MAGNETICALLY ACTUATED PARTICLE-BASED PROCEDURES IN CONTINUOUS FLOW

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ABSTRACT

We demonstrate a versatile multilaminar flow microfluidic device in which magnetic particles are used as mobile supports for performing two important applications, namely (i) a clinically relevant sandwich immunoassay, and (ii) polyelectrolyte coating of templates towards the fabrication of microcapsules for drug delivery applications. Furthermore, we demonstrate the use of a different force, diamagnetic repulsion, for deflecting polystyrene particles through a reagent stream with a view to performing multilaminar flow studies on diamagnetic material such as polymer particles and cells.

KEYWORDS: Magnetic particles, C-reactive protein, polyelectrolytes, diamagnetic repulsion, continuous flow

INTRODUCTION

Magnetic particles have become increasingly popular as solid supports for performing a number of (bio)chemical processes such as immunoassays and chemical reactions due to their small size, high surface-to-volume ratio, and their ease of manipulation using magnetic fields. However, conventional particle handling procedures require multiple, sequential reaction and washing steps that, despite yielding high capture efficiencies, render the process laborious and time-consuming. We have previously demonstrated that procedural times can be significantly reduced by using a continuous flow microfluidic platform to perform processes including mouse IgG immunoassays [1,2], DNA hybridization [3] and peptide bond formation [4]. This is achieved by deflecting functionalized magnetic particles through laminar flow streams containing alternating reagents and buffers, such that consecutive reaction and washing steps can be performed on the particle surface in one continuous process.

Here, we investigate the ability of the system for reducing procedural times of two important multi-step applications that are conventionally very time-consuming: (i) a clinically relevant C-reactive protein (CRP) sandwich immunoassay (Fig. 1a), and (ii) the deposition of polyelectrolyte layers onto magnetic templates (Fig. 1b). CRP is an inflammatory biomarker found in blood (typically in concentrations below 10 μg mL^{-1}) whose concentration increases with increasing inflammation or infection, making its detection very useful for clinical diagnostics. Polyelectrolytes have recently generated a great deal of interest for the fabrication of drug delivery vesicles [5]. Conventionally, such vessels are produced by layer-by-layer deposition of polyelectrolytes onto a sacrificial template that is later dissolved to yield a hollow capsule. Thus, drugs can be incorporated into these capsules and later released upon exposure to specific stimuli.

Additionally, we present an investigation towards the use of diamagnetic repulsion for performing multilaminar flow reactions. Previously, we reported the use of diamagnetic repulsion for the continuous flow separation of polystyrene particles [6], simply by using a permanent magnet and suspending the particles in a paramagnetic medium (aqueous manganese (II) chloride). We now extend the concept further by introducing particles into a reagent stream and allowing them to react, before deflecting them out of the reagent stream and into a buffer stream using diamagnetic repulsion, towards performing multilaminar flow reactions on materials without the need for magnetic labeling (Fig. 1c).

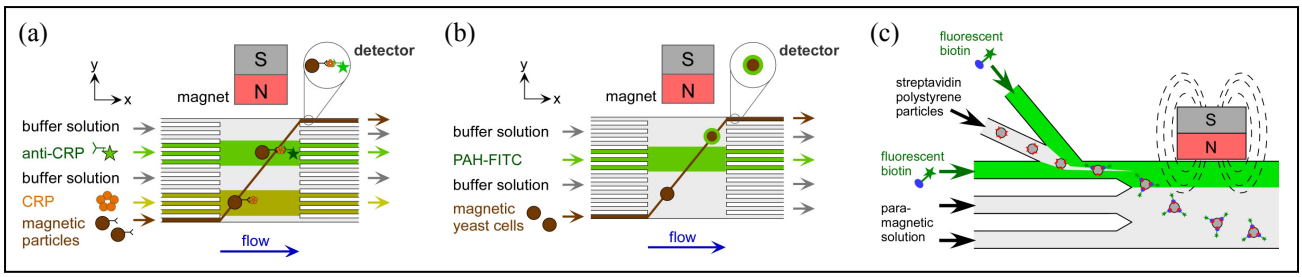

Figure 1: (a) Principle of the multilaminar flow system; here, a CRP sandwich assay is performed by deflecting particles through streams of CRP and fluorescently labeled antibody. (b) Multilaminar flow deposition of polyelectrolyte onto magnetic yeast cells. (c) Diamagnetic repulsion of streptavidin-coated particles out of a stream of biotin reagent.

