Electronic Quantification of Surface Proteins on Circulating Tumor Cells Based on Bead-CTC Aggregate Sizing

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Rapid quantification of surface markers on circulating tumor cells (CTCs) can allow for prediction of patient response to various cancer drugs. Preclinical studies targeting cells with an antibody to activated matriptase conjugated to a potent toxin show promise as a selective treatment for a variety of solid tumors. Here, we implemented an electrical-impedance based biochip for quantification of proteins on surfaces of cancer cells. We demonstrated proof-of-concept based on detection of activated matriptase proteins on the surface of CTCs. Figure 1 shows the basic device. Activated matriptase, a membrane-bound serine type II protease, is overexpressed in most epithelial CTCs. Our assay works by coating magnetic beads with an anti-matriptase monoclonal antibody (M69) that recognizes activated matriptase and then mixing the beads with isolated CTCs. The expression of matriptase on the membrane of CTCs results in bead-CTC aggregation. The use of multi-frequency electrical impedance cytometry allows for differentiating between unbound beads, non-target cells and bead-CTC aggregates. This method can be used for detection and quantification of surface membrane bound protein (i.e. matriptase) levels, as the size and quantity of peaks corresponding to bead-CTC aggregates is proportional to concentration of matriptase expressed on the CTCs.

This biochip (Fig 2a and Fig 2b) consists of two microelectrodes on a glass substrate with the channel above it formed in PDMS. Electrodes were patterned using lithography, gold evaporation, and lift-off processing. For proof of concept, 2.8 μm sheep anti-mouse IgG beads were coated with M69. Beads then were mixed with CTCs off chip. A portion of the beads and cells bind together, resulting in effectively large aggregates for the positive assay with M69 antibody (Fig 2C) compared to the negative control assay (Fig 2C) in which only beads are present. The mixture is then injected into the micro-channel. As beads and bead-CTC aggregates pass over the electrodes, which are connected to an AC voltage source and a multi-frequency lock-in amplifier, there will be a frequency dependent increase in impedance. The amplitude of the current peak is proportional to particle size as shown in the representative current trace (Fig 3). We plot the distribution of the peak amplitude for three sets of experiments including bare 1) 2.8μm beads, 2) bare cells, and 3) a mixture of beads and bead-CTC aggregates (Fig 4). The multi-frequency impedance 2-D scatter plot for Signal-to-Noise Ratio (SNR) at 500kHz and 20MHz is shown in (Fig 5), allowing for quantification of the number of bead-CTC aggregates along with the size of the aggregates allowing for estimation of matriptase expression levels.

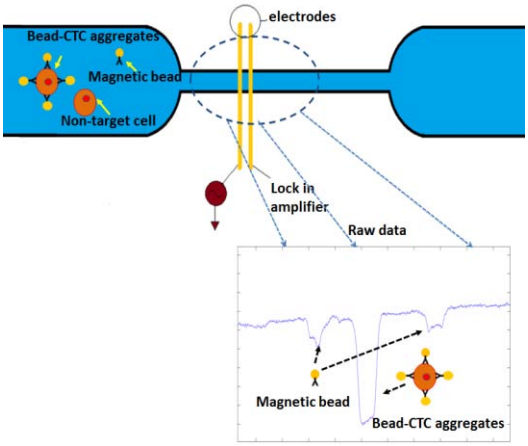

Figure 1: Schematic of biochip. Presence of matriptase expressing CTCs in the antibody coated beads mixture results in beads binding to the cell and aggregating. Impedance based sizing allows differentiation between Magnetic beads, and bead-CTC aggregates

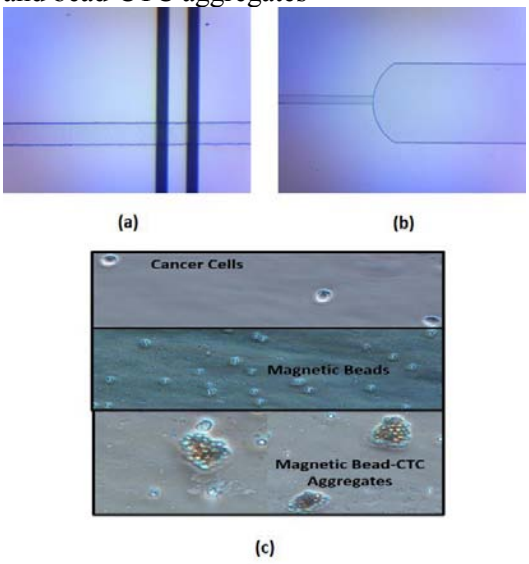

Figure 2: (a)Microfabricated electrodes (b)Entrance of micropore (c)Microscope image of Cancer Cells, Magnetic Beads and Magnetic Bead-CTC Aggregates.

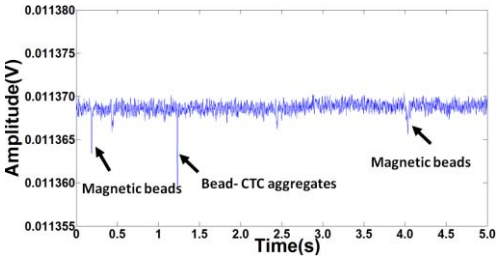

Figure 3: Magnetic bead's peak, and bead-CTC aggregates' peak in raw data. The Frequency and amplitude of the input AC voltage were 500kHz and 1V

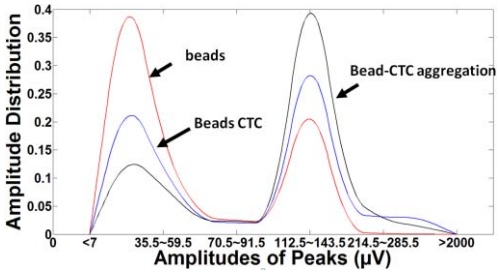

Figure 4: Probability distribution as a function of peak amplitude for experiments with 1) high concentration of 2.8 μm beads with low concentration of CTC bead aggregates(red), 2) smaller concentration of beads and larger CTC-bead mixture (blue), and 3) minority beads with majorityBead-CTC aggregation (black). The Frequency and amplitude of the input AC voltage were 20MHz and 1V

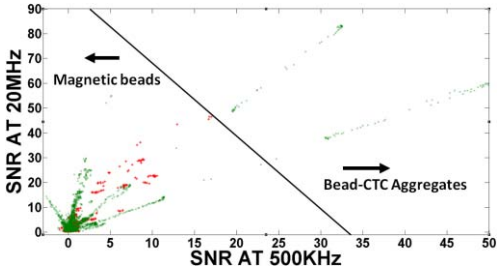

Figure 5: Scatter plots for SNR at 500K and SNR at 20MHz. Red dots correspond to bead only measurements, and green dots correspond to the mixture of beads and bead-CTC aggregates. Overlap of the two data sets corresponds to unbound beads. Everything to the right of the black line corresponds to bead-CTC aggregates.