[SUPPORTING INFORMATION]

Highly Efficient Assay of Circulating Tumor Cells by Selective Sedimentation with a Density Gradient Medium and Microfiltration from Whole Blood

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Materials.

Monoclonal anti-human EpCAM/TROP1 antibody was purchased from R&D Systems (Minneapolis, MN). The magnetic protein G (pG) modified microbeads (diameter = 2.8 μm), PBS buffer (pH 7.4), Dulbecco's Modified Eagle Medium (DMEM), RPMI 1640 medium, fetal bovine serum (FBS), and penicillin-streptomycin were purchased from Invitrogen (Carlsbad, CA). Ethanolamine (50 mM in pH 8.0 100mM sodium borate buffer), dimethyl pimelimidate dihydrochloride (DMP), paraformaldehyde, Triton X-100, and L-glutamine were obtained from Sigma-Aldrich (St. Louis, MO). Bovine serum albumin (BSA) was acquired from Amresco (Solon, OH). MCF-7 human breast cancer cell line and DMS-79 small cell lung cancer (SCLC) cell line were obtained from ATCC (Rockville, MD). Trypsin and EDTA solution were obtained from BioWhittaker (Lonza, Switzerland). CM-5206 was purchased from NOF (Tokyo, Japan). Ficoll-Paque Plus® was purchased from GE Healthcare Biosciences AB (Uppsala, Sweden). 4', 6'-Diamidino-2-phenylindole (DAPI), anti-cytokeratin phycoerythrin (PE), and anti-CD45 FITCwere acquired from BD Biosciences (San Jose, CA).

Fabrication of microfilter device and microfluidic experimental set-up.

The microfilter was fabricated by silicon-on-glass (SOG) technology to make an accurate and precise gap between filter slots. Briefly, silicon and glass wafers were bonded together by anodic bonding. Lapping and chemical mechanical polishing (CMP) were performed on the Si layer. This process determines the thickness of the filter device for the present study, a Si layer (50 μm thick) was retained. Photoresist (AZ 4330, NJ) was patterned to create accurate gaps; thus, the distance of the gap between slots is affected only by the precision of the photolithography. Deep reactive-ion etching (DRIE) was performed for 15 min to create filter slots. Using patterned silicon layer as a mask, the outer flow channel is easily patterned by isotropic wet etching of the glass layer up to 50 μm in hydrofluoric acid (HF) solution. The inner flow channel is patterned on a capping glass wafer using the same HF solution, and then a dry film photoresist, Ordyl BF 410 (Tokyo Oga Kogyo, Kanagawa, Japan), was used to laminate and pattern the wafer. When the sandblasting process was completed for an inlet and an outlet port, the capping glass wafer was aligned and bonded to the SOG wafer to finalize the device fabrication. (Figure S-1B)

