Circulating Cancer Cell Collection Device and Analyzing Student and Faculty Perceptions of Writing in the BME

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Abstract

Neuroblastoma is a deadly childhood cancer which accounts for 15% of all pediatric cancer deaths [1]. This cancer is especially hard to treat as it frequently metastasizes to locations such as bone, liver, and lymph nodes [2]. Neuroblastoma has been modeled by the use of perfusion-based bioreactors in the Coburn Functional Biomaterials Lab to study the progression of the tumor in various environments. However, capturing the populating metastatic or metastatic like cells to study remains a challenge. There are a few devices on the market which attempt to solve this challenge, but even fewer that work for research and modeling purposes. The goal for this project was to create a cell collection device that can capture non-anchored or metastatic like cancer cells from circulation within a small scale perfusion based bioreactor. The team created a system which utilized pluriStrainer mesh cell strainers inserted into filter holders which were in line with the bioreactor system. The filters were able to collect and begin to separate cell types within circulation. A microfluidic device was also proposed by the team to use in conjunction with this device for further cell separation.

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I. Introduction

Neuroblastoma is a pediatric cancer that accounts for 8% of all childhood cancers and 15% of pediatric cancer deaths [1]. Neuroblastoma appears early in development, often in embryos or shortly after birth [1]. The cancer is derived from cells in the neural crest, a grouping of cells which develop in the embryo and differentiate into a range of cell types in numerous parts of the body including in the peripheral nervous system and some smooth muscle and connective tissues. [1]. Primary neuroblastoma tumors can be located anywhere in the sympathetic nervous system, but are most commonly found on the adrenal glands and in the abdomen [2]. Patients with neuroblastoma can display a variety of symptoms, often directly related to the location of the tumors.

It is likely that high-risk neuroblastoma patients will have a recurrence of the cancer within the first five years after initial treatment. The risk level of neuroblastoma (determined on a scale of 1 to 4) is dependent on factors including the patient's age, location of the tumor, when the cancer was diagnosed, and whether or not the cancer has metastasized [2]. Metastatic neuroblastoma is especially difficult to treat, requiring multimodal therapy in forms such as chemotherapy, radiation therapy, immunotherapy, and other additional methods of treatment [3]. Approximately 70% of all patients diagnosed with neuroblastoma develop metastasis [2]. Metastasis is defined by cancer cells detaching from the primary tumor and forming a secondary tumor in a downstream location. Circulating tumor cells (CTCs) migrate through either the lymphatic system or the bloodstream, most often metastasizing to bone [2].

Multiple *in-vitro* 3D models of metastasis have been developed to better understand the progression of the disease. Perfusion-based bioreactors model the circulation of fluids in the body, and have subsequently been utilized to research how CTCs flow through the bloodstream or lymphatic system. There is currently a need for a device that can isolate and collect CTCs from 3D models of metastasis for further characterization and in order to better understand the process of metastasis. Our project will focus on creating a cell collection device for a small-scale perfusion based bioreactor that is capable of separating CTCs from circulating media based on size and deformability compared to other cells that can be found in the blood. Our device will utilize two filters in series to separate CTCs from other particles in circulation and subsequently isolate them. These collected CTCs will be removed and remain viable for further study and characterization. This device can be used to further research on the CTCs involved in neuroblastoma and metastasis.

II. Background

2.1 Neuroblastoma

Neuroblastoma is a deadly cancer that develops in the womb or very early in childhood. There are approximately 600 – 700 cases of neuroblastoma diagnosed every year in the United States alone. One out of every 100,000 children is affected with the disease. While it is only the eighth most commonly diagnosed pediatric cancer in the United States, it is the most fatal [1]. The signs and symptoms of neuroblastoma often appear early in development and the average age of patients at the time of diagnosis is around one to two years old. Children with neuroblastoma often show discoloration of the skin, have tenderness in their bones and on their skin, and develop masses in the abdomen [3]. Screening for neuroblastoma is commonly performed during pregnancy and on newborn infants. Signs of hypertension, discoloration of the skin and eyes, tenderness, and masses in the abdomen could be indicative of neuroblastoma [2].

Neuroblastoma develops from cells in the neural crest within the embryo. These cells differentiate into different key cell types throughout the body, including bone cells, neurons, and skin cells. These cells can become cancerous in part due to unregulated neural growth factor (NGF) and improper signaling of TRK receptors [6]. Primary tumors of neuroblastoma often develop in the abdomen in the adrenal gland above the kidneys, and metastasize to other parts of the body such as bones [3].

2.2 Metastasis

Neuroblastoma remains difficult to treat because of the metastatic nature of the cancer. Metastasis is the development of cancerous growths at a secondary location in the body.

Conditions need to be favorable for the tumor to spread. CTCs must detach from this primary tumor and can either proliferate and grow or remain migratory throughout the body. The process of how cancer metastasizes in other locations is called the invasion-metastasis cascade. The first process of this is the detachment of the cell from the primary tumor and into the bloodstream, that is said to be facilitated by the epithelial-mesenchymal transition. This can induce cancer cells to form tumors and disseminate throughout the body. Cells that are metastatic often have a marker such as E-cadherin, which promotes attachment between epithelial cells and the metastatic cells [6].

Understanding the mechanisms by which circulating cancer cells (CTCs), or metastatic cancer cells, migrate is crucial to understanding metastasis. In current research, there are two models of how metastatic cancer cells can spread, these are linear and parallel progression. The linear model describes the metastatic cancer cell detaching from the primary tumor only after the primary tumor is fully developed, while the parallel model describes the metastatic cell detaching early in the development of the primary tumor and developing almost in parallel with the tumor [7]. Studies have been done to try to determine if metastasized cells work together or compete to form metastatic tumors, and evidence of both has been found- some metastatic cells compete with others for favorable environments in which to grow, while others join with other metastatic cells before or upon reaching a favorable environment. There is also some research to suggest that cells from metastatic tumor sites might detach and attach back onto the primary tumor [7].

After entering circulation, CTCs often move into microvessels in different parts of the body. Due to their tendency to get caught in smaller vasculature because they are so small and deformable it is possible they are only in the main vasculature for as little as minutes.

Throughout their travels in the bloodstreams, CTCs can encounter forces, conditions, and environments that can affect their survival and phenotype [9]. CTCs must to be able to withstand blood flow and shear stress within the vessels in order to survive, which can often change their shape. CTCs also have to be able to evade immune cells. One way CTCs avoid being the target of attacks from T-cells is by interacting with platelets through platelet-derived cytokines within the blood [9]. Platelets help CTCs avoid recognition from the immune system by both physically protecting CTCs from white blood cells and expressing factors such as transforming growth factor (TGF) beta and platelet derived growth factor (PDGF) that stop the activity of Natural Killer T Cells, part of the body's innate immune response [9]. Platelets can also help tumors establish themselves in different locations by making the vasculature more permeable by interacting with P2Y2 receptors on endothelial cells [9]. CTCs also utilize neutrophils to survive. Neutrophils are designed to capture CTCs, but this might only help them survive in the vasculature even longer. They also can suppress the immune response, protecting CTCs even further [6].

Tumor cells also adapt to express important receptors within the body. In the case of breast cancer, primary tumor cells can adapt to express angiopoietin-like 4 (ANGPTL4), which can aid in metastatic growth by making the vasculature more permeable [6]. This is done through transforming growth factor (TGF) beta signaling ANGPTL4, which can disrupt cell to cell junctions in endothelial cells and form a path for CTCs to infiltrate [10]. Another way CTCs are able to infiltrate the bloodstream is through the expression of carbohydrate selectin ligands. These ligands allow them to enter blood vessels by facilitating their interaction with endothelial cells within the body [11].

There are several markers involved in metastatic cancer cells, including mutations in TP53, which is linked to increases in metastatic cancer [13]. Two surface markers that have been found on neuroblastoma calls are TALLA-1 and CD44. Surface markers are important to identify because they help with diagnosis of cancers and can show the difference between primary tumor cells and metastatic tumor cells. Surface markers on neuroblastoma cells can be indicative of their potential to metastasize. Neuroblastoma cells also expressing the surface marker CD44 have been proven to show changes when the cell transforms from primary cell to metastatic cell. Cells coming from metastatic cancer lines had an additional 162 amino acid in their CD44 proteins compared to those coming from non-metastatic cell lines [16]. Neuroblastoma tumors have been known to express GD12, and tumors have been targeted using anti-GD2 antibodies [A]. Understanding changes of surface markers when tumor cells change from primary to metastatic is important in cancer research.

2.3 CTC Characteristics

In addition to surface markers, there are several physical characteristics of CTCs that distinguish them from other cell types. CTCs generally larger than other cell types present in blood, with a diameter of 17-52 µm compared to 6-8 µm for red blood cells (RBCs), 12-15 µm for granulocytes, 7-20 µm for agranulocytes, and 15-25 µm for monocytes [17]. CTCs have an elongated shape compared to RBCs, which are shaped like biconcave disks [17]. CTCs have a more ruffled surface membrane than other cell types [18]. CTCs are also less dense than RBCs, with a density of <1.007 g/mL when compared with rbcs which have a density of >1.007 g/mL [17].

The deformability of CTCs is lower than red blood cells. CTCs have a young's modulus of 0.2 kPa; other blood cells have a young's modulus of around 3.7-150 kPa [17]. This indicates that CTCs are more stiff and less elastic than other components of blood. This was confirmed in a study by Byun et al. which determined that CTCs have cytoskeletons that are more deformable than red blood cells [19]. It has also been determined that metastatic cells are more deformable than their non-metastatic counterparts [17]. CTCs have been found to have distinct electrical properties when compared to other cells in blood. CTCs have a membrane capacitance of 26 +/- 4.2 mF/m^2 , higher than white blood cells which have a membrane capacitance of 11 +/- 4.2 mF/m^2 and RBCs which have a membrane capacitance of 9 +/- 0.8 mF/m^2 [17]. This indicates that CTC's can respond to membrane potential faster than other cells.

2.3 Bioreactors for Cancer Modeling

Bioreactors are closed systems that are used to model different biological processes. The system is a controlled environment with specific conditions designed to mimic different growth situations. Bioreactors typically contain a tank, a pump or motor, and sensors or other software to monitor the process. There are different types of bioreactors depending on what is being modeled. For cancer circulation models, it is important to model the effect of fluid flow on the cells. A perfused tumor model is an effective option for in-vitro modeling. Bioreactors are also helpful for monitoring cell-cell interactions, the effect of flow on tumor migration, and shear stress on cells in various conditions. Perfusion bioreactors can help visualize how cancer both grows and migrates [25].

While none have been developed for neuroblastoma, bioreactors have been used to study breast cancer bone metastasis [20]. These bioreactors are often used in conjunction with

membrane dialysis, which can help mimic processes in the body by delivering nutrients and removing wastes. Bioreactors have the advantage over some other metastasis models that they can be used for long term modeling, often up to 5 months. This longer culture time gives circulating tumor cells more time to seed in secondary locations. A drawback of using bioreactors to model metastasis is their limited volume, and there are too many cells in the bioreactor it could lead to mass transfer issues and cells may not be able to survive [20].

2.4 Separating Circulating Cancer Cells and Current Market Devices

Isolation and collection of metastatic cells within *in vitro* models allows for characterization and further analysis of CTCs. There are devices on the market for separating cancer cells from circulation. These devices utilized different methods for separation which can be split into two categories: separation based on physical properties of cells, and separation via targeting surface receptors. Physical properties such as density, deformity, size, and charge of cancer cells are used for separation. Density gradient centrifugation is a technique for separation that works by separating cells based on density. Red blood cells and neutrophils have a higher density, compared to immune cells and CTCs [17]. A centrifuge spins the solution containing the cells and cells of higher density will sink to the bottom while less dense cells with stay closer to the top, remaining in the supernatant. Centrifugation alone has shown to be relatively inefficient in separating cells, this led to the introduction of gradient media. Gradient media is a liquid which helps to separate cells that have similar density by forming a distinct layer between cell types. Ficoll-Hypaque, Percoll, and OncoQuick are examples of gradient medias, all used to improve efficiency of separation in cell centrifugation [17]. Recovery rate of tumor cells

separated with gradient media ranges between 70 and 90%, but it is important to note that these gradient medias are toxic to cells and the cells will not be viable after use [27].

Circulating tumor cells can be isolated through the use of various microfiltration methods. Several studies have captured and isolated circulating tumor cells by though the use of microfilters, targeting the cell of the tumor cell. D. L. Adams et. al, conducted a study which utilized microfilters with pore sizes ranging from 5 to 9 μm, the highest capture efficiency was achieved through use of a 160,000 porosity 7 μm filter, with a 98 ± 2% efficiency [28]. One of the biggest drawbacks to microfiltration is the possibility of clogging [17]. 3D microfiltration devices have been invented to combat this issue. Siyang Zheng et. all, created a 3D microfilter device for viable circulating tumor cell enrichment which allows for filtration of larger sample volumes with less clogging compared to traditional 2D microfilters. This device consists of two layers of porous membrane layered on top of each other. The top membrane has pore sizes of 9 μm and the bottom has pore sizes of 8 μm. This device was able to capture circulating tumor cells with an 86.5 ± 5.3% efficiency [29]. An additional method for separation based on particle size is bead packed filtration. Bead packed filtration works by flowing a sample through a column filled with uniform and non-uniform diameter beads which act as filters. Circulating tumor cells flow through the beads while RBCs and WBCs bind to beads [17]. However, this method of separation has been proven to show low circulating tumor cell capture efficiency, between 21% and 40% [30]. Deformability of circulating tumor cells compared to other cells in circulation is used as another method for separation. Red and white blood cells are typically highly deformable compared to CTCs [20]. Since tumor cells are typically less deformable than other cells in circulation, microfluidic devices can be manufactured to capture CTCs [17].

Charge is another property that can can be used as a separation techniques. Dielectrophoresis (DEP) separates circulating tumor cell by separating cells which are neutral but polarizable in nonuniform electric fields [21]. Compared to other cells in circulation, CTCs have high surface area giving them a larger capacitance. Studies have been conducted with DEP on colon, breast, lung, and prostate cancer cells [17]. The range of separation efficiency in these ranges from 70 to 90%[17].

