INERTIAL MICROFLUIDICS FOR MULTIPLEXED AFFINITY SEPARATION OF PROTEINS AND CELLS

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ABSTRACT

We present an inertial microfluidic platform for rapid, multiplexed affinity purification of proteins and cells from complex mixtures. Beads of different sizes, coated with different bait molecules bind specific proteins or cells in a single reaction step and the bound targets are then size-sorted by flowing in a spiral microchannel. We use this scheme to simultaneously purify antibodies specific to three different HIV antigens from HIV patient sera. We then demonstrate the simultaneous sorting of various cell types and also the isolation of rare antigen-specific cells such as HIV-specific cells from peripheral blood mononuclear cells (PBMC) from HIV patients.

KEYWORDS: Protein Separation, Cell Separation, Affinity Purification, Inertial Microfluidics, Multiplexing, HIV

INTRODUCTION

Isolation of specific proteins and cells from complex biological mixtures such as blood serves as the essential first step in analytical and preparative methods involved in a range of diagnostic, therapeutic and research applications. For a large class of target proteins and cells, such as antigen-specific antibodies or immune cells, binding affinity to the cognate antigen is their only distinguishing characteristic and forms the basis of current isolation methods for them. Traditional affinity purification involves serial binding, washing and elution for each antigen. Hours-long binding times and sample loss in each step makes it difficult to use for low-abundance species and low-availability clinical samples. For cells, commercial magnetic bead-based sorting allows only binary separation while microfluidic multi-target affinity-based sorting [1] was achieved only for bacteria. Multiplexed fluorescence activated cell-sorting (FACS) remains relatively inaccessible due to high equipment cost (usually >$100,000) and the throughput is still low for isolating rare cells (usually ~10,000 cells/second) [2]. In this work, we report an inertial microfluidic platform for rapid, multiplexed affinity purification of both proteins and cells from complex mixtures.

THEORY

The multiplexed affinity purification scheme, as shown in Figure 1, involves a single binding step in which the sample is incubated with a mixture of coated beads of a number of different sizes each coated with a different capture agent. After binding, the mixture is flowed through a spiral microchannel device,

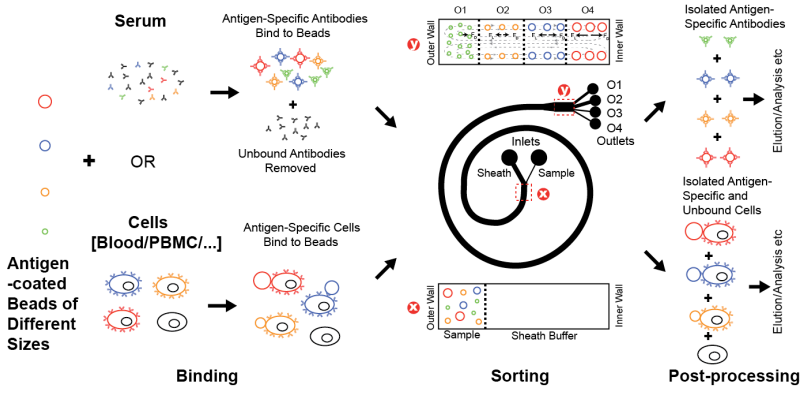

Figure 1: Principle of multiplexed inertial affinity separation of proteins or cells: Different affinity capture agents are coated on beads of different sizes and a mixture of beads is allowed to bind to the sample. When flowed through a spiral microchannel, the protein-bound or cell-bound beads are sorted by size. Separated species are eluted from beads.

which sorts particles in the mixture into different outlets based on their size. This device works on the principle of Dean Flow Fractionation (DFF) [3]. Particles above a certain size threshold, when flowing through a spiral channel ($d_p/h > 0.07$, where d_p is the effective particle diameter and h is the channel height) can be focused into distinct streams due to the superposition of size-dependent inertial lift forces (F_L) and a drag force (F_D) due to the Dean flow generated as a result of centrifugal acceleration of the fluid, shown here as the counter-rotating fluid vortices it generates. The device height, particle sizes and outlet positions can be designed to match a single outlet to each stream containing the different protein or cell-bound beads (Outlets 1-3). All particles below the focusing threshold remain entrained in the Dean flow and can also be collectively guided into a separate outlet (Outlet 4). The output streams of particles are collected and used for downstream processing of the bound cells or antibodies.

