Supplementary Information

Separable Bilayer Microfiltration Device for Viable Label-free Enrichment of Circulating Tumour Cells

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Feasibility study of clinical samples. Clinical samples were obtained with consent from cancer patients at the Penn State Hershey Medical Center according to IRB approved protocol. Samples were drawn into EDTA-coated Vacutainer tubes (Becton Dickinson) from peripheral venipuncture. Blood samples were processed immediately upon receiving to facilitate optimal filtration conditions.

The SB microfilters were washed with DPBS immediately after filtration and then fixed with 4% paraformaldehyde (VWR) for 20 minutes. The captured cells were permeabilized in 0.3% Triton X-100 (VWR) for 30 minutes. The devices were incubated with DAPI, and then blocked with 5% goat serum (Sigma-Aldrich). Cells were subsequently incubated with primary and secondary antibodies, 1 μg/mL mouse monoclonal anti-cytokeratin 8/18/19 (Abcam) and 10 μg/mL goat anti-mouse IgG conjugated to DyLight 550 (Thermo Scientific). After blocked again with 5% goat serum, the devices were incubated with 0.5 μg/mL monoclonal mouse anti-CD45 conjugated to Alexa Fluor 647 (Santa Cruz). Mouse monoclonal anti-cytokeratin was incubated at 4°C overnight. The rest blocking and antibody incubation steps were carried out for 1 hour at room temperature.

An Olympus IX-71 inverted microscope was used to carry out fluorescence imaging. Each fluorophore was imaged separately using an appropriate optical filter set. Images were acquired with a monochromatic camera (QImaging, Retiga EXi Blue), and composite images were re-created by applying corresponding pseudo color in QCapture Pro 7 software (QImaging).

An example of enrichment and on-chip immunocytochemical detection of CTCs from blood sample of a metastatic cancer patient is shown in Figure S1.

