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DEPARTMENT OF BIOENGINEERING

THE EFFECT OF BLOOD ON CIRCULATING TUMOR CELLS' EXPRESSION OF EPITHELIAL AND MESENCHYMAL CELL MARKERS

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ABSTRACT

This research aims to examine the variation in expression of the biological markers EpCAM, vimentin, and cytokeratin on the surface of circulating tumor cells (CTC) depending on the cells' time in blood. CTC detection, enumeration, and characterization offer potential prognostic information in a much less invasive form than current bone marrow biopsies. CTC that are especially of interest are ones that are undergoing the epithelial to mesenchymal transition (EMT), a process associated with the metastasis of tumor cells. During this transition, the markers EpCAM, vimentin, and cytokeratin are presumed to change their expression. The most popular, current technology used to capture CTC is the CellSearch® method. This immunoaffinity method relies on the expression of EpCAM, an epithelial cell marker, to capture CTC and may miss cells due to varied cell phenotypes and long processing times. The device used to capture the cells in this study was size based and therefore could capture cells without a positive EpCAM expression. NCI-H441 (lung cancer) cells were spiked into blood for various amounts of time before filtration, ranging from 0 to 24 hours. Cells were filtered, fixed, and stained using immunofluorescent antibodies. The intensity of the staining was related to expression of the marker. There was a significant decrease in EpCAM, cytokeratin, and vimentin expression from 0 to 4 hours, but expression remained similar from 4 to 24 hours. This illustrates that the phenotype of the cell is potentially changing due to either the EMT, the chemistry of the blood, or the life of the cell. The results suggest that immunoaffinity based capture methods may not provide correct information about the patient's cancer due to the time between blood draw and CTC capture and the cells' altered phenotypes. Future research should be performed to provide additional time points to determine the approximate time before this alteration, and therefore a desired maximum processing time.

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Chapter 1

Introduction

Cancer Staging

Millions of people throughout the world are affected yearly by many different forms of cancer. More people die from cancer than from AIDS, malaria, and tuberculosis combined. It is defined as the uncontrolled growth of abnormal cells in the body and will cause healthy cells to be overgrown by tumors. Cancer can be measured in stages from zero to four, with zero representing the most benign and four the most malignant. The method of determining the severity of the cancer is to examine whether the cancer has remained local to the affected tissue or it has traveled to other parts of the body. The classical marker of a stage four disease is metastasis.

Metastasis and CTC

Metastasis (shown in Figure 1) is the process of the cancer spreading from one organ to another non-adjacent one by the travel of tumor cells through the lymph nodes or blood vessels. Cells that spread to the lymph nodes and bone marrow are referred to as “disseminated tumor cells” (DTC). When these tumor cells flow through the peripheral blood, they are termed “circulating tumor cells” (CTC). Although some of these cells will never form a metastatic tumor, it is believed that the presence of DTC and CTC have a relation to the prognosis of the patient[1]. The cells can remain in the patient’s system even after the primary tumor has been

removed. If these remaining cells are able to penetrate a healthy tissue and reform the tumor, the patient is at the risk of a relapse.

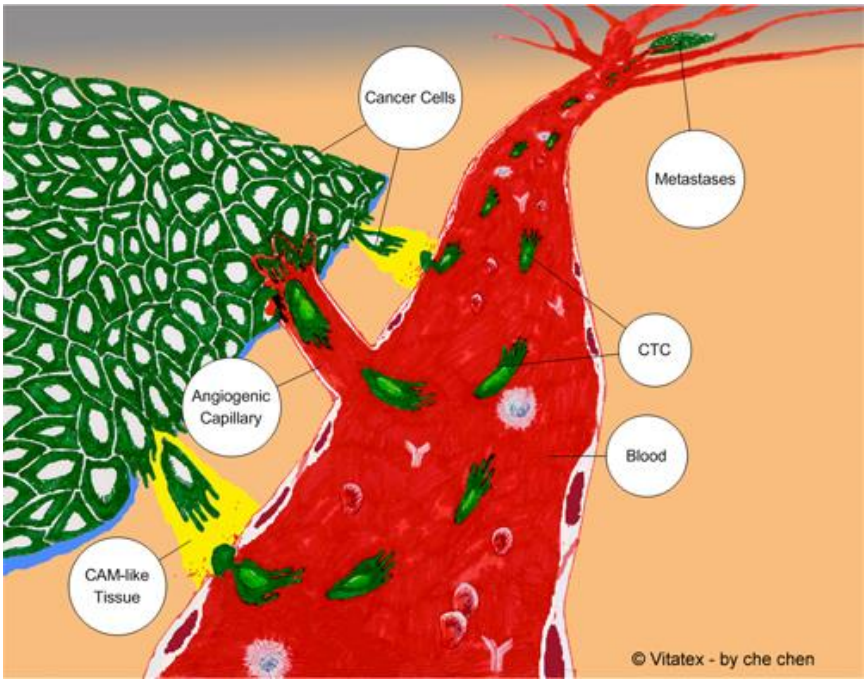


Figure 1: Circulating Tumor Cells Invading Bloodstream and Forming Metastases
In this figure, the green cancer cells represent the primary tumor. As they invade the bloodstream through the angiogenic capillary or CAM-like tissue, they are termed CTC and can travel to form metastases in distal locations. Figure adapted from reference [2].

Detection of CTC

Current imaging methods and laboratory testing cannot detect certain "micrometastases" which can lead to these relapses[3]. Therefore there is great interest in the detection and study of DTC and CTC. However, CTC are of an extremely small proportion in blood, as there are only a few CTC for approximately every 10 million leukocytes and 5 billion erythrocytes in 1 ml of blood[4]. Although rate of detection of DTC is much greater than that for CTC, the testing of peripheral blood is much less invasive than that of bone marrow[5]. The blood provides a simple way, a possible "liquid biopsy", that a patient's status could be tested frequently both during and

after their cancer treatment. Peripheral blood testing has fewer complications and has a higher patient compliance than blood marrow testing. Ideally, captured CTC can be taken from these blood samples, accurately counted, and used to determine prognosis as well as act as a marker for disease response in therapy [6]. However, a more accurate method of capture, enumeration, and analysis is necessary for this goal to be achieved.

Methods of Capture

Density Gradient Centrifugation

There are many different methods of capturing CTC. One of the earliest and the most established methods is density based and is performed by buoyant density gradient centrifugation using the Ficoll-Hypaque® solution[7]. During this centrifugation, the cells separate according to their buoyant density and result in distinct layers of cell types. Density separation centrifugation has also been advanced using a commercially available device called OncoQuick® to separate the blood sample from the separation medium before centrifugation. With this method, pictured in Figure 2, the tumor cells can easily be aspirated leaving the red blood cells below a porous liner[8]. However, these methods often leave lymphocytes in the same layer as the CTC.

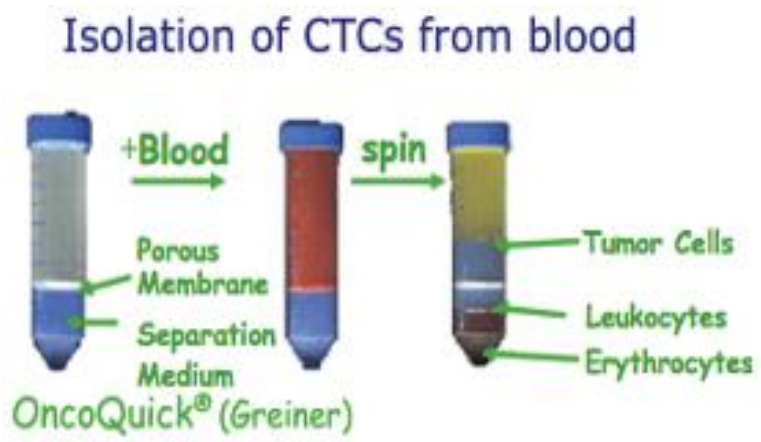


Figure 2: OncoQuick as a Separation Method
The OncoQuick method of CTC detection is shown in this figure. In the blood tube on the left, the blood and separation medium are separated by OncoQuick's porous membrane. When centrifuged, the result is a tube of blood with distinct layers for the erythrocytes, leukocytes, and tumor cells. The porous membrane separates the leukocytes and tumor cells. Figure adapted from reference [9].

Antibody-Based Detection

One of the most common, and the only Food and Drug Administration (FDA) approved method, is the CellSearch® method[10]. This method is based on the expression of the epithelial cell adhesion molecule (EpCAM) marker. EpCAM is a transmembrane glycoprotein involved in cell adhesion, migration, proliferation, and differentiation[11]. Although the extent of its role in cancer is still somewhat unknown, it has been a target as a diagnostic marker due to its oncogenic potential[12]. Overexpression is found in many carcinomas, and studies have shown that the overexpression of the protein is associated with decreased survival rates [12]. The CellSearch® method uses EpCAM-specific antibodies that are conjugated to magnetic particles. In Figure 3, the process is shown.

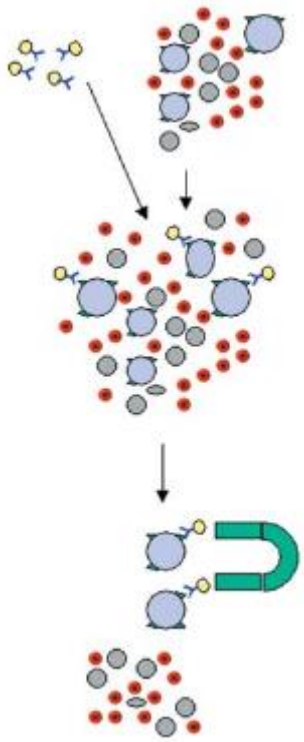


Figure 3:Tumor Cell Separation by Magnetic Particles
In this figure, the yellow circles represent the magnetic beads containing the anti-EpCAM antibodies. The large gray circles are the CTC that express EpCAM and bind to the magnetic particles. The magnet separates the CTC from the rest of the blood constituents. Figure adapted from reference [8].

The first step in the process of CellSearch® is to take a 7.5 mL blood sample and store it in a tube lined with anticoagulants. The blood is centrifuged and the magnetic nanoparticles separate the CTC from the red and white blood cells. The cells are also fluorescently stained for cytokeratin(CK) and the leukocyte common antigen (CD45) [13]. Cytokeratin is a protein found in the intermediate filaments of cytoskeleton of epithelial cells[14]. CD45 is a leukocyte marker that can distinguish the CTC from the white blood cells. According to the CellSearch® method, the EpCAM positive cells detected must also be cytokeratin positive and CD45 negative to be considered a CTC[10]. This standard of evaluating CTC is used in many other methods of detection as well. CellSearch® has had great success in correlating a higher CTC count to a worse prognosis[7]. However, clinical samples have shown that only 10% of patients have had a

CTC count that was 5 or higher[15]. While these patients statistically had a worse prognosis, the assay could not predict the status of the disease in the remaining 90%.

