SIMPLE CIRCULATING TUMOR CELL SEPARATION

J.G. Kralj,[1] C. Arya,[2] M.S. Munson,[1] T.P. Forbes,[1] A. Tona,[1] L. Sorbara,[3] S. Srivastava,[3] S.P. Forry[1]

[1]National Institute of Standards and Technology, USA, [2]University of Maryland, USA, [3]Early Detection Research Network, National Institutes of Health, USA

ABSTRACT

We developed a cell separation system to isolate rare cells from whole blood using off-the-shelf components and simple microfluidics. 148 μm diameter avidin-functionalized beads were used to generate a packed bed column for cell capture, rather than performing surface functionalization chemistry on chip. Using this system, we demonstrated rare cell enrichment by capturing cultured breast cancer cells (MCF-7) spiked into whole human blood at physiologically relevant levels

KEYWORDS

rare cell capture, circulating tumor cell, CTC, beads, EpCAM, MCF-7, whole blood

INTRODUCTION

Rare cell capture from whole blood (e.g. circulating tumor cells, stem cells) holds promise for emerging therapies and personalized medicine. Highly expressed surface proteins on cells of interest allow high surface-to-volume ratio (S/V) solid-phase extraction systems to capture the cells using antibodies. Microfluidic systems with antibody-functionalized device surfaces have demonstrated high capture efficiency,[1-4] but can be expensive to generate, irreproducible, and poorly amenable to quality control. In this work, we demonstrate a simple microfluidic approach using commercially available components and simple microfluidics to capture model circulating tumor cells (CTCs) spiked into whole blood.

EXPERIMENTAL

A packed bed of avidin-functionalized beads was generated within a simple microfluidic device using a single weir structure (Figure 1a-c). Biotinylated capture antibody (anti-EpCAM) was added to model CTC samples (fluorescently stained breast cancer cells spiked into whole human blood), and 200 μL were pumped through each channel over 1 h. After the column was washed, fluorescence images were taken (Figure 1d), and immobilized cells were counted along the length of the column (Figure 1e). Control samples (lacking the capture antibody) provided a limit of detection ($p<0.05$) of 15 cells captured.

RESULTS & CONCLUSIONS

Parallel analysis of multiple dilution series demonstrated high analysis reproducibility and good capture efficiency (Figure 2). In each series, a high correlation was observed between the level of cancer cells spiked into whole blood and the number of cells captured and counted on the packed bed. Further, capture efficiency was sufficient for detection CTCs at physiologically relevant levels (≈100 CTCs/ml).

However, we observed significant variation in the capture efficiency between different blood donors (65 ± 17% and 36 ± 12% [avg ± 95% confidence interval] for Figures 2a and 2b, respectively). When 3 different blood donor samples were tested side by side using the same cell passage and density, differences were observed in both the total cell capture as well as the rate of binding to the column (Figure 3). The overall average capture efficiency was 40% ± 23% (avg ± SD).

In conclusion, we designed and characterized a new system that achieved similar functionality to existing microfluidic rare cell capture devices, without chemically functionalizing the device surface in situ, and in a disposable format. This system allowed capture and enumeration of physiologically relevant levels of cancer cells spiked into whole human blood. Total capture correlated strongly with cell density, and significant variability in capture efficiency was observed between blood donors. The performance and accessibility of this simple approach will enable engineers and biologists to rapidly develop their own rare-cell capture experiments

