BEAD TRAPS IN CAPILLARY-DRIVEN MICROFLUIDICS FOR FLUORESCENCE IMMUNOASSAYS

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ABSTRACT

Polymeric microspheres represent an important class of components for biological assays because they can be fabricated from many materials, with well-controlled diameters from nanometers to hundreds of micrometers and functionalized with biomolecules. However, the incorporation of such microspheres inside microfluidic chips remains a challenge in particular when reproducibility and mass fabrication play an important role. We design and demonstrate how bead traps can assemble defined patterns of 10 µm-polystyrene microspheres in capillary-driven microfluidic chips and show the applicability of bead traps in the context of fluorescence immunoassays.

KEYWORDS

microfluidics, capillary system, reagent integration, immunoassay

INTRODUCTION

There currently are intense efforts on developing microfluidics for point-of-care diagnostics because microfluidics can preserve samples and reagents, shorten the time needed for results, and provide multiplexed analysis of samples with a small form factor device.[1] Ideally, such microfluidics should incorporate all reagents needed for an analysis so as to make their use simple. Integrating stationary receptors for analytes in microfluidic systems is however challenging and has mostly been done so far by patterning (protein) receptors on surfaces or across wicking matrices. Beads offer an interesting alternative to patterning receptors because they are commercially available with various dimensions, compositions, surface chemistries, and can be fluorescent or magnetic.[2]

Here, we describe strategies for trapping beads in capillary-driven microfluidic chips for ligand-receptor fluorescence assays, using a microliter or less of sample and within a few minutes. We demonstrate that simple-to-fabricate traps in microchannels can efficiently collect ~150 beads in a 10 pL volume element of a chip for assays utilizing capillary-driven flow. This work expands our concept of "one-step" assays using self-powered microfluidic chips[3] and is also simpler to implement than recent work based e.g. on manual insertion of large agarose-beads in microfluidics.[4]