Similar magnetic bead methods aimed to improve the efficacy of the method by binding more strongly to the cells bound to anti-EpCAM coated beads. One prototype called "MagSweeper" has achieved a capture rate of 61% when testing 9 mL blood samples[16]. Yet another method using magnetic beads is a series of assays called AdnaTest®. This product functionalizes the beads by using a cocktail of antibodies that are targeted towards specific cancers as well as markers of specific cell phenotypes[17].

A method that uses both selective size amplification (SSA) and a multi-obstacle architecture (MOA) film has been developed [18]. This method uses microbeads before filtration to differentiate between CTC and white blood cells [18]. The schematic of this system is shown in Figure 4. The pore size of the filter does not allow for the amplified CTC to pass through. This method was able to achieve a 92% recovery rate when MCF-7 breast cancer cells were amplified as opposed to a 20% rate for native cells[18].

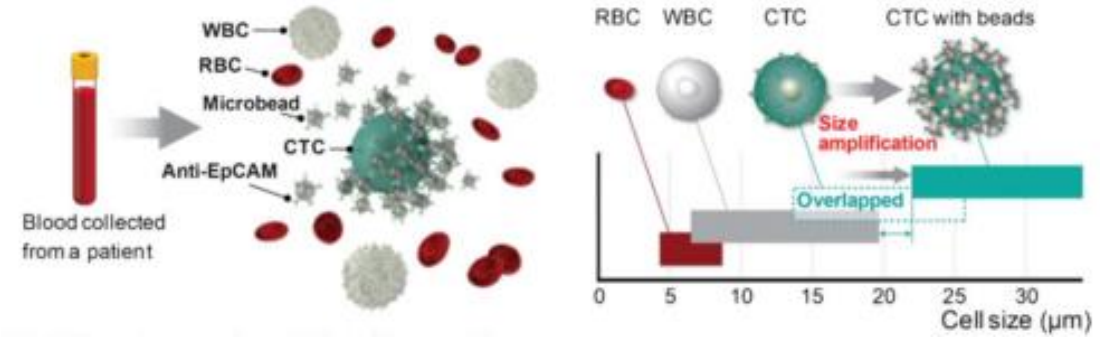


Figure 4: Illustration of Micobeads Used to Amplify CTC Size
This figure shows microbeads being attached to the CTC by targeting the EpCAM on the surface of the cell. The graph on the right shows that the CTC now have a larger size range and do not overlap with the RBC or WBC. Figure adapted from reference [18].

A similar method to the multi-obstacle architecture film called the “CTC-chip” uses an array of thousands of silicon micropillars and a very large surface area coated with anti-EpCAM antibodies to process whole blood samples[19]. This microfluidic chip was able to achieve a capture efficacy of over 60% and a sample purity of 50%[19]. Improvements on the chip method have been accomplished by designing a microvortex-generating “herringbone chip”[20]. This chip increases the number of interactions between the cells and the antibody-coated grooves of the device using passive mixing of the blood, and was able to increase the capture rate to 91.8%[20]. Nanoscale filtration chips have also been researched based on their high surface area to maximize CTC contact. One particular chip was found to have a capture efficacy ranging from 40-70% when used statically. Further addition of a powerful fluidic handling system to mix the cells as they pass through the chip was able to increase this value to over 95%[21].

All of the previously discussed immunoaffinity methods of capture are reliant on positive selection of antibodies. However, there are also negative capture methods. Negative selection methods are directed against surface markers that are overexpressed in hematopoietic cells[8]. They target leukocyte markers such as CD45 and CD14 and remove these cells from the solution. One study used depletion of leukocytes to capture CTC in 20 out of 26 cancer patients[22]. However, these have been shown to be less sensitive than the positive selection methods and may result in low sample purity.

The main issue with all immunoaffinity CTC capture is the reliance on EpCAM and CK expression. There is great speculation that all CTC will not express these markers due to the varying phenotypes of cancer cells and the possible loss of expression during the cells’ transition from the primary tumor to the bloodstream. In addition, a major limitation of antibody-based methods is the processing time for successful capture[7]. The time between blood draw and capture may be enough to cause a change in phenotype of the cells that could lead to

misdiagnosis. Also, it may be difficult to reversibly remove anti-EpCAM tags from the CTC in order to do qualitative studies.

Filtration by Physical Properties

Another way of capturing CTC is to filter them by size. Because CTC are much larger than regular leukocytes and red blood cells, they can be distinguished on a size basis. One method called ISET (isolation by size of epithelial cells) works by filtering blood through a filtering membrane with pore sizes of 8 μm [10]. This method categorizes CTC as being larger than 16 μm, having an irregular nuclear contour and cytoplasm and a large nucleus [10]. When comparing ISET and CellSearch, it was found that CTC were detected in 80% of patients using ISET, and only 23% using CellSearch [23].

Devices using microfluidic channels have also been created for enriching CTC. Tan, Yobas, and the rest of the research group at the University of Singapore developed a channel with multiple crescent shaped arrays that are able to isolate CTC. The channels have gaps of 5 μm that allow red blood constituents and deformable white blood cells to flow through, while retaining the CTC[24]. This method has obtained a capture efficacy of over 80% and has successfully detected CTC in blood samples from metastatic lung cancer patients[24]. Expanding on this method, high aspect-ratio microchannels coupled with pinched flow dynamics have been used to isolate low abundance cells from diluted blood samples[25]. The inertial forces and high throughput used in the device to minimize clogging and maximize flow rate[25].

The method of detection that this research uses is a flexible micro spring array filter that can capture viable CTC while minimizing cell damage. The filter is composed of a single layer of patterned parylene membrane containing arrays of microspring structures[26]. Figure 5 shows microscopic images of the design. To create the filter, 10 μm thick parylene was deposited on a

silicon wafer using a CVD process and patterned with photolithography. The sidewalls of the parylene are vertical and gap width is controlled to the desired value. To assemble the filtering device, a custom-made holder keeps the filter in place while the blood sample flows through. When the gap width is small enough (4 μm), the capture efficacy can be up to 94%[26].

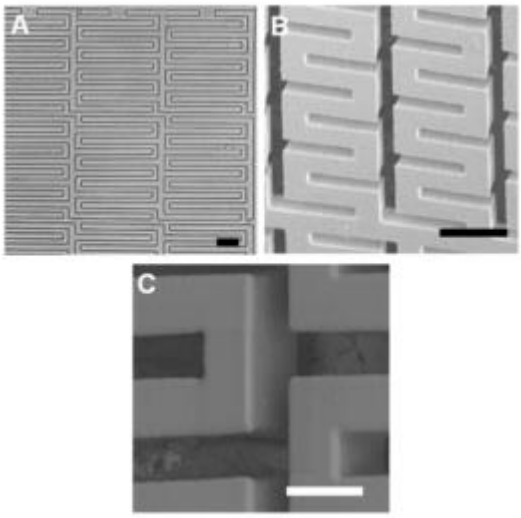


Figure 5: Microscopic Images of the FMSA Array
Image A is an optical microscopic image of the area while Images B and C are closer SEM images. The scale bar on images A and B represents 30 μm while the bar on C is 10 μm. Figure adapted from reference [26].

This method has the ability to capture CTC based on size and not the expression of EpCAM. The cells remain intact and viable during filtration with no anti-EpCAM microbeads attached, as opposed to the required fixation using other methods. Therefore, the filter allows for the study of the expression of EpCAM and of other biological markers. This study is especially valuable in cancer research due to the interest in the phenotypic differences between epithelial and mesenchymal cells.

Epithelial to Mesenchymal Transition

Some cells lack of EpCAM and CK expression is due to phenotypic differences between epithelial and mesenchymal cells. Epithelial cells phenotypically are less migratory and are associated with tissues that line the cavities of the body. Mesenchymal cells are more invasive and can rapidly change their properties to differentiate into a variety of cells. It has also been found that mesenchymal cells are more aggressive than the epithelial cells [27].

This change from epithelial to a mesenchymal phenotype, named the epithelial to mesenchymal transition(EMT), is of interest because it is presumed to occur during cancer metastasis [23] . Studies have shown that cells undergoing EMT are more resistant to chemotherapy and acquire stem cell like properties that may contribute to tumor growth[28]. The cells transform their phenotype in order to navigate from the primary tumor to the bloodstream. Epithelial cells lose their cell-cell adhesion, reorganize their cytoskeletons and have enhanced motility, as shown in Figure 6 [28]. Adherens and tight junctions as well as desmosomes are repressed and delocalized. The cadherin supporting molecule β-catenin often relocates from the membrane to the nucleus of the cell to participate in the signaling events involved in the transition[29]. EpCAM is downregulated to allow for this dissociation of the cells from the tumor, and CK is downregulated to increase the cells' plasticity and migration[30]. Expression of mesenchymal intermediate filament protein vimentin and the N-cadherin are then increased in the transformed cells[29].

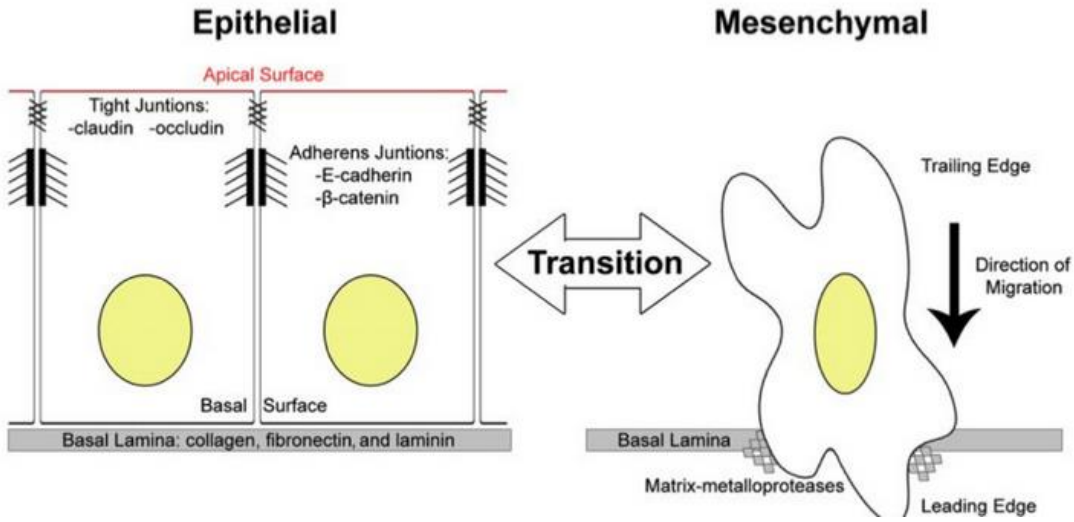


Figure 6: Epithelial to Mesenchymal Transition
In this figure, the transition from an epithelial to mesenchymal phenotype is shown. The left hand side shows an epithelial cell, containing both tight and adherens junctions. During the transition phenotypic changes occur, including the loss of these junctions, and the cell is able to travel through the body. Figure adapted from reference [31].

There is also evidence that the expression of cell markers continues to vary while in the blood stream and is sometimes inconsistent with its predicted phenotype. A study on breast cancer patients found that when treatment was performed, cells shifted towards an epithelial phenotype. However, inconsistent results shows that clusters of cells, as opposed to migratory, had a high expression of mesenchymal markers[32]. Inconsistencies are also predicted due to the ability of cancer cells to rapidly change their phenotype. The EMT is reversible, which could explain a cluster of cells still expressing vimentin if they recently established after traveling in circulation. A valuable prognostic tool would be if these inconsistences and tumor cell characterization could be linked to the patient's prognosis.

