INTEGRATED MICROFLUIDIC FLUIDIZED BED FOR SAMPLE PRECONCENTRATION AND IMMUNOEXTRACTION

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ABSTRACT

We present a magnetic microfluidic fluidized bed device for on-chip sample processing. As a first proof of concept, the immunoextraction and preconcentration of fluorescently labelled IgG based a beads plug in fluidized state were investigated. Starting from a relatively large volume (ranging from 20 to 200 µL), this device allows for IgG an efficient immunoextraction and a subsequent elution. The detection of 1 ng/mL (~ 6 pM) labelled IgG has been successfully demonstrated using a "stop and go" elution mode.

KEYWORDS

Microfluidic, magnetic beads, fluidized bed, preconcentration, immunoextraction.

INTRODUCTION

Functional magnetic microparticles in microfluidic device are often dedicated to immunoassays. They provide large specific surface for biochemical binding and therefore highly efficient interactions with target molecules can occur. Nevertheless the handling of such particles in confined geometries has to be carefully optimized to improve interactions with fluids and thus the efficiency of the interactions. Here, we investigated a novel microdevice that uses a miniaturized magnetic fluidized bed to perform immunoextraction. Earlier systems involved either an immobilized matrix of large magnetic particles[1], or a "plug" maintained in place by a pair of magnets facing each other across the channel. In the first system, the magnetic elements are large and immobile so the whole beads surface is not accessible while low flow rate are applied to avoid high backpressure. In the second system, a "fracture" regime occurs when the flow rate increases, inducing inhomogeneous perfusion through the plug and reduced efficiency[2, 3]. We propose here a new microchannel and field geometry that leads to the creation of a fluidized bed regime and confer fluid properties to a dispersed solid substance. This approach should overcome the above limitations while improving the assay efficiency by increasing the surface to volume ration and improving the target/beads mixing.

EXPERIMENT

The design of the microfluidic device is presented in Figure 1.a. The core part of the device is made of a 21 mm long and 50 µm high channel with lateral dimensions that linearly widen from 100 µm up to 2 mm. A magnet is placed close to the channel to create a uniformly oriented magnetic field and confine particles. As the magnetic field intensity decays along the channel, the magnetic field gradient generates a force towards the channel entrance that induces particles confinement. As the liquid flows through the bed, the hydrodynamic drag force compensates for the Magnetic force and leads to bed expansion and fluidization (see figure 1.b).

We investigated the hydrodynamic behaviour of the magnetic plug of 50 µg made of 2.8 µm beads by measuring the flow rate as a function of the pressure applied using a MAESFLO controller (Fluigent).

