Capturing a boulder using a field of grass : cancer cell capture optimisation

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Introduction

Cancer is a leading cause of death in Canada and will affect 2 of 5 Canadians at some point in their lifetime *(2)*. In 2016, there were an estimated 202, 400 new cases of cancer and 78, 000 deaths caused by cancer *(2)*. Due to its mode of action, cancer becomes increasingly difficult to treat as time goes on. If left unchecked, cancerous cells can migrate to other regions of the body to form secondary tumors, which is known as metastasis, making it very difficult to eliminate completely. Therefore, early detection is a key factor in the survival of cancer patients. According to Cancer Research UK, ten-year survival in patients diagnosed at stage one cancer is 90% compared to 5% in patients diagnosed at stage 5 *(3)*.
In order for cancer to spread, circulating tumor cells (CTC) are separated from the main tumor and reach systemic circulation, allowing them to travel through the body, at which point they can settle in another tissue and replicate to form a secondary, metastatic, tumor. A critical issue in cancer detection is the difficulty to positively identify a malignant tumor before it reaches a certain size, by which point the cancer has most likely spread to other areas of the body.

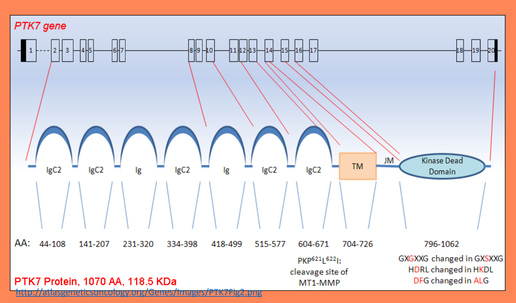
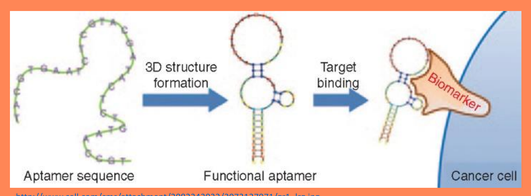


Figure 1: Schematic representation of PTK7's structure. Ig: extracellular immunoglobulin domains; TM: transmembrane domain; Kinase Dead Domain: intracellular domain.

Protein tyrosine kinase (PTK7) is a cell membrane protein which is highly expressed in CTCs. PTK7 has a major role in cancer cell motility and in the regulation of metastasis. DNA structures known as aptamers provide an excellent method of detection for the presence of characteristically high PTK7 presence on cell membranes and, consequently, a way to detect CTCs. This is possible because of the aptamer's specific folding and three-dimensional layout, allowing it to bind with very high affinity and specificity to its target protein. The aptamer with a complementary structure to PTK7 is known as Sgc8.



http://www.cell.com/cms/attachment/2083343032/2073137971/gr1_lrg.jpg

Figure 2: Schematic representation aptamer structure and binding to cell membrane biomarker.

Microfluidic channels (MFC), polymer chips made of polydimethylsiloxane (PDMS) containing microscopic flow channels, provide an excellent system to utilise the properties of aptamers. MFCs provide a high surface/volume ratio, meaning less fluid volume is needed and the particles in solution will have a higher rate of interaction with the walls of the channels. By binding these aptamers to the surface on the inside of the channel, it becomes possible to immobilise CTCs in the microfluidic channels and detect their presence in a blood sample. The aptamers are not tethered directly to the MFC walls, but to dendrimers, which are large branched polymers, allowing a much higher density of aptamers lining the MFC as a large number can be tethered to a single dendrimer.

First experiment

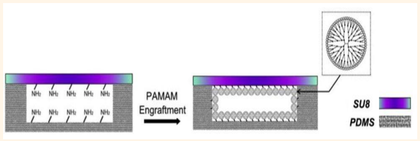


Figure 3: Dendrimer engraftment onto amine groups of microfluidic channel walls.

1. PAMAM dendrimers are bound to amino surface of the microfluidic channel lumen via carboxyl functional groups.
2. Aptamers are tethered to carboxyl groups on dendrimer branches via amine group present on aptamer tail.

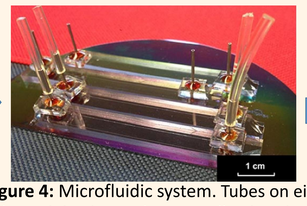


Figure 4: Microfluidic system. Tubes on either side are injection and waste collection sites on either side of MFC.