Through the EMT and the cells' loss of their expression of epithelial markers EpCAM and CK it may be impossible for CellSearch and other antibody-reliant methods to detect them. A study found that using this method, CTC were detected in only one third of patients with stage IV non-small cell lung cancer and in less than 5% in those with stage III [23]. The system was also

examined with breast cancer patients, and it was found that a higher proportion of metastatic patients with poor prognosis tested negative for CTC[27]. It is also likely that chemotherapy lowers the cells' expression of EpCAM, making it more difficult for antibody-based methods to detect CTC in patients that are undergoing treatment[30]. In order to investigate cells during this transition and those not expressing EpCAM or CK, other detection devices, such as the size-based filter, are necessary.

Research in detection of EpCAM and CK negative cells, although novel, has already been started by various research groups. Mikolajczyk and colleagues have used a unique microchannel with specifically designed posts to capture the CTC as the blood sample flows through[30]. A mixture of antibodies that may include, but is not limited to, EpCAM was employed and an enhanced staining method that can detect the captured cells regardless of their phenotype was used. They concluded that using a combination of antibodies was much more effective in capturing cells than by using EpCAM alone. This research group also acknowledged that it is still unclear whether the loss of CK and EpCAM expression is due to the EMT or due to independent oncogenic processes[30]. To make CTC capture and analysis a valuable tool in cancer treatment, cells undergoing this transition should be captured. Using the unique FMSA filter that is able to capture CTC regardless of EpCAM expression, examination of biological markers involved in this transition is possible.

Expression of Markers after Varying Times in Bloodstream

One hypothesis of this study is that the time spent in the bloodstream contributes to the EMT and therefore the representative epithelial and mesenchymal cell markers will vary their expression as they spend more time in circulation. This hypothesis has been supported by studies including one which found that gene expression changes very fast in CTC when they contact

endothelial surfaces or organ systems[33]. One gene expression change they found was the rapid downregulation of EpCAM. The study proposes that the reason for this could be the cleavage and shedding of the extracellular domain during the process. Cytokeratin was another marker that showed a significant decrease, although not as substantial as EpCAM[33]. While EpCAM and CK are downregulated, mesenchymal markers such as vimentin would then be expected to increase to assist in the EMT. In the study, 100% of the tested cells tested had a positive expression of vimentin. However, it may also be likely that it is not vimentin that regulates the transition to a mesenchymal cell and the expression will remain the same. A study has supported this by showing that EpCAM was indeed down-regulated while vimentin expression did not change [34]. Using the FMSA filter, it is possible to observe the expression level of these markers after cells have been in blood for various amounts of time and aim to confirm these results. To do so, cells can be suspended in blood samples for varying amounts of time before filtration.

The second hypothesis of this study is that the life of a cell in a collected blood sample is short and that this will affect the effectiveness of antibody-based methods of detection. For results from an antibody-based capture method to be used as an accurate representation of a patient's prognosis, the EpCAM expression levels should remain the same over the period of time that it takes to process the sample. The half-life of a CTC after surgical resection of the primary tumor has been previously estimated to be less than 24 hours[35]. Therefore, in order to make peripheral blood samples a viable testing method for a patient's prognosis, it is likely that the test should be completed within a few hours of blood draw.

In order to test these two hypotheses, this research focuses on what influence the chemical properties of blood have on the phenotype of the cell by study of the markers EpCAM, vimentin, and cytokeratin. The cells were analyzed for their levels of expression of each of these markers at various time points using fluorescent staining and image analysis.

Chapter 2

Methods

Fixation Techniques

This research project measured the expression of EpCAM, vimentin, and cytokeratin on the membrane of fixed circulating tumor cells. Fixation was necessary to prepare the samples for fluorescent staining and microscopy. Some forms of fixation permeablize the membrane using detergents such as Triton-X 100. Some methods also use formaldehyde, causing the proteins to alter their properties and to cross-link. It is possible that for either of these reasons, the fluorescent signal from the cell markers could be decreased. To achieve the best results, and to ensure that the strongest signal was retained, several different methods of fixation were tested.

NCI-H441 cells were seeded into a 96 well plate. The first solution tested was Formalin with 0.3% Triton X-100. This method includes permeablization. 100 µL of solution was applied to two wells for 10 minutes. The formalin is used to fix the cells while the Triton X permeablizes the membrane. The next method tested was 1:1 methanol acetone added for 20 minutes. 100 µL was applied to two wells for 20 minutes. Neutral formalin buffer solution and formalin in PBS were the final two combinations tested, again by adding 100 µL to two wells for 20 minutes.

The cells fixed by each of these methods were tested for the relative EpCAM staining intensity levels. To stain the cells for EpCAM, they were first blocked with 100 µL of goat block buffer for one hour. 100 µL of a 1:11 dilution of mouse anti-EpCAM FITC in goat buffer was then added for one hour. 100 µL of PBS was added for five minutes three separate times to wash the cells before they were ready for imaging. Cells from each of the two wells per method were imaged at 10x and a 500ms exposure using the FITC channel of the fluorescent microscope.

Relative intensity and size data was collected from five cells in each of the wells using ImageJ softeware and plotted. The data was analyzed to choose the method of fixation that retained the highest EpCAM intensity.

Antibody Optimization

Once the fixation protocol was optimized, the antibodies used to mark EpCAM, cytokeratin, vimentin, and CD45 were tested. The antibodies tested were mouse anti-epcam FITC (Green), mouse-anti CK (Primary), Goat anti-mouse (Near IR secondary) AF 750, mouse anti-CD45 AF 650 (Far Red) , and mouse-anti vimentin (Red).

Four cell lines were tested including NCI-H441, NCI-H661, MCF-7, and MDA-MB 231. NCI-H441 and NCI-H661 are lung cancer cell lines and MCF-7 and MDA-MB 231 are breast cancer lines. NCI-H441 is known to have a negative expression of vimentin, but a positive expression of EpCAM and CK. NCI-H661 has a more mesenchymal phenotype and therefore will positively express vimentin. MCF-7 has similar properties to NCI-H441, and MDA-MB 231 was used similar to NCI-H661.

Each of these cell lines were fixed in a 96 well plate with 100 µL of 1:1 methanol acetone for 20 minutes. They were washed using 100 µL of PBS. 100 µL of DAPI was used for 10 minutes to label the nuclei of the cells. The cells were blocked for one hour with 100 µL of goat block buffer. Block was removed and 50 µL of a 1:100 dilution of mouse anti-CK in goat block buffer was added for one hour. Samples were washed three times with PBS. A 50 µL sample of 1:100 dilution of goat anti-mouse AF 750 was added for one hour and then washed again three times. 100 µL of a 1:11 dilution of mouse anti-epcam FITC, a 1:100 dilution of mouse anti-CD45 AF 650, and a 1:200 dilution of mouse anti-vimentin were added for one hour. All dilutions were

in goat block buffer. Samples were washed three times and 100 µL of PBS was added before imaging.

Filtration and Time Points

After the antibodies were optimized, experiments using the filtration device with the flexible micro spring array (FMSA) filter were performed. The filters are flexible enough to allow red blood cells to pass through while they capture the tumor cells. First, 1500 NCI-H441 cells were each spiked into 6 tubes of 7.5 mL of healthy donor blood. Until needed, the cells were kept in RPMI-1640 media in an incubator and maintained by Merisa Nisic. The cells were passed and suspended in PBS within an hour before they were spiked. Two tubes were filtered immediately, two after 4 hours, and two after 24 hours. The samples that were filtered past the initial time point were kept in an incubator at 37°C until their filtration. A custom-made holder stabilized the filter as the blood flowed through. The filter remained in a PDMS gasket that is clamped between the holder. The blood flowed steadily, typically without pressure to ensure that the cells remained intact. Once the blood had flowed through the device, 5 mL of PBS was filtered through to wash the excess red blood cells off of the filter. If necessary, the washing was repeated until the filter was clear and free of red blood cells. Filtration was completed by Waleed Khan. A diagram of this part of the experimental procedure can be found in Figure 7.

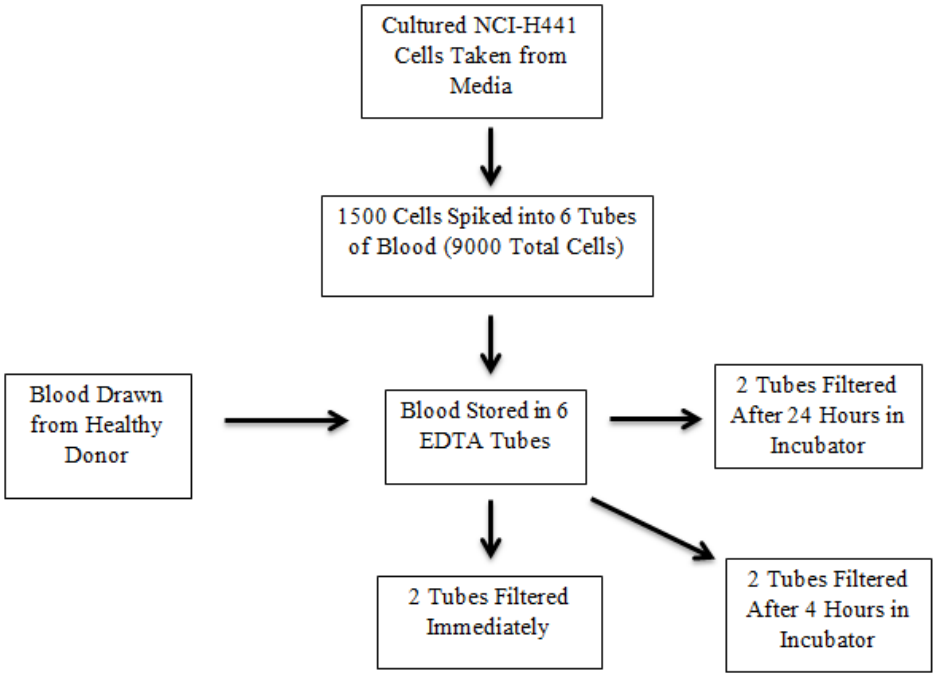


Figure 7: Diagram of Experimental Procedure
This figure shows a basic diagram of the experimental procedure. First the blood is drawn from a healthy donor. 6 tubes of blood were obtained, each containing 7.5 mL. Each tube was spiked with 1500 NCI-H441 cells taken from culture. 2 of these tubes were filtered immediately, 2 after 4 hours, and 2 after 24 hours.

Filter Fixing and Staining

The filters were fixed and stained with a similar protocol to the one developed when testing the antibodies. The fixation method used was 50 µL of ice cold 1:1 methanol acetone for 20 minutes. The cells were on filters originally contained in a PDMS gasket as opposed to the 96 well plate used previously. In addition, all volumes of solutions used on the filters were 50 µL instead of 100 of µL. Once staining was completed, the 6 filters were each transferred to a clean microscope slide and mounted using Life Technologies Prolong Antifade Gold.