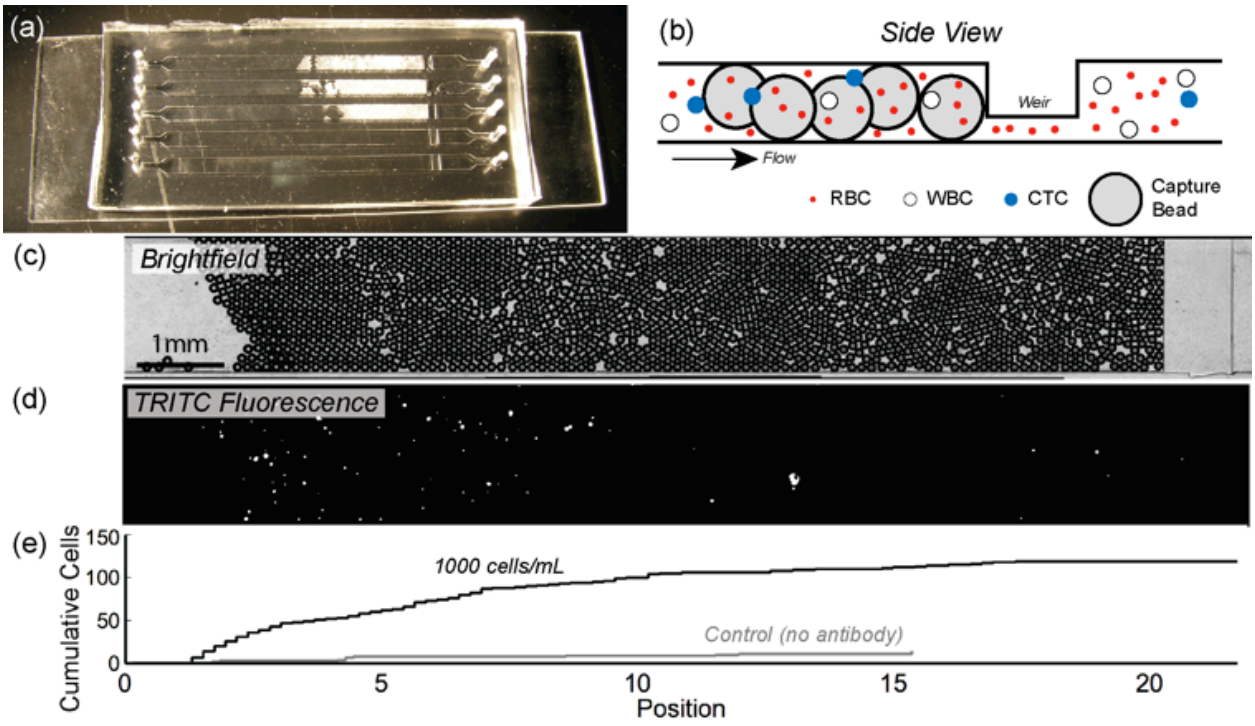

Figure 1 *Experimental design and execution. A photograph (a) and schematic (b) are shown of the 5-channel microfluidic device with weir. This geometry allowed the generation a 2D packed bed of large (148 µm diameter), commercially available beads. Human cancer cells (MCF-7) were spiked into whole human blood and pumped through this packed bed. The large bead size and gaps allowed cells and fluids to pass while ensuring significant solid phase contact. Brightfield (c) and fluorescence (d) imaging displayed the 2D packed bed and captured cancer cells. (e) EpCAM-mediated cell capture was quantified by enumerating the bright spots along the length of the column (black line). Minimal capture was observed in control experiments where no cancer cells (not shown) or no capture antibodies (gray line) were added to the whole blood. The total volume of blood analyzed on each column was 200 µL.*

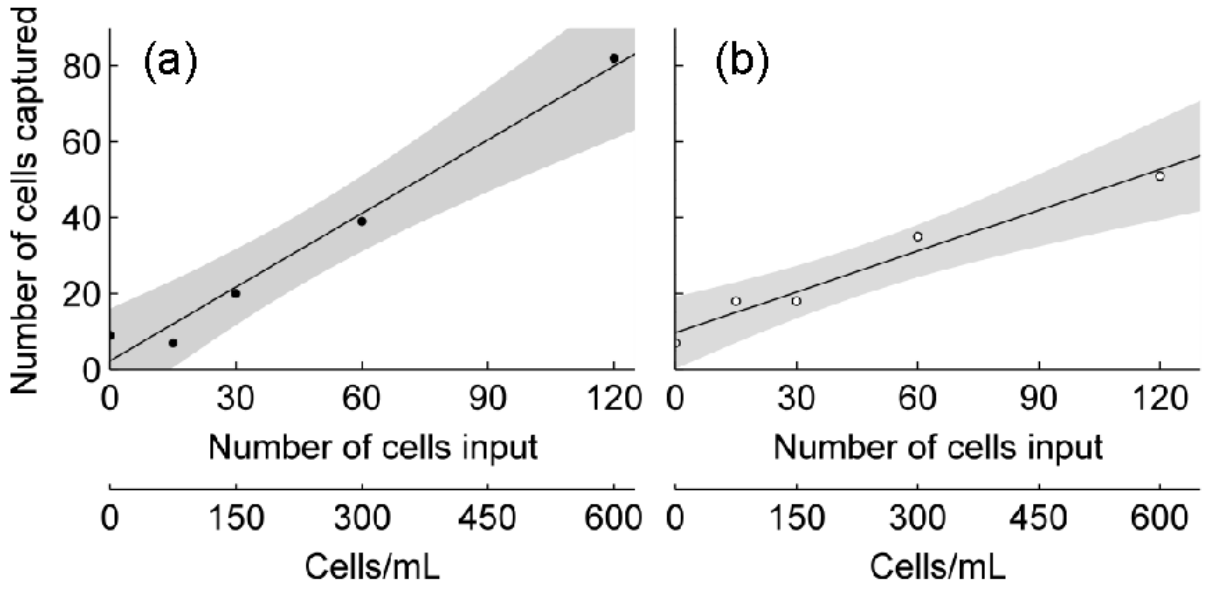

Figure 2 *Capture reproducibility and efficiency. The total number of cells captured across a whole column correlated strongly with the density of cancer cells spiked into whole blood at physiologically relevant levels. The shaded regions show the Working-Hotelling 95% confidence bands for the regressions from two different blood donors (a) and (b). The sensitivity in these analyses was 15 captured cells ($p<0.05$).*