All of the aforementioned physical properties (size, shape, charge and deformability of CTCs) have been targeted in methods for isolation of CTCs in many microfluidic devices on the market. The Parsortix™, developed by Angle PLC, targets the deformability of CTCs to separate them from other components in circulation [33]. The Celsee PREP 400 and Celsee ANALYZER by Celsee diagnostics is a device which separates CTCs from other components through sized based separation and filtration [34]. VTX-1 by Vortex Bioscience is a device which separates CTCs based on their size and deformability [35].

Aside from physical properties, circulating tumor cells can be tagged with immunofluorescence, magnetic particles, and specific antibodies to separate them from other cells. Fluorescence-activated cell sorting or FACS is a method which works by tagging cells with fluorescent labels. The labeled cells are isolated using devices such as FACSort or FACStar and then studied for characterization and viability [36]. This method has the ability to separate highly specific populations of cells with great precision and viability, but a major disadvantage is that this method is not universally available for separating all cancer types because it works through targeting cancers derived from epithelial cells that express EpCAM [32]. Magnetic activated cell separation is another way to manipulate CTCs for isolation. This method works by

labeling cancer cells with magnetic beads and running them through a microfluidic device with a magnetic to draw the CTCs out from circulation [37]. This method is able to maintain about 70% cell viability [37]. There are many market devices utilizing methods of tagging for cell separation. Target Selector™ platform (CEE microfluidic chip) by Biocept, Inc. utilizes a combination of antibody labeling and microfluidic channels for CTC enrichment [38]. The IsoFlux CTC system and IsoFlux Cytation Imager by Fluxion Bioscience separate CTCs based on EpCAM and EGFR markers. LiquidBiopsy® Platform (ClearID® Clinical Testing) by Cynvenio Biosystems, Inc. both separates CTCs using the magnetic activated cell sorting method [39].

2.5 Clinical and Research Applications

Few devices have been effectively implemented into bioreactors for modeling purposes. Even fewer are specifically designed to separate and remove CTCs from circulation within a bioreactor system. Bioreactors have been used to model how tissues will grow and react in certain environments. They are important for standardizing research models, testing reproducible products, and establishing *in vitro* models for testing drug effectiveness [40]. Perfusion bioreactors are used to represent blood circulation and its effects on tissues and scaffolds by adding and removing media simultaneously. They also have potential to show how tumor metastasis happens, and to what extent.

For *in vitro* testing and modeling specifically, connecting the separation and collection device to the bioreactor will allow researchers to better analyze the cells that break off and continue in circulation. This allows for cells to be collected and analyzed over time, creating a more accurate model of circulation in the body. This will also test the accuracy and

reproducibility of cell-based therapy methods to stop tumor circulation and metastasis [41]. Having the results of cells that begin circulation from a model tumor can help predict the metastatic potential of certain tumors in various environments. If scientists had the ability to use a bioreactor and collection device in tandem or separate, they will further understand how certain tumors spread in circulation and be able to test different factors, like flow rate, tumor size, cancer type, length of circulation, temperature, and blood vessel size [43]. As discussed in section 2.4, there are marketed devices that achieve isolation and collection; however, the process is lengthy, the collected cells are no longer viable, or the devices are not integratable into bioreactors.

In clinical research, it is important to have a diagnostic tool that can detect the number and properties of CTCS and extract them from a patient's blood sample; however, there are few methods that are proven to be effective for this purpose. Bioreactors can be another way to model the effectiveness of such a device before it can be used in clinical settings. If CTCs could be accuarately isolated and collected from patient's blood, they can be counted, sized, and analyzed further. The clinical potential involves testing viable CTCs that have been collected from patients to determine what treatment options will work best for their specific cancer type [27]. When trying to predict the rate of metastasis, it can be helpful to know the amount of cells that may have entered circulation during tumor removal surgery. Further testing of the surgery's effectiveness and predicting metastasis potential can be crucial for modeling the probability of metastasis for patients in early stages if location and size of tumor is known.

III. Project Strategy

3.1 Initial Client Statement

The initial client statement given to the team was to develop a circulating cell collection device to capture non-anchored or metastatic-like cells that can be integrated within a perfusion bioreactor system.

3.2 Design Requirements -Technical

The goal for this project was to create a device with the ability to separate cancer cells from circulation of a perfusion bioreactor for characterization. Design of this device will be based on the following objectives:

1. Design a device that will collect circulating cells
2. Design a device which can separate circulating cells
3. Integrate this device with a perfusion-based bioreactor.

The cell line used within the bioreactor was SK-N-AS cells, a line of neuroblastoma cells. The diameter and shape of these cells had to be determined in order to isolate based on size. The bioreactor utilized for this project was run at a flow rate of 248.2 mL/hr creating a shear stress of 3.82e-005 - 0.03 Pa generated by a peristaltic pump setting of 100 rpm within the tubing of the bioreactor. Therefore, our device must be able to withstand a flow rate of 248.2 mL/hr and shear stress of at least 0.2 Pa. Capillary conditions within the body have a flow rate of 126 mL/hr and a wall shear stress of 2.8 dyn/cm^2 (0.28 Pa) [42]. This means the perfusion bioreactor system used

was compatible with these parameters. The flow rate of the device dictates the shear stress on the capillary walls, and therefore only the flow rate within the bioreactor needed to be controlled to achieve the appropriate shear force. In order to be further characterize cells after collection, the cells must remain viable. To be comparable to other devices currently on the market, this device must maintain 80% at least cell viability.The size of CTCs have been cited in literature to range in size from 17 to 52 µm while red blood cells, one of the most abundant components of blood, were significantly smaller. This device must be able to separate cells based on size.

In order to be comparable to similar devices on the market, this device must separate cells from other components with 80% efficiency, this means collect 80% of the sample passing though. In order to mimic conditions in the body, our device must be able to separate at least one CTC in every one milliliter of medium. The criteria for engineering specification of the device can be seen in Table 3.1 in the following section.

3.2.1 Specifications of the Device

Table 3.1 Engineering Criteria

Specifications	Criteria
Must maintain/withstand flow rate of bioreactor and capillaries	Must withstand/maintain flow rate of 248.2 mL/hr and 128 mL/hr
Must not cause flow to become turbulent	The Reynolds number for fluid flow must be <2100
Must maintain cell viability	Must maintain at least 80% cell viability
Must be able to differentiate cells based on size	Must be able to distinguish cells of varying sizes in between 6-52 micrometers
Must be able to differentiate cells based on deformability	Must be able to deform cells based on stiffness (0.2 kPa and 3.7-150 kPa)
Must be able to efficiently separate cells comparable to products currently on the market	Must be able to separate at least one CTC for every 1 mL of media.

3.3 Design Requirements - Standards

One of the goals for the cell collection device was integration into the bioreactor system. Bioreactors require a sterile environment to replicate *in vivo* conditions without contamination. ISO standard, ISO 14937:2009, specifies the characterization of sterilizing agents as well as development, validation and monitoring of the sterilization process [43]. This standard will need to be followed when sterilizing the components of the cell collection device as they are integrated into the bioreactor. ISO 10993-1:2018, is a standard for biological evaluation of medical devices. This standard covers the biocompatibility of medical devices. It is important for the device we have created to followed standards for biocompatibility because it is part of an in

vitro bioreactor model. Being part of an in vitro bioreactor system it is important for components of the device to maintain viability of the cells and not have any cytotoxic effects on cells within the system.

Standards regarding sterilization are also important to follow while creating this device. Sterilization is an important process for maintaining the environment of the bioreactor system and avoiding external contamination. Ethylene oxide sterilize standards can be followed to ensure a sterile system. There are four important standards to follow to achieve sterilization through ethylene oxide: gas concentration at 450 to 1200 mg/l, temperature at 37 to 63°C, relative humidity at 40 to 80% and exposure time 1 to 6 hours [46].

While creating this device, it is important to follow the standards of manufacturing/ software associated with CAD files. SolidWorks software will most likely be utilized in drafting designs for this device, and the drafts must be to standard. COMSOL multiphysics modeling software will also be utilized for creating visualizations throughout this project. The appropriate standards for modeling in COMSOL are expected to be followed as well.

3.4 Revised Client Statement

Develop a cell collection device that can identify and isolate non-anchored or metastatic like cells out of a circulating blood substitute. The device must be able to both separate and collect these cells. The device must be able to keep these cells alive for further characterization testing. The device must be able to be integrated with a perfusion bioreactor system.

3.5 Management Approach

Throughout our project, there are many different components we have to consider, including separation, collection, and integration into the bioreactor. These include, but are not limited to: background research, initial prototyping, initial testing, revised prototyping, and revised testing. The Gantt chart below (Table 3.2) shows an approximated breakdown of when we plan to work on and complete these portions of our project. The yellow sections denote “in progress”, while the green is when we plan to have these phases completed.

The key below indicates what is in the left column on the chart. The top row, C1 through D7 represents weeks in C and D term with C1 being week one in C term.

Table 3.2 Gantt Chart[1]

	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7
A															
B															
C															
D															
E															
F															
G															

[1] A - Filter Integration with Bioreactor, B - Testing of Filter in Bioreactor, C - Testing of Filter with additional cells, D - Microfluidic Component, E - Testing of Entire System, F - Design Improvements, G - Report/Presentation

IV. Design Process

4.1 Needs Analysis

Our device must collect and isolate CTCs from other components in media and be able to be integrated with a perfusion based bioreactor while maintaining cell viability. In addition to these needs, some important factors to consider are safety, efficiency, ease of use, accuracy, and precision of the device. In order to determine the relative importance of these factors, we created a pairwise comparison chart. Efficiency is defined in this context as the speed at which the procedure takes place. In the pairwise comparison chart, a one on in the row of a factor indicates that it was determined by the team to be a more important than the factor in the column it is being compared to. For example, “safety” was determined to be more important than “ease of use”, so in the row with safety and the column of ease of use there is a 1, indicating safety was more important. Conversely, the row with ease of use has a 0 in the column with safety, indicating it was less important. “X”s denote where factors would have been compared to each other.

Table 4.1 Pairwise comparison chart

	Ease of use	Safety	Accuracy	Precision	Affordability	Total
Ease of use	x	0	0	0	0	**0**
Safety	1	x	1	1	1	**4**
Accuracy	1	0	x	1	1	**3**
Precision	1	0	0	x	1	**2**
Affordability	1	0	0	0	x	**1**

Based on these factors, we determined needs for our project. To ensure safety, no toxic material was used and safety precautions were taken in the lab environment. Accuracy and precision will be met by both separating and collection cells in an efficient process - potential means for accomplishing this are detailed in Table 4.2 below.

The device needs to be accurate and precise in accomplishing the main objectives of the project - cell collection, separation, and integration into the bioreactor. In order to meet the needs of for our devices, we determined several functions that must be achieved. These functions and the means we plan to use to ensure our device meets these functions, can be seen in Table 4.2 below.

Table 4.2 Design Function Means Table

Design function	Possible means of accomplishing functions		
Separate circulating tumor cells	Centrifuge	Microfluidics (magnetic/immuno tagging/cell deformity)	SEC - Size Exclusion Chromatography
Collect circulating tumor cells	Using a filter to differentiate cells by size	Having a separate tube attachment to divert cells to a collection area	
The ability to be adapted into a small scale bioreactor model	Tubing to connect device in line with bioreactor	Device is built so it can be put inside and taken out of bioreactor	Media is collected and run through device externally

4.2 Potential Conceptual Designs

Conceptual designs were created to target three main functions of the device: cell collection, cell separation, and device integration. The main design concepts were created to satisfy our objectives and functions.

4.2.1 Cell Collection and Separation

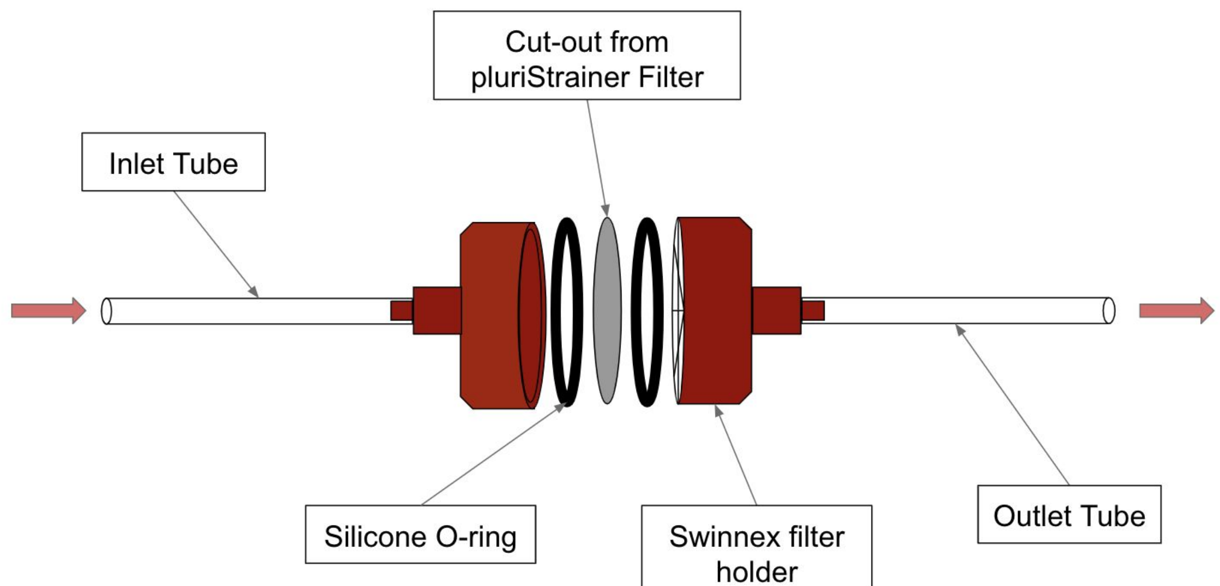


Figure 4.1: Cell Collection Conceptual Design

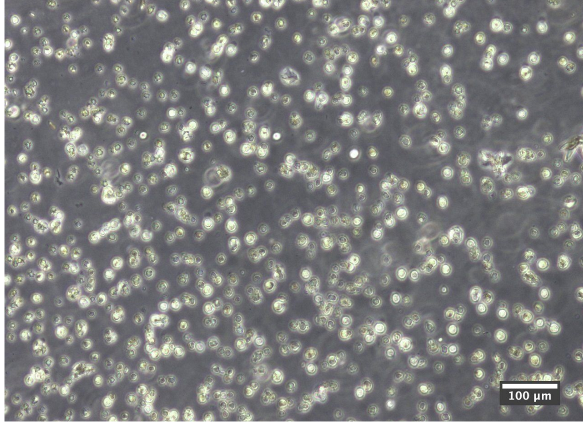
Figure 4.1 above, represents a conceptual design of the cell collection device. The device consists of two tubes with cell strainers at each inlet. The sample will flow through the tube on the left and target cells will be captured by the cell strainer. Determining which pore size for the

cell strainer and potentially how many cell strainers will need to be determined by the team through preliminary testing. The tubes are connected to create an alternative route if the primary filter in the leftmost tube becomes clogged. Since other cells in circulation could potentially be very close in size to the targeted circulating tumor cells, the team predicts another method for further separation will be needed. Two different filter sizes, 1 µm and 10 µm were placed in line with each other. The 10 µm filter is able to capture and collect larger cells such as fibroblasts (HFF) and the smaller 1 µm filter is able to separate the smaller SK-N-AS cells.

