QUANTIFYING THE METASTATIC PROPENSITY OF CANCER CELLS THAT UNDERGO PERITONEAL METASTASIS AS A PROCESS

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Abstract

The treatment of cancers that use peritoneal metastasis is difficult, primarily because of the positive feed forward manner of its rapid metastasis. The tumour microenvironment plays a big role in controlling it's growth and metastasis. The current gold standard for quantifying the cells that migrate through the channel is by counting them manually from the microscopic images. This experiment shows the optimisation of the adhesion process to make it a high throughput analysis. Cells are classified as adhered or non-adhered based on time series photos taken at 1s intervals. Furthermore, it aims test an algorithm to automate the process of cell counting. Finally, it aims to use various theories from software psychology to create a platform that allows researchers to automate the cell counting process. In this experiment, it is seen that no clear relationship exists between using different ratios of Highly Metastatic (HM) and Non-Metastatic (NM) cells, and that the optimum concentration to use in experiments is $1x10^5$cells/ml. Furthermore, some calibration is required for the algorithm such as using machine learning techniques and object tracking to accurately display the cell count.

Introduction

Among the ways of death from gynaecology malignancies, the leading cause of death appears to be ovarian cancer. The tumour cells may detach from the tumour and metastasize by being carried in the peritoneal fluid to the omentum and peritoneal surfaces where they may seed[1]. Different organs have different barriers to metastasis. The ovarian cancer cells in the peritoneum are under constant exposure of shear stress created by ascites making studying the mechanical mechanisms of this adhesion very difficult. Therefore, not much research is done in this area[1].

Selective binding between selectin and ligand determines where the tumour colonises[1]. Previous studies have shown a significant number of cells adhere to P-selectins, in comparison to E-selectin and L-selectin highlighting P-selectin's dominant role in cell adhesion[1].

A microfluidic platform is used in this study for a more high-throughput analysis of cellular behaviour under shear stress[3]. By altering the flow rate, we are able to see the relationship between shear stress and gene expression.

However, a product of experiments that mimic these dynamic systems is a set of time lapsed images of the stained cells. The current gold standard for quantifying the cells that migrate through the channel is by counting them manually from the microscopic images. These images may contain hundreds of cells depending on the concentration of the sample and so manually counting them is not a trivial task and may involve a lot of human errors. Image cytometry is one solution to this bottleneck as it provides highly reliable results within minutes and saves the researcher days of tedious inspection.

The aim of this study is to use bioinformatics and laboratory techniques to quantify the metastatic propensity of these cells. Furthermore, it is to optimise the adhesion process, as well to further make it a higher throughput analysis by automating the cell counting process. This will be done by testing the algorithm against the gold standard of manual counting. Furthermore, a software will be created with cognitive psychology theories in mind to show the utility of this software by various researchers for this type of experiment.

Materials and Method

The microfluidic chip will first be washed and coated with P-selectin. The cells will be grown and maintained. The cells will be dissolved in binding buffer and inserted into the channel using a syringe pump. 3 sets of images will be taken for each channel at a different location where each set has 40 images taken at 1s intervals. The number of cells will be classified as adhered or non-adhered. ImageJ will be used to trace the cells. The process of tracing those cells flowing through will be automated by accumulating data to train the algorithm. This will help to measure the kinetics as to understand the mechanisms that allow the cells to metastasize and colonise in a particular organ.

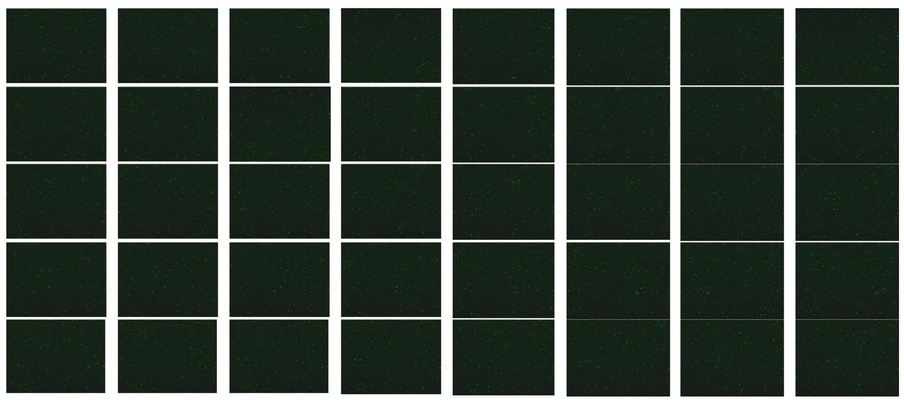
The algorithm received will then be tested against the manual counting to analyse the average cut off point between adhesion and non adhesion. After which the set of images will be run in the algorithm to receive the number of adhered vs non adhered cells.

A software is created using Java that allows the user to input various parameters such as cell size into it and allows the user to preview the process to ensure all cells are correctly selected. After which the algorithm can be run to return the result of adhered vs non adhered cells as well as the results in terms of average speed of cell, orientation of cell and location of the cell within the images.