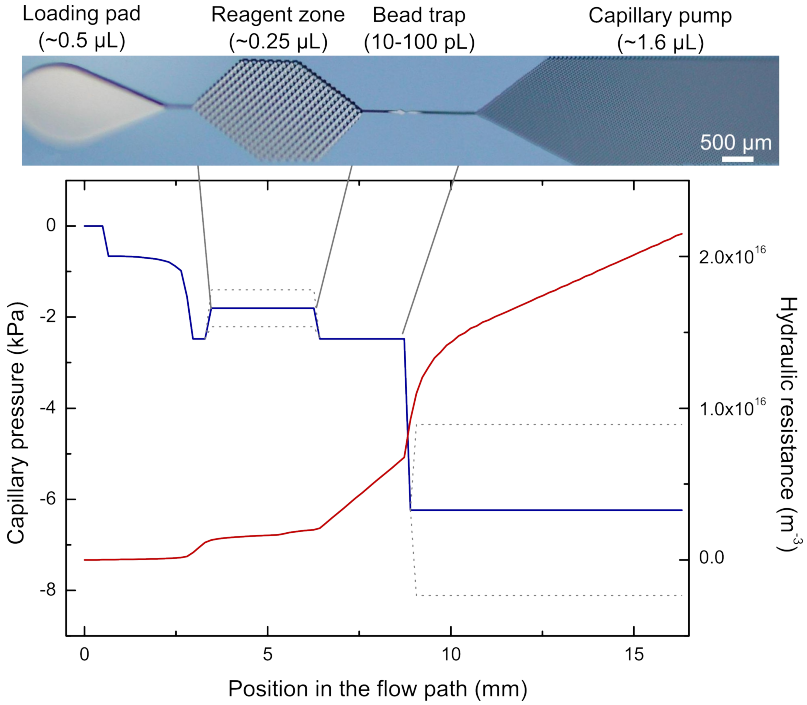


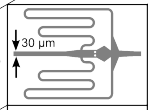
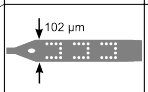
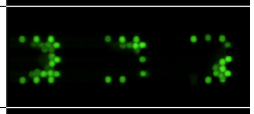
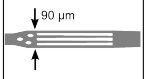
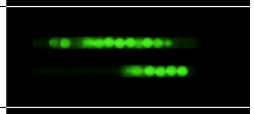
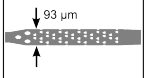
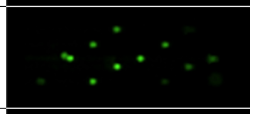
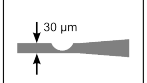
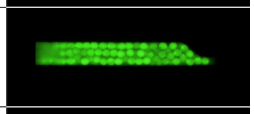
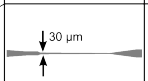
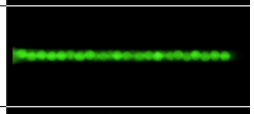
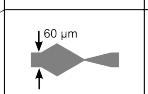
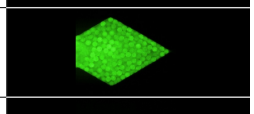
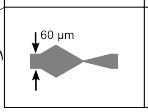
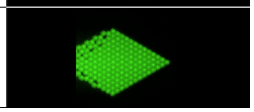
Figure 1. *Capillary-driven microfluidic chip incorporating a bead trap. The trap of this Si chip has a 7 µm lateral constriction and retains 10 µm beads. Beads are trapped before sealing the chip with a PDMS cover and drawing via capillary forces a sample from a loading pad, through the bead chamber and throughout a capillary pump. Here, the capillary pressure and hydraulic resistance are modeled considering a 30-µm-wide channel instead of a specific bead trap (see Table 1).*

EXPERIMENT

Patterns having microfluidic channels were defined on 4 inch Si wafers using two-layer optical lithography with photoresist AZ 6612 (Microchemicals, Ulm, Germany) and a SiO_2 as soft mask and hard mask, respectively. The patterns were transferred into the silicon using deep reactive ion etching (AMS-200SE, Alcatel Micro Machining Systems) to a depth of 60 µm for channels and pumps and to a depth of 12 µm for shallow traps. Before use, chips were cleaned with ethanol and ultrapure water (Millipore, Billerica, MA), blow dried with nitrogen and treated with an air-based plasma (200 W, 0.7 torr base pressure for 2 min; Tepla microwave-plasma system 100, Asslar, Germany). Subsequently, chips were immersed into 1 % (w/v) solution of Pluronic® F108 (BASF, Ludwigshafen, Germany) for 30 min, rinsed with ultrapure water and blow-dried. Trapping of beads was visualized using 10 µm fluorescent microspheres (Fluoro-Max®, Fisher Scientific AG, Wohlen, Switzerland).

For assay experiments streptavidin-coated microspheres (Bangs Laboratories, Chemie Brunschwig AG, Basel, Switzerland) were used. Bead concentrations in the solution were adjusted to match the desired number of trapped beads for each design. The chip was covered with a piece of 1-mm-thick PDMS. The surface facing the chip was previously passivated with bovine serum albumin. A sample solution (1 µL) containing Atto 590 biotin in PBS was then loaded onto the chip and the fluorescent signal accumulated onto the streptavidin-coated beads was detected using a fluorescence microscope (Eclipse 90i, Nikon, Japan).

Table 1. *Many traps can be designed and implemented to vary the architecture of immobilized beads, hydraulic properties and fabrication requirements of the chip, and geometry of the zones where fluorescence signal is measured. Images reveal the packing of 10 µm green fluorescent microspheres.*

design	hydraulic resistance [m^{-3}] no beads	hydraulic resistance [m^{-3}] filled with beads	max. theor. # of trapped beads (10 µm)	comments	fluorescence image
double bypass 	~2.9 10^{15}	~2.7 10^{16}	300 ± 15	- add. surface chemistry needed for creating efficient capillary delay valves - prone to air bubbles	
pockets 	~4.5 10^{14}	~1.2 10^{16}	880 ± 40	- only few beads trapped	
line array 	~2.4 10^{16}	~3.5 10^{16}	270 ± 5	- only few beads trapped	
single traps 	~1.1 10^{16}	~1.6 10^{16}	130 ± 5	- low trapping efficiency	
simple 	~1.2 10^{16}	~1.7 10^{18}	at least 250	- simple to fabricate - very high flow resistance	
line 	~4.3 10^{16}	~3.5 10^{18}	155 ± 5	- prone to air bubbles - very high flow resistance	
chamber (60 µm deep) 	~2.4 10^{14}	~3.6 10^{17}	1100 ± 50	- simple to fabricate - efficient trapping - high number of trapped beads	
chamber (12 µm deep) 	~1.7 10^{16}	~2.2 10^{18}	160 ± 20	- 2-layer lithography - efficient trapping - high number of trapped beads - well-defined fluorescence signal	

