (R&D systems), lane 4 contained 200 μg mL^{-1} of FITC-tagged secondary anti-CRP antibody (Abcam, UK), and lanes 3 and 5 held PBS buffer solution for washing unbound reagents from the surface of the magnetic beads following the binding steps. As before, a NdFeB magnet was placed over lane 1 and then moved across the chip to lane 5 (Fig. 2c), and the fluorescence intensity of the beads was measured before and after passing through the lanes of fluorescently labeled secondary antibody.

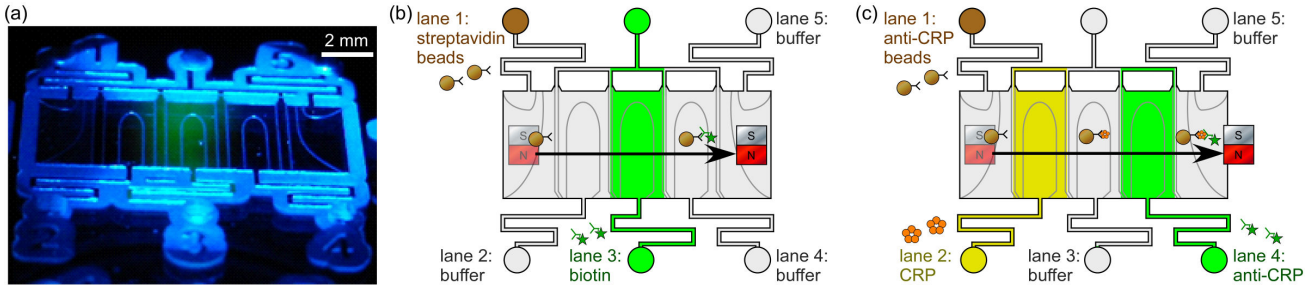

Figure 2: Magnetic bead-based assays on the multi-lane platform. (a) Photograph of the glass device, featuring a central lane of fluorescent biotin. (b) Principle of the streptavidin-biotin binding assay, in which streptavidin coated magnetic beads are pulled through a lane of biotin via a moving magnet. (c) Principle of the CRP sandwich immunoassay, whereby primary antibody coated magnetic beads are pulled through a stream of CRP sample, before being pulled through a stream of fluorescently labeled secondary antibody.

RESULTS AND DISCUSSION

The multi-lane system was first employed for a streptavidin-biotin binding assay. Upon magnetic actuation, the beads were transferred across the entire chamber as they followed the moving magnet, passing through the central biotin lane (3) and into the buffer lane (5) for fluorescence detection. The magnetic beads exhibited higher fluorescence intensities after passing through the biotin reagent, indicating successful binding to the streptavidin coated beads (Fig. 3a). Moreover, the assay displayed a linear response when the biotin concentration was varied from 1 to 100 μg mL^{-1} (Fig. 3b).

Subsequently, a sandwich CRP immunoassay was performed by pulling primary antibody coated magnetic beads through CRP sample solution, wash solution, FITC-tagged secondary antibody solution, and into a final buffer solution. Comparison of the fluorescence intensities of the beads before and after passing through the lanes showed an increase in fluorescence signal in the final wash solution, thereby demonstrating successful detection of CRP. CRP concentrations were analysed in the range of 0.1 to 10 μg mL^{-1}, with the fluorescence intensity of the beads increasing as the concentration increased (Fig. 3c). This indicates the potential of the multi-lane system for the measurement of CRP in clinical diagnostics. Importantly, both the streptavidin-biotin assay and the CRP sandwich immunoassay were performed in less than 8 seconds, the time taken to pull the magnetic beads from lane 1 to lane 5, yielding a significant decrease in reaction/washing times that would usually require tens of minutes for the former assay and several hours for the latter.

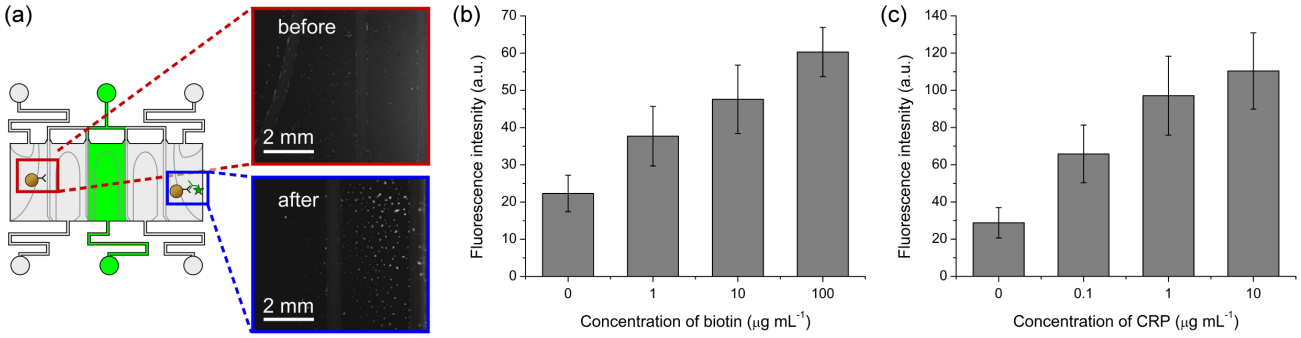

Figure 3: (a) Photographs showing the fluorescence intensity of streptavidin coated magnetic beads before and after passing through a lane of fluorescently labeled biotin. (b) Fluorescence intensities of streptavidin coated magnetic beads after passing through different concentrations of fluorescently labeled biotin. (c) Fluorescence intensities of primary CRP antibody coated magnetic beads after passing through different concentrations of CRP.

CONCLUSION

We have, for the first time, demonstrated the sequential lamination of five aqueous liquids within a rectangular micro-fluidic chamber. Magnetic bead-based assays have shown the platform's potential for performing rapid immunoassays (< 8 s), while extension to nucleic acid assays and solid-phase extraction protocols would be straightforward. Importantly, the system can be operated without the need for the fluid pumping. The fact that such complex microfluidic operations could be performed in a single chamber supports our claim that phaseguides enable black-box type microfluidics without any concession to the geometry.

ACKNOWLEDGEMENTS

C.P. thanks the Royal Thai Government for financial support. E.Y. thanks the Scientific and Technological Research Council of Turkey for financial support.

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