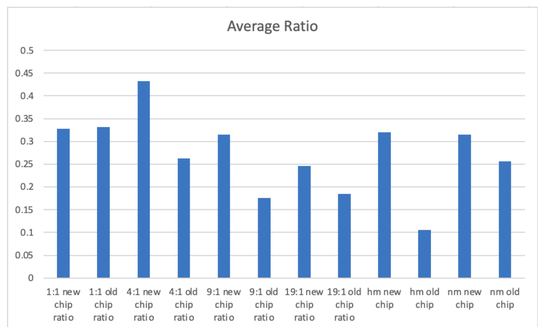
Results

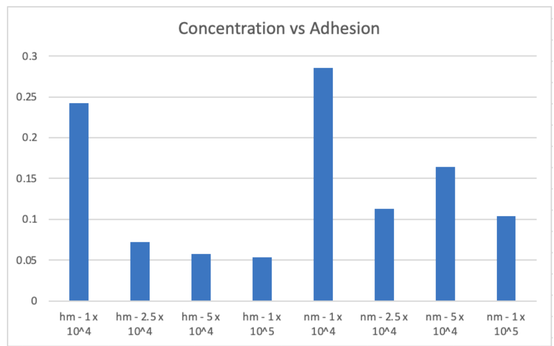
The current results we have received are to optimize the adhesion process involving cell concentration, duration and counting, the data analysis of the algorithm and a software design section.

1) Photos of cell counts from microscope

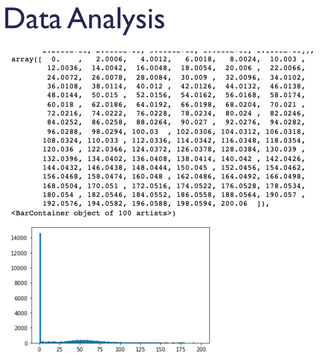


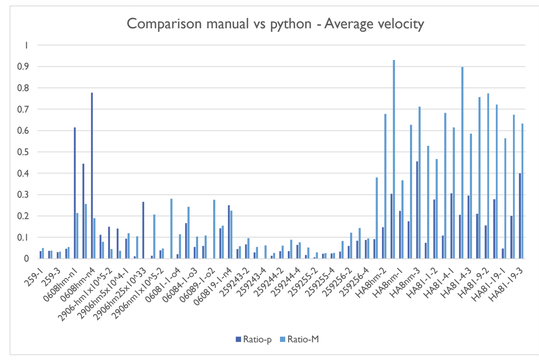
2) Graphs obtained





3) Data Analysis





4) Software Design

Discussion

It was seen that adding a higher volume of DMSO to dissolve the cell tracker, ultimately killed the cells during incubation and so the optimized protocol had the same concentration of cell tracker (2.5µg/ml) but only 20µg of DMSO was used instead of the initial 100µg. It was also seen that the optimum staining time should be 3 hours prior to the perfusion assay.

Furthermore, manual counting has some human errors, due to speed and size of cell or clarity of stain. This sometimes amount to the significant standard deviations present in the data. Therefore, further research will be done to automate the cell counting process by creating an algorithm.

Due to the nature of the experiment, when the velocity of the cell was calculated, a bimodal distribution was expected, where the first peak would indicate the cutoff point of the adhered to non adhered cells. In particular, it was seen that regardless of using the 5th, 10^{th}, or 50^{th} percentile, the cutoff point came to be the same at 2.006 pixels per second.

As we can see, the prediction was not very accurate so instead have values specific to the experimental condition. In that case, although the results were a little mixed, we did see that a lot of the results gained from the algorithm has a large standard deviation.

Various software psychology concepts such as placement of text, layout of screen, amount of information present on screen were taken into consideration when building the software as the concepts of human-computer interaction are crucial in making the usage of the software easy.

Future Research

Further research includes doing various HM and NM comparisons using other cancer types and other models to get an overall idea of all cancers that use peritoneal metastasis as a process. Furthermore, an algorithm will be created to automate the cell counting process and limit all the errors observed above. Finally, upon the success of method optimization and adhesion process optimization, we will conduct drug screening where we can see the effect of a particular drug on adhesion, to further see whether cancer metastasis can be blocked. This methodology can, therefore, be used as a drug development tool as well.

Moreover, more data analysis and modifications need to be done to the algorithm such as object tracking and machine learning to receive a value that reflects to the gold standard more.

References

1. Li, S., Ip, C. K., Tang, M. Y., Tang, M. K., Tong, Y., Zhang, J., . . . Wong, A. S. (2019). Sialyl lewisx-p-selectin Cascade Mediates Tumor–mesothelial adhesion in ascitic fluid SHEAR FLOW. Nature Communications, 10(1). doi:10.1038/s41467-019-10334-6
2. Dong H, Tulley S, Zhao Q, Cho L, Chen D, Pearl ML, Chen WT. The propensity of invasive circulating tumor cells (iCTCs) in metastatic progression and therapeutic responsiveness. Cancer Med. 2019 Jul;8(8):3864-3874. doi: 10.1002/cam4.2218. Epub 2019 May 21. PMID: 31115187; PMCID: PMC6639176.
3. Ip, C. K. M., Li, S.-S., Tang, M. Y. H., Sy, S. K. H., Ren, Y., Shum, H. C., & Wong, A. S. T. (2016, June 1). *Stemness and Chemoresistance IN epithelial ovarian carcinoma cells under shear stress*. Nature News. Retrieved September 28, 2021, from https://www.nature.com/articles/srep26788.