THEORY

The principle of magnetic deflection of particles through multilaminar flow streams is shown in Fig. 1a and 1b. Alternating streams of reagent and buffer are generated across a microfluidic chamber in the x-direction, and functionalized magnetic particles are deflected through each stream in the y-direction via an external magnetic field, allowing consecutive reactions to take place on the particle surface. The extent to which a particle deflects across the microfluidic chamber depends on the magnetic force on the particle ($\mathbf{F}_{mag}$), which in turn depends on the difference in magnetic susceptibility between the particle (χ_p) and the medium (χ_m), the volume (V) of material in the particle that is affected by the

magnetic field, and the strength (**B**) and the gradient of the magnetic field (∇**B**). In the case of magnetic particles, V describes the volume of iron oxide present, whilst for the diamagnetic repulsion of polystyrene particles, V is the volume of the entire particle since the whole particle is affected by the field (Eq. 1). Typical $\mathbf{F_{mag}}$ values are in the order of pN.

$$\mathbf{F_{mag}} = \frac{(\chi_p - \chi_m)V(\mathbf{B}\cdot\nabla)\mathbf{B}}{\mu_0} \quad (1)$$

For magnetic particles, the difference in magnetic susceptibility with the medium is positive ($\chi_p - \chi_m > 0$), indicating that the particle will experience attraction to the field, whereas a diamagnetic particle suspended in paramagnetic medium gives a negative value ($\chi_p - \chi_m < 0$), resulting in repulsion of the particle from the field (Fig. 1c). Thus, both attraction and repulsion forces can be used to deflect particles across a microfluidic chip, simply by tailoring the relevant properties.

EXPERIMENTAL

For magnetic attraction, chip design A was used, while for the repulsion, chip design B was employed. Both designs were fabricated in glass to a depth of 20 μm using conventional photolithography and wet etching techniques. Chip design A, used for the CRP assay and the polyelectrolyte coating, featured an 8 x 3 mm^2 reaction chamber with 5 inlets and 5 outlets (Fig. 2a), and was coated internally with a thin layer of agarose gel to prevent particles sticking. For the CRP assay, 2.8 μm magnetic particles (Dynabeads, Invitrogen) functionalized with primary CRP antibody were introduced into inlet 1, CRP solution (10 μg mL^{-1}) into inlet 2, secondary CRP antibody labeled with FITC into inlet 4, and buffer solutions into inlets 3 and 5 (see Fig. 1a). For polyelectrolyte coating, yeast cells coated with cationic polyelectrolyte (poly(allylamine hydrochloride) (PAH)), and anionic tetramethylammonium stabilized magnetic nanoparticles [7] were introduced via inlet 1, poly(allylamine hydrochloride) tagged with FITC fluorescent label (PAH-FITC) via inlet 3, and sodium chloride solution via inlets 2, 4 and 5 (Fig. 1b). In both experiments, a 4 x 4 x 5 mm^3 neodymium-iron-boron (NdFeB) magnet was placed on top of the chip, next to the chamber (Fig. 2b).

Chip design B featured 10 inlets and 9 outlets, and incorporated a particle focusing design (Fig. 2a). All buffer and reagent solutions were prepared in paramagnetic $MnCl_2$ solution (10 % w/v). Streptavidin-coated polystyrene particles (10 μm, Polysciences) were introduced into the focusing section of the chip, where they were surrounded by two reagent streams of fluorescently labeled biotin, with paramagnetic solution introduced via the remaining inlets. A 20 mm Ø x 5 mm NdFeB disc magnet was placed on the chip, next to the chamber and nearest the side of the reagent inlets (Fig. 2b).

