HEMI-FUNCTIONALIZED SILICON FILTERS FOR SIMULTANEOUS CAPTURING AND TYPING OF CIRCULATING TUMOR CELLS

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Abstract
- We propose hemi-functionalization of microfilters as a method for simultaneous capture and rapid characterization of circulating tumor cells (Figure 1).
- Silicon filters were selectively functionalized with anti-EpCAM IgG only on one half.
- We found that the amount of EpCAM expressing cells captured is significantly higher on the anti-EpCAM half of the filters (Figure 7).

Schematic diagram of the filter hemi-functionalization

Hemi-functionalization of the filters
- The microfilters (Figure 4) have a circular active area of 10 mm, and a thickness of 15 μm. They were fabricated by photolithography techniques.
- An apparatus for hemi-functionalization was custom-built using microscope glass slides (Figure 3).
- The hemi-functionalization apparatus pins the antibody solution by capillary forces, requiring only 84 μL to coat one half of a filter with antibodies.
- Antibody was covalently coupled to the silicon surface by EDC/NHS chemistry.

See-through filter cartridge
- We also fabricated a custom filter cartridge that directs the sample fluid flow (Figure 4) without additional manipulation of the filters.
- The cartridge is fabricated on transparent acrylic, which allows for real-time monitoring of the cell capturing process.

Circulating tumor cell models
- Two breast cancer cell lines were selected as CTC models: MCF-7 and MDA-MB-231.
- MCF-7 is an EpCAM+ cell. Average diameter is 18 μm.
- MDA-MB-231 is an EpCAM+ cell. Average diameter is 14 μm.
- All cells were stained with Vybrant Green dye before flowing them at a rate of 0.5 mL/min through the filter.
- Counting and visualization of the captured cells was done through an inverted fluorescence microscope (Figure 6).

Control experiments

MCF-7 capture experiments
- MCF-7 cancer cells captured on a hemi-functionalized filter.
- Arrows indicate the captured cells. A higher number of captured cells is evident on (a) the anti-EpCAM functionalized half (26 cells) when compared with (b) the non-functionalized half (9 cells).

Results from MCF-7 capture experiments

Figure 5: (Left) Results from control experiments of MCF-7 cells with non-coated filters and (right) MDA-MB-231 cells on hemi-functionalized filters. Captured cells are equally distributed in both cases regardless of the filter pore size. Error bars represent standard deviation (N = 3).

Figure 7: Results from capture of MCF-7 cells using hemi-functionalized filters. A clear capture distribution trend is observed in comparison to the control experiments. Significance of these results was evaluated using t-test analysis (*** = p-value < 0.01; ** = p-value < 0.05). Error bars represent standard deviation (N = 3).

Figure 3: (a) Schematic showing a filter installed in the hemi-functionalization device. (b) Selective incubation with EpCAM antibody solution only on one half of the filter.

Figure 4: (a) Cartridge schematic showing the custom cartridge for live imaging. (b) The silicon filter patterned with (D) uniform pores.