4.3 Preliminary Feasibility Studies for Cell Separation

To determine the proper method for collection cells, preliminary feasibility studies were used. First, the team needed to determine if SK-N-AS cells could be captured by cell strainers. An initial verification experiment was conducted to confirmed the use of strainers as the method of collection. For this experiment, 1 million SK-N-AS cells suspended in 5 mL of DPBS were placed in a 5 mL syringe. The syringe was placed in a syringe pump and flowed the cells through tubing connected to the Swinnex filter holder holding the 5 µm filter at 2.133 mL/min. The filtered DPBS was collected in an outlet tube. Once the entire solution passed through the system, the filter was removed from the system and rinsed with DPBS to collect the cells for counting. Figures 4.2 and 4.3 below show images from the cells the 5 µm filter caught and and the cells left over in the filtered DPBS that ran through the system. 10 µL samples from both the collected cells and cells that made it through the filter were placed in a hemocytometer for quantification. The filtered DPBS had 62,500 cells, meaning the 5 µm filter was able to capture ~94% of SK-N-AS cells passed through in this trial, thus confirming the ability for the cell filter

to be used as a cell collection method. Further verification of the filters as a method for collection and separation can be seen in Chapter 5 Design Verification.



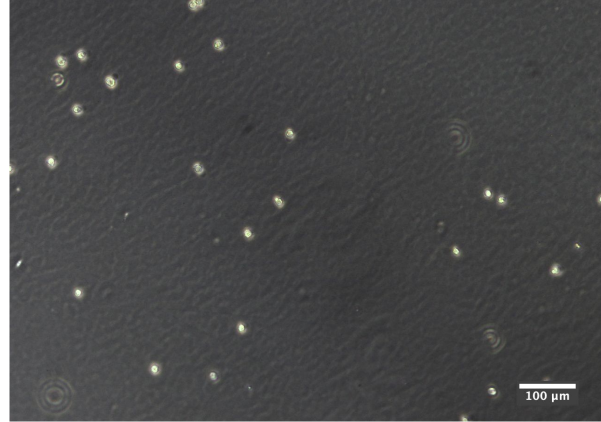


Figure 4.2: SK-NA-S cells Collected from 5 μm filter   *Figure 4.3: SK-NA-S cells in Filtered DPBS*

The material of the strainer needed to be biocompatible with the CTCs to preserve viability of the cells for further characterization. Biocompatibility tests ensured the material of the filter would not impact viability of cells. The team conducted preliminary imaging of HFF and SK-N-AS cells to determine the appropriate pore size for the cell strainer. Preliminary tests of flowing the HFF and SK-N-AS cells through the cell strainer proved that cell strainers were an effective method for collecting cells.

Since other cells that could potentially be in circulation in tandem with the CTCs in the bioreactor will likely be very close in size to CTCs, the team predicts that the filters will not be 100% accurate with catching only CTCs and no other cells. As a result, the team suggests using a microfluidic device to separate out the CTCs from other cells. The device will target deformability of CTCs and flow the sample through a series of pillars/channels to isolate the CTCs.

4.4 Device Integration

Conceptual designs for potential integration methods are shown in Figure 4.4 and 4.5. This potential design shows the the collection device placed in line with the bioreactor with flow that can be turned on or off. This would model the actual circulation of metastatic cells that detach during circulation. The design includes the filtration through gravity or pressure concept. The filter would be placed directly after the tumor scaffold and collect any cells that detach right away. The filter would also be removable to allow the team to preserve and analyze the metastatic like cells that are caught during circulation. The blood components that are not caught in the filter would continue on in the cycle back to the media reservoir. Circulation could occur for a long period of time before adding the filter and collecting the CTCs; or the filter could start collecting right away. Both of these concepts need to be able to separate the cancer cells during flow and safely remove them from circulation. The feasibility of each concept can be confirmed by creating parameters for the bioreactor (flow rate, circulation time, distance of collection from tumor model, etc) and testing the accuracy of separation and collection, as well as the viability of the cells after collection. Figure 4.4 shows a schematic of the design of the bioreactor system before integration of the cell collection device and Figure 4.5 shows the integration of the device.

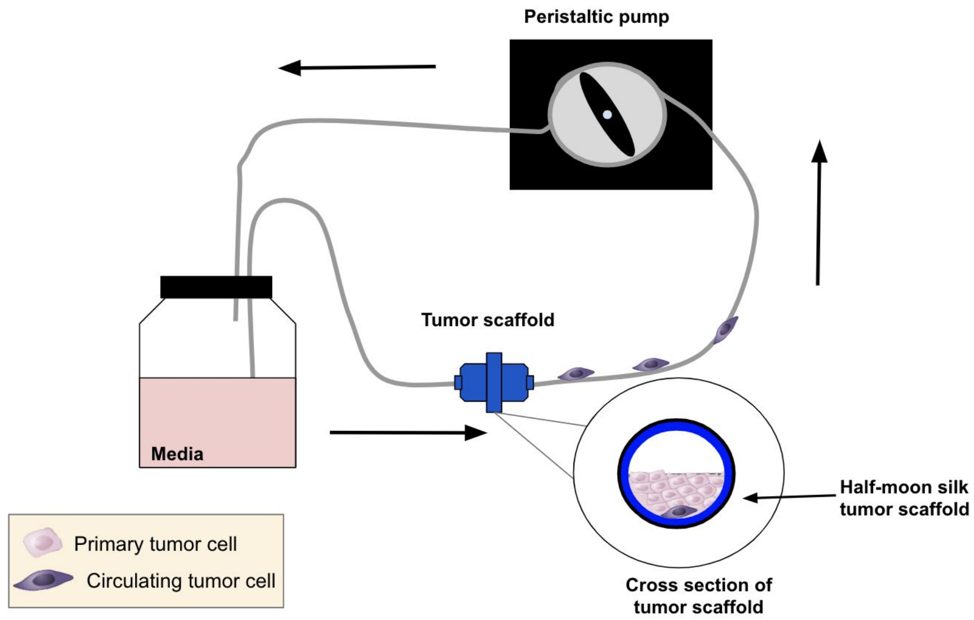


Figure 4.4 Bioreactor Design Before Integration of Cell Collection Device

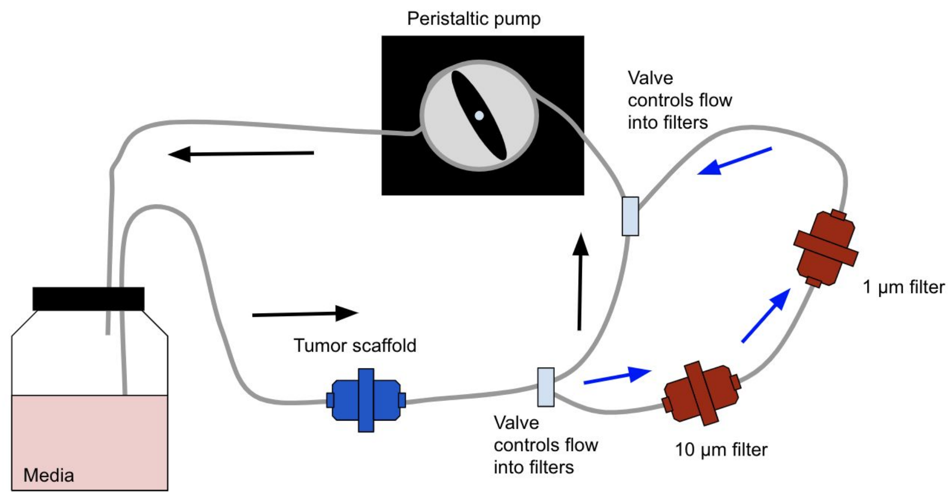


Figure 4.5: Device Integration to a Bioreactor Conceptual Design

4.5 Alternative Designs

The team considered multiple alternative designs before selecting the final. The designs included bead packed filtration, cell strainers for collection and separation, and microfluidic separation based on cell deformability. Table 4.3 below is a comparison of the different designs which could accomplish the functions discussed above. The designs were ranked against each other for how well they accomplished the functions. Overall, we determined design 2: microfluidics and design 3: filters in sequence would best fit the needs of our device. Dark red on the table below indicates that the design does not meet the need for the function, light red indicates the device somewhat meets the need, and gray indicates the device does meet the need. From evaluation of the potential alternative designs, filters in sequence and microfluidics had the highest scores for addressing the needs. Filters in sequence was selected by the team as the final design to move forward with designing.

Table 4.3 Comparison of Alternative Designs

	Function 1: Separate Tumor from Cells	Function 2: Collect Tumor Cells from Circulation	Function 3: The ability to be adapted into a small scale bioreactor model
Design 1: Density Based Gradient Centrifuge	Cells are separated through centrifugation, low separation efficiency	Another step would be needed to collect cells before centrifugation	Will require collection of media to be transferred to centrifuge
Design 2: Microfluidics	Highest separation efficiency (~98% efficient [17])	Cells flowed through microfluidic devices will be collected in outlet vessels	Requires multiple inputs for cross flows to separate particles, would be very difficult to integrate into bioreactor system
Design 3: Filters in sequence	Separation efficiency depends on filter type	Cells will collect on the filter surface	Filters can be put added to bioreactor tubing through adaptors and filter holders
Design 4: Bead Packed Filtration	Lowest separation efficiency (21-40% efficient [17])	Target cells would be captured by beads in the column	Will require adapter to ensure CTCs are eluting at the correct time

Originally, the team considered bead packed filtration as the method for separation and collection of the cells. This method was selected as it satisfied the design requirements provided appropriate means to accomplish the necessary functions of this device. Ultimately this method was not selected by the team since literature showed very low separation efficiency (~20-40%) [17].

Next, the team considered use of cell strainers as the method for collection and separation. Figure 4.6 below shows multiple pluriStrainers with pore sizes ranging from 1 to 30

µm . These strainers accomplished all of the functions necessary in theory. The strainers could be filter holders which could be put in line with the bioreactor to collect and separate cells in circulation.

Figure 4.6 pluriStrainer 1 µm, 5µm, 10 µm, 15µm, 20 µm and 30 µm

The third design considered by the team was a microfluidic method for separation and collection based on deformity. This method satisfies all of the functions for the device in terms of separation and collection of cells, but a major challenge would be integrating this device into the bioreactor. Since this device would be a small chip, placing it in line with the bioreactor would cause a major change in pressure which could cause harm to the cells, impacting viability of the cells collected. This design was not selected for this reason.

4.6 Final Design Selection

Out of the potential designs considered by the team, the cell strainers accomplished cell collection, cell separation and had the best potential to be placed in line with the bioreactor system. This design had the most potential for satisfying the needs of this device and preliminary feasibility studies showed promise for this device to be effective for accomplishing the required functions. The following section details verification of various aspects of this design.

V. Design Verification

This chapter outlines the experiments conducted to determine the device's attainment of previously specified design objectives, requirements, and specifications. The design verification process is necessary to ensure the basic design will meet the criteria before moving forward with more expensive materials or detailed methods. The results of testing on cell imaging, biocompatibility, and cell collection were gathered and recorded for further analysis.

5.1 Cell Size Verification

SK-NA-S, a neuroblastoma cell line, and human foreskin fibroblast (HFF) cells were used in experiments for verification of the device. Using two different cell types allowed the team to test the ability of the device to separate cells of different sizes and properties. Three different bright field images were taken of each cell type in suspension at 10x magnification and ImageJ software was used to analyze the diameter of the cells in micrometers (µm), images shown in figures 5.1 and 5.2 below.

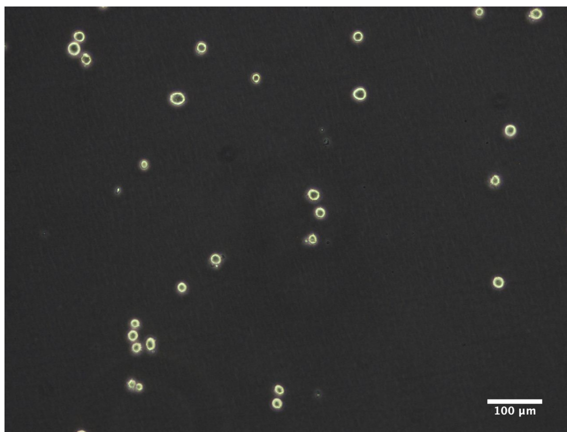


Figure 5.1: HFF Cells in Suspension

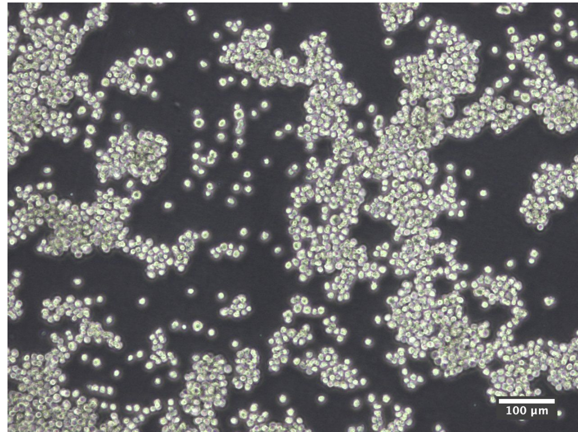


Figure 5.2: SKNAS Cells in Suspension

Thirty cells were measured from each image, a summary of these results can be seen below in Table 5.1. The SK-N-AS cells were on average 8.46 ± 0.2 µm and the HFF cells were 14.5± 1.1 µm , based off these results the team was able to determine the need for two different filter sizes and purchased a set of pluriStrainer filters ranging from 1 µm filters to 30 µm to continue with further experimentation and development of the cell collection device.