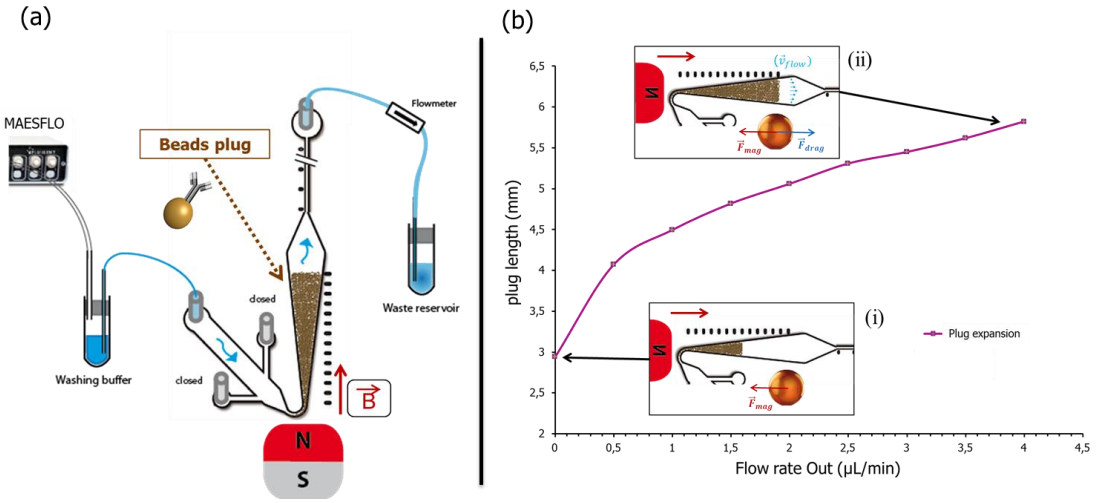

Figure 1: (a) Schematic representation of the magnetic microfluidic fluidized bed device. (b) Variation of the plug length versus the flow rate with a schematic view of a packed bead of 2.8µm magnetic particles within PDMS chip in two different regimes (i) in absence of flow rate: compaction into a dense and static bed. (ii) Equilibrium state where each particle satisfies the condition $\vec{F}_{mag} \approx \vec{F}_{drag}$*, the fluidized bed expands.*

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Figure 2 describes the evolution of the flow rate when increasing the applied pressure in the chip. Different hydrodynamic regimes can be distinguished: first, below a pressure threshold, there is no flow which could be measured, and secondly, above 90 mbar the pressure is sufficient to induce a drag force overcoming the magnetic one, causing an abrupt decrease of flow resistance by unpacking of the bed and a sudden jump of the flow rate (black curve). Recirculation flows are generated, causing a net particle influx in the centre of the channel and backflows close to the channel walls. After this initial "opening" of the plug, the pressure can be decreased down to 25 mbar, and a linear and reversible dependence of the flow versus pressure is obtained from 4 µl/min down to 0 (red curve). It parallels the behaviour of the empty channel, this behaviour is typical of fluidized bed. The geometry of the device was carefully optimized to favour uniform plug dynamics while maintaining a close to uniform beads density.

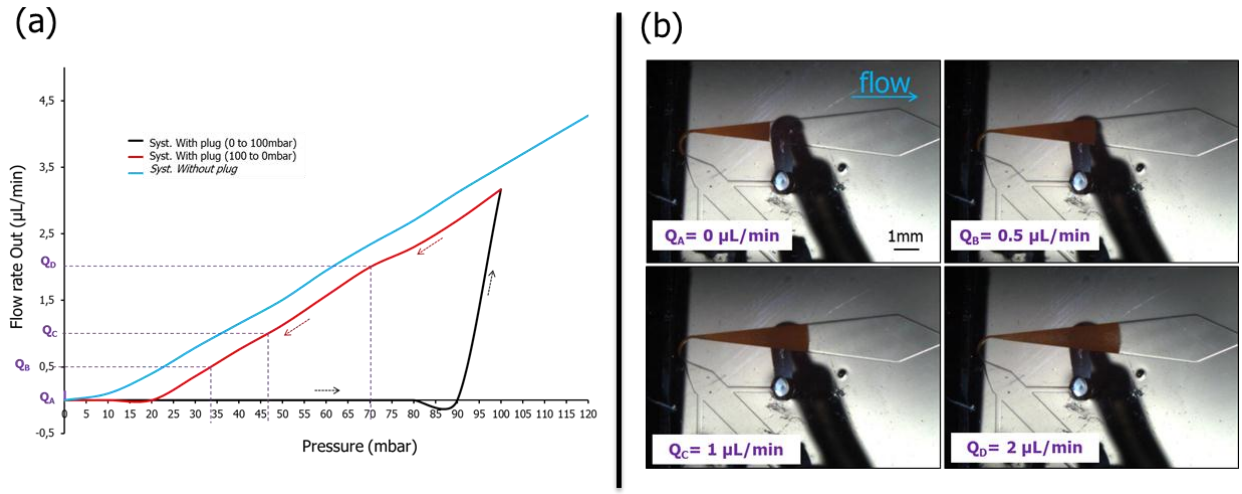

Figure 2: (a) Variation of the flow rate versus the pressure for a magnetic plug of 50 µg of Ø 2.8 µm beads (Dynal) (b) Plug pictures at different flow rates, each one corresponding to a sample processing step.