Imaging and Analyzing Images

Imaging was performed using a 5-channel Olympus IX-17 inverted microscope and a connected camera. Using the QCapture Pro 7 software, images were taken of the cells' DAPI, EpCAM, vimentin, CD45, and cytokeratin expression. DAPI and EpCAM were imaged with an exposure time of 1.5 seconds. Vimentin was imaged with an exposure time of 100 milliseconds, CD45 with 2.5 seconds, and cytokeratin with 8 seconds. Approximately 60 cells per time point were imaged (30 per sample), all at 40x magnification. The images could be composited to view qualitative data, as shown in Figure 8.

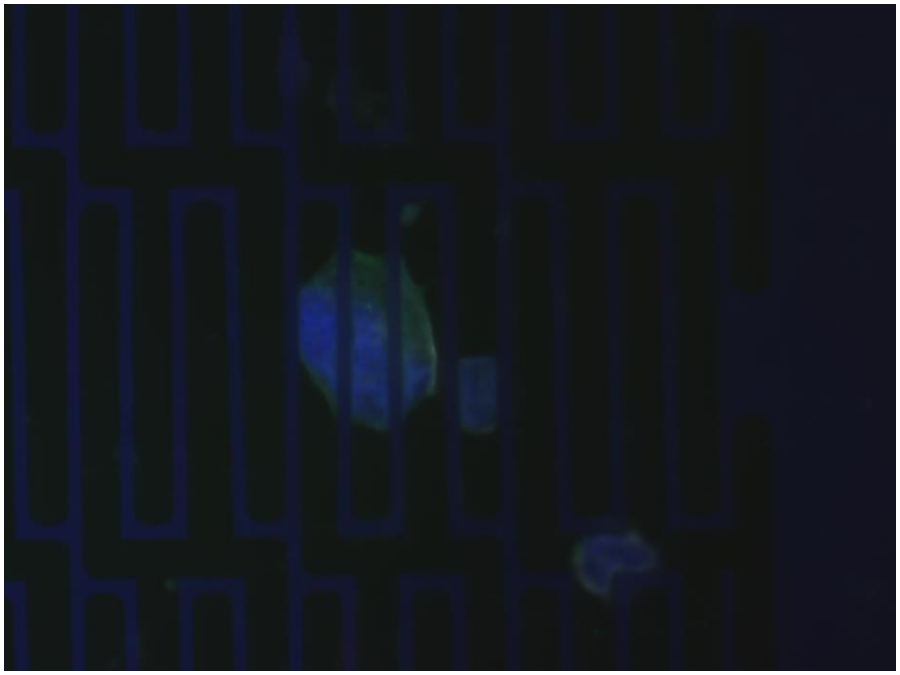


Figure 8: Composite Image of a CTC
In this overlay of images from different channels of the microscope, the blue tint represents DAPI and the green represents EpCAM. Although less visible, vimentin was stained red, CD45 was magenta, and cytokeratin remained white.

The images were analyzing using ImageJ software. Each cell was selected either by adjusting the threshold to select the cells or if necessary by using the freehand selection tool. The selections were first chosen on the DAPI images, and were consistently used on the images of the other surface markers by using the region of interest manager. Figures 9 and 10 demonstrate the region of interest manager and show two different images that have the same area selected in yellow.

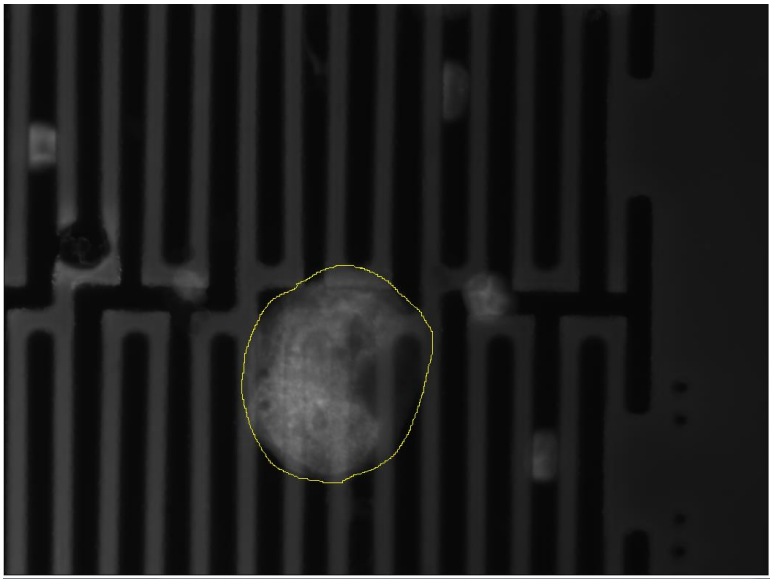


Figure 9: Image of DAPI Staining
The area selected in yellow was drawn using the freehand tool and represents the area that ImageJ will calculate the average gray value and size for.

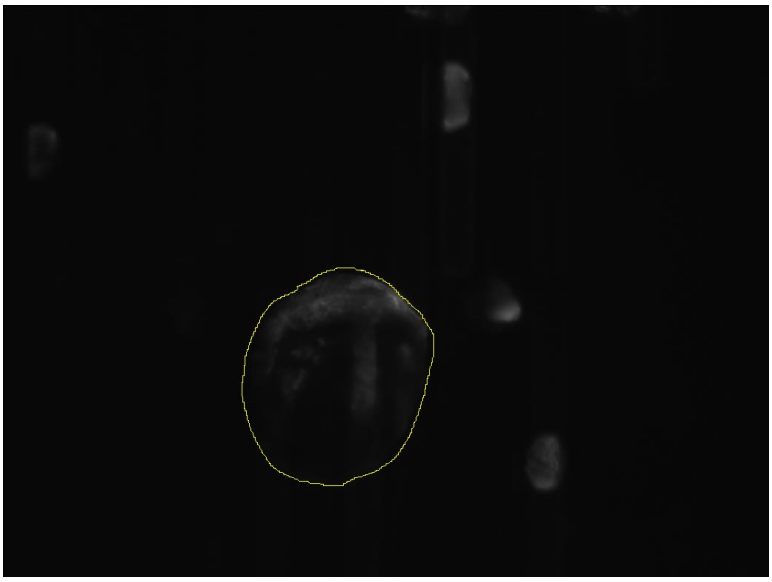


Figure 10: Image of CK Staining
This figure is an image of the CK staining and has the same area selected as Figure 9. The region of interest manager was used to maintain the area between the two images.

Data for the mean gray value and the area of the cells were collected from the selections on ImageJ. The gray value was calculated by summing the brightness of each pixel and dividing by the total number of pixels. This value corresponds to the signal received from the camera for each marker and therefore the average intensity of the staining.

Statistical Analysis

After data collection, tests for difference in means of size and staining intensity for each surface marker were performed. A two sample t-test for difference of means was performed for each variable between the data at 0 and 4 hours, 4 and 24 hours, and 0 and 24 hours. ANOVA testing was also used to compare the data from all three time points at once. Samples were grouped using the post hoc Tukey method. Using a 95% confidence interval, results were chosen to be significant with p values less than 0.05.

Chapter 3

Results

EpCAM Intensities of Fixation Methods

The first results obtained were the effects of various cell fixing methods on the expression of EpCAM on the surfaces of the cells. The intensity of the staining for EpCAM was examined for each of the four types of fixing including formalin with 0.3% Triton-X, 1:1 methanol acetone, neutral formalin buffer solution and formalin in PBS. Figure 11 below shows images of the cells from each type of fixation.

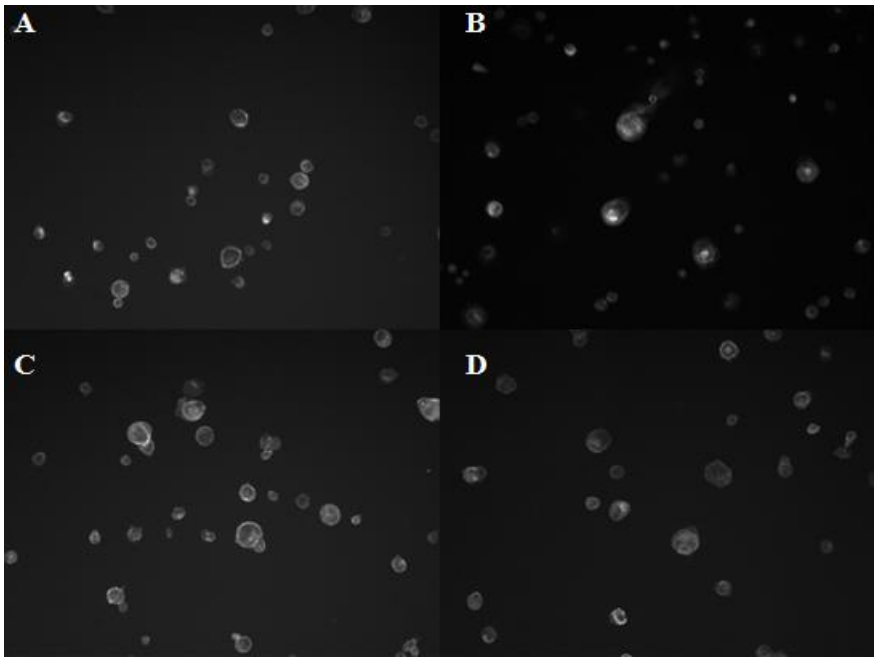


Figure 11: EpCAM Staining Results from Fixation with Formalin with Triton X (A), 1:1 Methanol Acetone (B), Formalin (Buffer) (C), and Formalin with PBS (D)
This figure shows the contrast between the different methods of fixation. The methanol acetone image shows the highest contrast between the background and the cells.

Table 1 below gives the data taken from image analysis with ImageJ software. 10 cells were analyzed for each method of fixing. The average cell size and EpCAM staining intensity is given. The intensity is in arbitrary units taken from ImageJ for relative comparison only.

Table 1: Staining Intensity Data for Fixing Techniques

Fixing Technique	Average Cell Size (μm^2) ± SD	Average Staining Intensity (arbitrary units) ± SD
Formalin with 10% Triton X	1031 ± 374	686 ± 54
1:1 methanol acetone	1028 ± 678	1307 ± 391
Neutral formalin buffer	911 ± 613	677 ± 66
Formalin in PBS	985 ± 405	798 ± 93

It was found that 1:1 methanol acetone had the highest average staining intensity at 1307, but also the largest standard deviation for both cell size and intensity. The individual data was graphed to observe the trends of staining intensity. Figure 12 shows the average intensity for each method of fixing based on size for the 10 cells examined.

