Supplementary Material (ESI) for Lab on a Chip

Manuscript reference: B916199F

No Cellular Contamination in Serum Samples

Materials and methods

Two samples of 10 mL of peripheral blood from a healthy volunteer were collected by venipuncture in a serum separation tube (BD Biosciences, Franklin Lakes, NJ) and a K_2EDTA vacutainer tube (BD Biosciences). Serum sample was prepared according to the manufacture's protocol and followed by passage through a 0.8 µm filter. 100 µL serum or whole blood was introduced into anti-CD63-antibody-coated microchannels of dimensions 5 cm (L) x4 mm (W) x30 µm (H) at a flow rate of 1.8 µL/min followed with 50 µL PBS rinsing at a flow rate of 7.2 µL/min to remove non-specifically attached cells. The microchannels were then stained with anti-CD36 antibody (BD Biosciences) and DAPI (Invitrogen, Carlsban, CA) to inspect platelets and nucleated cells, respectively.

Figures and Captions

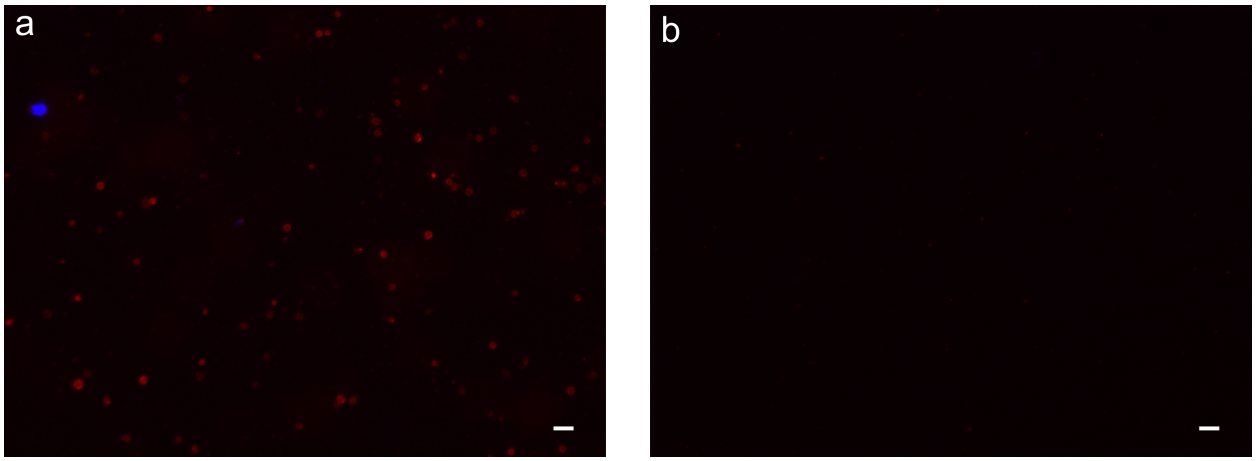

Figure S1. Representative images showing no cellular contamination in the serum sample. Anti-CD63-antibody-coated microchannels were injected with 100 µL of (a) whole blood or (b) serum from a healthy donor, respectively. Platelets (stained by PE-anti-CD36 (red)) and nucleated cells (stained by DAPI (blue)) were captured on the surface of the microchannel in (a). There are few, if any, platelets and nucleated cells were detected in the serum sample (b). (scalebars: 10 µm)

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Microfluidic Capture of PKH67-labeled Microvesicles

Materials and methods

PKH67 labeled microvesicles

Differential centrifugation purified glioblastoma microvesicles were labeled with PKH67 Green fluorescent labeling kit (Sigma-Aldrich, St. Louis, MO) and measured for their protein content using DC protein assay (Bio-Rad, Hercules, CA) according to the instruction manuals. In brief, purified glioblastoma microvesicles in 100 µl of PBS was resuspended in 1 ml of diluent C, then mixed rapidly with a freshly prepared PKH67 solution in diluent C (final concentration during labelling step: 5 x 10^{-6} M) and incubated for exactly 3 min to ensure homogeneous staining. The labelling step was stopped by addition of an equal volume of dFBS for 1 min, followed by an equal volume of complete PBS. After two washes in PBS by ultracentrifugation, the microvesicles were resuspended in 100 µl of PBS and measured for their protein content.

Image analysis

The fluorescence images were analyzed using an image processing program (ImageJ, National Institutes of Health). Differences between fluorescence intensities inside the microfluidic channel and the PDMS background after PBS rinse were measured.

Figures and Captions

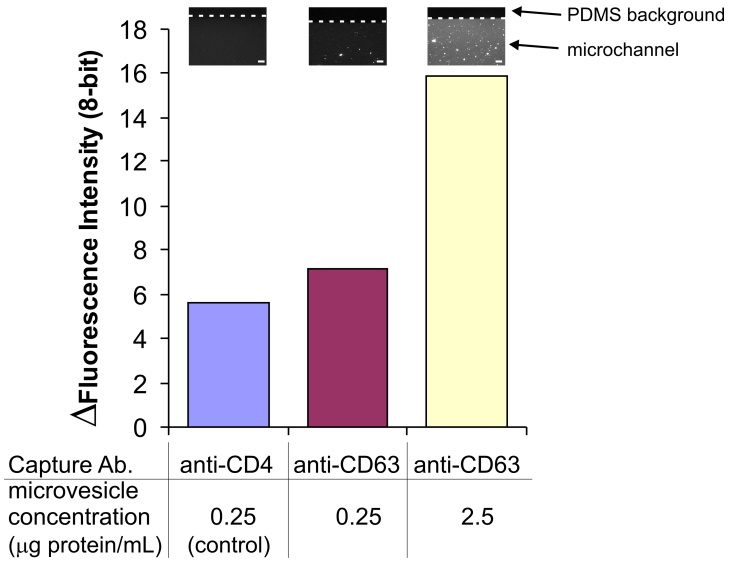

Figure S2. Fluorescently-labeled microvesicles are captured on anti-CD63-IgG-coated microchannels. 10 µL PKH67-labeled microvesicle-containing solution and its 10-time dilutions were flowed through microchannels of dimensions 5 cm (L) x4 mm (W) x30 µm (H) coated with

anti-CD63 antibodies or anti-CD4 antibodies, respectively. The changes in fluorescence intensity were measured after microchannels were washed with 50 μL PBS. Fluorescence images of each microchannel were shown in the insets (scale bar = 100 μm).