VERIFAST: AN INTEGRATED SYSTEM FOR FLEXIBLE CTC ISOLATION AND ANALYSIS

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ABSTRACT

Circulating tumor cells (CTCs) are rare cellular events that are relevant as a prognostic for many epithelial origin cancers. CTCs offer a unique insight into the metastatic cascade and are accessible by a simple blood draw. Here, we demonstrate a method for high efficiency and purity CTC isolation that leaves these cells accessible for downstream analysis in order to study this highly relevant tumor cell population for targets or responses to cancer therapies. We present the VerIFAST, a method and device incorporating all facets of CTC analysis from isolation to enumeration and intracellular staining.

KEYWORD

CTC, Cancer, Cell Isolation, IFAST, Buffycoat

INTRODUCTION

The isolation of cells from a heterogeneous population is necessary for a range of research and diagnostic tools. Specifically, isolation of circulating tumor cells (CTCs) from the buffycoat formed from a patient blood draw has shown clinical relevance. CTCs are cells within the circulation of patients with metastatic cancer, and are very rare events, with approximately one CTC per billion background cells. Further, the prognostically relevant bar for determining overall survival and disease-free progression is 5 CTCs per 7.5 mLs of whole blood [1]. As such, CTC capture is an isolation method requiring both high sensitivity (5 cells) and high specificity (7.5 billion background cells) coupled with the need to perform clinically relevant cellular analyses downstream of isolation.

