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Precision Microfilters for Capture and Culture of Circulating Tumor Cells

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ABSTRACT

A promising method to achieve personalized cancer therapy is to isolate viable circulating tumor cells (CTCs) from the patient and culture them to test their response to drugs. Methods to culture CTCs is receiving extensive interest for research purposes[1]. CTCs can be isolated by size-exclusion using microfilters[2]. Here we present microfilters with precision pores with uniform distribution. Combining cell isolation with culture in one device eliminates cell loss, minimizes cell damage, and facilitates cell growth with simple and rapid workflow.

INTRODUCTION

CellSieve™ Microfilter

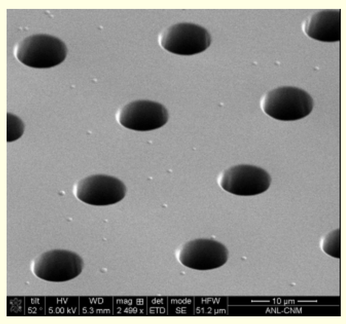


Figure 1. SEM image.
Pore diameter: 7 µm
Thickness: 10 µm

FILTER FEATURES	BENEFITS
Precision pore size and distribution	High capture efficiency; Low standard deviation
High porosity (160,000 pores)	Rapid filtration; Low contamination
Nonfluorescent	Highly detailed fluorescent images
Lies flat on slides	Easy microscope imaging
Transparent	Histological staining
Strong	Protects valuable samples
Hydrophobic	Enables culture in holder

MATERIAL & METHODS

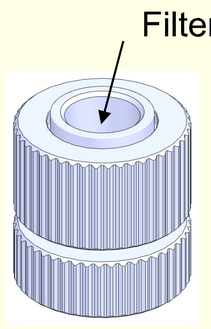
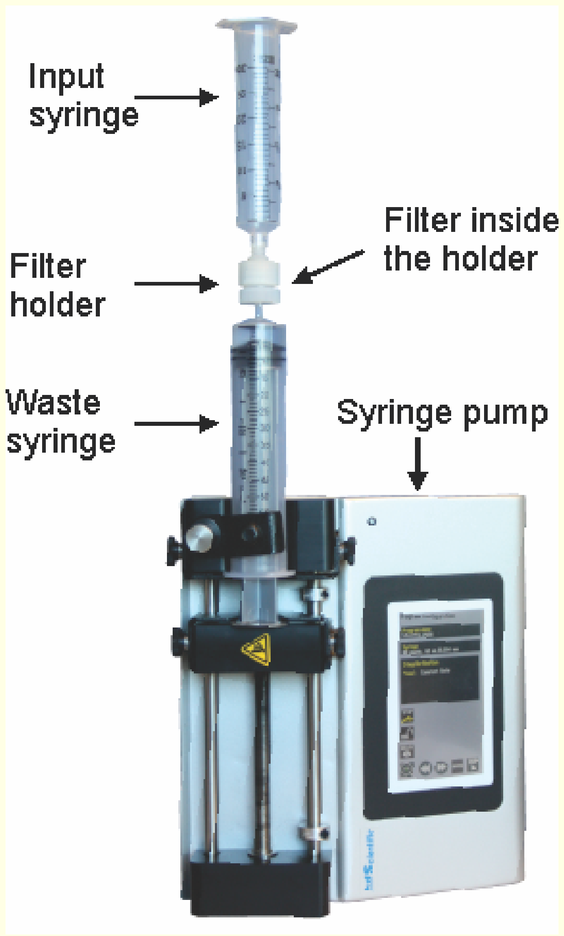
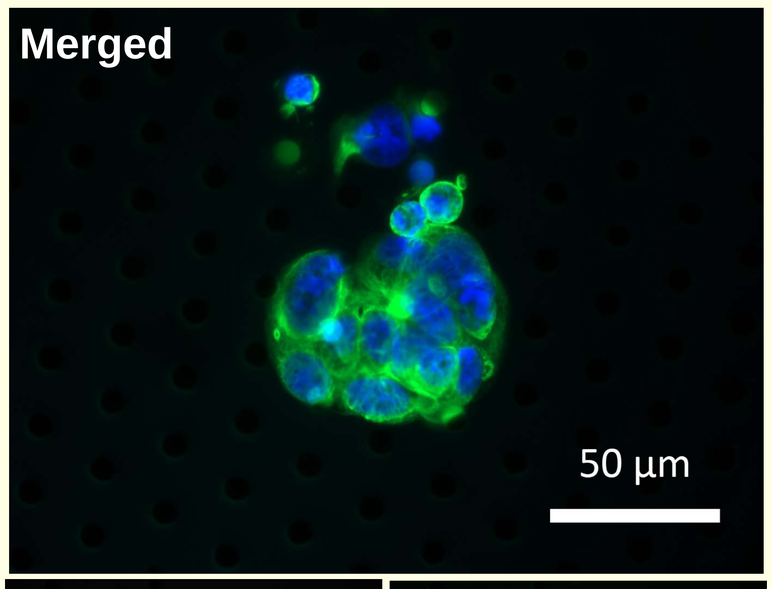