Table 5.1 Cell Size Comparison Results using ImageJ to Determine Diameter

Cell Type	Image Number	Average Diameter of 30 cells (µm)
SK-N-AS	Image 1	8.3
	Image 2	8.7
	Image 3	8.4
	Average ± St. Dev.	8.46 ± 0.2
HFF	Image 1	13.8
	Image 2	15.9
	Image 3	13.9
	Average ± St. Dev.	14.5± 1.1

5.2 Biocompatibility Results

The team completed two studies with a focus of biocompatibility. First, biocompatibility of the pluriStrainer filters was examined to ensure that contact with the filter materials would not

be toxic to the cells. Second, a biocompatibility study was run examining viability of the cells after circulation through the sterilized bioreactor system.

5.2.1 Biocompatibility of pluriStrainer Filters

To set up this experiment, a 12-well plate was used. Three wells contained untreated SK-N-AS cells with growth media to serve as a control. Three wells contained SK-N-AS cells, growth media with a 10 mm diameter mesh biopsy punch of from 1 μm filter layed on the well. Three more wells contained SK-N-AS cells, growth media, and a piece of the plastic filter casing. Media was added to the rest of the wells and the plate was left in a 37 ℃ incubator for 24 hours.

A viability assay was completed to verify biocompatibility. Ten wells were seeded with 200,000 SK-N-AS cells per well in a 12 well plate. 0.5 mL of a mixture of 10 mL of media and 2 mL of resazurin was added to each well and a well with just resazurin alone was added to act as a control. The plate was placed back in the 37℃ incubator for 3 hours. Next, a sample of 100 μL was taken from each well and transferred into a 96 well plate. The 96 well plate was read using microplate reader. The results of this test are shown in Figure 5.3 below. This graph compares the average fluorescence intensity from each condition, control well with just SK-NA-S cells, cell in contact with mesh filter, and cells in contact with the filter casing. The value of the fluorescence intensity from the resazurin control well was subtracted from each condition. Results from this study confirms the pluriStrainer filters were not toxic to the SK-N-AS cells. The percent standard deviation among the trials is within limits denoted in literature to be considered non-toxic.

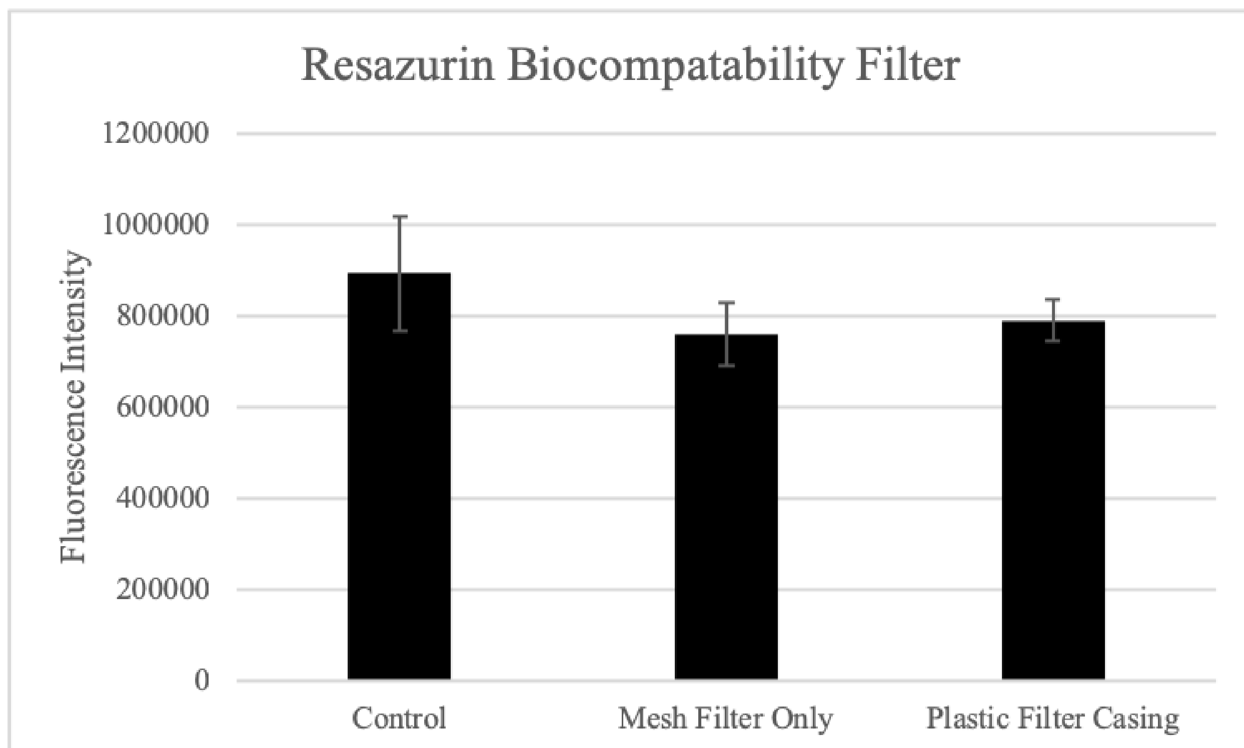


Figure 5.3: Results of Cell Viability Assay with SK Cells

5.2.2 Sterile Bioreactor Study Biocompatibility Results

The next experiment studying biocompatibility involves integration of the cell collection device into the bioreactor system. The cell collection device was integrated into the bioreactor system through use of swinnex filter holders, tubing, and valves that can control flow through the collection device. The entire system was sterilized following standard protocol using ethylene oxide. Following sterilization, the bioreactor system was set up in the cell culture hood to maintain sterility. 9 million SK-N-AS cell were run through the system for 15 minutes. After completing circulation for 15 minutes, cells were recovered from the 1 µm filter, the 10 µm

filter, and the media. The cells were plated in two 24 well plates at 15,000 cells/well. Set up for the plate can be seen below in Figure 5.4.

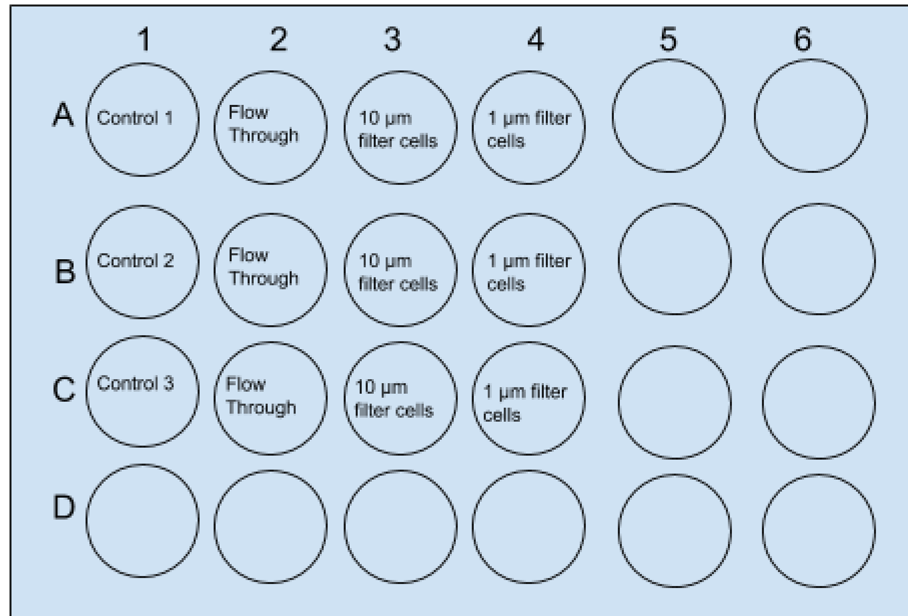


Figure 5.4 Resazurin Viability Test Plate Set Up

The plates were left in an incubator at 37 ℃. The first plate was analyzed after 24 hours, results from this reading can be seen in Figure 5.5 below. The next plate was read after 3 days of initial plating. Results from this can be seen in Figure 5.6 below. This shows the relative viability of cells on the 1 µm filter decreasing, suggesting that the 1 µm filter may be harmful to the viability of cells. Further studies can be conducted to investigate the long term viability of cells from the 1 µm filter.

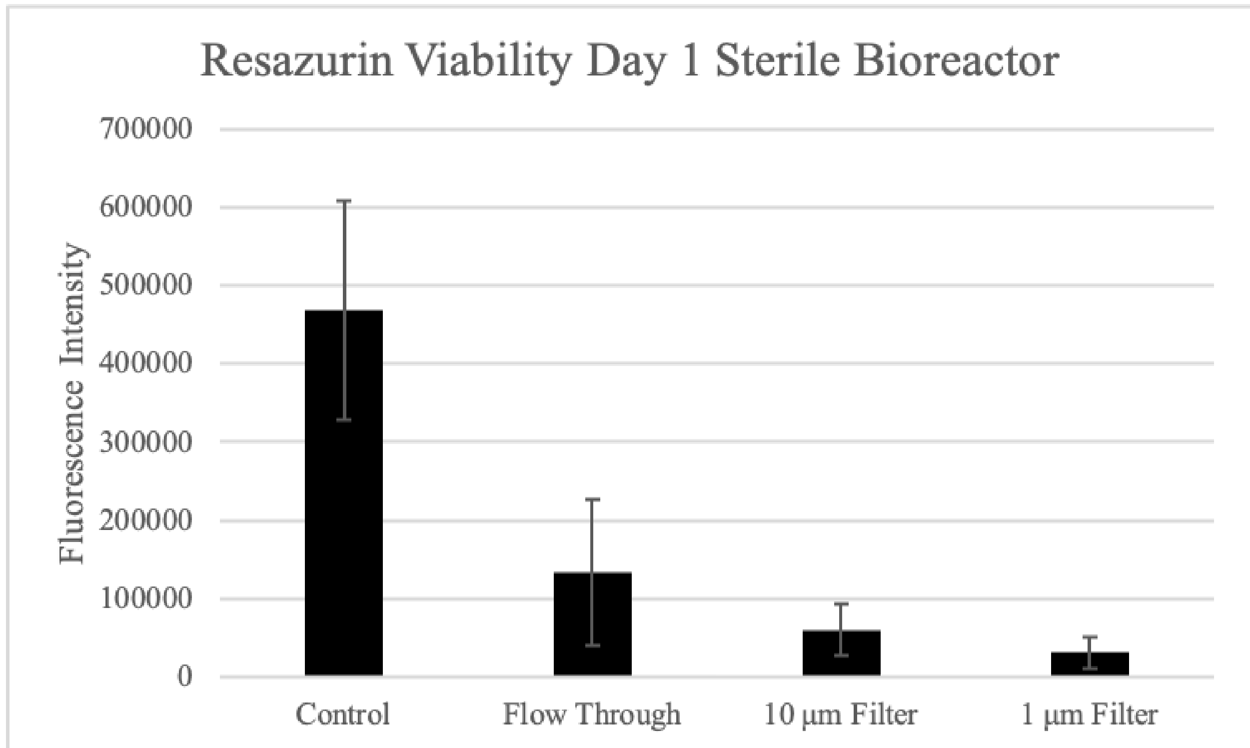


Figure 5.5: Day 1 Resazurin Viability, Sterile Bioreactor Study

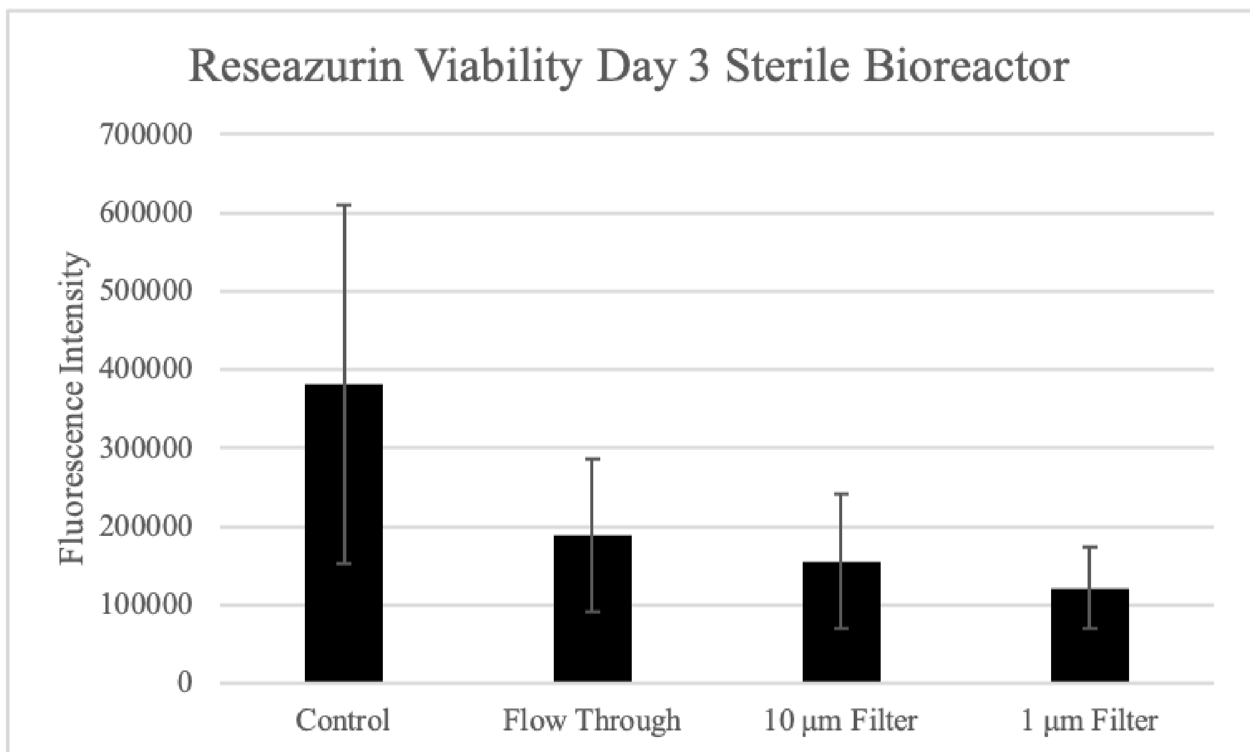


Figure 5.6: Day 3 Resazurin Viability, Sterile Bioreactor Study

5.3 Filter Verification - Cell Collection

To test the functionality of the filtration method, a flow through system was set up. A 1 µm filter inserted into a swinnex filter holder and tubing were used to set up the system for a syringe pump as shown in Figure 5.7. The syringe pump would flow the cells suspended in media through the filter at a given rate. The filter flow when modeled correctly, has a flow rate of 2.133 mL per minute, which is 128 mL/hour. For each trial, 1 million cells, in either 5 mL of media, were flowed through the filter and collected in a conical tube. The filter was then removed immediately and placed in a dish and rinsed with 10 mL PBS to resuspend any cells that were caught on the filter. The cells in the dish and the cells in the conical tube were then put into a 6-well plate for imaging. This process was completed for both HFF and SK-N-AS cells.