RESULTS AND DISCUSSION

Spiral micro-channel devices (Fig. 2a) were designed and fabricated which focus particles of different sizes into distinct positions (Fig. 2b). Particle sizes (d_1= 10µm, d_2=6µm, d_3=4.5µm, d_4=1µm) were chosen which could be reliably directed into distinct streams at a range of flow rates (Fig 2c). Outlets of the device were then designed to capture these separate streams. The sorting efficiency for beads of four different sizes was verified by flow cytometry (Fig. 2d,e).

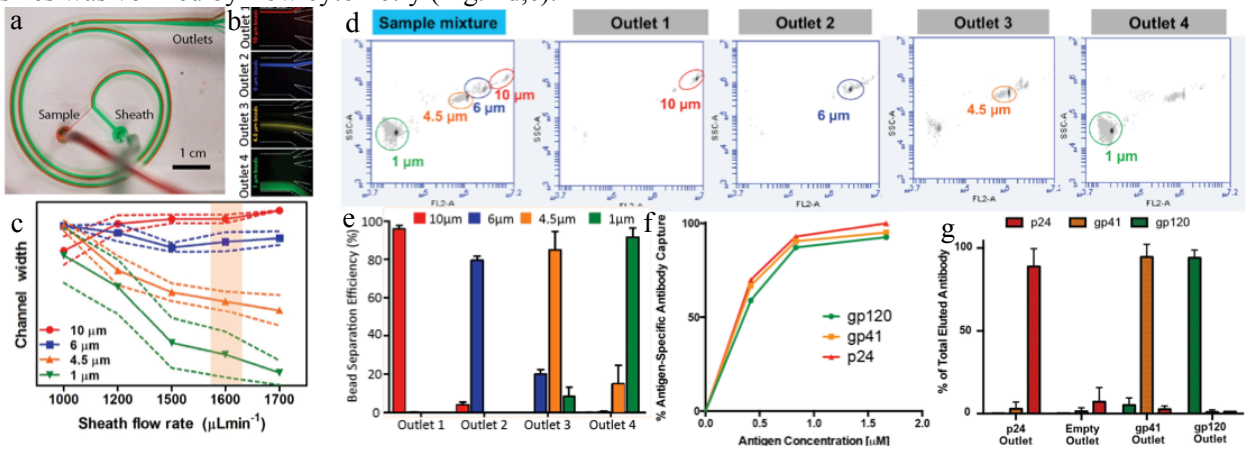

Figure 2a. Fabricated PDMS-glass spiral microchannel b. Separate focused streams of 10µm, 6µm, 4.5µm and 1µm particles c. Optimization of flow rates for particle separation. d. Flow cytometry based evaluation of input mixture of beads and separated output streams. e. Separation efficiency for mixture of beads of four sizes. f. Simultaneous capture of three antibodies in a single binding step from HIV patient sera using a mixture of beads coated with different antigens. g. Purity of antibodies eluted from separated beads evaluated using an antigen-binding ELISA.

Beads coated with three different HIV proteins p24, gp41 and gp120 were used to simultaneously capture antibodies against them from HIV patient sera in a single binding step. This was verified by performing an antigen-binding ELISA of the depleted serum after binding which showed a bead-concentration, and hence effective antigen-concentration, dependent capture of all three different antibodies (Fig. 2f). The antibody-bound beads were washed and flowed into the microfluidic device and antibodies were eluted from beads obtained at each device outlet. The purity of isolated antibodies was verified by performing an antigen-binding ELISA of these elutes (Fig. 2g).