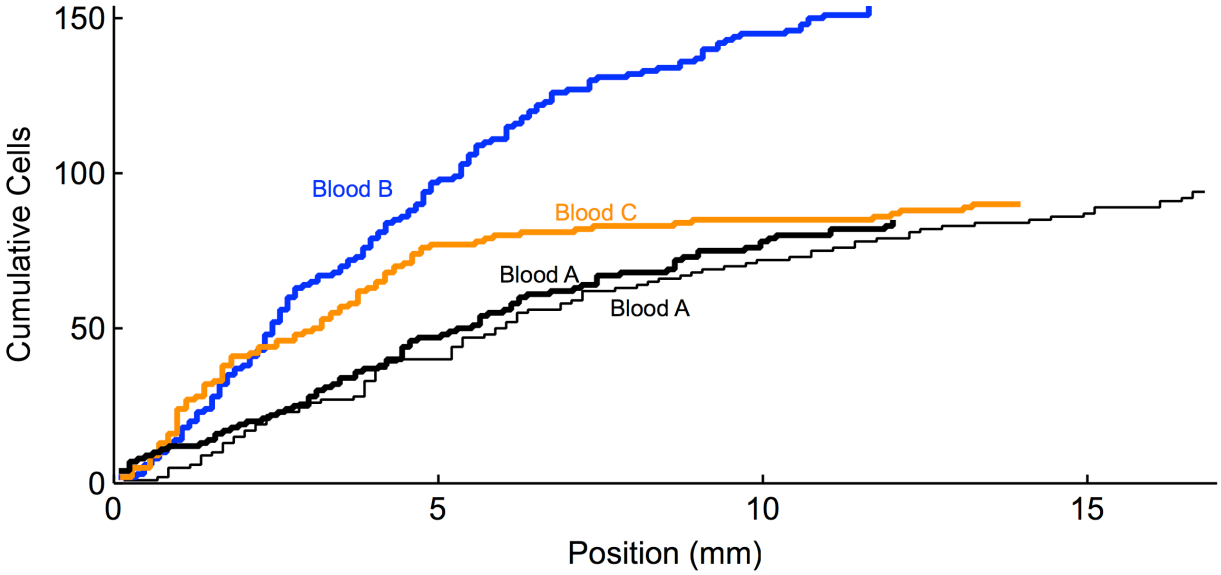

Figure 3 *Capture variability between blood donors. Although replicate analyses were highly repeatable for a single blood source, significant variability in capture efficiency was observed between different blood donors with 1000 MCF-7 cells/mL (approximately 200 cells total used per experiment). Replicate experiments using Blood A show good reproducibility. Bloods B and C show different binding characteristics along the columns.*

REFERENCES

[1] S Nagrath, LV Sequist, S Maheswaran, DW Bell, D Irimia, L Ulkus, MR Smith, EL Kwak, S Digumarthy, A Muzikansky, P Ryan, UJ Balis, RG Tompkins, DA Haber, M Toner, *Isolation of rare circulating tumour cells in cancer patients by microchip technology,* Nature (2007), **450**, 1235.

[2] SL Stott, C-H Hsu, DI Tsukrov, M Yu, DT Miyamoto, BA Waltman, SM Rothenberg, AM Shah, ME Smas, GK Korir, FP Floyd, Jr., AJ Gilman, JB Lord, D Winokur, S Springer, D Irimia, S Nagrath, LV Sequist, RJ Lee, KJ Isselbacher, S Maheswaran, DA Haber, M Toner, *Isolation of circulating tumor cells using a microvortex-generating herringbone-chip*, PNAS (2010), **107**, 18392.

[3] JP Gleghorn, ED Pratt, D Denning, H Liu, NH Bander, ST Tagawa, DM Nanus, PA Giannakakou, BJ Kirby, *Capture of circulating tumor cells from whole blood of prostate cancer patients using geometrically enhanced differential immunocapture (GEDI) and a prostate-specific antibody*, Lab Chip (2010), **10**, 27.

[4] G Hansmann, BD Plouffe, A Hatch, A von Gise, H Sallmon, RT Zamanian, SK Murthy, *Design and validation of an endothelial progenitor ell capture chip and its application in patients with pulmonary arterial hypertension*, J Molec Med-JMM (2011), **89**, 971.