IMMUNOMAGNETIC PURIFICATION OF CANCER CELLS FROM WHOLE BLOOD ON A CENTRIFUGAL MICROFLUIDIC PLATFORM

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ABSTRACT

Based on our pioneering work on multi-force, centrifugo-magnetic particle separation presented at µTAS'11 [1] [2], we have substantially improved design (Fig. 1) and functionality to enable the direct immuno-separation of cancer cells from whole blood. The system presented here is shown to successfully extract a population of less than a hundred cancer cells from a background of many hundreds of thousands of blood cells. We outline the novel system design and notably refined working principle. The ability to "pick out" rare cells from whole blood on our platform bears the promise to leverage a host of interesting applications in biomedical diagnostics, therapeutics and point-of-care systems.

KEYWORDS

Cell separation, magnetic, cancer cells, centrifugal microfluidics

PRINCIPLE OF OPERATION

Similar to work by Pamme *et al.* on a flow-based scheme [3], our threefold differential centrifugo-magnetic separation of magnetically tagged cancer cells from a background of blood cells and unbound magnetic tagging beads (Fig. 1) is governed by the interplay of the centrifugal force F_ω, the magnetic force F_m, and the Stokes drag F_d. Table 1 compiles estimates of typical values for the corresponding magnitudes of the centrifugal and magnetic forces.

Table 1. Relevant forces present on the three particles in the system at a given point. Rotational frequency is 11.25 Hz.

Particle	F_ω (N)	F_m (N)
1-µm magnetic bead	5.2×10^{-13}	8.0×10^{-14}
Blood cell	1.4×10^{-10}	0
Tagged MCF7 cell	1.9×10^{-10}	1.6×10^{-11}

All cells and particles will be exposed to a radial centrifugal force F_ω and the Stokes drag F_d. In addition, unbound, 1-µm magnetic tagging beads will experience a relatively large magnetic force F_m resulting in a strong lateral deflection. The magnetically tagged cancer cells will experience both a smaller lateral force due to its reduced, volume-averaged magnetic moment compared to the particles and a higher Stokes drag F_d, eventually resulting in an intermediate deflection path into the collection chamber C (Fig. 2b).

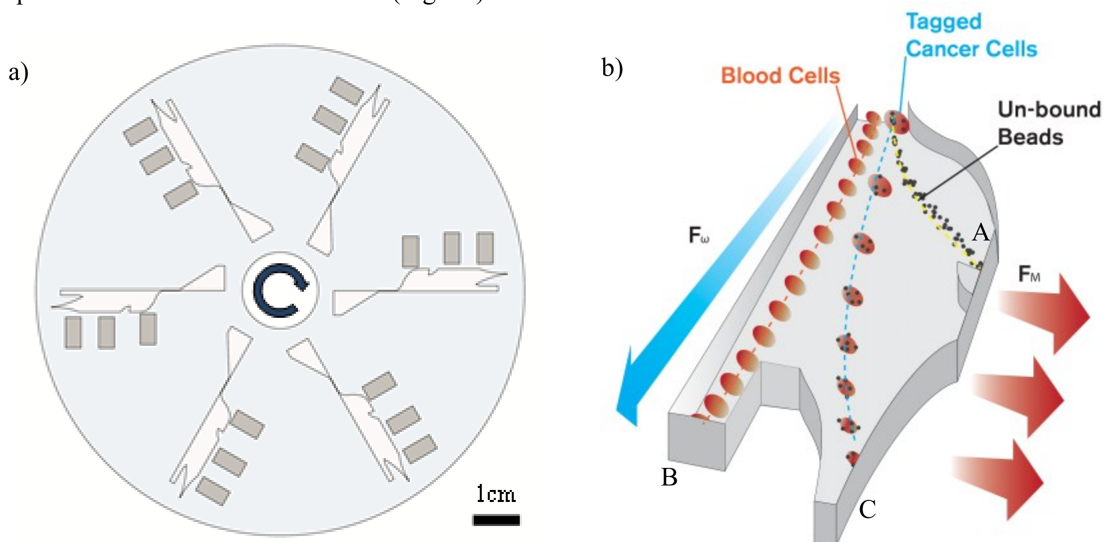

Fig. 1a) Schematic showing entire disk which contains six separate chambers with three permanent magnets aligned along their respective radial direction. b) Magnified 3-D schematic (not to scale) of a single chamber outlining the principle of separation of blood cells, cancer cells and unbound tagging beads as the sample enters the separation chamber (Fig. 2). The separation structure features an inlet as well as designated receiving chambers for unbound magnetic beads (A), blood cells (B) and the target cancer cells (C).