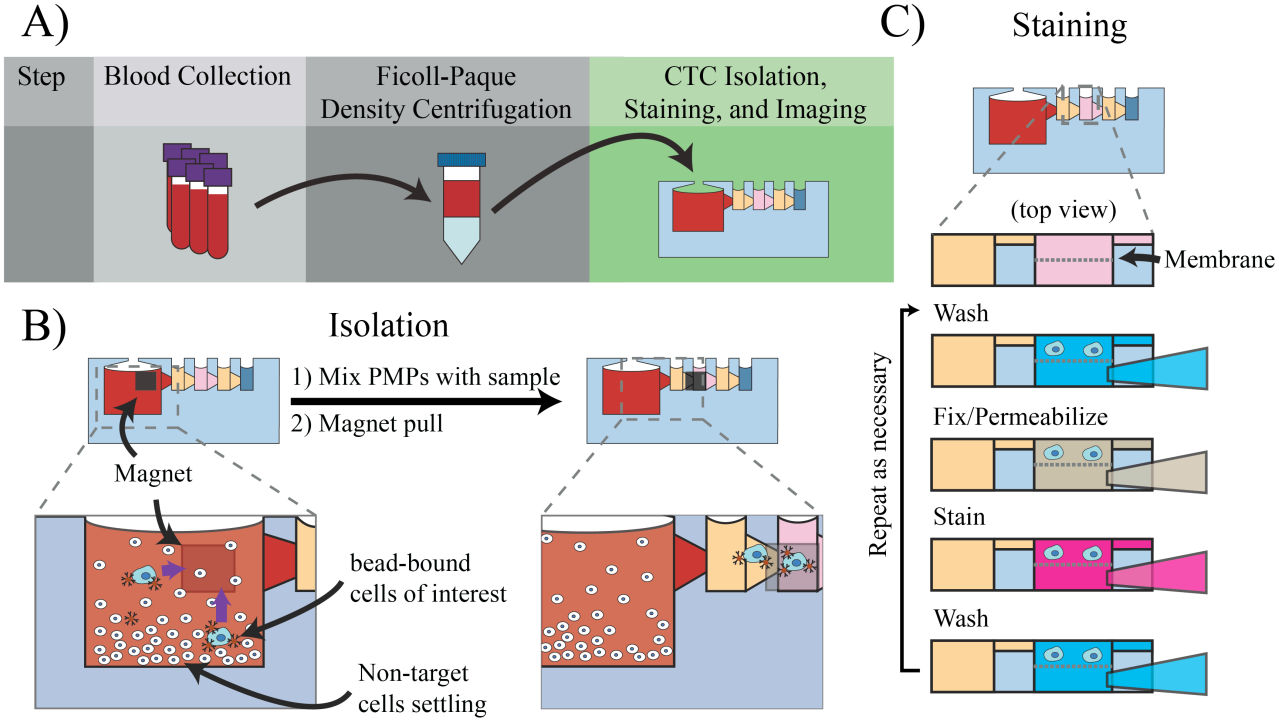

Figure 1: Processing Schematic. A) Methodology outlining blood collection through analysis and readout using the VerIFAST methodology. B) Schematic illustrating the isolation process with PMBCs settling out of the magnetic path of the paramagnetic particle (PMP) bound LNCaP cells. The magnet is used to pull PMP-bound cells to and across the virtual wall created by the oil well. C) Top view of the VerIFAST device shown. Staining methodology involves a membrane separating two wells of fluid with a porous membrane allowing iterative wash steps for staining endpoints.

Macroscale methods to perform this isolation feature high cell loss due to wasteful transfer steps or centrifugation and resuspension steps, but offer the flexibility to perform a wide range of downstream assays. To overcome cell loss, microfluidic methods have arisen that feature high capture efficiency by leveraging functionalized micropost arrays, patterned surfaces, or physical cellular characteristics to isolate CTCs from background peripheral blood mononuclear cells (PBMCs). Although these methods have proven to be enabling compared to macroscale isolation techniques, device

flexibility can be limiting, restricting methods of CTC analysis downstream of isolation to a few specific analytical techniques. **For use with CTCs, there is a specific need for an integrated isolation and analysis technique within a microfluidic system that enables downstream flexibility.**

Here, we present a methodology that uses Immiscible Filtration Assisted by Surface Tension (previously shown [2,3]) to isolate CTCs from background PBMCs and further perform extra- and intracellular staining without ever having to transfer the sample out of the device. EpCAM-bound paramagnetic particles are added to the first well of the device and allowed to bind to the target cells (LNCaP cells). The bead-bound cells are then pulled to and across the 'virtual wall' of the oil phase into a wash well, where staining techniques can be applied (Figure 1).

RESULTS AND CONCLUSION

This specific embodiment of the IFAST, termed VerIFAST, features vertically oriented chambers that allow PBMCs to passively settle out of the operational path of the device. Instead of cells settling to the bottom of the Horizontal IFAST device and a magnetic force pulling beads and target cells in the same direction, the VerIFAST changes the directions of the two force vectors such that the beads and target cells are pulled away from the background cells (Figure 2A). As such, >77% purity (representing a 6-fold depletion of PBMCs) and >70% isolation efficiency can be achieved with a single oil traverse, as opposed to three traverses with the previous IFAST embodiment (Figure 2B). This is directly important and enabling for DNA and RNA isolation from purified CTC samples, as less background nucleic acids interfere with the signal generated from CTCs. This vertically oriented device allows the VerIFAST to be highly arrayable, and can be conceived as a bulk-processing method to isolate and analyze multiple samples at one time.

To expand the utility of captured CTCs, a novel method to perform more complex, intracellular and multiple antibody analyses using CTCs, a specialized chamber was added to the VerIFAST in order to facilitate wash steps and staining within this device. This chamber features a unique wash well fabricated into the device featuring two fluid chambers separated by an 8-micron porous membrane. By adding sample into one side of the membrane, washes and aspiration steps can be performed on the other side of the membrane without perturbing, binding or removing the sample, with demonstrated staining complexity for surface proteins (EpCAM), intracellular proteins (pan-cytokeratins and androgen receptors) and a Hoescht nuclear stain (Figure 2D).

