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Isolation of Circulating Tumor Cells by Size Exclusion using Lithography Fabricated Precision Microfilters

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ABSTRACT

We present a novel precision microfilter, CellSieve™, that 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 tumor cells without reliance on cell surface expression markers. For many years CTC filtration has relied on track-etch filters with randomly distributed pores and low-porosity. Our lithography based fabrication method makes precise, high porosity microfilters suitable for microfiltration applications. These microfilters are strong, transparent, and have high CTC capture efficiency.

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 among10^9 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 are inexpensive, but have angled pores, low porosity and random overlapping pores, resulting in larger effective pore size.

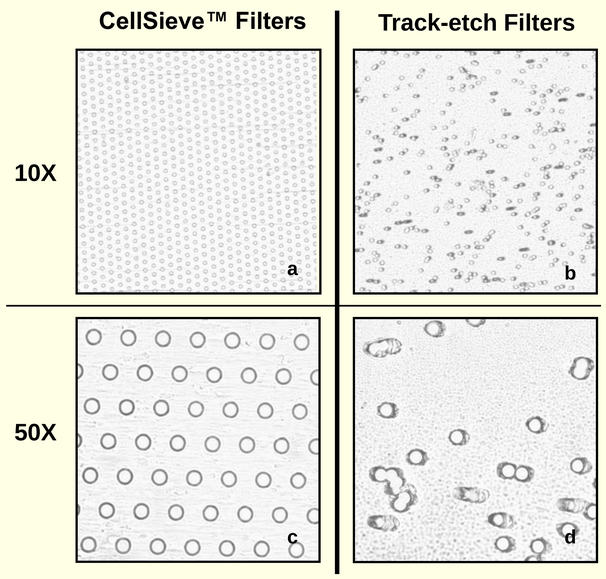


Figure 1.
CellSieve™ filter under magnification:
(a) 10X, (**c**) 50X;
Track-etch filter under magnification:
(**b**) 10X, (**d**) 50X

Lithography based microfabrication allows for efficient mass production of highly uniform precision microfilters. This method yields increased porosity and pore uniformity.

MATERIALS & METHODS

We describe an assay to capture and enumerate both previously fixed and live CTCs on CellSieve™ filters. MCF-7 human breast adenocarcinoma cells (ATCC) were stained in a fixative/staining solution containing paraformaldehyde (PFA), acridine orange, and DAPI dilacetate. After incubation, cells were individually counted (to obtain exact inputs), spiked into 7.5 mL whole human blood with 7.5 mL PBS with PFA 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 PFA fixative to capture live MCF-7 cells.

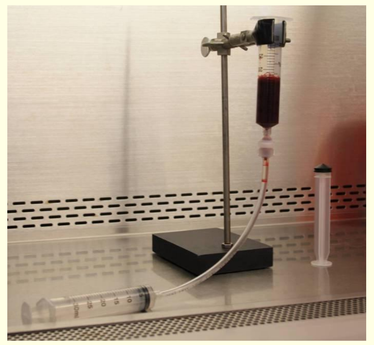
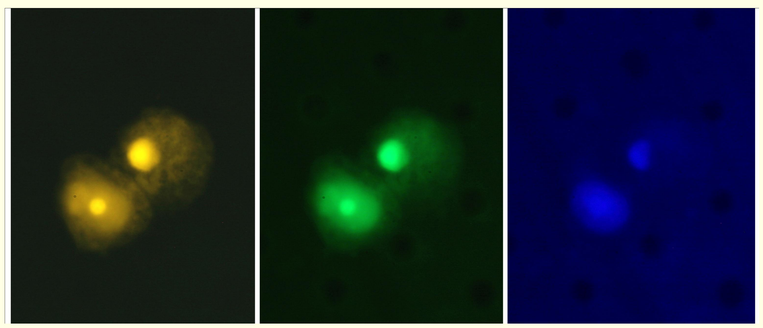


Figure 2. Filtration set up. One syringe holds blood sample with filter in a filter holder. A second syringe acts to draw sample through the microfilter.

The above experiments were then run with 8 µm pore size track etch filters. Both types of filters were run with live and fixed 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.

CellSieve™ filters were able to capture MCF-7 cells spiked into 7.5 mL human blood with a recovery rate of 98±2% for fixed cells, and 85±3% for unfixed cells. The filtration was performed in 2 minutes, and cell counts were obtained in 10 minutes. By contrast, track-etch filters captured only 73%±10% of fixed cells and 50%±14% for unfixed cells. Cell counts required >20 minutes.

Table 1. Recovery of CTCs on CellSieve™ filters

	Input	Cell counts on Filter	% Recovery on Filter
Paraformadehyde Fixed	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	Cell counts on Filter	Recovery on Filter
Paraformadehyde Fixed	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%**

CONCLUSIONS

- CellSieve™ filters can capture 98% of spiked fixed MCF-7 cells from fixed whole human blood.
- CellSieve™ filters recover MCF-7 cells more efficiently than track-etched filters.
- Manual cell counts are faster and more accurate on CellSieve™ filters because of pore uniformity and lower background.

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