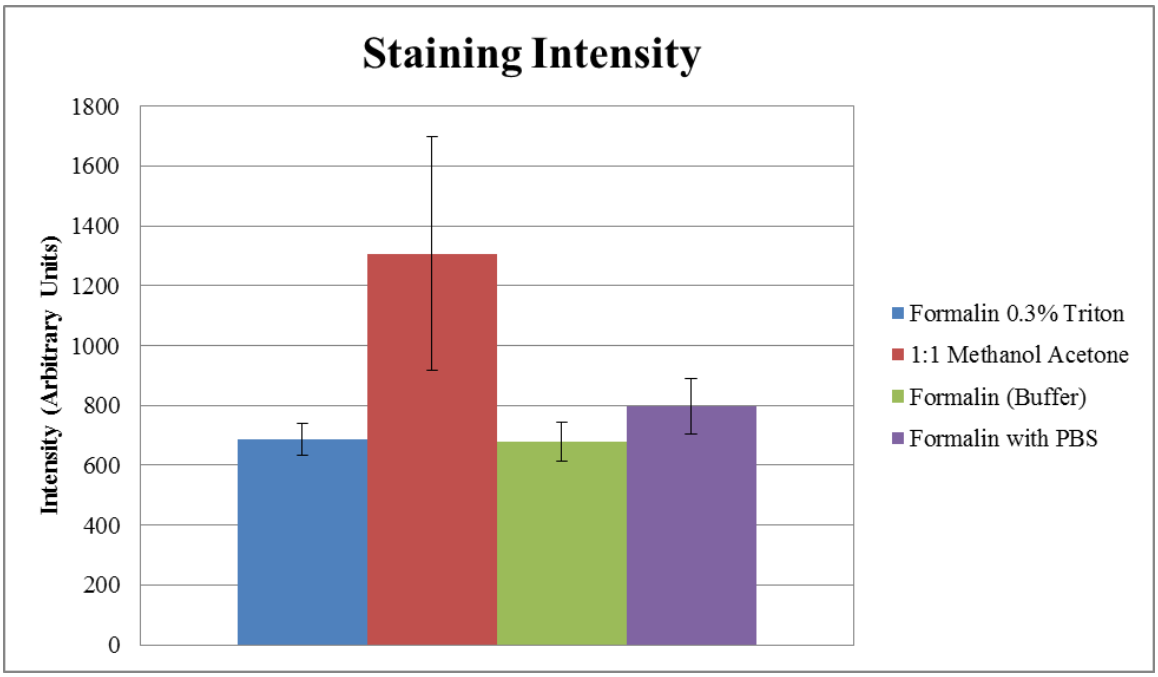


Figure 12: EpCAM Staining Intensity of Fixation Methods
This graph shows the average staining intensity for each method of fixation. Averages were based on 10 cells per method. The error bars on the graph represent the standard deviation for each sample. Methanol acetone had the highest intensity, but also the highest standard deviation.

Antibody Optimization

The figures below show the expression of all four cell lines for EpCAM, vimentin, and CK. Cell line NCI-H441 stained positive for CK and EpCAM, MCF-7 stained positive for CK, NCI-H661 stained positive for EpCAM and vimentin, and MDA-MB 231 stained positive for all markers. All cell lines tested negative for the leukocyte marker CD45 at an exposure time of 2.5 seconds.

Figure 13 shows the EpCAM images for each of the four cell lines. Cell lines other than MCF-7 tested positive for the marker. The images were all taken on the green channel at 500 milliseconds.

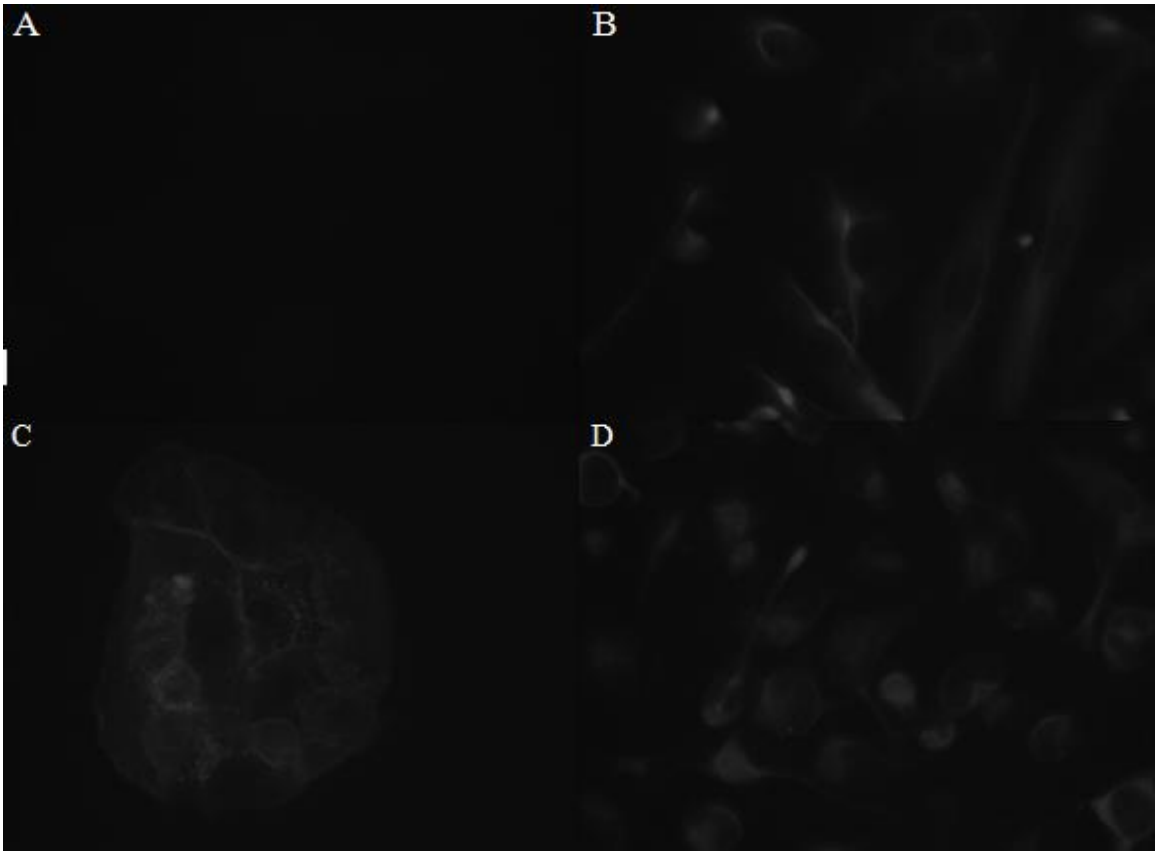


Figure 13: EpCAM Images for MCF-7 (A), MDA-MB 231 (B), NCI-H441 (C), and NCI-H661 (D)

This figure shows the images for the EpCAM staining of the four cell lines. All cell lines besides MCF-7 tested positive for the marker when imaging at a 500 millisecond exposure time.

The results for the staining of vimentin are shown in Figure 14. Cell lines NCI-H441 and MCF-7 tested negative for the marker while NCI-H661 and MDA-MB 231 tested positive. When analyzed in ImageJ, the stainings of the positive cells were over 10 times stronger than the intensity of the intensity of the negative cells. Images were taken on the red channel and all exposure times were kept consistent at 100 milliseconds.

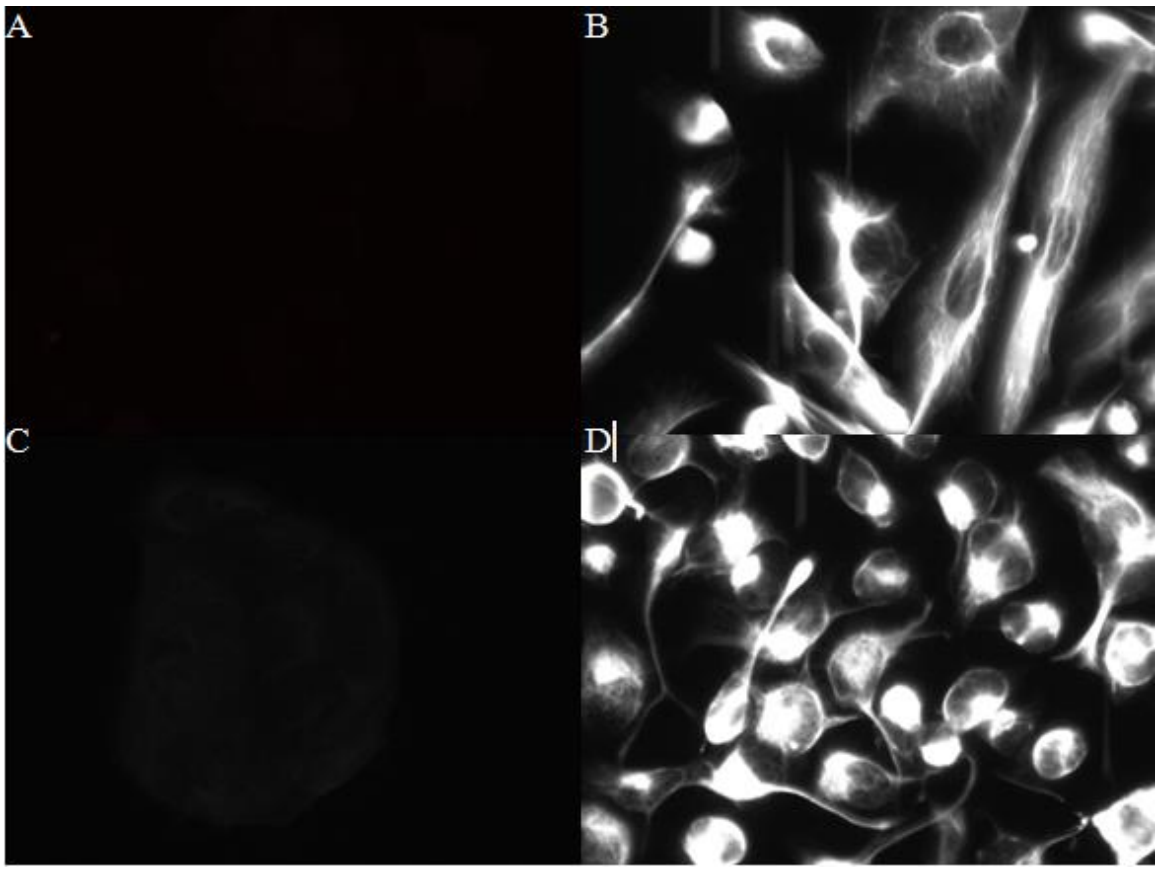


Figure 14: Vimentin Images for MCF-7 (A), MDA-MB 231 (B), NCI-H441 (C), and NCI-H661 (D)

This figure shows the images for the vimentin staining of the four cell lines. Cell lines MDA-MB 231 and NCI-H661 stained positive while MCF-7 and NCI-H441 stained negative when imaging at a 100 millisecond exposure time.

Images were also collected for CK expression and are shown below in Figure 15.The cells were positive for the marker other than the NCI-H661, which stained negative. Images were taken on the near-IR channel with an exposure time of 8 seconds.