This system was applied to heterogeneous magnetic immunoextraction of model compounds (Fluorescently-labeled IgG; *Alexa fluo 488 Rabbit IgG antibodies*) based on the interaction between antibody-coated beads and target antigens. The target IgG was first captured on particles in fluidized bed regime and subsequent to a washing step, IgG will be eluted by an acidic buffer (citrate buffer). An original approach is proposed to perform the elution step ("stop and go" elution): the microchip is first prefilled with 2 µL of the solution, and then the flow rate is set at 0 µL/min (stop mode) during 10 min. During this period of time, the plug is maintained in packed-bed state. This stop flow step should allow to improve the elution as this gives more time to the dissociation to take place and should thus allow to get higher target recovery. It can also be expected that performing the elution in packed bed regime should limit the target dispersion and consequently improve the assay sensitivity. For an IgG sample at 100 ng/mL the signal to noise ratio achieved with this "stop an go" elution is 3.5 higher than the one obtained by conventional continuous elution (see Figure 3). A sequential elution was performed (four successive elutions) and as expected the band intensity of these sharp bands decreases as the number of elution step increases.

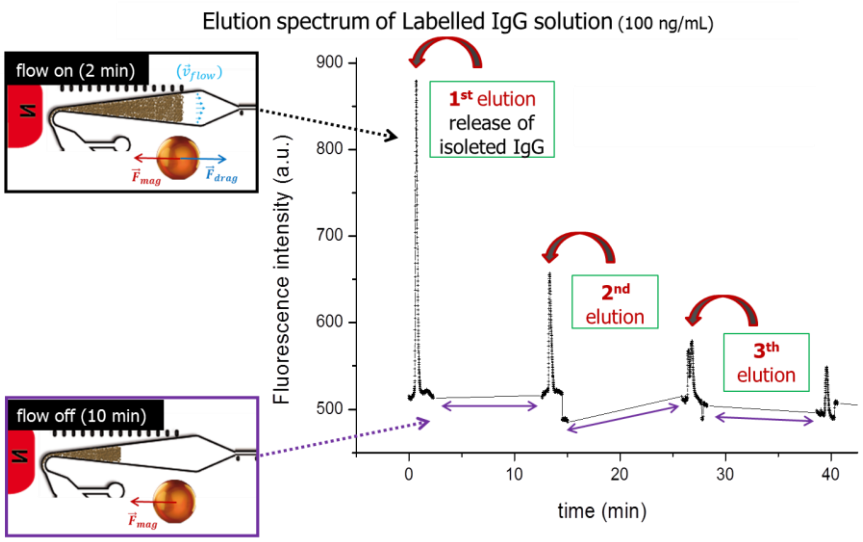

Figure 3: "stop and go" elution modes using citrate buffer pH 2 as eluting buffer. Direct fluorescence detection of labeled IgG released from the plug ([IgG] =100 ng/mL, injected volume 20 µL). "Stop and go" elution mode at flow rate of 0, 5µL/min: fractionated elution with four sequential elution steps.

As shown in Figure 4, we could obtain a LOD below 1 ng/mL (≈ 6 pM), a sensitivity at least ten times lower than that achieved earlier configurations[4]. This approach provides an efficient and easy to use dynamic capture platform. In contrast with previous magnetic microfluidic systems, this device allows for a dense, fluidized and self-mixing steady-state bed, yield optimal fluid-particles interactions, capture capacity. It also offers the possibility to modulate the plug density by tuning the flow and the magnetic field. The current flow rate is a few hundred µL/h, but with suitable scale up of the chamber, it can be increased to several mL/h in order to capture trace elements from large volume samples.

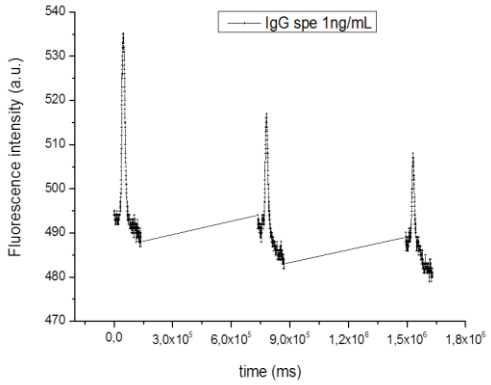

Figure 4: Direct fluorescence detection of labeled IgG released from the plug ([IgG] = 1 ng/mL, injected volume 200 µL). "Stop and go" elution mode at flow rate of 0.5µL/min: fractionated elution with three sequential elution steps.

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