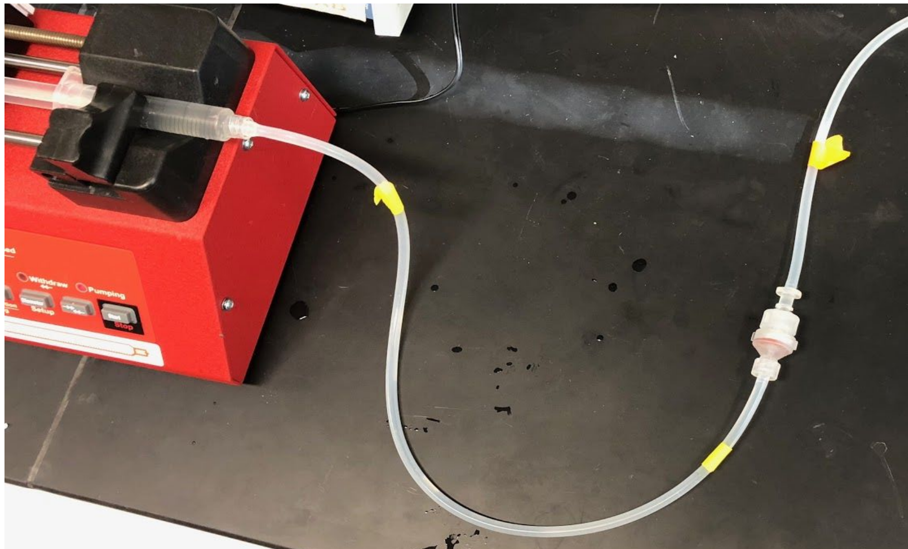


Figure 5.7: Setup of Filtration with Pump for 5µm Filter

Figure 5.8 Setup of Filtration with Pump for 1 µm and 10 µm Filter in Series

The results of these flow tests can be seen in Figure 5.8. Because the HFF and SK cells were previously determined to be different enough in size, two filter chambers were added in series to preliminarily separate the HFF cells from the SK-N-AS cells, to allow for only SK-N-AS cells to be collected. To achieve this, a 1 µm filter and a 10 µm filter were used in descending order as shown in 5.8. Based on our results, there were a few remaining cells that were captured in the flow through.Tables 5.2 and 5.3 show the breakdown of the cells that have flowed through the system at various conditions.

Table 5.2 Filtration Results for SK-N-AS Cells

Filter Size	Number of Cells Input	Number of Cells Counted in Output
1 µm Filter	1,000,000	173,000
5 µm Filter	1,000,000	372,000

Table 5.3 Filtration Results for HFF Cells

Filter Size	Number of Cells Input	Number of Cells Counted in Output
1 µm Filter	1,000,000	86,000

5.4 Visualization of Cell Collection - Cell Staining

To determine the accuracy of our filters for collecting and separating CTCs from other cells of different sizes, two different cell stains were used. SK-N-AS cells were stained with calcein (green) and HFF cells are stained with hoechst (blue). Both cells were flowed through the system (1 million cells each). Cells that were caught on the 10 µm filter and 1 µm filter were imaged before and after PBS washings. The cells that did make it through the series filtration and into the output were also imaged. To begin staining the cells, calcein was added to 2.5 million SK-N-AS cells at 10 µL of calcein/ 1 million cells/ mL and left for 20 min, 3 PBS washes were then performed and 5 mL of media was added before flowing the cells through the system. Next, 1 million HFF cells were stained using hoechst stain at 1 µL of hoechst/ 5 mL, 3 washed of PBS were performed on the HFF cells and the stained cells were resuspended in 5 mL of media. Both cell types were run through the 10 µm and 1 µm filters in series using a syringe pump to pump the 10 mL of the cell mixtures through the system. After running the cells through the system the filters were taken out of the filter holder ands the filtered media was collected. The filters and flow through media were imaged with different filters to visualize which type of cell was present, this is shown in Figures 5.9 and 5.10. Figure 5.9 shows the large amount of SK-N-AS cells that are still stuck on the 1 µm filter, which shows promise for collection. Figure 5.11 shows that both SK-N-AS and HFF cells were stopped by the 10 µm filter, but there are more

HFF cells, which is concurrent with our cell imaging results. To accurately count the number of cells that are on each filter, as well as the distribution of size for each cell type, flow cytometry of each condition will be needed.

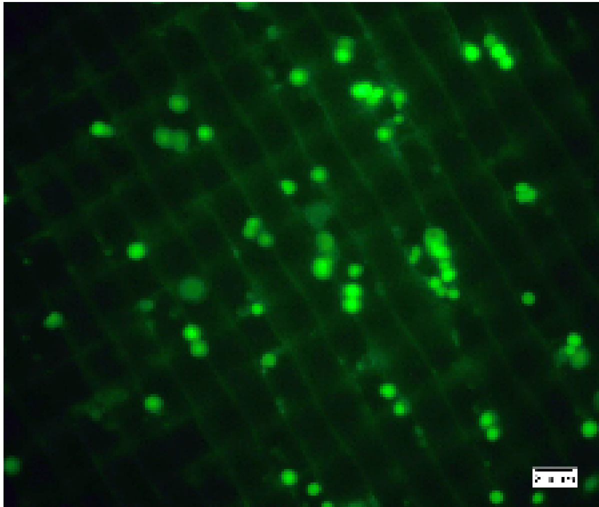


Figure 5.9: Calcein Stain-SK cells, 1 µm

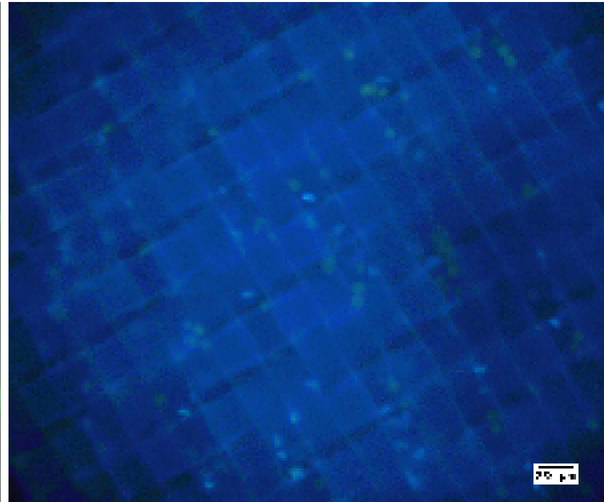


Figure 5.10: Hoechst Stain-HFF cells, 10 µm

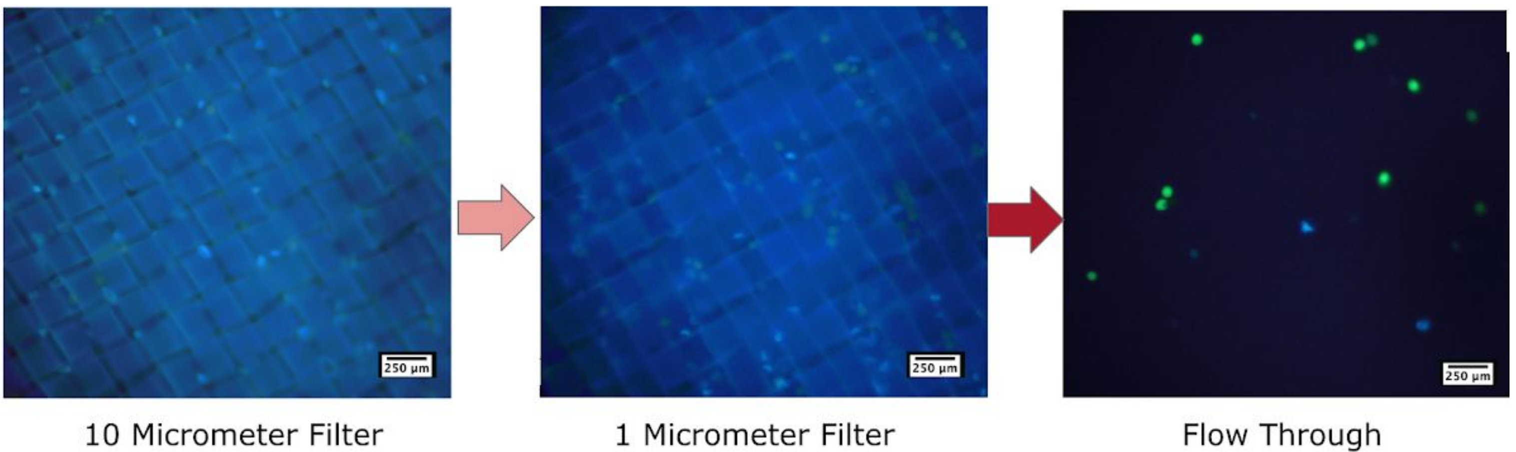
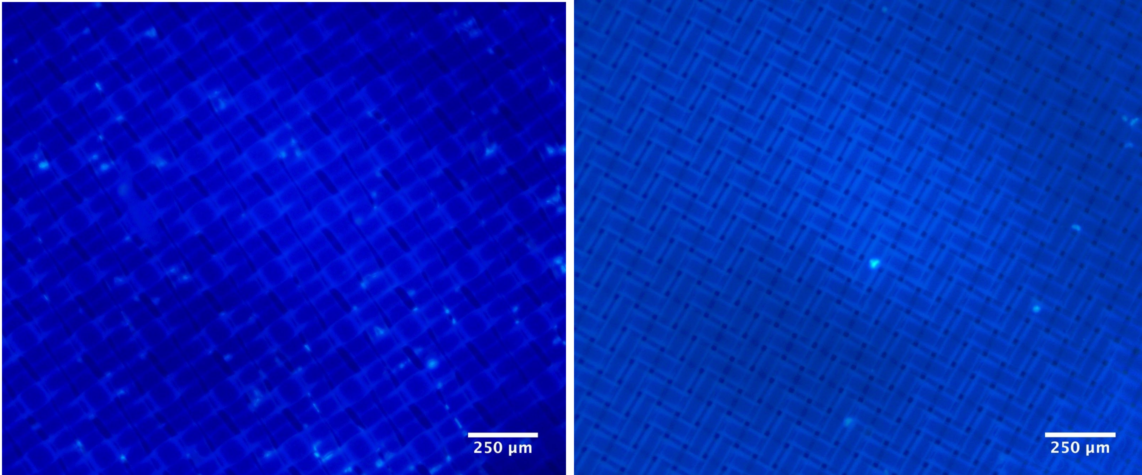


Figure 5.11: Calcein and Hoechst Stain of SK and HFF Cells

An addition test was completed to further test the collection functionality of the device. The 1 µm and 10 µm filters were placed in line and connected to with 1/16 diameter tubing and run through a Watson Marlow 323 series peristaltic pump. Different concentrations of cells

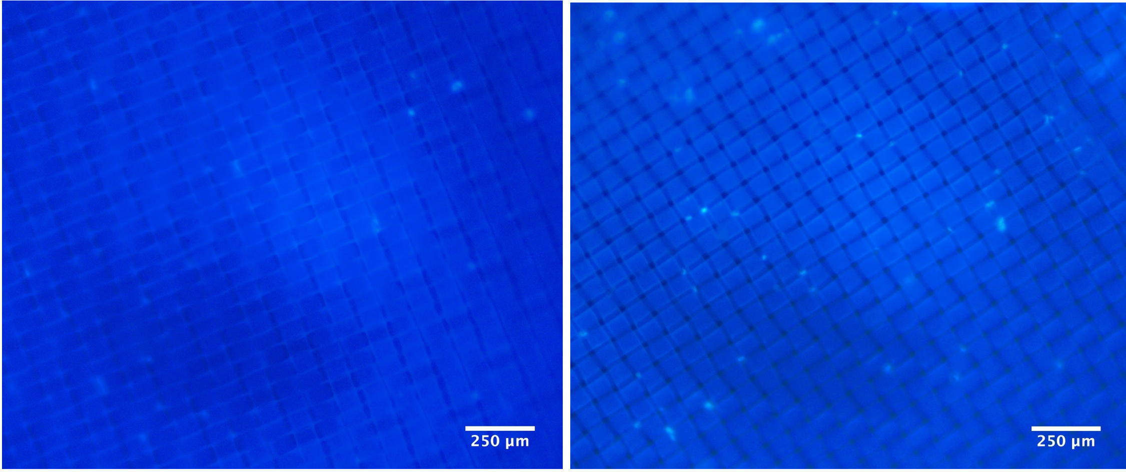
stained with hoechst nuclear stain were passed through the system and the filters removed in between trials for imaging. First SK-N-AS cells were circulated through the system for 10 minutes each at concentrations of 10,000, 100,000, and 1,000,000 cells in 20 mL of media. The same procedure was followed for the HFF cells next. Figure 5.12 below shows images taken from the filters at each concentration of the two cell types.