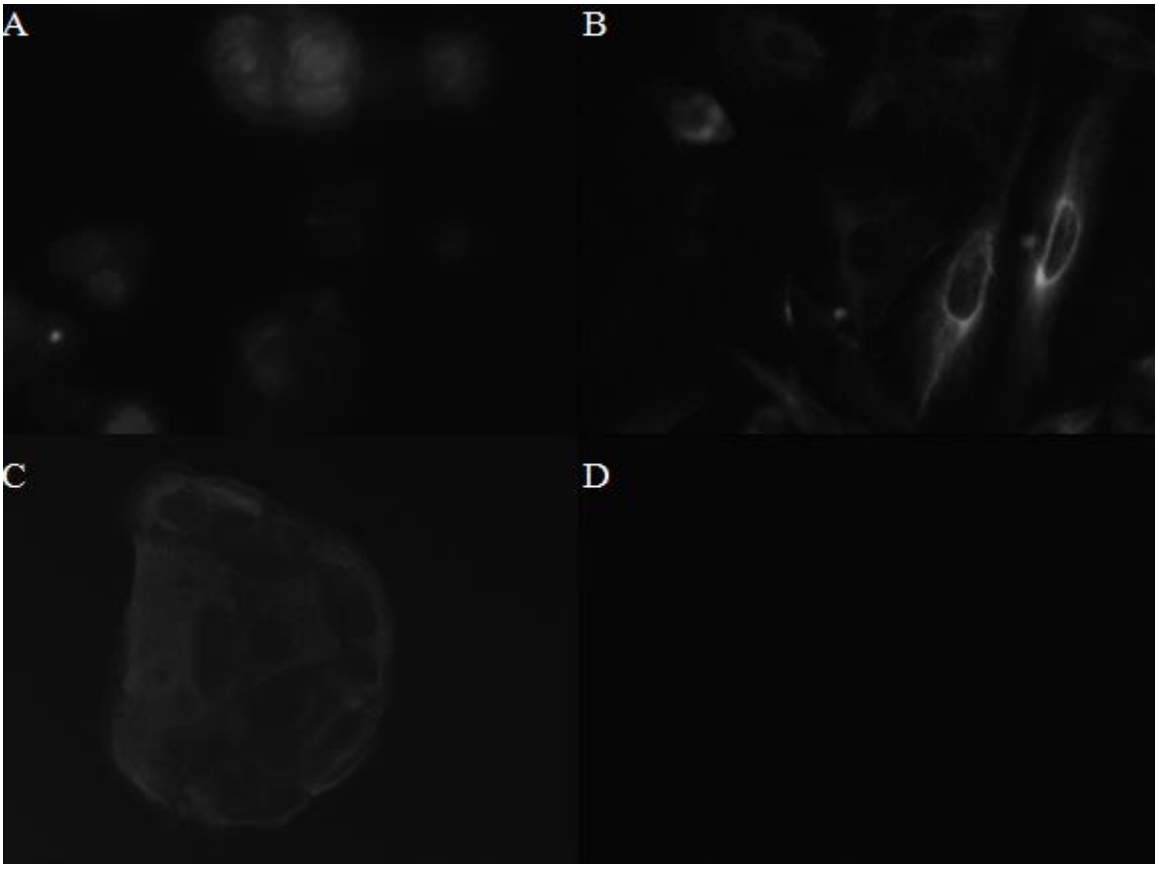


Figure 15: CK Images for MCF-7 (A), MDA-MB 231 (B), NCI-H441 (C), and NCI-H661 (D)

This figure shows the images for the CK staining of the four cell lines. All cell lines besides NCI-H661 tested positive for the marker when imaging at an 8 second exposure time.

Expression of EpCAM, Vimentin, and CK

Intensity data was collected for biological markers including EpCAM, vimentin, and cytokeratin. DAPI images were used to collect nuclear sizes. CD45 expression was confirmed as negative on all CTC. Table 2 below summarizes the results for the average nuclear size and number of cells that were analyzed for each filtration time.

Table 2: Number of Cells Analyzed and Average Cell Size for Each Time Point

Filtration Time Point	Number of Cells Analyzed	Average Nuclear Size (μm^2) ± SD
0 hours	58	578 ± 671
4 hours	61	773 ± 787
24 hours	49	949 ± 822

It was found that the size of the nuclei of the cells significantly increases on average from 587 to 949 μm when the time point changes from 0 to 24 hours ($p=0.002$). The increases from 578 to 773 μm and from 773 to 949 μm (when the time point changed from 0 to 4 hours and from 4 to 24 hours) were not significant.

Table 3 summarizes the intensity of the staining for the images of EpCAM, vimentin, and CK. The details of each cell marker's staining are in the sections below. The complete statistical analysis can be found in Appendix A.

Table 3: Staining Intensity for EpCAM, Vimentin, and CK

Filtration Time Point	Average EpCAM Intensity (arbitrary units) ± SD	Average Vimentin Intensity (arbitrary units) ± SD	Average CK Intensity (arbitrary units) ± SD
0 hours (n=58)	31.6 ± 13.3	9.9 ± 6.9	19.4 ± 11.9
4 hours (n=61)	25.3 ± 7.5	6.8 ± 0.9	10.6 ± 4.7
24 hours (n=49)	24.2 ± 8.3	7.3 ± 1.8	10.5 ± 4.2

EpCAM Expression

It was found that the EpCAM expression decreased significantly from 31.6 to 25.3 when the filtration time changed from 0 to 4 hours (p=0.002). The difference between 0 and 24 hours, a decrease from 31.6 to 24.2, was also significant (p=0.001). When the filtration time varied from 4 hours to 24, the decrease was only from 25.3 to 24.2 and therefore was not significant. The distribution of the cell's staining intensities at each time point is shown in Figure 16.

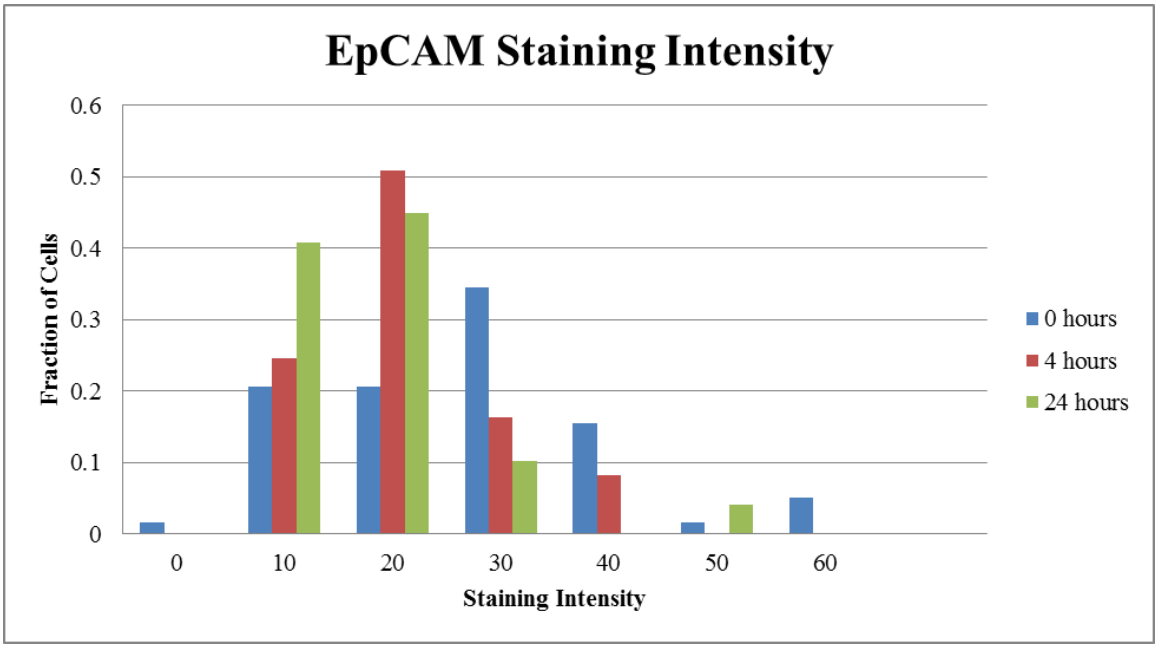


Figure 16: Fraction of Cells with Varying EpCAM Staining Intensities
This figure shows the fraction of the cells that had varying staining intensities. It can be seen that the staining intensities at the 4 and 24 hour time points are more commonly in the lower range than at the 0 hour time point.

Vimentin Expression

The vimentin expression when filtration time was changed from 0 to 4 hours was shown to significantly decrease from 9.9 to 6.8 ($p=0.002$). From 0 to 24 hours, the decrease was from 9.9 to 7.3 was still significant, but less than the decrease from 0 to 4 hours ($p=0.009$). The increase from 4 hours to 24 hours was not significant ($p=0.088$). The distribution of the cell's staining intensities at each time point is shown in Figure 17.

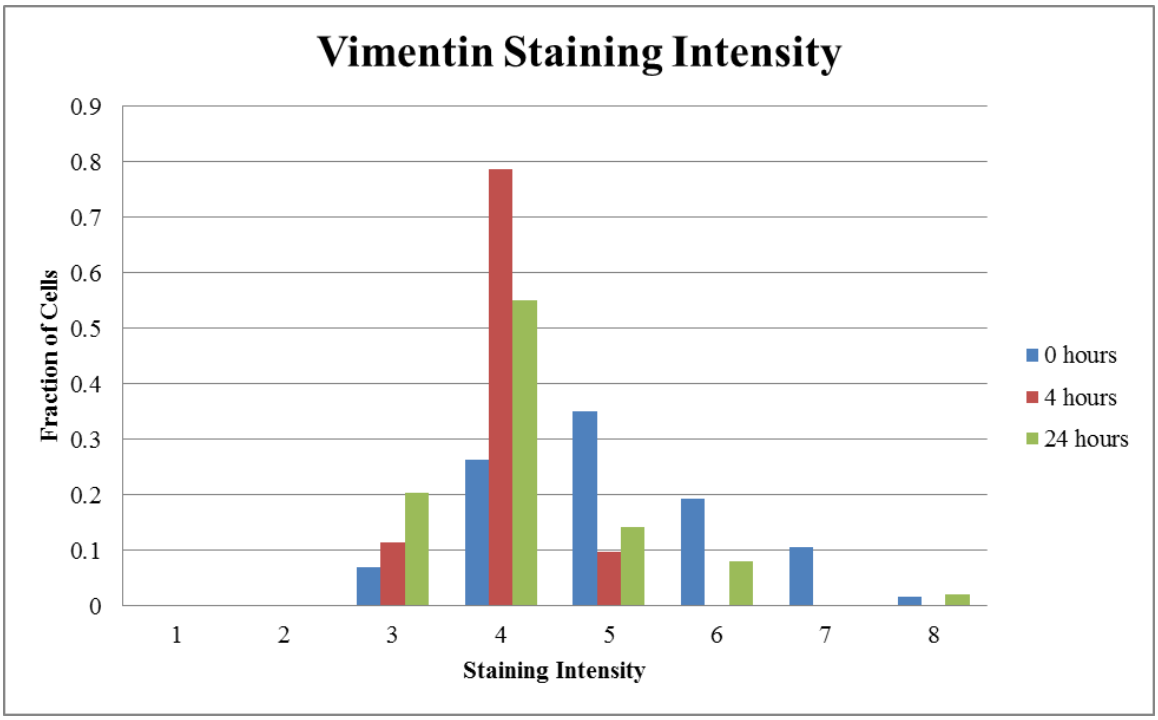


Figure 17: Fraction of Cells with Varying Vimentin Intensities
This figure shows the fraction of the cells that had varying staining intensities. It can be seen that the staining intensities at the 4 and 24 hour time points are more commonly in the lower range than at the 0 hour time point.