3. Microfluidic channel rinsed with PBS buffer.
4. Solution containing varying concentrations of CCRF-CEM cells (leukemia) is flowed through MFCs at a rate of 0.05mL/hour.
5. Microfluidic channel is flushed with PBS buffer to eliminate non-bound cells.

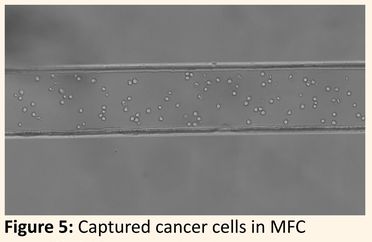


Figure 5: Captured cancer cells in MFC magnified 10x. The width of one channel is approximately 150µm. Small translucent circles are cancer cells captured by surface-tethered aptamers.

6. Microfluidic channel is observed under a microscope and captured cells are counted.

Results

Analysis of the microfluidic channels after various assays shows a relatively stable capture efficiency between target-cell concentrations of 1, 000 – 100, 000 cells/mL, averaging at 85.9% total cells captured. However this value plummets as the concentration of cells diminishes to 100 cells/mL. This is not ideal as the goal is to efficiently capture cells at low concentrations in order to detect a cancerous presence as early on as possible. The capture efficiency at higher concentrations also leaves a less than ideal margin of error.

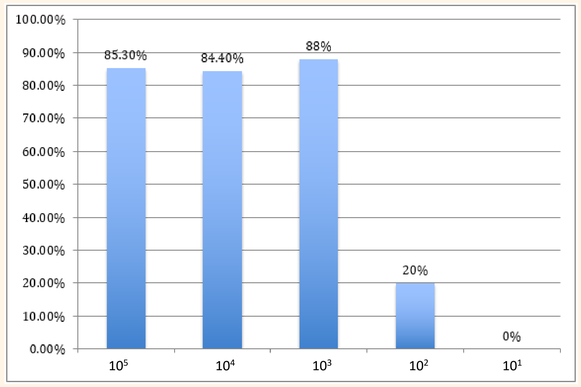


Figure 6: Percentage of CCRT-CEM cells captured by surface-tethered Sgc8 aptamer relative to total cell concentration in solution. X axis represents cell concentration in solution, with 10^5 being 100,000 cells/mL; 10^4 being 10, 000 cells/mL and so on and so forth.

Conclusion

In this first experiment, we have determined that it is in fact possible to capture cancerous cells expressing PTK7 in a microfluidic system using surface-bound dendrimer-aptamer complexes. However, optimisation of this process is required if it is to be used as an early detection method for cancerous presence in plasma.

Next step

Optimisation

Spacers

In order to allow optimal aptamer binding to a target protein, it must adopt its native folding structure. However, when an aptamer is restrained to a surface, the connection that binds said aptamer to the surface does not offer enough flexibility for this to occur, thus decreasing the aptamer's affinity for the target protein.

In order to compensate for this, a spacer will be used to give more flexibility to the aptamer's (Sgc8) structure, allowing it to adopt a conformation optimal for protein (PTK7) recognition and binding. Based on previous studies *(1) (4)*, it is known that the use of oligo-T spacers, thymine chain, with the Sgc8 aptamer have been particularly useful in increasing affinity for PTK7. These studies used free, non-tethered aptamers in solution, though the concept is much the same.

The current area of focus in this project is optimal spacer length; too short and the spacer does not allow maximum flexibility for aptamer folding, too long and the spacer will bend causing the aptamer to be flattened back against the surface rather than branching out, decreasing its ability to capture oncoming cancer cells.

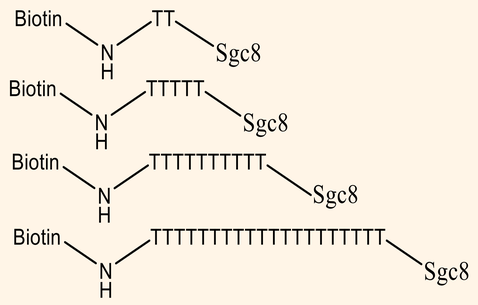
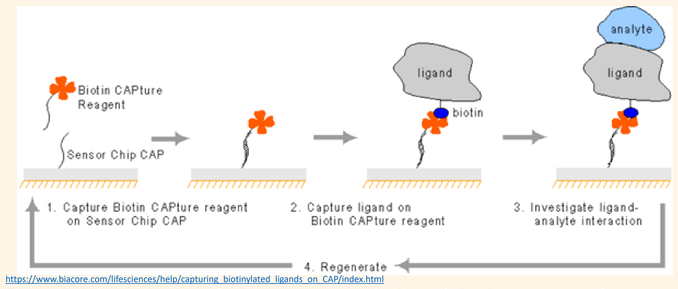


Figure 7: Representation of spacer-aptamer complexes. T sequences represent oligo-T spacers (T2, T5, T10 and T20 respectively) bound to Sgc8 aptamer on the right and biotin on the left via amine group.