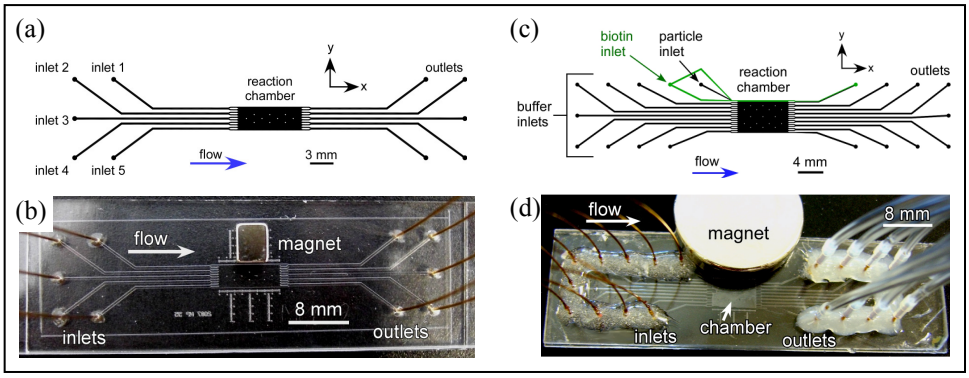

Figure 2: (a) AutoCAD design of the multilaminar flow device. (b) Photograph of the multi-flow chip, with the magnet placed next to the chamber. (c) Schematic of the diamagnetic repulsion setup, showing particle/reagent inlets. (d) Photograph of the repulsion setup, showing the position of the magnet.

RESULTS AND DISCUSSION

CRP immunoassay: The functionalized magnetic particles were deflected through each of the reagent and washing streams, with CRP binding to the particles in the first reagent stream before being fluorescently labeled in the second reagent stream. Fig. 3a shows the average increase in fluorescence intensity of 20 particles after the assay, indicating the successful detection of 10 μg mL^{-1} CRP that would suggest mild inflammation or viral infection in the human body. Each particle required only 90 seconds to cross the chamber and perform the assay, a significant reduction in procedural times compared to the hours required conventionally. Future work will involve an investigation into a calibration range, and CRP detection in real human serum samples.

Polyelectrolyte coating: Negatively charged, magnetically functionalized yeast cells [7] were deflected through a stream of cationic polyelectrolyte, PAH-FITC. The cells displayed an increase in their fluorescence after crossing the stream of polyelectrolyte solution, indicating successful adsorption of a layer of PAH-FITC onto the cell surface (Fig. 3b,c) and demonstrating great potential for performing fast layer-by-layer deposition of materials onto magnetic templates for various applications, including fabrication of drug containing microcapsules.

Diamagnetic repulsion: Streptavidin functionalized polystyrene particles entered the microfluidic chamber between two streams of fluorescently labeled biotin, where the particles were exposed to the biotin. Fig. 4a shows particles first entering the flow focusing section of the chip where they were exposed to biotin, before traversing the chamber and being deflected out of the reagent stream and into the washing stream by a magnetic field. The particles displayed an increase in fluorescence after exiting the biotin stream, showing that the reaction was successful (Fig. 4b). These initial results show great promise

that, with an optimized chip design and magnetic set-up, diamagnetic repulsion could be used to perform a number of multi-laminar flow processes on particles and cells without the need for magnetic labeling.

