Supporting Information

Effective Reduction of Non-Specific Binding of Blood Cells in a Microfluidic

Chip for Isolation of Rare Cancer Cells

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Figure S1. Chemical structures of PDDA and BALG. Alginate biopolymer was functionalized using biotin moieties (blue). Alginate can be degraded by bacterial enzyme alginate lyase (red) which cleaves the backbone of the biopolymer.



Figure S2. Schematic of the structure of layer-by-layer film of PDDA/BALG, modified with neutravidin and biotinylated antibody/ PEG molecules.



Figure S3-a. Stepwise growth of PDDA/BALG multilayer film, obtained by data from quartz crystal microbalance with dissipation (QCM-D). Decrease in the resonance frequency (ΔF , left axis) of a QCM-D sensor reflects the increase of mass deposited on the surface. Changes in the dissipation factor (ΔD , right axis) are primarily related to the viscoelasticity (softness) of the film.



Figure S3-b. QCM-D monitoring of neutravidin and PEG molecules deposited on the PDDA/BALG LbL film.



Figure S3-c. Mass deposited on the substrate during the LbL assembly process, calculated from the change of (Δ F) from Figure S3-a and S3-b.



Figure S4. Images of captured PC-3 cells and non-specifically adhered blood cells in a $^{\rm HB}$ CTC-chip with molar ratio of antibody to PEG as 1:1 (left) and 1:100 (right). PC-3 cells were stained with the CellTracker Green, RBCs were pointed by dark red arrows and non-RBCs were pointed by blue arrows. The scale bar is 100 µm.



Figure S5. Captured MCF-7 and SK-BR-3 cells and non-specific binding of RBCs and non-RBCs on a ^{HB}CTC-chip. The surface of microchip was modified with mixture of PEG molecules and anti-EpCAM (or anti-HER2) antibody with ratio 1:0 and 1:10. Left axis represents the number of blood cells (cells/mm²) attached on the surface of microchip. Dark red circles and blue circles are for RBCs and non-RBCs, respectively. Right axis and star symbols represent the capture efficiency (%) for various modification conditions.



Figure S6. Captured mixture of MCF-7 and SK-BR-3 cells by a cocktail approach and non-specific binding of RBCs and non-RBCs on a ^{HB}CTC-chip. The surface of microchip was modified with mixture of PEG and a mixture of antibodies (anti-HER2, anti-EpCAM, anti-Musin1). The molar ratio between antibodies to PEG molecules were (1:0, left) and (1:10, right). MCF-7 cells were stained with CellTracker Blue while SKBR-3 cells were stained CellTracker Deep Red (pseudo-pink color). RBCs were pointed by dark red arrows and non-RBCs were pointed by blue arrows. The scale bar is 100 μ m.