Methodology

In this experiment, the spacer-aptamer complex will be bound to a sensor chip surface via biotin rather than amino group to allow chip regeneration to run multiple assays.
Rather than using whole cells, only the extracellular domain of PTK7 will be used as analyte in solution to measure specific PTK7-Sgc8 interaction kinetics. Solution containing PTK7 is injected into Biacore microfluidic chip.



https://www.biacore.com/lifesciences/help/capturing_biotinylated_ligands_on_CAP/index.html

Figure 8: Schematisation of a Biacore assay cycle. Ligand: Sgc8-spacer complex; analyte: PTK7 in solution. Surface is activated with biotin CAPture reagent; biotinylated Sgc8-aptamer complex is injected into microfluidic system and binds to surface via CAPture reagent; analyte in solution is flowed through channel and interaction is measured by plasmon resonance; PBS buffer is then flowed through to regenerate the surface; cycle is repeated.

Biacore

Quantitative kinetic data on the interaction between ligand (Sgc8) and analyte (PTK7) can be obtained with Biacore, equipment which measures mass fluctuations near a detection surface. This is achieved by measuring the incidence angle at which light is absorbed by the thin gold layer of the detector's surface. Light is absorbed by this gold film when the oscillations of the gold's electrons (plasmon oscillation) are in phase with the incident light beam, causing resonance. This only occurs at a specific incidence angle which is measured by photodetectors in Biacore. Further, plasmon oscillation is very sensitive to surrounding mass fluctuations. Therefore, a fluctuation in mass, such as when PTK7 binds to the surface-tethered aptamer-spacer complex, causes a change in the incidence angle at which light is absorbed by the gold film, which can be measured by Biacore.

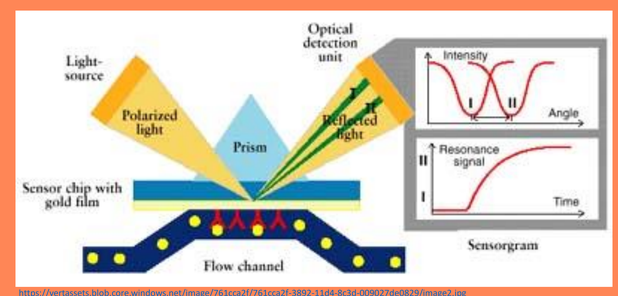


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Figure 9: Schematic representation of surface plasmon resonance detection by Biacore. Incidence angle of absorption is measured by low reflection intensity. Angle variation, as analyte binds and dissociated from surface-tethered Sgc8-spacer complexes is expressed as a function of time.

The data obtained by Biacore is a variation of the angle at which light is absorbed, representing mass fluctuations close to the detector's surface, relative to the initial mass of the ligand (aptamer-spacer complex). Kinetic parameters of the PTK7-Sgc8 interaction such as affinity and dissociation constants can then be determined by analysing the progression of relative mass fluctuations as a function of time.

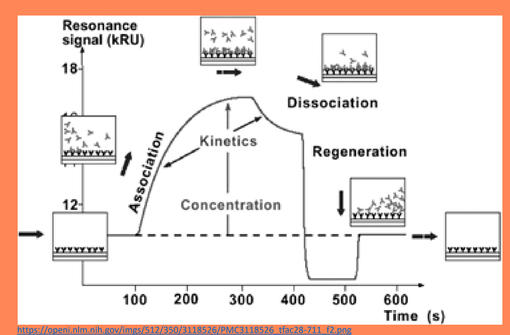

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Figure 10: Representation of a typical resonance signal variation graph. Pictures at each major stage of the assay are shown in relation to the graph to illustrate relation between analyte-ligand interaction and resonance signal graph.

References

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Acknowledgements

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