Figure 2. Filter is installed inside the holder, which also serves as a reaction well and culture chamber.

Figure 3. Sample is placed into the input syringe. CTCs are retained on the filter as the syringe draws the sample into the waste syringe. The filtration system provides constant flow at low pressure. The process is gentle and rapid, maintaining cell viability. 7.5 mL of blood diluted 1:1 is filtered in 3 min.



RESULTS



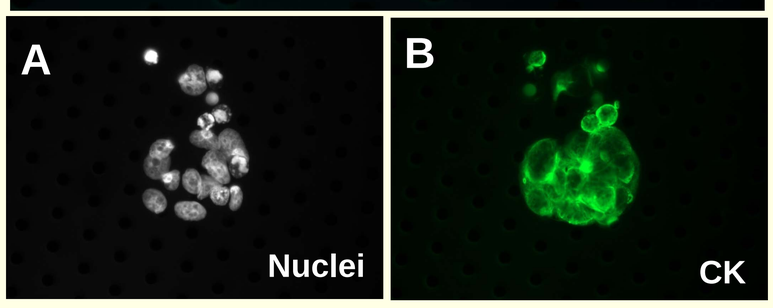


Figure 4. Culture of filter-captured MCF-7 breast cancer cells in a standard cell culture plate. Following filtration, the holder was dissembled. The filter membrane was placed in an individual well of 12-well culture plate. The cells captured on the membrane were cultured in EMEM medium for 4 days. (A) Nuclei were stained with DAPI (grey). (B) Cytokeratins 8, 18 were stained with the FITC-conjugated antibody (green). DAPI staining is shown as blue in the merged image.

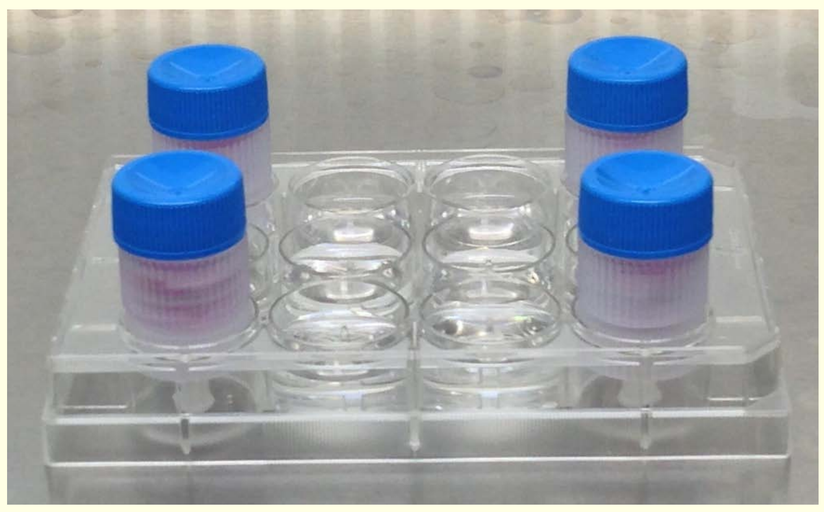
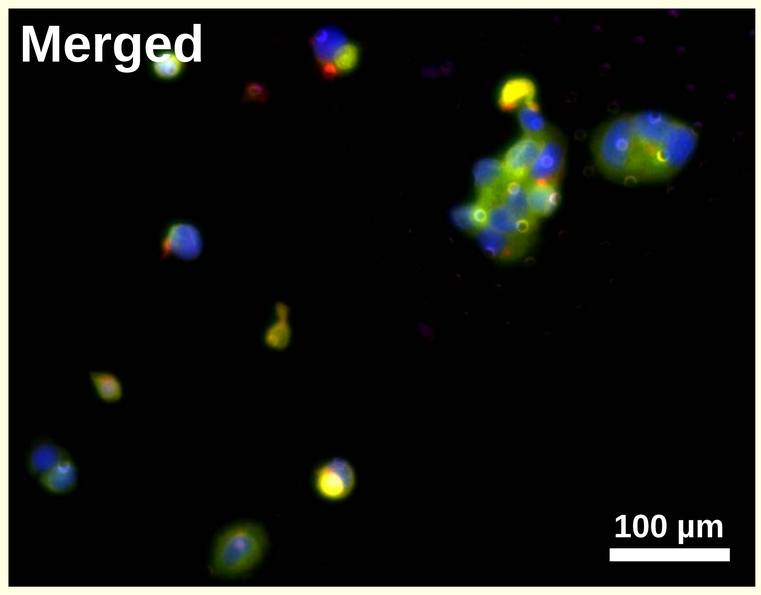