It is important to note that this separation takes place in a stopped-flow system, that is, the chambers are completely filled with buffer composed of Phosphate buffered saline (PBS) containing 0.1% bovine serum albumin (BSA) before the sample is loaded. The cells and beads in the system then sediment through the buffer liquid in the same manner as in a standard centrifuge. This sedimentation under stagnant conditions allows very stable operation due to the absence of divergent fluid flow lines and hydrodynamic instabilities.

MATERIALS AND METHODS

Figure 1b shows a magnified view of one of the six separation structures on each CD-sized disc which is molded in PDMS (Polydimethylsiloxane). The disk is formed in two parts. The main structures on the disk are formed from a lithographically patterned mould which consists of an SU-8 negative of the microfluidic channels and a 3D printed mould for forming the larger 3 mm × 6 mm magnet holes. PDMS at a ratio of 5:1 (base to curing agent) was used for this part of the disk. The floor of the disk was formed from a laser cut piece of PMMA polymer with a thin layer of of 10:1 PDMS which was spin-coated onto the PMMA. Once this was performed, both sections of the disk were cured in an oven at 70°C for 30-40 minutes. Once both sections had hardened they were placed on top of each other and put in the oven where an irreversible bond was formed due to the mismatch of curing agent ratios at the border [4].

Each separation structure is 100 µm deep and consists of a loading chamber and an inlet channel leading into the main separation chamber which exhibits designated receiving chambers for unbound magnetic beads (A), blood cells (B) and the target cancer cells (C). The chambers are first primed with PBS w/0.1% BSA through degas flow and then placed on a spindle motor as part of a stroboscopic imaging platform [5]. Meanwhile, a sample of MCF7 breast cancer cells is incubated on a rotator with a solution of 1-µm magnetic anti-EpCAM coated beads (which bind very specifically with the MCF7 cancer cells) for 20 minutes at room temperature [1]. The suspension of beads and cells is then spiked into whole blood and delivered to the system via a micropipette. The experiments take place at a spin rate of 675 RPM.

RESULTS AND DISCUSSION

Our experiments established a three-way cell separation which can be seen in Fig. 2. Figures 2a and 2b display a photograph of the system and a schematic the different trajectories for the three particle types resolved. Figure 2b also shows the magnetic field lines and field intensity is indicated by colour. Figures 2c and 2d feature the trajectories of the three particle types as captured by our stroboscopic imaging system during disk rotation. Blood can be observed to sediment strictly radially outward until entering area B (Fig.3) while tagged MCF7 target cells are deflected into the funnel C (Fig. 2c). Figure 2d shows the separation of whole blood from 1.43-µm magnetic tagging beads into the retention zone A.