In optimizing bead sizes for cell sorting, it was observed that bound bead-cell pairs focused at positions set by the bigger of the two sizes if they had unequal sizes or by the sum of the two sizes if they had similar size. This is seen in the enrichment of the T cell fraction of PBMC, bound to anti-CD3 coated beads into different outlets depending on bead size (Fig. 3a-d). Consequently, beads bigger than cells were chosen for multiplexed cell sorting. Flow rates were tuned to obtain the separation of B Cells and T Cells from rest of PBMC using antibody-coated beads (Fig. 3e). Similarly CD4+ and CD8+ cells were sorted from other cells (Fig. 3f). Isolation of rare antigen-specific cells is demonstrated by the isolation of HIV-specific cells (<1 in

10,000 cells) from PBMC from HIV patients (Fig. 3g) and tetanus-specific cells (<1 in 100,000 cells) from PBMC healthy donors (Fig. 3h).

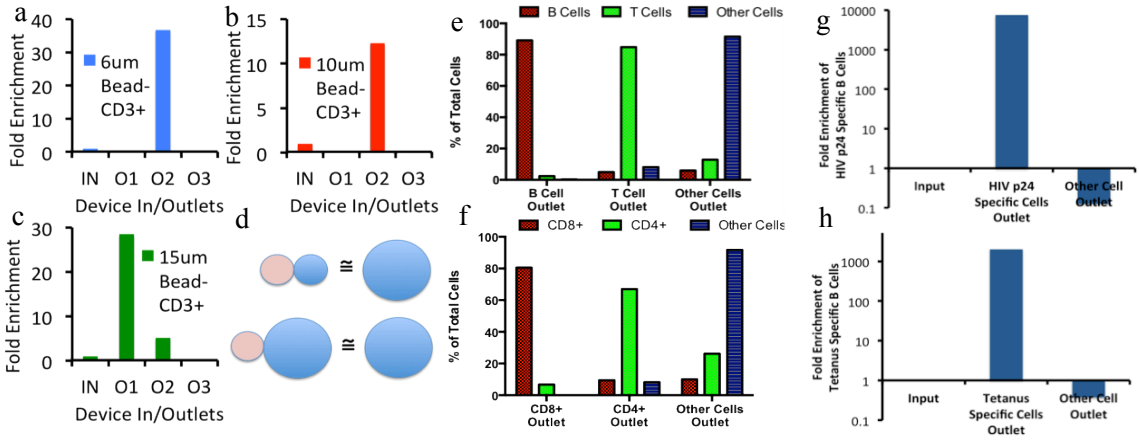

Figure 3 a) T Cells (nominal diameter~6-8μm) bound to 6μm beads are enriched in the 10μm outlet (O2) b) T Cells bound to 10μm beads enrich in O2 as well. c) T Cells bound to 15μm beads enrich in the 15μm outlet (O1) d) Effective diameter of bead-cell pair for focusing. e) Separation of T Cells (labeled with PE-anti-CD3) and B Cells (labeled with FITC-anti-CD19) from rest of PBMC by binding to 10μm anti-PE coated bead and 15μm anti-FITC coated beads at an optimized sample flow rate of 170μL/min and sheath flow rate of 1750μL/min. f) Separation of CD4+ and CD8+ cells from rest of PBMC by bead binding and flow rate as optimized above. g) Isolation of rare HIV protein p24-specific B cells from HIV+ PBMC and h) tetanus-specific B cells from healthy PBMC by binding to a 15μm bead coated via the respective antigen-tetramers coated on the cells.

CONCLUSION

This rapid separation of antibodies demonstrated here enables correlation of disease states to molecular characteristics, such as glycosylation [4], of antibodies helping the understanding of determinants of immunity and eventually the design of vaccines. Rapid, inexpensive sorting of rare antigen-specific cells will accelerate the discovery and development of novel antibodies [5] for prophylaxis and therapy. Even beyond applications demonstrated here, this scheme is widely applicable in analytical or preparative methods in a range of research and clinical applications and it is amenable to massive parallelization for high throughput and integration into sample-to-answer lab-on-chip platforms.

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