Figure 5. CellSieve™ filter housings support short-term culture of CTCs following capture. In-housing culture limits post-capture handling of rare cells.



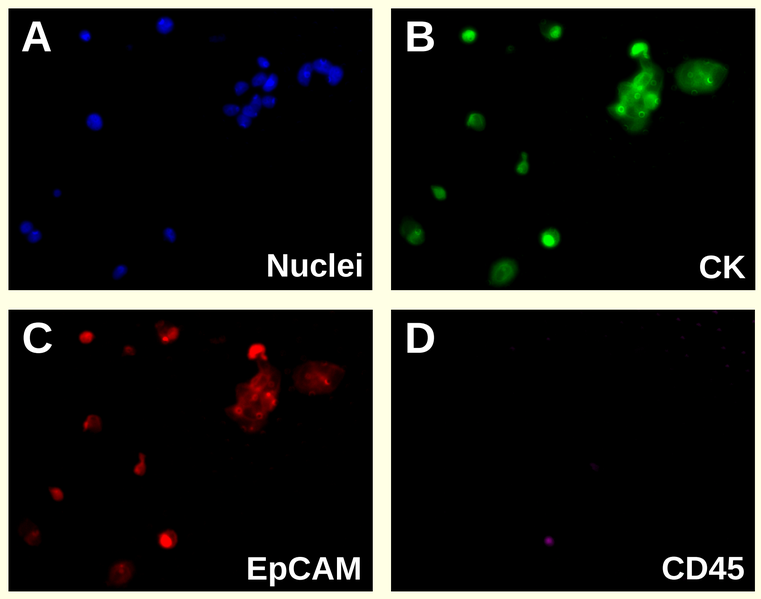


Figure 6. Microfilter membranes support tumor cell culture following isolation. MCF-7 cells were spiked into blood drawn into EDTA tubes from healthy donors. 7.5 mL spiked samples were diluted 1:1 and immediately filtered at 5 mL/min. Following filtration, filter membranes were washed with cell culture media and incubated at 37° C for 72 h. Cells were subsequently fixed, permeabilized, and stained for nuclei (Hoechst 33342; A), cytokeratins (B), EpCAM (C), and CD45 (D). The lack of CD45 staining indicates the absence of immune lineage cells 72 h after filtration.

CONCLUSIONS

Functional analysis of CTCs requires isolation of viable tumor cells from peripheral patient blood. We demonstrated that microfiltration can enrich viable MCF-7 cells from whole blood.

- Capture efficiency for unfixed MCF-7 cells spiked into blood was 74%.
- The workflow is easy and rapid.
- The captured MCF-7 cells could subsequently be cultured on the filter membrane by using both in-holder and standard culture methods.
- Contaminating normal blood cells did not affect the cell culture.
- The cultured cells could be characterized by immunofluorescence antibody staining.

Isolation and culture of CTCs using the CellSieve™ microfiltration system provide a novel tool for studying biological function of viable CTCs and for assessing drug sensitivity.

references

1. Zhang, L., *et al* (2013) ”The Identification and Characterization of Breast Cancer CTCs Competent for Brain Metastasis”, Sci. Transl Med 5, 180ra48.
2. 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.