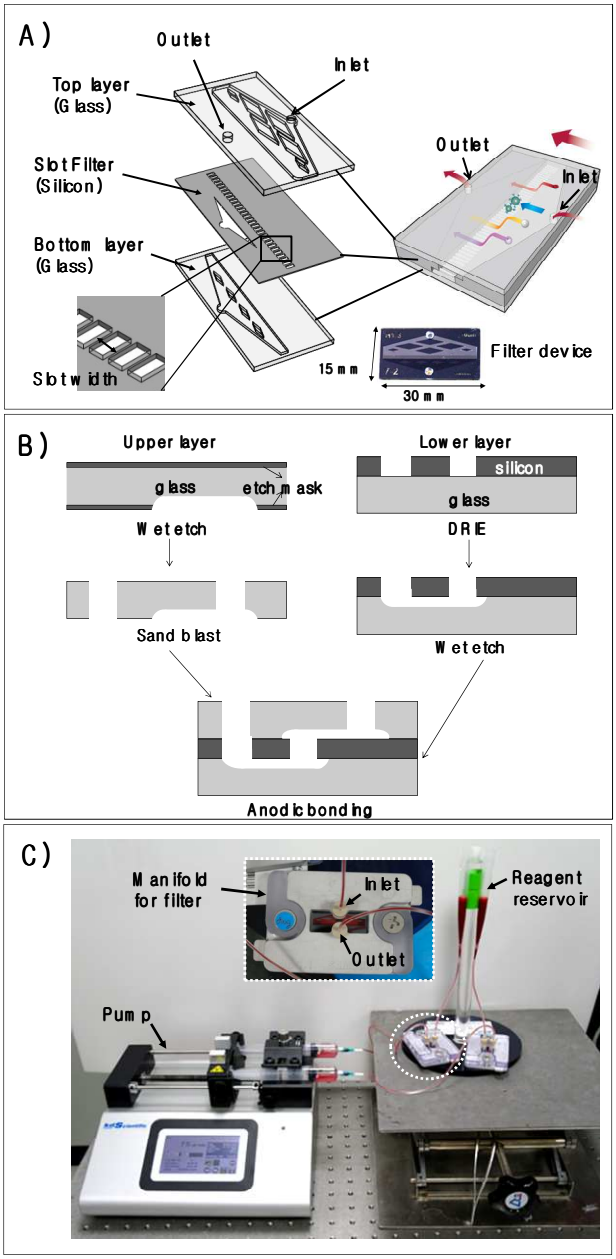

Figure S-1 The schematic diagram of filter device, fabrication process, and photographic images of microfluidic device used for CTC filtration. (A) Filter device was composed three parts: a top layer of glass, a silicon membrane filter, and a bottom layer of glass. The silicon-glass was assembled by precision alignment and anodic bonding. Inlet and outlet were placed on the same side. Slot width was either 10, 12, or 14 μm. (B) Schematic illustration of the fabrication process of the filter device. (C) A photograph showing the microfluidic experimental set-up for measuring the recovery rate of CTCs. Inset shows an enlarged photograph of a manifold with microfilter device.

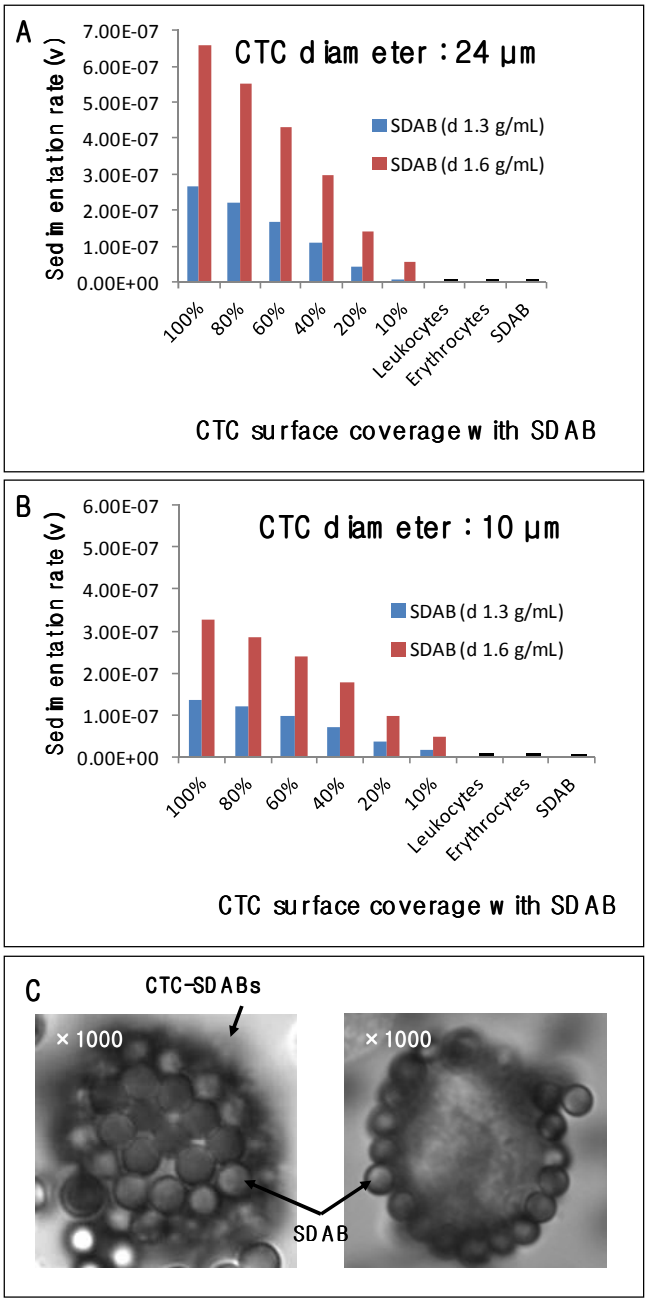

Figure S-2. Sedimentation rates as CTC surface coverage with SDAB and a TIRF (total internal reflection fluorescence) microscopoc image of CTC-SDABs. (A)-(B) Comparison of the sedimentation rates as CTC surface coverage with SDAB when SDAB has 1.3 g/mL (blue) and 1.6 g/mL (red), respectively. (C) TIRF microscopy image shows that CTC surface is covered with SDAB (2.8 μm diameter) at ×1000 magnification. Top (left) and middle (right) focused on CTC-SDAB images.