Cytokeratin Expression

When the filtration time was changed both from 0 to 4 and from 0 to 24 hours the CK staining intensity was shown to significantly decrease from 19.4 to 10.6 and to 10.5(p=0.000). From 4 to 24 hours, the mean intensity remained virtually the same, varying only from 10.6 to 10.5. The distribution of the cell's staining intensities at each time point is shown in Figure 18.

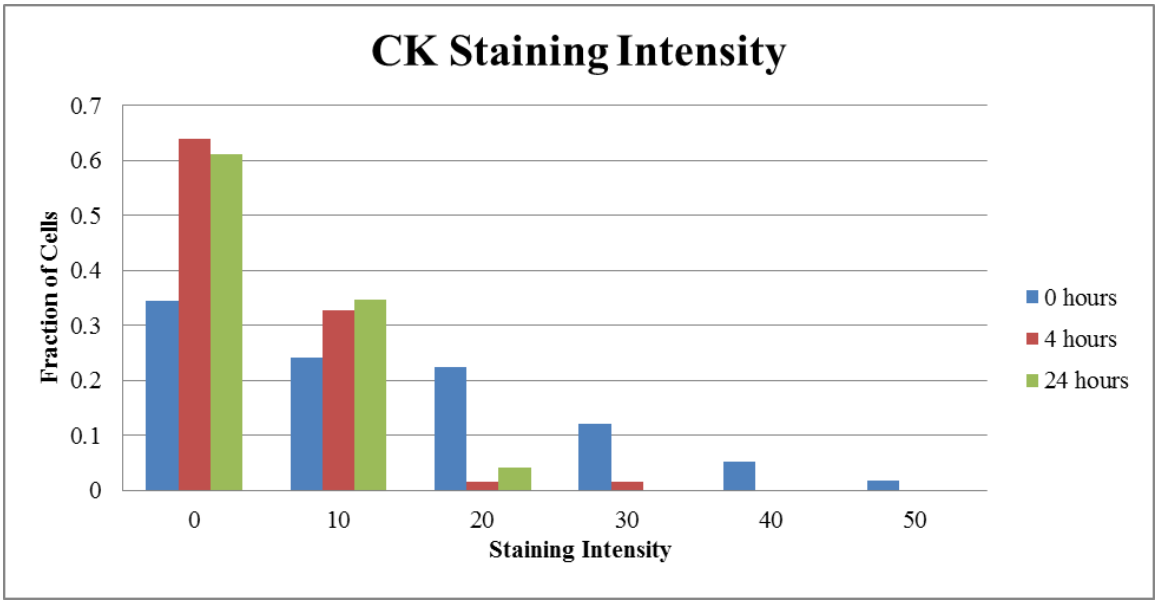


Figure 18: Fraction of Cells with Varying CK Staining Intensities
This figure shows the fraction of the cells that had varying staining intensities. It can be seen that the staining intensities at the 4 and 24 hour time points are more commonly in the lower range than at the 0 hour time point**.**

Chapter 4

Discussion

The goal of this study was to examine how a cell's time spent in blood affects the expression of biological markers. To do so, a fixation method that was found to have the greatest signal to noise ratio was chosen. After examining the results from the fixation methods, it was determined that the 1:1 methanol acetone fixation retained the highest EpCAM signal. The graph in Figure 15 clearly shows the higher intensity trend for this type of fixation. The higher level of contrast between the cells and the background for the methanol acetone can also be observed in Figure 12. The exact reason that this method had the highest signal is not known, but it may be due to the lack of permeabilization by use of a detergent. Although results from previous studies have varied, there have been a few that have agreed with these results. One study done by Louis Weiswald and his colleagues found that permeabilization using a detergent was much less successful at achieving a strong and uniform staining than dehydration of the membrane using an alcohol[36]. They presumed that one possible reason could be that the methanol acetone mixture was more effective dissolvent, and allowed the antibodies to more easily penetrate the membrane. Another hypothesis stated that the detergent causes the antigens to delocalize during permeabilization, causing brighter staining in some areas and darker in others.

The images taken for each of the four cell lines to optimize the antibodies show that they can successfully bind to the cells and their expression agreed with predictions. All cell lines were expected to be positive for both EpCAM and CK, while only mesenchymal cell lines NCI-H661 and NCI-H441 were expected to be positive for vimentin. In general, results agreed with this, but due to cell variation the levels of EpCAM and CK expression varied. NCI-H661 was found to be

negative for CK, which disagreed with the expected results. A possible reason for this difference could be due to the fact that this cell line expresses a high level of vimentin and has a very mesenchymal phenotype.

EpCAM signals were positive, but weak. MCF-7 showed to be negative despite predictions. When studying EpCAM expression in the later experiment, exposure time was increased from 500 milliseconds to 1.5 seconds to increase the signal and improve results. Overall, the antibodies were found to be successful in correctly depicting the expression of the desired surface markers.

The results of the staining intensity for EpCAM, vimentin, and CK in general agreed with the hypotheses. According to the hypothesis that the cells may undergo EMT as they are suspended in blood, the expression of EpCAM and CK was predicted to decrease, while the expression of vimentin was expected increase or remain the same due to the cells changing phenotype from epithelial to mesenchymal. The values of EpCAM and CK did decrease significantly from 0 to 4 hours, providing possible evidence that the EMT is still occurring while the cells are in circulation. However, it was expected that the vimentin expression would increase or remain the same, which it did not.

Variation in experimental data and predictions could be due to the limitations of the imaging. The camera has a very high resolution and can focus to 1 μm; however there is still a minimal amount of inconsistency due to this variable. It is also extremely difficult to analyze in ImageJ only within the exact confines of the cell. If areas of the cell are darker and do not appear in the image, ImageJ will not contribute this to the size of the cell or the average staining intensity. The expression of vimentin is typically much lower than EpCAM and CK in the cell line examined, which could have caused the inherent error of imaging to be more significant. If performing this experiment again, the exposure time of the camera could be increased to create a stronger signal for comparison reasons. It is also possible that if the decrease in vimentin

expression is not due to error, it could be attributed to the cells losing a portion of all of their marker expression when kept in a tube of blood. This agrees with the second hypothesis that the cells may not survive in blood for a significant amount of time without the attachment to or the presence of a primary tumor. Additional research would need to be performed to conclude if expression does in fact decrease.

Although the expression of all three markers significantly decreased after spending only 4 hours in blood, the expression levels did not vary significantly from 4 to 24 hours. This suggests that cells could change their phenotype in blood quickly initially, which agrees with the previous studies. It also appears as if there may be a steady state expression level and a specific time after the cells enter circulation at which this is reached. This research shows that because the phenotypes are altered significantly within the first 4 hours, it is ideal that methods of detection capture cells at least within that time frame. To test this hypothesis and to find a possible plateau time, more data points between 0 and 4 hours should be gathered. That time would be the goal for maximum cell capture process time in order to achieve accurate results.

Another interesting result was that the size of the nuclei of the cells increased significantly from 0 to 24 hours. According to the hypothesis that the cells cannot survive in blood for an extended period of time without a primary tumor, the size of the nucleus might be expected to decrease over time. If the cell is dying, it may be presumed that the nuclear information would be degrading or shrinking. This result also disagreed with initial testing in which the nuclear size decreased over time. However, it is likely that the DNA is simply spreading over a larger area due to the pressure of the blood without growing. One contributing factor to this data could be the error due to the difficulty of filtration due to the age of the blood. Freshly drawn blood flows easier through the filter, reducing the pressure on the cells and keeping them rounder and more compact. The cells that were suspended in blood for 24 hours likely required more pressure to flow through the filter, flattening and expanding them. Research

when testing the filter used in this experiment showed that blood kept in an EDTA tube should be filtered within 4 hours in order to keep the pressure low enough to maintain cell viability[26]. Because the older blood was also thicker, it may have required more washes with PBS that could have damaged the cells. Further studies on this hypothesis could include viability assays at each time point and a comparison to the nuclear size. These studies would also be useful in determining if the reason the expression levels are decreasing is due to the EMT or to the life of the cell.

Although some data disagreed with predictions, it is clear that alterations in the cell's phenotype are occurring during the time spent in the blood. It is very likely that the transition happens rapidly showing that viable cell capture without fixation is necessary to achieve ideal results and accurate correlations to patients' prognoses.

Chapter 5

Conclusion

After performing this research, it can be presumed that the time CTC spend in blood has an effect on their phenotype and therefore may negatively affect the ability of detection methods to capture them. It was found that all three cellular markers, EpCAM, vimetin, and CK decreased over time spent in blood suggesting that the cells may not survive for extended periods of time. The decrease happened in the first 4 hours of suspension, and expression remained similar between 4 and 24 hours. These results show that there may be a point at which expression plateaus, and this time point could be valuable for setting a maximum process time for capture methods. Further testing should analyze data points between 0 and 4 hours to determine this point.

This research also provides evidence that capture methods that maintain the cells' viability would be an ideal way of determining the patient's prognosis and best method of treatment. Future possibilities could include finding the relationship, if any, between EpCAM, vimentin, or CK expression and the status of the patient's disease. It would also be a great diagnostic leap if cells' transitions and phenotypes could be examined in real time. Because the EMT may be directly associated with cancer metastasis, observing the live transition could provide a target for therapeutic drugs and prevent late stage cancers from occurring.

