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

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ABSTRACT

We have developed CellSieve™ microfilters for the isolation of circulating tumor cells (CTCs) from patient peripheral blood. Previously presented data has shown that CellSieve™ microfiltration achieved rapid (<2 min) and highly efficient isolation (>98%) of pre-stained tumor cells spiked into human blood. Here we demonstrate a clinically relevant assay for isolation and identification of tumor cells from patient samples. This assay captures CTCs on the CellSieve™ microfilter, then stains the cells on the filter to identify intracellular and extracellular cancer markers. CTCs are identified as being cytokeratins 8, 18 & 19 positive, DAPI positive, and CD45 negative. Additional confirmation is provided by the extracellular cancer marker, EpCAM.

INTRODUCTION

CTCs are cancer cells disseminated from primary, or metastatic tumors, which can be used to monitor therapy response and predict disease outcome.[1] Efficient collection of CTCs from peripheral blood is challenging, due to their extreme rarity, approximately 1-100 CTCs among 10^9 total blood cells.

Isolation of CTCs by size exclusion is a widely researched technique with the advantage of capturing cells without reliance on surface marker expression.[1-4] For many years CTC filtration technology has relied on track-etch microfilters, which have randomly located pores and low-porosity (Figure 1b).

We have developed CellSieve™ microfilters that are more suitable for CTC isolation. They are strong, transparent, biocompatible, and non-fluorescent with precision pores and high porosity. CellSieve™ microfilters have 8 µm diameter pores patterned in an uniform array with approximately 90,000 pores within a 9 mm diameter area in a 13 mm filter format (Figure 1a).

Microfiltration using CellSieve™ microfilters is a simple method for the isolation of CTCs from large volumes of whole human blood. Filtration can be performed in less than 2 minutes with consistently high capture efficiency and low blood cell contamination.

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

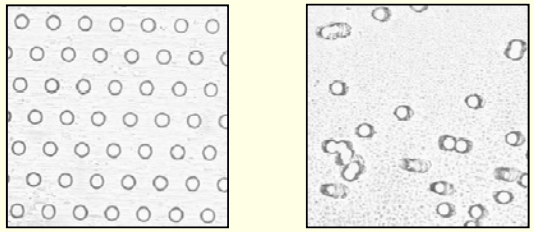

Figure 1. (a) CellSieve™ microfilter fabricated by lithography method with regularly distributed pores, and (b) track-etch filter with randomly distributed pores.

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MATERIALS & METHODS

We describe an assay to capture and enumerate CTCs spiked into whole human blood using CellSieve™ microfilters. MCF-7 human adenocarcinoma cells (ATCC) were used to evaluate the assay performance.

Cells were first enumerated to obtain precise input, then spiked into 7.5 mL whole human blood, mixed with 7.5 mL fixative buffer, and placed into a syringe. Filtration was performed using a microfilter mounted in a filter holder, with the sample drawn by negative pressure at a flow rate of ~10 mL/min. Cells captured on the filter were fixed a second time, permeabilized, and blocked inside the filter holder apparatus. Cells were then stained by antibodies against cytokeratins 8, 18, & 19 conjugated to FITC, EpCAM conjugated to phycoerythrin, and CD45 conjugated to Cy5. Following a wash, the microfilter was removed, mounted onto a microscope slide, and stained with DAPI. CTCs were identified by the presence of DAPI, cytokeratin, and EpCAM, while showing no CD45 marker. Contaminating blood cells were identified by the presence of CD45, and/or DAPI cells without EpCAM and cytokeratin. The above experiments were performed in triplicate.

RESULTS

Consistent with previously presented data, the CTC capture efficiency averaged 97% (Table 1). Total assay time, from capture through staining, was less than 2 hours. In addition to high capture efficiency, we demonstrated that CTC isolation and immuno-staining of EpCAM cytokeratin 8,18, & 19 and CD45 can be performed rapidly and easily on the CellSieve™ microfilter. The fluorescence imaging was clear and simple to interpret as the background fluorescence was negligible (Figure 3).

Contamination by red blood cells, which are easily visualized under white light, was not observed. Other blood cell contamination fell into two groups: cells that were only DAPI positive, averaging 855 cells per filter, and cells that were both DAPI positive and CD45 presenting, averaging 589 cells per filter (Table 2).

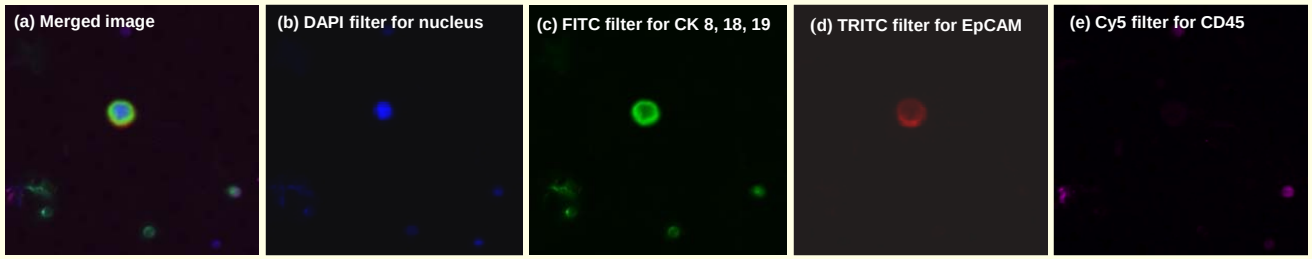
Table 1. Recovery of CTCs on CellSieve™ Microfilters

MCF-7	Input cell count	Cell count on microfilter	% Recovery on microfilter
Spiked samples	101	99	98
	89	89	100
	28	26	93
Average			**97±4%**

Table 2. Blood Cell Contamination on CellSieve™ Microfilters

Cell Counts	DAPI positive on microfilter	CD45 positive on microfilter
Sample 1	352	181
Sample 2	1650	1105
Sample 3	562	482
Average	**855**	**589**

Figure 2. Individual and merged fluorescent images of stained MCF-7 cell and white blood cells on CellSieve™ microfilter: (a) Overlay image, (b) DAPI filter for nucleus, (c) FITC filter for CK 8, 18, 19, (d) TRITC filter for EpCAM, and (e) Cy5 filter for CD45.



CONCLUSIONS

- Filtration of whole blood using CellSieve™ takes < 2 min.
- CTC isolation and immuno-staining can be performed in < 2 hours
- CellSieve™ can capture 97±4% of MCF-7 cells from whole blood
- Red blood cell contamination was not observed.
- White blood cells on CellSieve™ microfilter average 500-1000 per sample or 0.000001% of input.

References

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