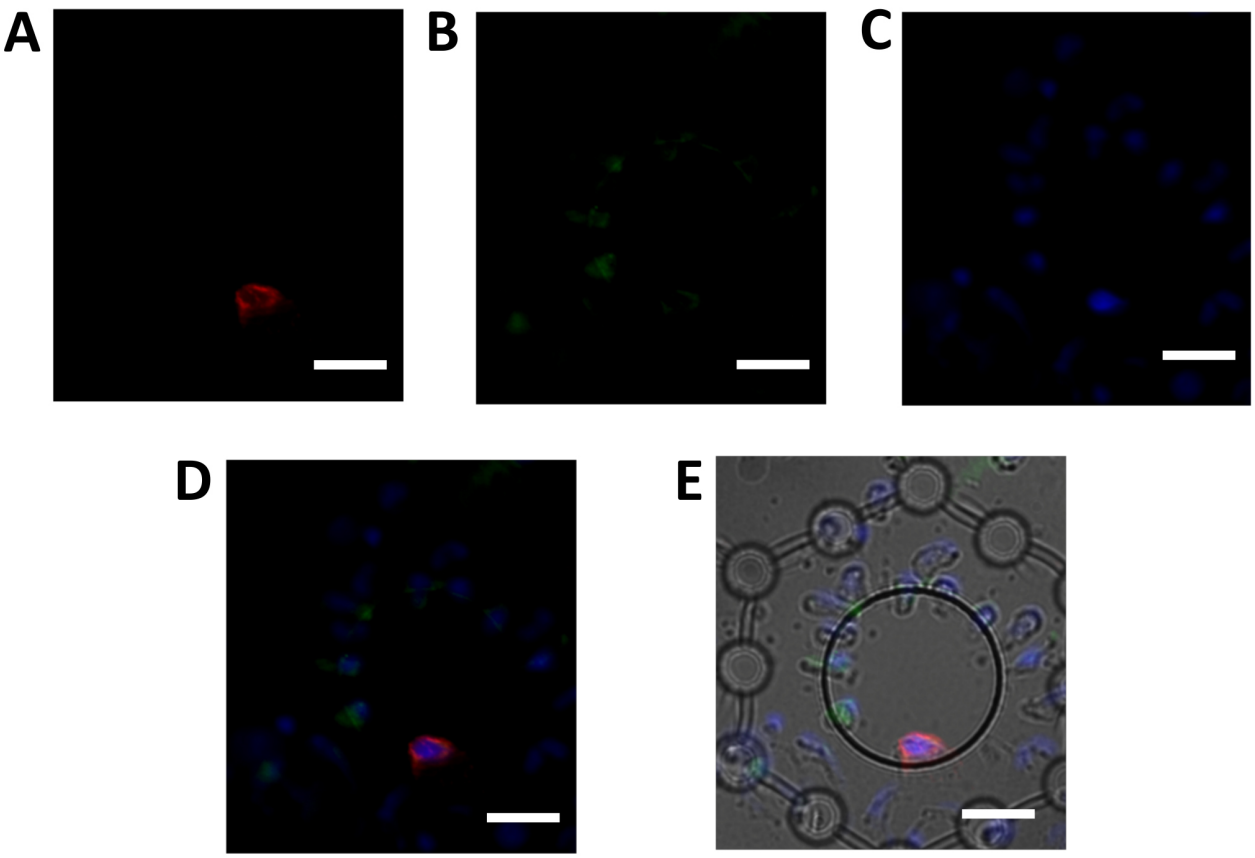

Figure S1: On-chip immunocytochemical detection of captured CTCs in clinical blood samples. Fluorescence images were taken using a monochromatic camera. Pseudo color were applied for cytokeratin 8/18/19 (red), CD45 (green) and DAPI (blue). Representative areas of fluorescence composite images of one CTC detected in red CK channel (A), green CD45 channel (B), blue DAPI (C), composite of three channels (D), and differential interference contrast (DIC)/fluorescence composite images (E). The scale bar is 10 μm.

Blood sample capacity of the SB microfilter. For a 1 cm2 device, the practical processing capacity reaches ~1 mL of whole blood sample. We demonstrated reliable and robust performance of the SB device. There was no incidence of clogging in the tested fresh whole

blood samples. In addition, we also challenged the processing capacity of the 1 cm2 SB device since current clinical samples require testing of a full tube of blood (7.5 mL).

In Figure S2, we summarized the time used to process the blood sample using the 1 cm^2 SB microfilter. A tube (7.5 mL) of undiluted whole blood can pass through the SB filter in ~4 hours without clogging the device. After the successful filtration, the device was crowded with blood cells, which might prevent further analysis and recover of enriched CTCs. Figure S3 represents an example of the SB device after processing a tube of whole blood. Using 1:1 (blood:DPBS) diluted whole blood, we can process up to 15 mL diluted blood in ~1.5 hours. For future clinical testing, the surface area needs to be enlarged to avoid clogging the device when processing larger whole blood volume.

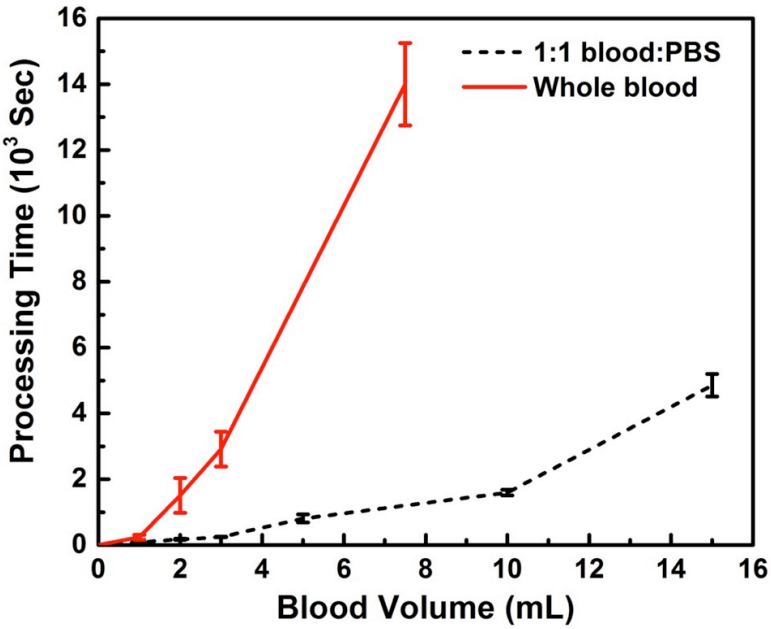

Figure S2: Filtration time of 1:1 DPBS diluted blood and whole blood using the SB microfilters (n = 3).

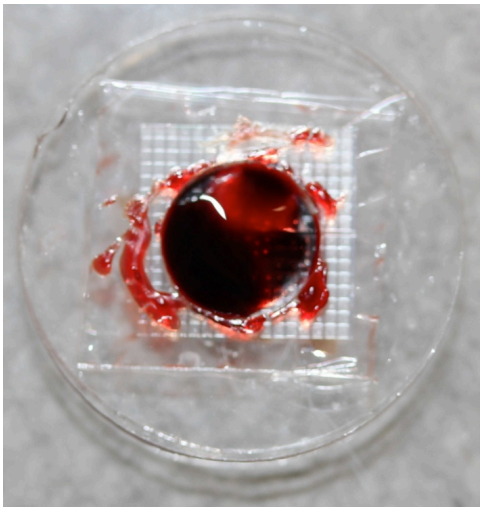

Figure S3: Example of one SB microfilter after processed 7.5 mL whole blood.