APPENDICES

Appendix A- Statistical Analysis

Cytokeratin

Two-Sample T-Test and CI: CK 0hr, CK 4hr

```
Two-sample T for CK 0hr vs CK 4hr

         N   Mean  StDev  SE Mean
CK 0hr  58   19.4   11.9      1.6
CK 4hr  61  10.55   4.75     0.61


Difference = mu (CK 0hr) - mu (CK 4hr)
Estimate for difference:  8.81
95% CI for difference:  (5.48, 12.14)
T-Test of difference = 0 (vs not =): T-Value = 5.27  P-Value = 0.000  DF = 74
```

Two-Sample T-Test and CI: CK 0hr, CK 24hr

```
Two-sample T for CK 0hr vs CK 24hr

          N   Mean  StDev  SE Mean
CK 0hr   58   19.4   11.9      1.6
CK 24hr  49  10.50   4.27     0.61


Difference = mu (CK 0hr) - mu (CK 24hr)
Estimate for difference:  8.86
95% CI for difference:  (5.53, 12.19)
T-Test of difference = 0 (vs not =): T-Value = 5.30  P-Value = 0.000  DF = 73
```

Two-Sample T-Test and CI: CK 4hr, CK 24hr

```
Two-sample T for CK 4hr vs CK 24hr

          N   Mean  StDev  SE Mean
CK 4hr   61  10.55   4.75     0.61
CK 24hr  49  10.50   4.27     0.61


Difference = mu (CK 4hr) - mu (CK 24hr)
Estimate for difference:  0.052
95% CI for difference:  (-1.656, 1.759)
T-Test of difference = 0 (vs not =): T-Value = 0.06  P-Value = 0.952  DF = 106
```

One-way ANOVA: CK 0hr, CK 4hr, CK 24hr

```
Source   DF       SS      MS      F      P
Factor    2   2962.3  1481.1  23.88  0.000
Error   165  10234.9    62.0
Total   167  13197.2

S = 7.876   R-Sq = 22.45%   R-Sq(adj) = 21.51%


                            Individual 95% CIs For Mean Based on
                            Pooled StDev
Level     N    Mean   StDev  ------+---------+---------+---------+---
CK 0hr   58  19.361  11.852                          (-----*-----)
CK 4hr   61  10.553   4.750  (-----*-----)
CK 24hr  49  10.501   4.269  (-----*-----)
                            ------+---------+---------+---------+---
                               10.5      14.0      17.5      21.0

Pooled StDev = 7.876


Grouping Information Using Tukey Method

          N    Mean  Grouping
CK 0hr   58  19.361  A
CK 4hr   61  10.553    B
CK 24hr  49  10.501    B

Means that do not share a letter are significantly different.


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons

Individual confidence level = 98.06%


CK 0hr subtracted from:

          Lower  Center   Upper  -----+---------+---------+---------+----
CK 4hr   -12.220  -8.809  -5.398   (-----*------)
CK 24hr  -12.470  -8.861  -5.251  (------*------)
                                 -----+---------+---------+---------+----
                                  -10.0      -5.0       0.0       5.0


CK 4hr subtracted from:

          Lower  Center  Upper  -----+---------+---------+---------+----
CK 24hr  -3.620  -0.052  3.517                   (------*------)
                                 -----+---------+---------+---------+----
                                  -10.0      -5.0       0.0       5.0
```

EpCAM

Two-Sample T-Test and CI: EpCAM 0hr, EpCAM 4hr

```
Two-sample T for EpCAM 0hr vs EpCAM 4hr

            N   Mean  StDev  SE Mean
EpCAM 0hr  58   31.6   13.3      1.7
EpCAM 4hr  61  25.31   7.54     0.97


Difference = mu (EpCAM 0hr) - mu (EpCAM 4hr)
Estimate for difference:  6.30
95% CI for difference:  (2.33, 10.27)
T-Test of difference = 0 (vs not =): T-Value = 3.16  P-Value = 0.002  DF = 89
```

Two-Sample T-Test and CI: EpCAM 0hr, EpCAM 24hr

```
Two-sample T for EpCAM 0hr vs EpCAM 24hr

             N   Mean  StDev  SE Mean
EpCAM 0hr   58   31.6   13.3      1.7
EpCAM 24hr  49  24.23   8.27      1.2


Difference = mu (EpCAM 0hr) - mu (EpCAM 24hr)
Estimate for difference:  7.38
95% CI for difference:  (3.19, 11.57)
T-Test of difference = 0 (vs not =): T-Value = 3.50  P-Value = 0.001  DF = 96
```

Two-Sample T-Test and CI: EpCAM 4hr, EpCAM 24hr

```
Two-sample T for EpCAM 4hr vs EpCAM 24hr

             N   Mean  StDev  SE Mean
EpCAM 4hr   61  25.31   7.54     0.97
EpCAM 24hr  49  24.23   8.27      1.2


Difference = mu (EpCAM 4hr) - mu (EpCAM 24hr)
Estimate for difference:  1.08
95% CI for difference:  (-1.95, 4.11)
T-Test of difference = 0 (vs not =): T-Value = 0.71  P-Value = 0.482  DF = 98
```

One-way ANOVA: EpCAM 0hr, EpCAM 4hr, EpCAM 24hr

```
Source   DF     SS   MS     F      P
Factor    2   1779  889  8.73  0.000
Error   165  16801  102
Total   167  18580

S = 10.09   R-Sq = 9.57%   R-Sq(adj) = 8.48%


                                Individual 95% CIs For Mean Based on
                                Pooled StDev
Level        N   Mean  StDev  ---------+---------+---------+---------+
EpCAM 0hr   58  31.61  13.31                       (------*-------)
EpCAM 4hr   61  25.31   7.54      (------*-------)
EpCAM 24hr  49  24.23   8.27  (-------*-------)
                                ---------+---------+---------+---------+
                                      24.5      28.0      31.5      35.0

Pooled StDev = 10.09

Grouping Information Using Tukey Method

             N   Mean  Grouping
EpCAM 0hr   58  31.61  A
EpCAM 4hr   61  25.31    B
EpCAM 24hr  49  24.23    B

Means that do not share a letter are significantly different.


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons

Individual confidence level = 98.06%


EpCAM 0hr subtracted from:

             Lower  Center  Upper  ----+---------+---------+---------+-----
EpCAM 4hr   -10.67   -6.30  -1.93      (-------*--------)
EpCAM 24hr  -12.00   -7.38  -2.76  (--------*--------)
                                   ----+---------+---------+---------+-----
                                   -10.0      -5.0       0.0       5.0


EpCAM 4hr subtracted from:

            Lower  Center  Upper  ----+---------+---------+---------+-----
EpCAM 24hr  -5.65   -1.08   3.49               (--------*--------)
                                   ----+---------+---------+---------+-----
                                   -10.0      -5.0       0.0       5.0
```

Vimentin

Two-Sample T-Test and CI: Vimentin 0hr, Vimentin 4hr

```
Two-sample T for Vimentin 0hr vs Vimentin 4hr

               N   Mean  StDev  SE Mean
Vimentin 0hr  58   9.86   6.87     0.90
Vimentin 4hr  61  6.834  0.914     0.12


Difference = mu (Vimentin 0hr) - mu (Vimentin 4hr)
Estimate for difference:  3.024
95% CI for difference:  (1.204, 4.845)
T-Test of difference = 0 (vs not =): T-Value = 3.33  P-Value = 0.002  DF = 58
```

Two-Sample T-Test and CI: Vimentin 0hr, Vimentin 24hr

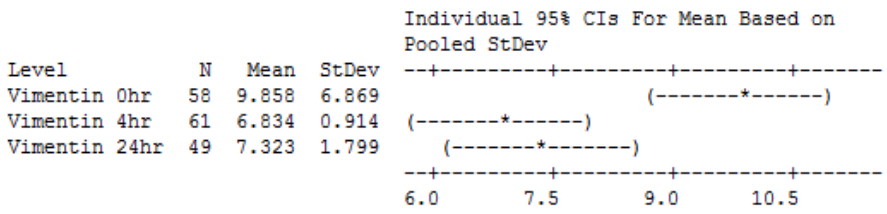
```
Two-sample T for Vimentin 0hr vs Vimentin 24hr

               N  Mean  StDev  SE Mean
Vimentin 0hr   58  9.86   6.87     0.90
Vimentin 24hr  49  7.32   1.80     0.26


Difference = mu (Vimentin 0hr) - mu (Vimentin 24hr)
Estimate for difference:  2.535
95% CI for difference:  (0.662, 4.407)
T-Test of difference = 0 (vs not =): T-Value = 2.70  P-Value = 0.009  DF = 66
```

Two-Sample T-Test and CI: Vimentin 4hr, Vimentin 24hr

```
Two-sample T for Vimentin 4hr vs Vimentin 24hr

               N   Mean  StDev  SE Mean
Vimentin 4hr   61  6.834  0.914     0.12
Vimentin 24hr  49   7.32   1.80     0.26


Difference = mu (Vimentin 4hr) - mu (Vimentin 24hr)
Estimate for difference:  -0.489
95% CI for difference:  (-1.053, 0.074)
T-Test of difference = 0 (vs not =): T-Value = -1.73  P-Value = 0.088  DF = 67
```

One-way ANOVA: Vimentin 0hr, Vimentin 4hr, Vimentin 24hr

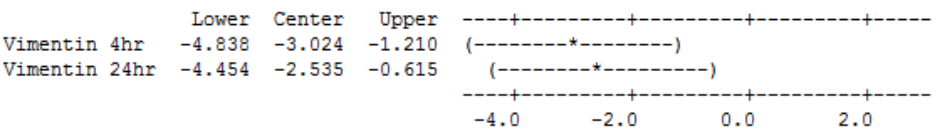
```
Source   DF      SS     MS     F      P
Factor    2   305.6  152.8  8.71  0.000
Error   165  2894.8   17.5
Total   167  3200.3

S = 4.189   R-Sq = 9.55%   R-Sq(adj) = 8.45%
```



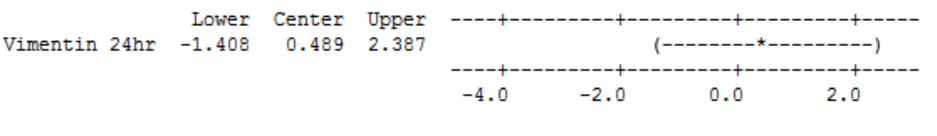

```
Pooled StDev = 4.189
```

```
Grouping Information Using Tukey Method

               N   Mean  Grouping
Vimentin 0hr   58  9.858  A
Vimentin 24hr  49  7.323    B
Vimentin 4hr   61  6.834    B

Means that do not share a letter are significantly different.
```

```
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons

Individual confidence level = 98.06%

Vimentin 0hr subtracted from:

               Lower  Center   Upper
Vimentin 4hr   -4.838  -3.024  -1.210
Vimentin 24hr  -4.454  -2.535  -0.615
```




```
Vimentin 4hr subtracted from:

               Lower  Center  Upper
Vimentin 24hr  -1.408   0.489  2.387
```



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ACADEMIC VITA

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EDUCATION

B.S., Bioengineering
Option: Material Science
Schreyer Honors College
The Pennsylvania State University
University Park, PA
Anticipated Graduation: May 2014

Elective Coursework:
Tissue Engineering
Biomaterials
Biological Response to Surfaces

EXPERIENCE

Undergraduate Research Assistant Fall 2012-Current
Micro and Nano Integrated Biosystems Lab

- Performing research on the filtration and detection of circulating tumor cells from blood samples
- Developing skills in cell culture and immunofluorescence
- Achieving familiarity with sterile lab techniques
- Using statistical analysis including T-Tests and ANOVA

Estimator and Office Assistant
National Glass and Metal Co., Inc. Summers 2010-2012

- Estimated costs of materials and constructed proposals for approximately 40 projects
- Communicated with clients for specific project needs
- Created Microsoft Excel libraries of past projects to be used for reference

ACTIVITIES

Penn State Ski Club
President Fall 2013-Current

- Responsible for holding monthly meetings, coordinating trips with a travel agent and team of officers, corresponding with club members, and delegating tasks

Promotions Chair Fall 2012-Spring 2013

- Recruited approximately 300 members through information tables, fliers, and a presence at involvement fairs

Penn State THON Rules and Regulations Committee Fall 2012-Spring 2013

- Committee involved in THON, a 46 hour dance marathon benefitting children with pediatric cancer
- Responsible for managing access to the dance floor and enforcing rules during the marathon

SKILLS

Minitab SolidWorks ImageJ
COMSOL MATLAB