RESULTS AND DISCUSSION

Figure 1 shows a capillary-driven microfluidic chip for fluorescence immunoassays produced in silicon and a simulation of the capillary pressure and accumulated hydraulic resistance along the network disregarding any specific bead trap. The trap is located after a reagent zone and before a capillary pump. Various types of traps can be fabricated so as to entrap beads physically with varying arrangements, number of trapped beads and contribution to the hydraulic resistance of the network, Table 1. Using a single constriction, "cups" made of pillars, or arrays of pillars, many geometries of bead ensembles can be created by simply adding 0.5 µL of a bead suspension to the loading pad of a chip and rinsing with 0.5 µL of deionized water. Fluorescence images of the resulting bead architectures in various traps are shown in Table 1. Traps ending with a 7-µm-wide outlet and lateral walls forming a 60° angle were found optimal and resulted in hexagons of self-assembled beads. By reducing the depth of such a chamber from 60 µm to 12 µm, a single layer of beads was achieved. This facilitated optical reading of signal and direct counting of beads. In this case, ~160 beads are trapped in a 10 pL chamber. The utility of bead traps is demonstrated in Figure 2 by detecting fluorescently-labeled biotin in PBS using avidin-functionalized beads. The total signal measured on beads in 60-µm-deep traps is significantly higher than with the 12-µm-deep traps but it also exhibits a larger standard deviation presumably due to optical effects arising from the multilayer packing of the beads. As a result, the calculated lower limit of detection of the shallow trap (12 µm) is slightly lower (55 pM) than for the 60-µm-deep trap (129 pM).

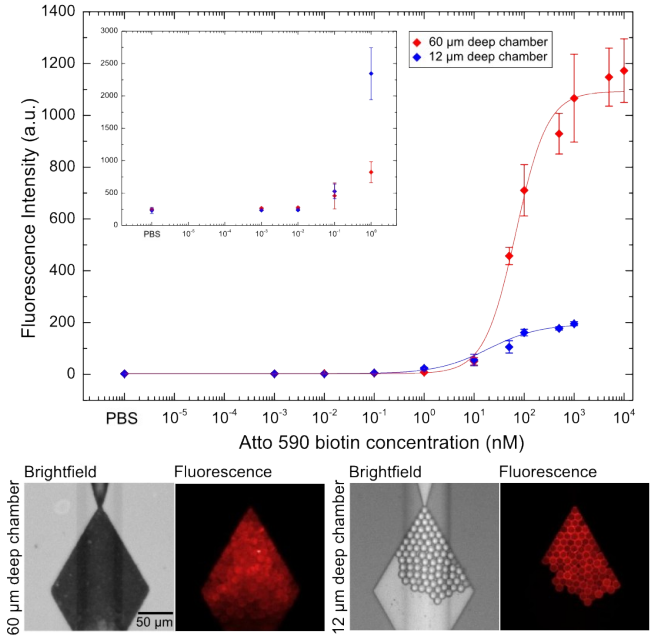


Figure 2. *Assay showing the fluorescence signal corresponding to the detection of Atto 590 biotin in PBS using avidin on 10 µm polystyrene beads packed in 60 or 12-µm-deep traps.*

CONCLUSION

We suggest that trapping beads carrying surface receptors for analytes as demonstrated here is greatly complementary to the concept of capillary-driven microfluidic chips: beads are commonly used for immunoassays and physically trapping them in microfluidics relieves from patterning receptors in the chip or on its cover. This strategy may therefore support the economic mass-manufacture of capillary-driven chips for diagnostic applications.

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