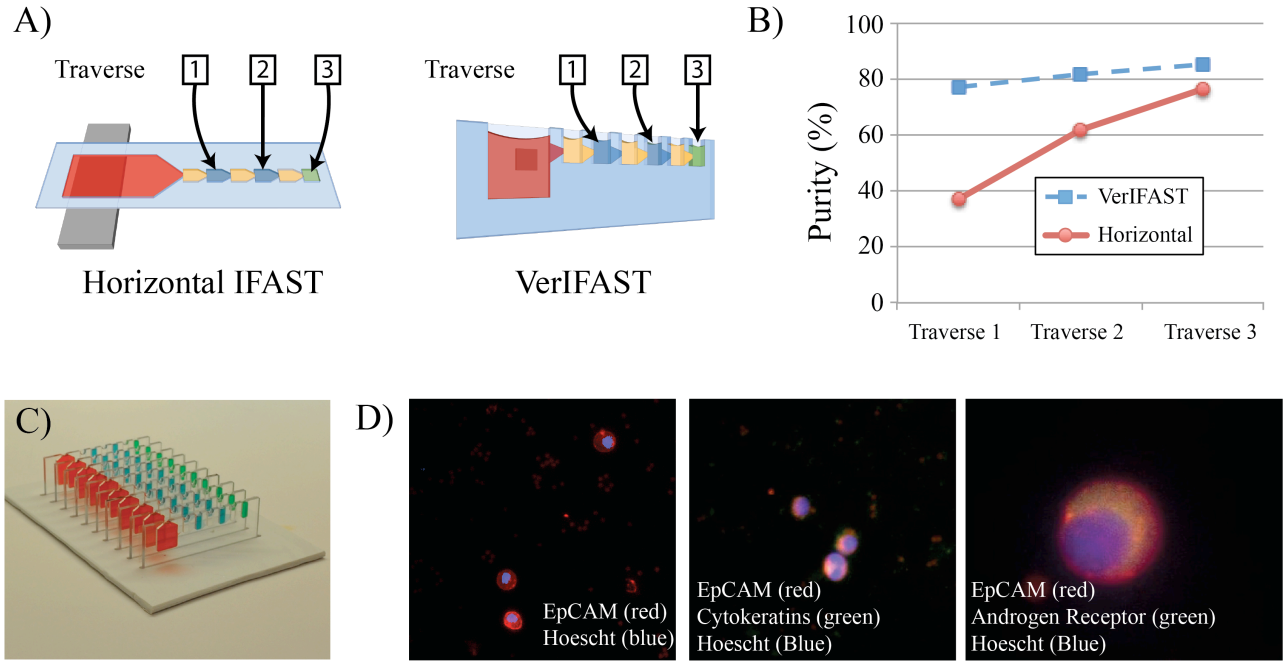

Figure 2: A) The original IFAST device (left) and the VerIFAST (right, shown with more wash wells) are shown with oil traverses highlighted. B) Increasing the number of traverses increases the purity of the resulting sample, however >70% purity can be achieved in a single oil traverse with the VerIFAST device compared to three with the original IFAST device. C) An array of VerIFAST devices shows the ability to streamline sample processing for clinical application. D) Three costains are shown to demonstrate the flexibility of the platform to different staining techniques: (right) EpCAM-PE (red) Hoescht (blue), (middle) EpCAM-PE (red) pan-cytokeratins-FITC (green) Hoescht (blue), (right) EpCAM-PE (red) anti-rabbit Androgen Receptor primary antibody with a Alexa488 goat anti-mouse secondary (green) and Hoescht (blue).

In conclusion, a method leveraging benefits of the microscale for specificity and sensitivity in CTC detection has been demonstrated, creating an integrated system for both CTC isolation and flexible downstream cellular analysis. We propose to use this method to expand CTC isolation beyond enumeration and surface staining to include intracellular

staining. With these methods, a more complete evaluation of CTCs can be employed to evaluate treatment efficacy by targeting and tracking proteins related to cellular function through the course of a patient's cancer treatment.

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