Efficient Capture of Suspended Tumor Cells with a Novel Immunocytochemical Microfluidic Device

Mary Dickson[†, ‡], Pavel Tsinberg[†], Zhongliang Tang[†], Timothy Wilson[†], Edward Leonard[‡]

[†]Biocept, Inc., San Diego, CA
[‡]Department of Chemical Engineering, Columbia University, New York, NY

Introduction

Capture of rare cells such as:

1.Circulating tumor cells (CTCs) in the blood of cancer patients

2.Fetal cells in the blood of expectant mothers

and their subsequent genetic investigation is crucial for the developing of individualized diagnoses & treatments.

A highly efficient, high purity separation of these cells (as rare as $1{:}10^9$) has been achieved by a method developed in the authors' lab. Here investigated are the optimal flow parameters for capture.

Methodology

1. Tag CTCs with precise ratio of biotinylated antibodies

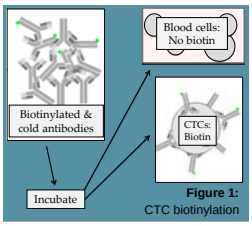


Figure 1: CTC biotinylation

2. Flow blood and CTCs through streptavidin coated micro-channel lined with ~9,000 posts.

- *Biotin – streptvidin bond only adheres CTCs to post*

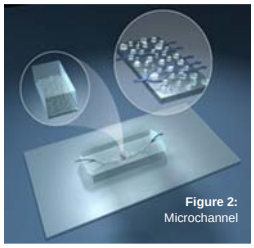


Figure 2: Microchannel

3. CTCs are located via fluorescent microscopy & cytogenetically interrogated in-situ (FISH, etc.)

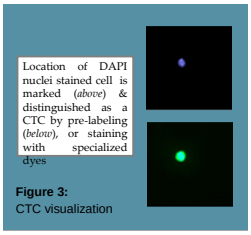


Figure 3: CTC visualization

Results

Capture efficiency, C: The number of CTCs captured vs. the total number of CTCs.

$$C = \frac{n_{cap}}{n_0}$$

C depends on surface antigen density (x), & can be characterized as:

$$y = \frac{x}{a + x}$$

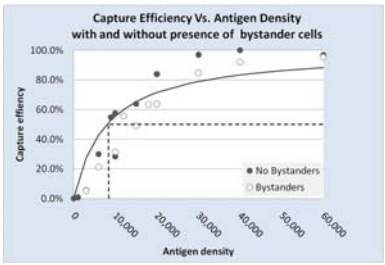


Figure 4: Series: Trials with and without other blood (bystander) cells. C is greater than 70% for antigen densities above 30,000. C is about 50% at antigen densities of 10,000.

How does antigen density affect cell binding?

1)Chance of adhesion

2)Strength of cell-post bond

*Both scale with number of biotin ligands per cell

Probability of Capture, k: Likelihood of a cell to capture within a given row. In a given row (r) the change in number of cells in suspension (n) is proportional to n.

$$k = \frac{\ln[1 - C]}{r}$$

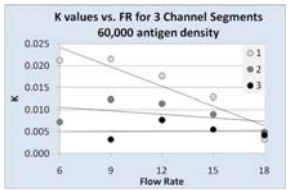
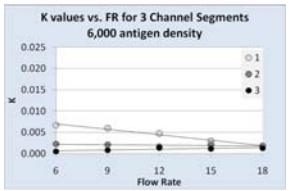


Figure 6 : Parameter: Distance along channel, each segment is 50 rows.

Left: High antigen density, k values decrease along channel at low flow rates.



Right: Low antigen density, k values are relatively constant

How does flow rate affect cell binding?

1)Higher flow-rates:

- Increased shear rate
  Displacement of cells from posts?
- Increased speed in higher flow-rate regimes
  Not enough time for binding to occur?

2) Low flow-rates:

- Streamlines develop which are farther from posts
  Some cells will never make contact?

Conclusions

Extremely rare cells can be separated with high efficiency and high purity for in-situ inspection. This will prove useful in

- Advanced diagnostics
- Individualized treatments
- Targeted cell removal

Higher antigen densities yield higher capture. Middle flow rates yield constant k values, avoiding streamline effect illustrated below.

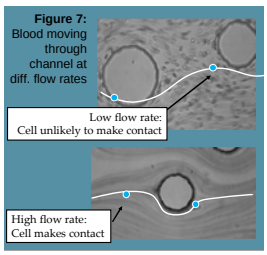


Figure 7: Blood moving through channel at diff. flow rates

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Acknowledgements

Many thanks to Lina Ebio for her skilled technical work, as well as my advisors: Tom Burns & Kathe Houghtaling

More information?

Biocept, Inc., San Diego, CA.
www.biocept.com

Mary Dickson
Columbia University Department of Chemcal Engineering
mary.dickson@gmail.com

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COLUMBIA UNIVERSITY IN THE CITY OF NEW YORK