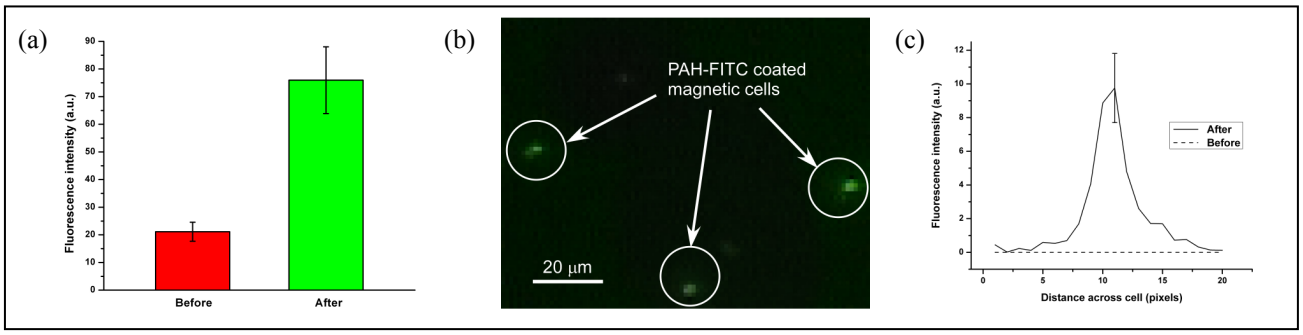

Figure 3: (a) CRP sandwich immunoassay: average fluorescence intensities of microparticles before and after crossing the reagent streams. (b) Photograph of PAH-FITC coated yeast cells after crossing the polyelectrolyte stream, and (c) fluorescence increase of yeast cells having traversed the PAH-FITC streams. Uncoated cells exhibited no fluorescence.

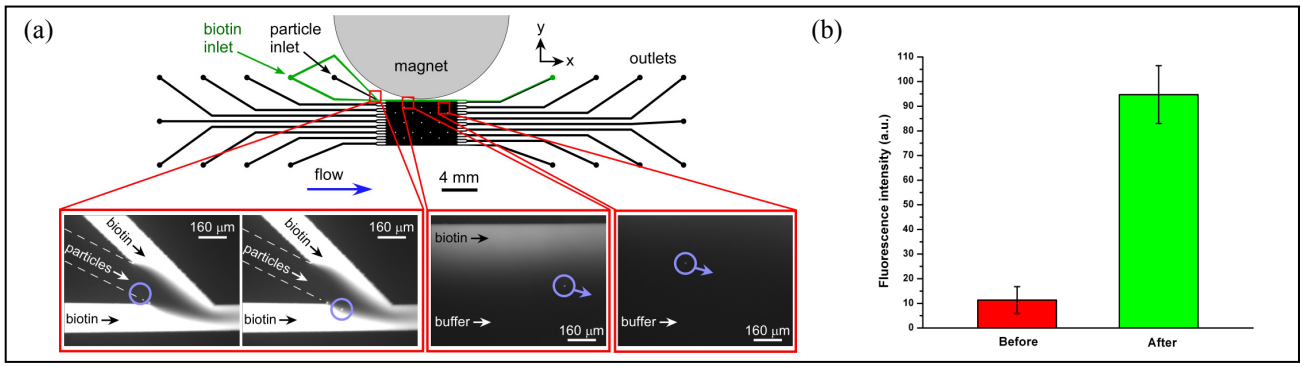

Figure 4: (a) Photographs showing the migration of microparticles into a reagent stream and their diamagnetic repulsion out of it. (b) Fluorescence increase of streptavidin-coated particles after their deflection out of the biotin stream.

CONCLUSION

We have demonstrated the use of our multilaminar flow microfluidic platform for significantly reducing procedural times of two important applications, namely (i) clinically relevant CRP immunoassays, and (ii) the deposition of materials onto templates for drug delivery vesicle formation We have also presented proof-of-principle results for the use of diamagnetic forces in deflecting materials out of reagent streams, towards performing multilaminar flow processes based on the intrinsic properties of materials, including microparticles and cells.

ACKNOWLEDGEMENTS

The authors thank the Engineering and Physical Sciences Research Council (EPSRC) for funding, and Leigh Madden and Prof. John Greenman (Clinical Biosciences Institute, Hull, UK) for their advice on the CRP assay. The support from the Government of the Republic of Tatarstan (Algarish Grantlar Programmasi) is gratefully acknowledged by R.F.F.

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