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Rapid and Efficient Isolation of Circulating Tumor Cells using High Porosity Precision Microfilters

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ABSTRACT

We present a novel precision microfilter, CellSieve™, which achieves rapid and highly efficient isolation of circulating tumor cells (CTCs) from peripheral blood. Isolation of CTCs by size exclusion is a widely researched technique with the advantage of capturing cells without reliance on cell surface expression markers.[1-3] For many years CTC filtration technology has relied on track-etch microfilters with randomly located pores and low-porosity. We have developed a new technology to fabricate precision, high porosity microfilters that are strong, transparent, and non-fluorescent. Microfiltration using CellSieve™ microfilters is a simple method to isolate circulating tumor cells from large volumes of human whole blood. This filtration can be done in less than 2 minutes with consistently high capture efficiency, while retaining a low rate of blood cell contamination.

INTRODUCTION

CTCs are cancer cells disseminated from primary, or metastatic tumors. CTCs are now used primarily to monitor therapy response and predict disease outcome, though CTCs can potentially be used to determine a patient's therapy. Efficient collection of CTCs from peripheral blood is crucial for these applications.

Isolation of CTCs is challenging because of their extreme rarity, approximately 1-10 CTCs among 10^9 total blood cells. Size based isolation of CTCs by microfiltration has been shown to rapidly capture CTCs from peripheral blood. Isolation of CTCs by size exclusion has typically employed track-etch filter membranes. Alternative microfilters have been developed, but are costly and not available commercially. Track-etch filters have angled pores, low porosity and random overlapping pores, resulting in larger effective pore size.

CellSieve™ microfilters have uniform 8 µm diameter pores in a clear polymer, with approximately 90,000 pores imprinted on a 9 mm diameter area in a standard a 13 mm filter format.

(a) CellSieve™ Filters **(b) Track-etch Filters**

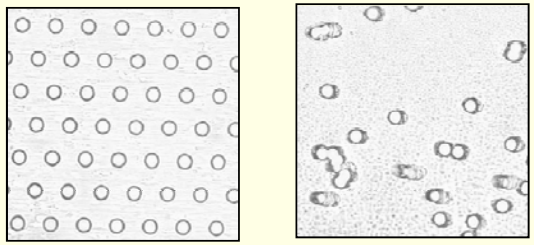


Figure 1. (a) CellSieve™ microfilter fabricated by lithography method with regularly distributed pores, and (b) track-etch filter with random pore distributed pores.

MATERIALS & METHODS

We describe an assay to capture and enumerate both previously fixed and unfixed CTCs on CellSieve™ filters. MCF-7 human breast adenocarcinoma cells (ATCC) were stained in a fixative/staining solution containing mild fixation agent, acridine orange, and DAPI dilacetat. After incubation, cells were individually counted (to obtain exact inputs), spiked into 7.5 mL whole human blood with 7.5 mL fixative and placed into a syringe (Figure 2).

An 8 µm pore microfilter was placed into filter holder and the sample drawn by negative pressure through the filter at ~10 mL/min (Figure 2). The microfilter was removed from the holder, mounted onto a microscope slide and counted using a fluorescence microscope under TRITC, FITC and DAPI settings. The procedure was then repeated using a staining solution without the fixative to capture unfixed MCF-7 cells.

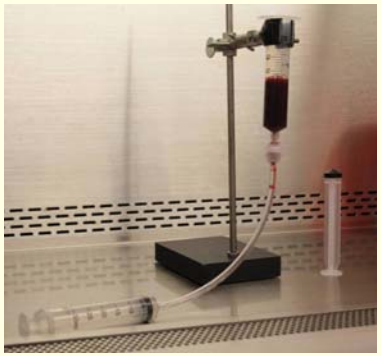
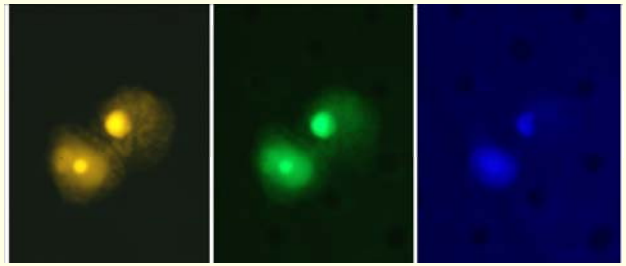


Figure 2. Filtration set up. One syringe holds blood sample with filter in a filter holder. A second syringe acts to draw sample through the microfilter.

The above experiments were then run with 8 µm pore size track etch filters. Both types of filters were run with fixed and unfixed MCF-7 cells, and all isolations were performed in triplicate.

RESULTS



(a) (b) (c)

Figure 3. MCF-7 stained cells captured on CellSieve™ filter shown under (a) TRITC filter, (b) FITC filter and (c) DAPI filter.

Table 1. Recovery of CTCs on CellSieve™ filters

	Input (MCF-7)	Cell counts on Filter	% Recovery on Filter
Fixed Cells	73	73	100%
	60	59	98%
	49	47	96%
Average			**98±2%**
Unfixed Cells	84	70	83%
	70	62	89%
	52	43	83%
Average			**85±3%**

Table 2. Recovery of CTCs on track etch membranes

	Input (MCF-7)	Cell counts on Filter	Recovery on Filter
Fixed Cells	80	50	63%
	72	54	75%
	71	58	82%
Average			**73±10%**
Unfixed Cells	76	32	42%
	80	34	43%
	53	35	66%
Average			**50±14%**

Contamination

To evaluate blood cell contamination retained on the filter, fixed or unfixed whole blood were filtered. The filters were then stained with DAPI and antibody against CD45 conjugated to PE. Contaminated blood cell counts are identified by the presence of CD45 positive cells and/or DAPI positive cells on the CellSieve™ filter, the count ranges from 500 to a few thousand, < 0.00001% from the original 7.5 ml of whole blood. Red blood cells are not retained on the filter.

CONCLUSIONS

- Filtration of whole blood using CellSieve™ is rapid taking < 2 min.
- Filtrations using CellSieve™ were efficient and reproducible: 98%±2% for fixed and 85%±2% for unfixed MCF-7 cells.
- Track etch microfilters have lower capture efficiencies and higher standard deviations than CellSieve™ microfilters.
- Contaminating blood cells on the CellSieve™ microfilter average under 1000 cells per sample, and zero for red blood cells.

References

1. Vona, G, et *al.* (2000). "Isolation by Size of Epithelial Tumor Cells A New Method for the Immunomorphological and Molecular Characterization of Circulating Tumor Cells." American Journal of Pathology **156**(1): 57-63
2. Zheng, S., et al. (2007). "Membrane microfilter device for selective capture, electrolysis and genomic analysis of human circulating tumor cells." J. Chromatogr. A. **1162**:154-61.
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