100,0000 HFF



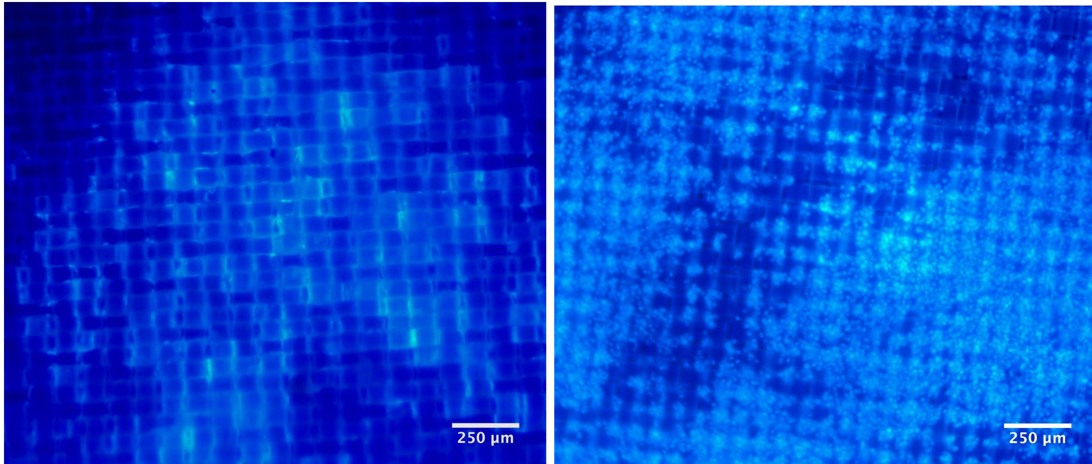
1 µm Filter 10 µm Filter

10,000 HFF



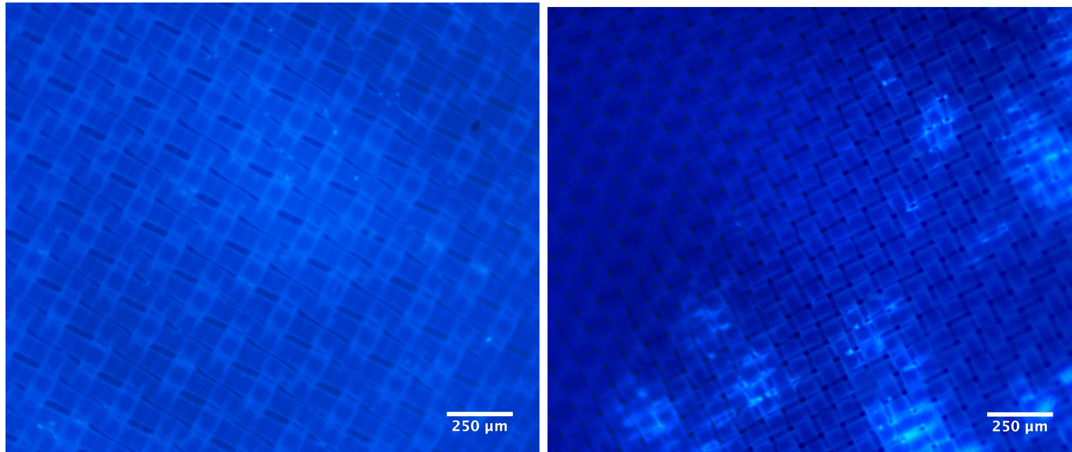
1 µm Filter 10 µm Filter

1,000,000 SK-N-AS



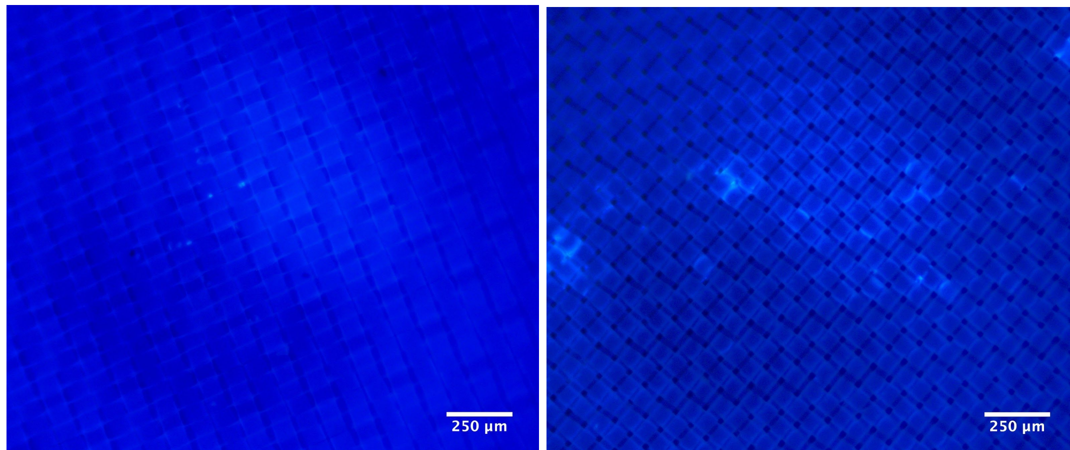
1 µm Filter 10 µm Filter

100,000 SK-N-AS



1 µm Filter 10 µm Filter

10,000 SK-N-AS



1 µm Filter 10 µm Filter

Figure 5.12: Visualization of Cell Collection and Separation at Different Concentrations

Results from this experiment confirmed that the filters were able to collect the two different cell types. It allowed us to determine the impact of different concentrations of cells and how selecting the correct filters for a specific cell type is very important for the overall system's level of effectiveness.

5.5 Pressure Testing Verification of Filters

To test the pressure and flow rates that our cells and system can withstand, we used a pressure transducer and computer software. Using set up in Figure 5.13, we inserted a 26 gauge catheter into the tubing directly opposite of the flow direction to produce the most accurate pressure results. We inserted the catheter directly in front of the 10 μm filter chamber, because the larger filter is collecting the fibroblasts and that's where the highest pressure would be read. The pressure transducer was calibrated before each trial. For each trial, 10 mL of media and cells were flowed through the series of filters (10 μm and 1 μm), increasing from 10,000 cells to 100,000 cells, to 1 million cells for each cell type. Because SK-NA-S cells are smaller, the concentration was increased to 20 million cells to test the system limitations. Figure 5.13 shows the set up of this testing.

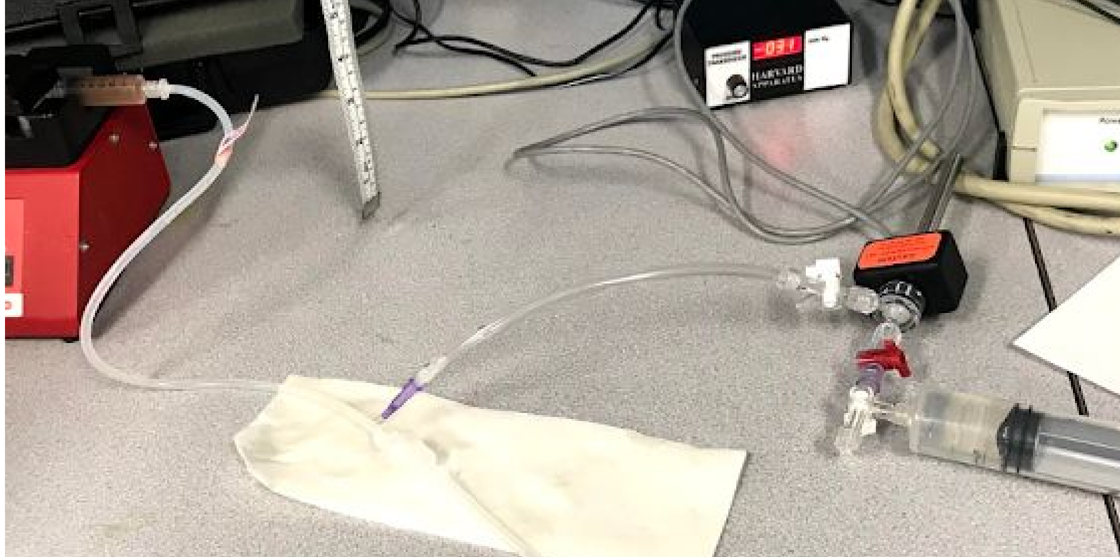


Figure 5.13: Pressure Testing Setup

Using Acknowledge Software, the results of the pressure was captured for 120 seconds and shown in Figure 5.14. As the figure shows, the pressure increased slightly, then decreased once the system overcame surface tension and eventually plateaued. The average pressure for the system without cells was 2.1 mmHg, as shown in Figure 5.15. The slight difference at each peak correlates to the sensitivity of the pressure transducer, it was picking up the slight movement of the syringe pump as it rotates around the screw that pushes the syringe. This variation was evident on every trial that was run, which is why averages were taken to compare each trial, as shown in Figure 5.15.

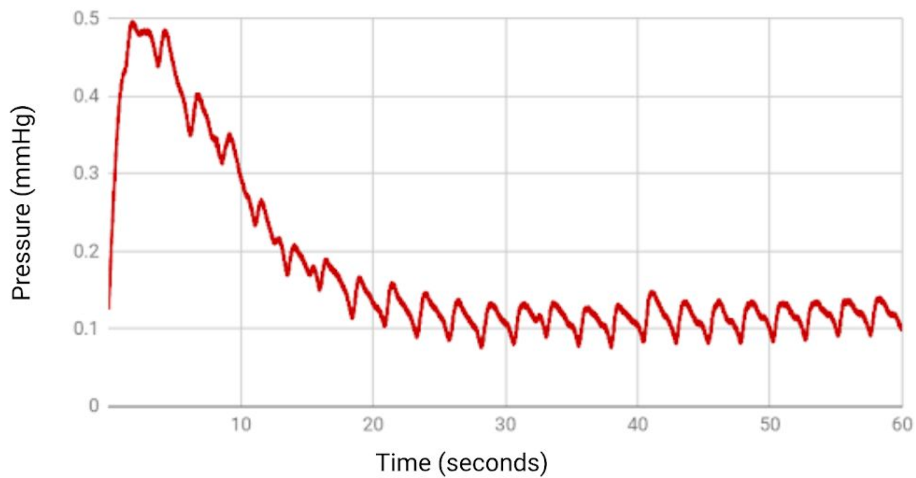


Figure 5.14: Control with no cells

In order to determine the limitations and averages for various concentrations of cells that would be potentially running through our device, we tested three different concentrations with both cell types and a control. Figure 5.15 shows the average pressure of each test after the surface tension was overcome and it reached equilibrium. The pressure was recorded right in front of the larger filter. HFF cells at a concentration of 10,000 cells/10mL of media was 66.4 mmHg on average. While SK-N-AS cells at that same concentration only caused a back pressure of 23.1 mmHg. At a higher concentration of 1 million cells per 10 mL, HFF cells caused an average pressure reading of 83.5 mmHg. While this is high, it is still less the pressure conditions of cells in the body. The data showed an expected trend between the concentrations by declining and between the two cell types. A higher pressure for a larger mass going through a fixed opening is consistent with literature, and is showed with our collection system in Figure 5.15

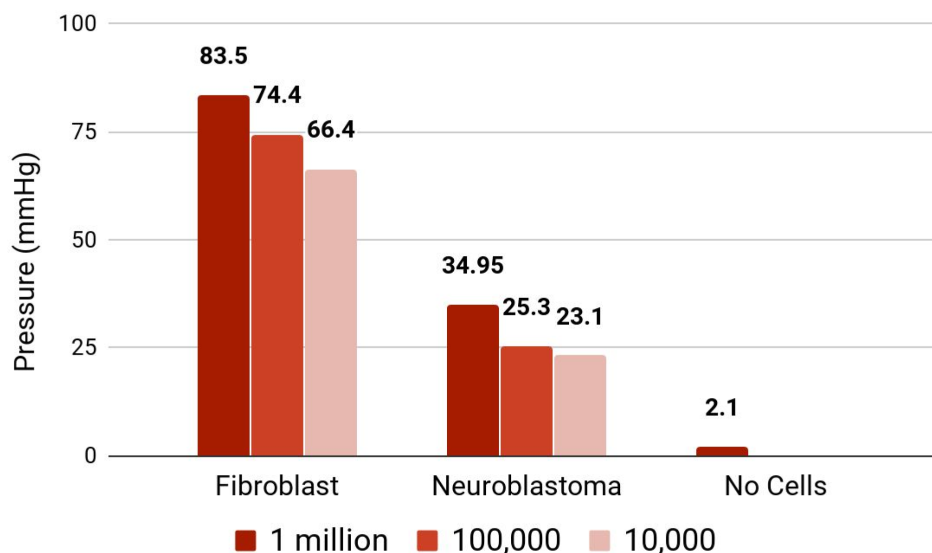


Figure 5.15: Comparison of pressures of 10,000, 100,000, and 1,000,000 cells in 10 mL

Once each cell type was tested at reasonable concentrations, we wanted to define the limitations of our device. Therefore, the concentration was increased to 10 million and 20 million cells per 10 mL. Since SK-N-AS cells were our main focus, we used these cells to test the upper limitations of the system. We also found growing fibroblasts to anything more than 1.5 million to be extremely difficult and most often resulted in contamination. To set up this test, a syringe pump was connected to the system and only the 1 µm filter was used. Figure 5.16 shows what happens when a concentration of 10 million cells per 10 mL of media attempts to go through our system. For the first 95 seconds, the pressure was increases and steadying out at equilibrium at an average pressure of 111.5 mmHg. However, at approximately 100 seconds, the pressure suddenly drops to near zero and continues to hover around 3.5 mmHg for the rest of the trial.

After opening our filter holders to clean the filters for the next trial, we discovered that the filter had popped out and was no longer being held in place. After looking at the data (Figure 5.16), we were able to see that the pressure for 10 million cells reached a maximum of 121.5 mmHg. The 20 million cells per 10 mL of media was also run, but a similar issue happened. The filter did not stay in place for the higher concentration either. The upper limitation of our device is 10 million SK-N-AS cells per 10 mL of media, and since HFF cells are larger, their upper limitation would likely be less than 10 million per 10 mL.