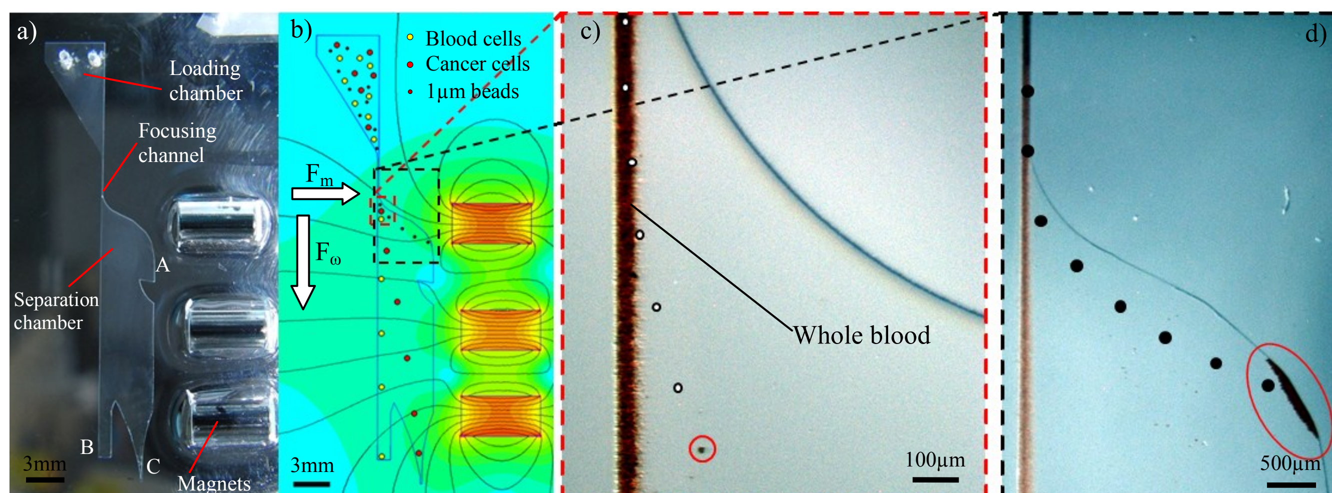

Fig. 2a) Photograph showing one of the microfluidic chambers with relevant parts labeled. Also labeled are the three separate capture areas. b) Schematic of microfluidic chamber showing the three particle trajectories indicated by colored circles. Also, magnet field intensity is encoded by colour. c) Image showing cancer cell deflection out of whole blood as outlined in the schematic. The cancer cell path is indicated by the white circles and the cell is circled in red. d) Image showing capture of many hundreds of thousands of background tagging beads from whole blood with bead trajectory indicated by the black circles and captured beads circled in red.

A theoretical analysis of the relevant forces acting on each of the particles in the system was carried out [1] for the three main forces present in the system, Stokes drag, centrifugal force and magnetic force and the results are presented in Table 1. Image tracking software [6] was also used to calculate the velocities and deflections of both the tagged MCF7 cells and the single beads (table 2). This data confirms the observed trajectories and shows the large difference in forces between the two particles which allows for high quality separation by our system with 62 tagged MCF7 cancer cells being pulled out of whole blood and arriving either directly in capture area C or at the wall of its entrance (Fig. 3). Roughly 350,000 blood cells are trapped in capture area B (Fig. 3) with no false positives, i.e. blood cells deflected into area C.

Table.2. Measured velocities and relative deflections of single magnetic beads and tagged MCF7 cells from fig 2.b) (n=4)

Particle	Velocity(μm s^{-1})	Deflection (AU)
1-μm magnetic bead	41.3	77
Tagged MCF7 cell	224.5	19

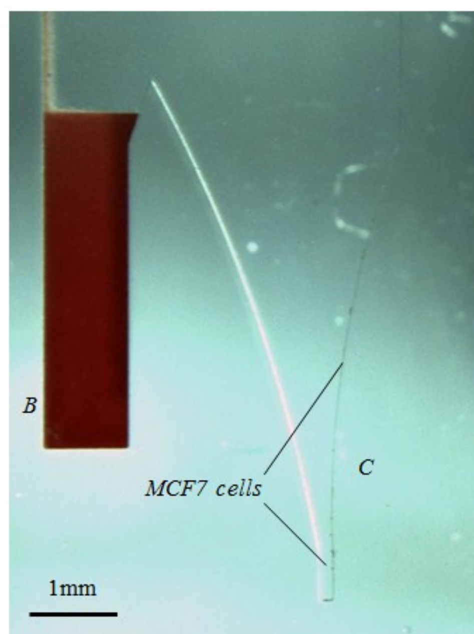

Fig. 4. It was observed that chamber B captured ~350,000 blood cells (volume based calculation) and did not capture any cancer cells. And chamber C captured 62 cancer cells and did not capture any blood cells. This 100% separation success shows great promise for this system for rare cell separation.

CONCLUSIONS

We have presented strong evidence that our centrifugo-magnetic separation scheme is able to very accurately retrieve rare target cells from a background of whole blood. The interplay of magnetic, centrifugal and drag forces allow extremely high affinity separation of cancer cells from blood. This low-cost and compact system could be applied to many biological applications for antibody based selection of cells from a very large background. This system also lends its self to inclusion of further technologies such as on-disk cell counting, incubation and assaying of samples [7][8] to move it towards being a fully integrated sample-to-answer device.

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