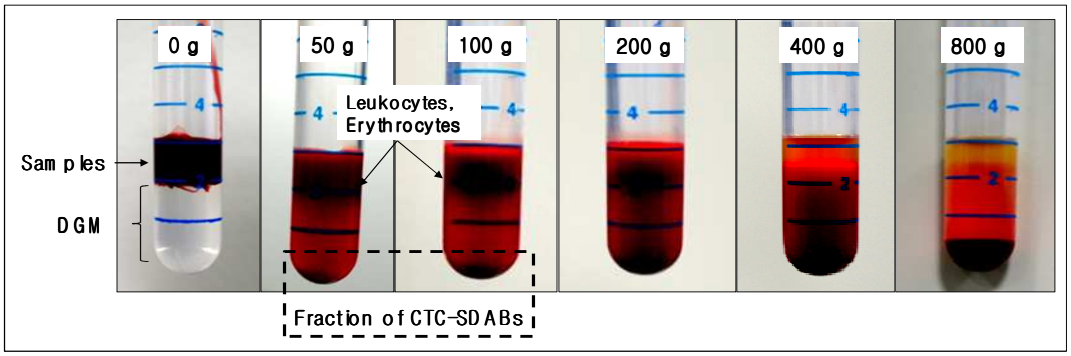

Figure S-3. Photographs of centrifuge tubes after centrifugation at specified g force for 2 min.

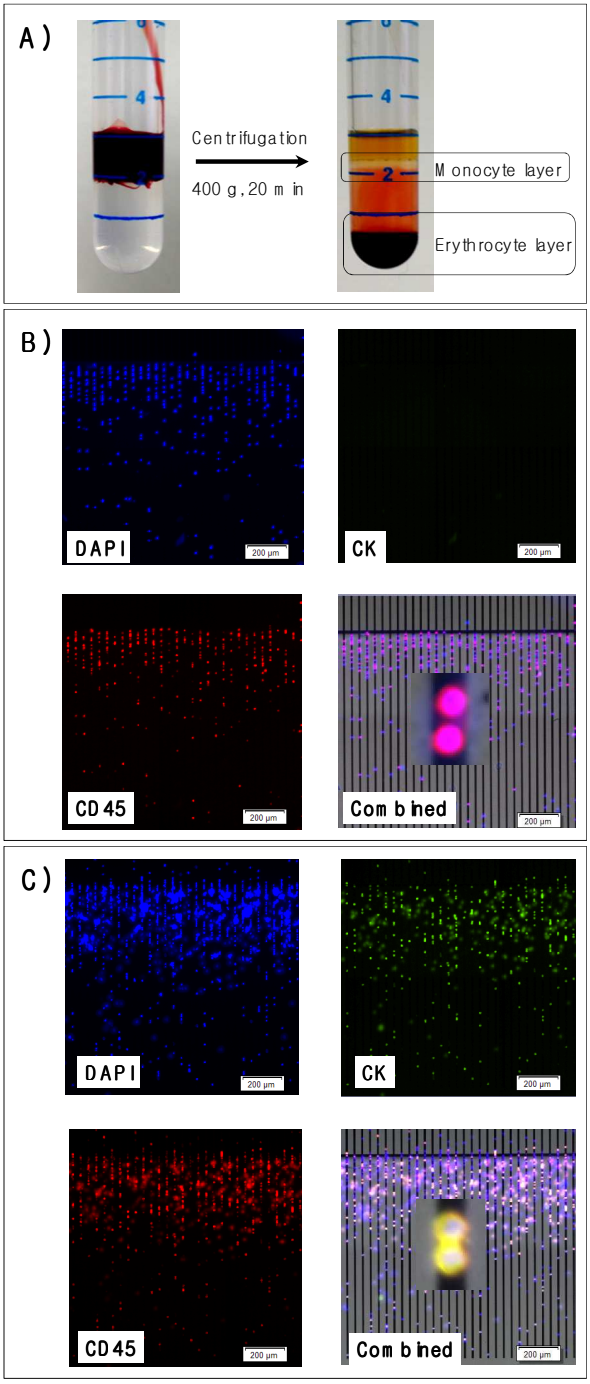

Figure S-4. (A) Photographs before and after monocyte separation by Ficoll-Paques centrifugal cell separation. (B) Stained images of the filter after filtration with fraction of monocyte layer (uppermost layer from (A)). (C) Stained images of the filter after filtration with erythrocyte layer (lowermost layer from (A)).