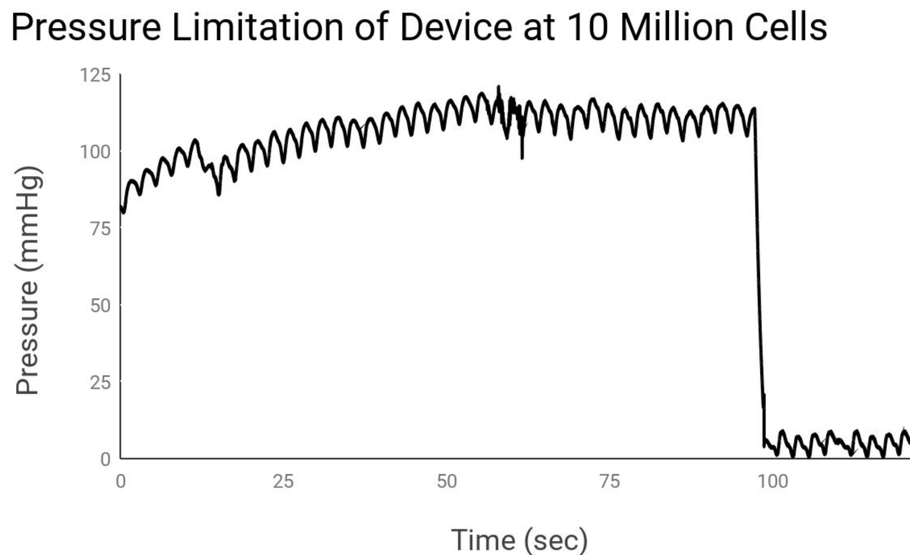


Figure 5.16: Pressure Limitations of Device using 10 million cells in 10 mL of media

5.6 Additional Cell Separation Method Identified

In the case that the filters collect more than just the CTCs from circulation, a microfluidic device could be implemented as the separation method. Figure 5.17 below show a conceptual design of the device. The sample will be loaded through the inlet at a flow rate which will not

harm the cells. The cells that are less deformable will not be able to pass through the pillars in the device and will be collected in a vessel at the bottom of the device. The cells which are more deformable will be able to pass through the pillars and will be collected in a vessel at the right side of the device [45]

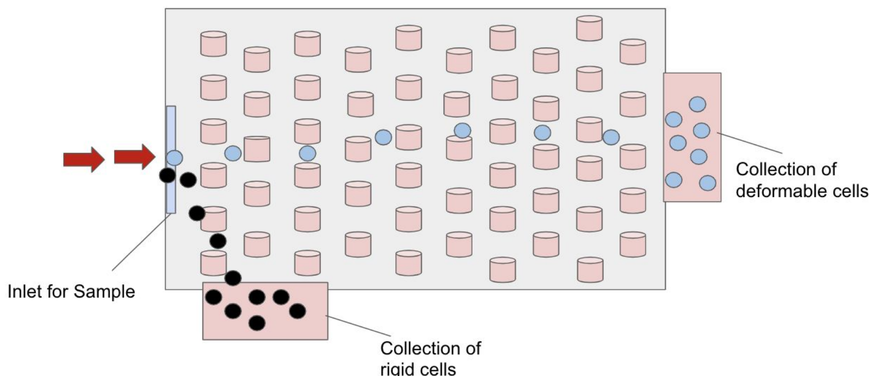


Figure 5.17: Conceptual design of microfluidic deformability separation device

This device does not need to be integrated within the bioreactor because cells could already be collected by the cell strainers. The cell strainers could be removed from the Swinnex filter holders and the cells collected in them will be resuspended. This resuspended solution would be ran through the separation device.

5.6.1 COMSOL 3D Multi-Physics Modeling of Additional Cell Separation Prototype

A model of the microfluidic device was created in COMSOL, a multiphysics modeling software. Results from the fluid flow analysis can be seen in Figure 5.18 below. This shows the velocity of the flow with blue being highest and red being lowest.

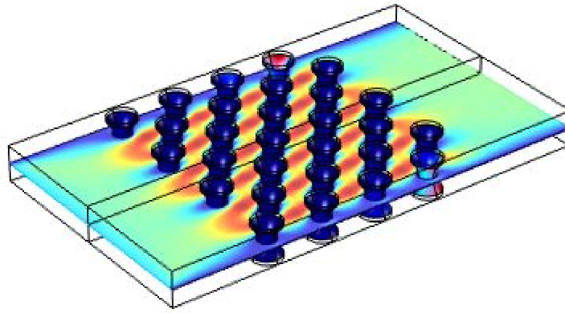


Figure 5.18: COMSOL model of microfluidic device

The team assumed the inflow velocity to be 0.045 m/s, the to be fluid density of 1000 kg/m3, and the dynamic viscosity to be 0.00078 Pa-s. For preliminary purposes of this model, an example was followed of a microfluidic device in COMSOL. This model can be used as a reference for creating and printing a working prototype of the

VI. Final Design and Validation

6.1 Summary of Final Design

The final design for this project includes two filtration chambers in series. This design allows for a two-step preliminary separation of CTCs before they are collected at the end of the system. In order to do this, two filters (10 μm and 1 μm) were cut into 6 mm diameter circles and placed inside two Swinnex filter holders. The tubing connects the filters to the rest of the bioreactor. The collection system will be connected using three way valves to allow and prevent the flow of media and cells either though the collection system during circulation or to bypass the collection system. The collection system can be removed and reattached when the bioreactor flow is bypassing it. This allows for easy access to the cells left on the filters. This also allows the bioreactor to remain continuous flow and sterility while the CTCs that were caught are tested and analyzed. Figure 6.1 shows an image of the full set-up with the blue arrows indicating direction of flow through the system. In order to simplify what is shown in Figure 6.1, a schematic of the full system is shown in figure 6.2.

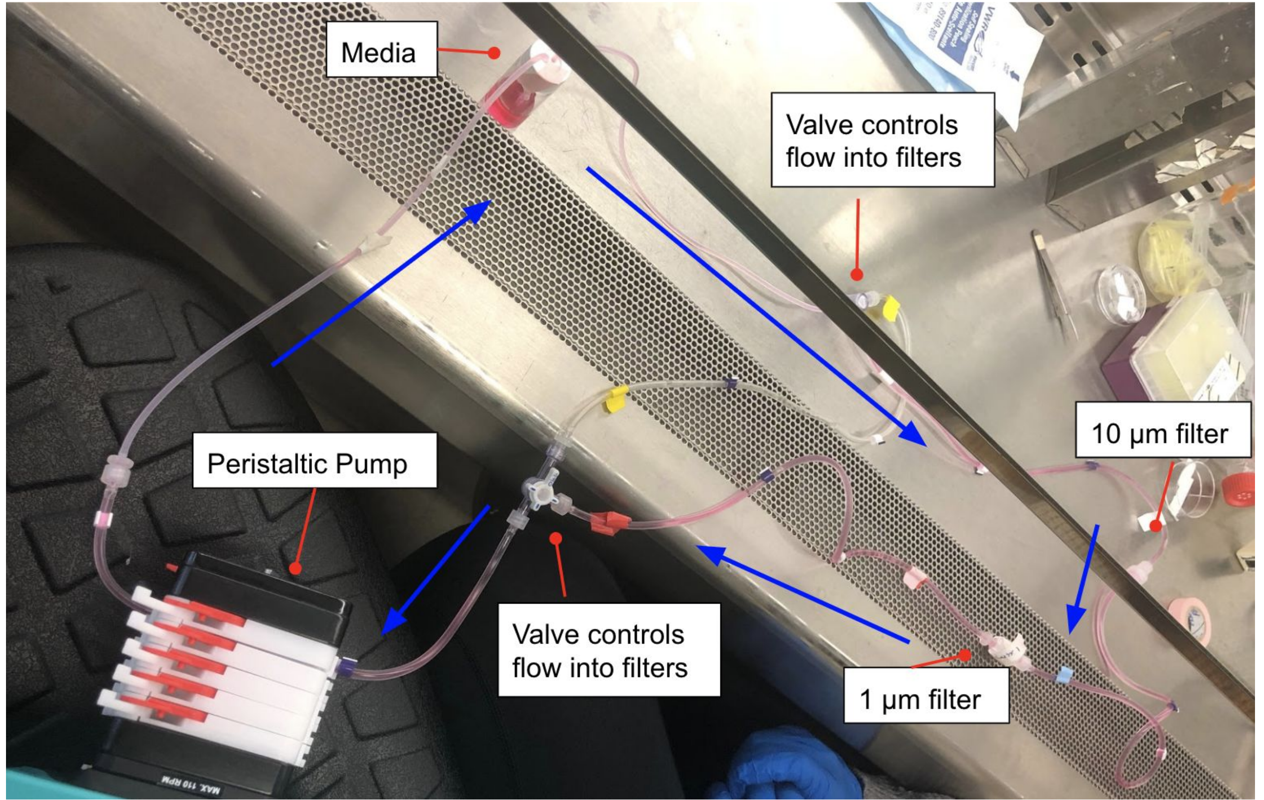


Figure 6.1: Final Collection System with Filters in Series

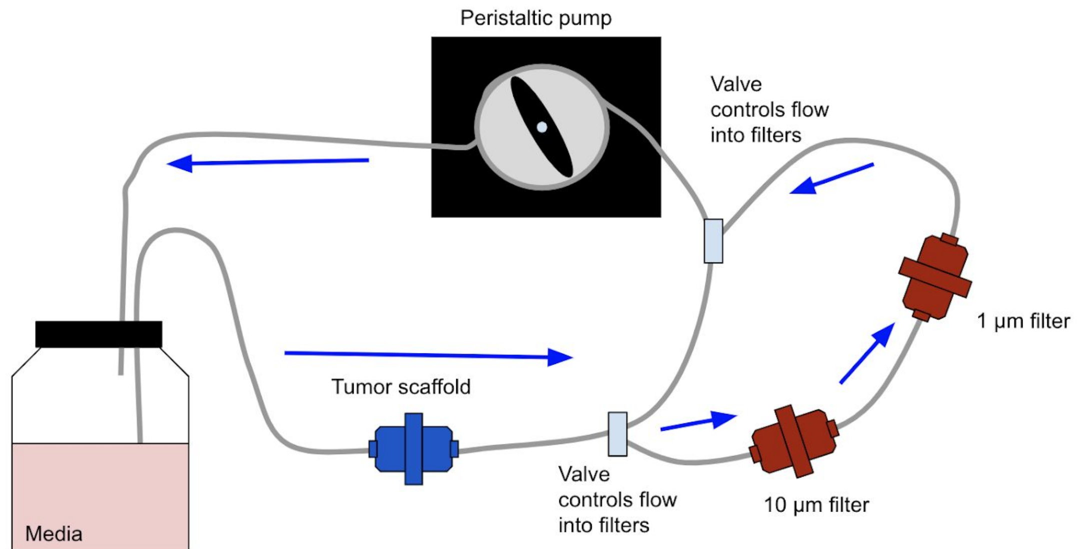


Figure 6.2: Final Collection System in Connection with the Bioreactor

6.2 Economical Consideration

Current market devices for circulating tumor cell collection and separation are extremely expensive to create and use. Our device offers a lower cost solution for cell separation and collection. The current market devices for CTC separation cost upwards of $125,000. Our team was given a budget of $750 for the project. This budget was used to purchase two sets of pluriStrainer filters and a set of swinnex filter holders which cost $446 total. One device which consists of one 10 μm filter and 1 μm filter, two swinnex filter holders, and tubing costs approximately $31.39 ($8.80 two pluriStrainer filters, $18.80 for two swinnex filter holders, and $3.79 for McMaster Carr tubing). The cost of our device means it can be utilized more extensively than other higher cost instruments in metastatic cancer research. The current treatment for metastatic neuroblastoma is multimodal therapy which is extremely expensive to the patient and expensive for the pharmaceutical companies creating the therapies. Use of our device could lead to better development of treatment for metastatic cancer, helping to alleviate the cost for patients and pharmaceutical companies.

6.2 Environmental Impact

The cell separation and collection device will likely have little impact on the natural environment. The separation device was created using tubing and connectors that can be placed in an autoclave to be sterilized. Use of reusable parts provides an environmental advantage compared to disposable parts. During the experimentation and creation of this device the team recycled paper materials and disposed of plastic sharps in designated receptacles where they would later be recycled.

6.3 Societal Influence

Researchers will most likely be the only people to interact with our device, so it will likely not have a significant immediate societal impact. This device can be used to research and characterize metastatic cancer cells in models of neuroblastoma metastasis, which has implications for the future research and potential treatment of the disease. While the device will not immediately impact the lives of everyday people, it will be able to further knowledge about a disease that impacts millions around the globe and may eventually contribute towards treatment of cancer.

6.4 Political Ramifications

This project will most likely have little to no political ramifications. It may contribute to research in other countries and eventually contribute towards a potential cure for cancer, but that is years in the future and not a current essential consideration of the team. Researchers in other countries would potentially be able to combine this device with their own developed research tools and collaborate to work towards a cure for cancer.

6.5 Ethical Concerns

Our project has the potential to aid in cancer research and a potential eventual cure for cancer, but remains a research tool. Researchers will be able to utilize this device to characterize metastatic cancer cells in models of cancer. Ethical concerns are not likely to play a role in the use of the device itself, if it is used and operated with lab safety and correct cell line usage guidelines. Anyone using this model may be able to use this system as a way to model how fast a

tumor is growing, given the number and size of CTCs in circulation, which may mean that they may be ethically obligated to use that information to notify the owner of the cells they are using. They will also potentially be able to see if drug treatments have an effect on metastatic cells in circulation. This project will aid in research for the treatment of cancer, which could eventually aid in improving quality of life for millions around the world.

6.6 Health and Safety Issues

Our project is designed for benchtop research and tumor modeling. Because this devices is not coming into contact with actual patients or health providers, there is minimal risk to those operating the device, including exposure to cells and media while removing the filters from the device. This model is an *in vitro* model of circulation and the neuroblastoma environment. The device does not pose any health or safety issues to the patient whose tumor is being modeled; however, if the device is not operated under current lab safety guidelines, there is some minimal risk to the operator. This model will hopefully reduce the risk of costly testing procedures and the possibility of predicting the rate of metastasis incorrectly.

6.7 Manufacturability

The collection system is made of a filter, a filter holder, and some tubing. The minimal materials needed will keep the cost low and it is able to be scaled up given that the materials can be purchased in bulk. The tubing sections were connected with female and male luer lock tube fittings. The Swinnex filters used to hold the filters come in packages of 12 at a time, allowing various circulation model systems to be made at one time within the bioreactor. The filters used

(pluriStrainer) also came in a multi pack (4 per size, 8 sizes), to allow for variability if using different cell types. To adapt our device for large scale models, few aspects would change: the cells used in the system, filter size, and pump size. However, because of the various filter sizes, this system is adaptable even in large quantities and for different cell sizes. If a large scale industry model is needed, Swinnex holders and filters come in larger sizes to allow more media and cells to flow through at a given time. A larger pump would also be required for large scale industry requirements.

6.8 Sustainability

Similar to environmental impact, the production of our device will likely have little effect on biology/ ecology in terms of renewable energy. Our device was created using reusable parts and filters which use minimal energy. The development of the perfusion bioreactor means that the system runs on a continuous loop. This continuous loop allows for less use of cell culture media run through the system as a whole which allows for a smaller environmental impact. Unfortunately, the electricity to run the pump and the power source needed do pose some concern. Also, the use of pipette tips and sterile cell culture materials are not renewable and are packaged in non-recyclable plastic.

VII. Discussion

7.1 Cell Collection Device

The created device was able to separate and collect circulating tumor cells by utilizing two filters in series. The first filter (10 μm) separated larger HFF cells in circulation from the CTCs by capturing the larger cell types on the surface or in the pores of the filter. The CTCs were then separated from smaller cells by a secondary filter (1 μm). Smaller particles were able to maneuver through the pores of the filter, while the CTCs were caught either on the surface or in the pores of the web.

While separation of CTCs from other cell types based on size is not unique to our project as it has been studied in literature before, the novel aspect of the device created for this project is that it is able to separate CTCs from other particles while in circulation within a perfusion bioreactor system. The device was incorporated into a small-scale perfusion based bioreactor, previously used to model *in vitro* metastatic neuroblastoma. Collection of the cells by the device allows future researchers to characterize metastatic cells in these models for further characterization. A cell collection device that has the ability to isolate SK-N-AS and similarly sized cells on through use of a 1 μm and 10 μm sized filters was created. These cells were able to be quantified through and viability of the cells was studied. This device separated SK-N-AS cells from larger cells in a model of circulation in the body, and a framework for a microfluidic model for further future separation was developed. This device meets the need for a cell collection

device for *in vitro* modeling, and can help further the characterization of model metastatic cells in future research.

The development of a microfluidic model for further CTC separation from other similarly sized cell types was also suggested as a method for further separation. This microfluidic device would separate cells based on deformability and utilize variously sized pathways and openings as well as multiple cross-stream flow rates. Future projects could research the feasibility of such a device.

7.2 Project Limitations

There were several limitations to our project. We only used one neuroblastoma cell line, SK-N-AS. This cell line was slightly smaller than the size of CTCs found in literature. This could have potentially impacted the size of the filters we used. The deformability of this cell line also likely had an effect on our experiments - despite size at around 9 μm, the cells were still able to maneuver through the 5 μm and 1 μm filters. While we did not have the time or resources to determine the young's modulus or stiffness of the cells, it is reasonable to assume they were able to deform enough to maneuver through holes smaller than their size. The weave of the filters themselves could also be to blame for cells making it through. Since we had to cut the cells from their original filter holders the weave of the filters could have been deformed or loosened over time. In future directions of this project a less damaging method for cutting the filters could be used or obtaining different filters that don't need to be cut at all could help to avoid this issue.

We were only able to separate the SK-N-AS cells from one other cell type - human foreskin fibroblasts (HFFs). These were larger than SK-N-AS cells on average that we were able

to separate the two, but separating cells closer in size to each other might pose as a challenge to our device.

The team encountered issues trying to remove both HFF and SK-N-AS cells from their respective filters. While flow-through cells could be counted and quantified, the cells on and in the filter were often stuck and the team could not accurately count the number of cells in the filers. Several different methods of removing cells from the filters such as rinsing the filters, running media through the filters backwards, and leaving the filters on a shaker plate, were all attempted to remove the cells from the filters for quantification. There is room for improvement in this area of our device.

Circulating tumor cells normally only move through the vasculature in a quantity of one CTC per milliliter of blood. This was difficult to visualize, so the team instead worked with quantities of CTCs in the hundred thousands or even millions per milliliter. This showed that our device worked in extreme circumstances, but it does not mimic conditions in the body. Similarly, there are often significantly less CTCs circulating in the blood when compared to other cells types, and we were testing with similar numbers of CTCs and HFFs in circulation. Future models could be adjusted to further test the ability of our device when working with cell counts that more accurately represent those in the body.

VIII. Conclusions and Recommendations

8.1 Conclusions

The goal for this project was to create a device that can collect and separate circulating metastatic like tumor cells from circulation following three main objectives:

1. Design a device that will collect circulating cells
2. Design a device which can separate circulating cells
3. Integrate this device with a perfusion-based bioreactor.

The team was able to develop a cell collection device which consisted of two pluriStrianer filters, 1 µm and 10 µm in series connected by tubing compatible with the bioreactor system. The filters were able to collect SK-N-AS cells in circulation within the bioreactor system, satisfying functions one and three. Human foreskin fibroblast (HFF) cells were also flowed through the filter system with SK-N-AS to model conditions of different cell types in the bioreactor. This demonstrated the ability for our device to collect cells from circulation and begin to separate different cell types based on size. Since the cell strainers were not able to separate cells with a high efficiency, the team developed a computational model for a microfluidic device which separates cell types based on deformity. To test the efficiency of our collection device, the team ran multiple verification tests including: initial sizing of the different cell types and pore sizes of the cell strainer using imageJ, pressure testing of the filters at various cell concentrations,

biocompatibility testing of the cell strainer material, long term viability study of the sterile bioreactor system and counting and staining of cells run through the filters.

8.2 Future Recommendations

The conclusions of this project point to many future directions for this research. The first being further development and refinement of the cell collection device. The collection device was created with filters that were cut from their original filter holders, but a future model of this device could be developed in a way where the filters do not need to be cut from their holders. Additionally, the filter sizes and geometry could be further reduced to sizes that could capture the cells in circulation more efficiently than the device we have created. Isolating cells from the filters is a multistep process which includes disconnecting the device from the bioreactor system, opening the filter holders, and washing the filters. There is room for improvement in this area of the device as a less disruptive process for obtaining the cells could be developed.

Further development of the microfluidic device is another future direction of this project. Due to time, resource, and budget constraints of this project the team was not able to develop a full working prototype of the microfluidic device for testing. Fabricating multiple variations of the microfluidic device changing the number, distribution, and size of the pillars as well as the cross flows provides lots of future research for this aspect of the project.

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Chapter 2: Analyzing Undergraduate Student and Faculty Perceptions of Writing in the BME Curriculum at WPI

Authorship Table

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I. Introduction	Sydney Hurley (Editor: Kelly Borden)
II. Background	**Primary Editor: Kelly Borden**
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Abstract

Our project focused on analyzing faculty and student perceptions of the teaching of writing within the BME Department. Through faculty and undergraduate (UG) student surveys, interviews, and focus groups, we discovered that the most writing core competencies occur at the 3000 course level, and more specifically, in lab courses. We then investigated the difference in student perceptions of a writing intensive (WI) lab course when compared to a lab course that was not writing intensive. We also completed a review of similar universities and their Biomedical Engineering departments to analyze how writing is taught and incorporated into other curriculums.

I.Introduction

Writing pedagogy in science and technology has been a source of research and debate. Courses in STEM fields often focus on the teaching of technical skills such as math, engineering, and lab techniques, and often neglect teaching about humanities and writing. However, knowledge of writing skills is essential for students to become effective communicators in industry. Researchers and professionals have argued about the benefits and costs of implicit compared to explicit instruction in the field, as well as whether Writing in the Discipline (WID) or Writing Across the Curriculum (WAC) is a more effective way to teach writing.

Worcester Polytechnic Institute (WPI) is a predominantly science, technology, engineering, and mathematics (STEM) based school. Because the school is predominantly technical majors, there is a much larger emphasis on STEM courses than humanities courses in the undergraduate curriculum. WPI is somewhat unique in that it does not require students to enroll in a writing class during their undergraduate studies.

The Biomedical Engineering Department at WPI has been internally evaluating their curriculum over the past several years to best prepare students for further graduate studies, future work in industry, and to best meet Accreditation Board for Engineering and Technology (ABET) requirements. As re-accreditation through ABET is occurring in the near future, the department is again evaluating their curriculum, and is looking at writing in their department.

ABET requirements for writing in technology are vague, simply requiring “An ability to communicate effectively with a range of audiences” (ABET). WPI’s lack of a required writing

course puts the department in a unique situation while trying to determine how, how much, and where writing should be incorporated into the curriculum.

WPI has no writing class requirements, but other similar universities differ in how they structure writing with in their programs. Northeastern's Undergraduate bioengineering program requires students to take two writing classes – "First Year Writing" their freshman year and "Advanced Writing in the Technical Professions" their junior year. The first course is an example of WAC – students in multiple different disciplines all must take this class. The second is more of an example of WID – it is more specific to STEM, but not as specific as a class based solely on Biomedical Engineering would be.

MIT's biomedical engineering program is more similar to that of WPI in that it only requires students take their choice of humanities courses, but requires at least two of these courses be communication intensive. RPI also doesn't require students take a specific writing course, but does require a writing-intensive capstone class.

Other similar universities seem to have more writing requirements than WPI, as well as offer more writing intensive (WI) classes. The BME department currently offers one writing intensive course which began in 2013 and in the past has kept track of writing and communication skills in courses based on "core competencies" for writing determined by the department. The department is evaluating writing in their department to both meet ABET requirements and ensure students are prepared for industry.

As students in the BME curriculum, we have experienced how writing is taught and assigned within our department. As the department is reviewing writing in their curriculum, we believe we can help to identify areas where the teaching of writing can be improved. The goal of

our project was to analyze where writing was currently happening in the Biomedical Engineering Department, and evaluate faculty and undergraduate student perceptions of writing within the department. Our research is novel in that it focuses more on student perspectives in conjunction with faculty perceptions, and uses our unique position as students in the department to provide a more involved insight to writing in the curriculum. Through identifying where writing pedagogy and assignments were occurring in the department as well as the perceptions of the people involved in that writing, we hoped to determine if there was a need for any changes to the writing curriculum and provide recommendations to the department based on our research.

II. Background

2.1 Writing Across the Curriculum (WAC) and Writing in the Disciplines (WID)

Students in the BME department at WPI require not only with technical knowledge of the theories learned in courses but also the ability to communicate as professional biomedical engineers. Writing across the curriculum (WAC) and writing in the disciplines (WID) are two different forms of teaching writing within college curriculums. WAC is a more general form of writing – an example of this could be a general writing course all students enrolled in a particular university are required to take. Writing in the disciplines (WID) is more specific to each discipline – an example could be a course called "Writing in the Life Sciences" that only biology students are required to enroll in. Programs that focus on integrating written and verbal communication skills have been designated as communication across the curriculum (CAC) - pulling from the same form of instruction and motivations seen in WAC programs (Bean, 2011). WPI has a form of a communication across the curriculum program to integrate writing into undergraduate courses.

WAC programs rely on assumptions in order to succeed, some of those being that writing aids in the learning process and that it is separate enough from English to be its own subject area of focus (Mcleod et al., 1992). WAC has been described as more of a writing to learn technique of teaching rather than a learning to write form of study (Herrington, 1981). The technique of teaching through WAC leads students to participate in a variety of writing activities helping them to dive deeper into ideas and concepts in a course (Bean, 2011)

WID is more specific to writing within a particular field and aims to develop student's

skills for writing in discipline-specific genres (Bean, 2011). Disciplines vary not only in style but also in sources and the formation of arguments (Hyland, 2009). With so many variations across disciplines, some argue that it is difficult to teach a general writing course across the curriculum that encompasses all the details students may need to know for their field. However, WID is much more narrow and requires more genre-specific resources than WAC.

At WPI, there is a Communication Across The Curriculum (CxC) university-wide program that aims to promote writing and other communication-based learning initiatives across the WPI curriculum ("Writing Intensive Courses", n.d.). One of the outcomes of this program was the implementation of writing intensive (WI) courses in at WPI.

We consulted with Professor Lorraine Higgins, Director of Communication Across the Curriculum, to gather more information about writing intensive courses at WPI. The discussion for writing intensive courses began at WPI in 2008. This led to the creation of the Summer Institute on Teaching Writing. The institute was designed to encourage professors to integrate writing into their courses and give professors instruction on teaching writing in technical courses.

The motion to approve the WI label of courses at WPI happened in 2012. There is an application process for a course to officially become writing intensive. At least 30% of the grade for the course must be based on writing, there must be a revision and feedback process for writing assignments, and there needs to be lectures including direct writing instruction.

Currently, there is only one WI designated course in the BME curriculum. These WI courses aim to integrate writing as a way to teach course content through writing and develop student communication skills, "As they write and revise in specialized genres, they learn to talk

and think like professionals in the field" ("Communication Across the Curriculum", n.d.). David J. Bartholomae, an American scholar in composition, literacy and pedagogy widely cited in literature about the pedagogy of writing, studied this transition from student to professional through mastering navigating the discourse of the professional community.

Bartholomae describes the different sets of conventions a student should master in order to be successful in the transition from student to professional (Bartholomae, 2005). These conventions can be taught through the methods described within writing across the curriculum (WAC) and writing in the disciplines (WID) programs. In describing the first set of conventions a student must master to translate their academic knowledge into professionalism, Bartholomae describes the general notions of writing that WAC style courses may cover, "He must master, to be successful, the conventions of written discourse rules of grammar and syntax, rules of style and diction, rules of structure and organization all varying with purpose and audience" (Bartholomae, 2005).

Bartholomae continues his discussion of necessary conventions to master along the journey from student to professional through describing the need for a student to have control over field specific conventions. "To perform successfully as a practitioner, one must be more than reasonable: one must have more than information about the subject" (Bartholomae, 2005) Bartholomae is speaking about the understanding that in order to perform as professionals, students need more than knowledge and theories about their field, they need to understand the way communication takes place in the field and they ways that mark it as discipline specific.

WID courses have been implemented with varying levels of success. A 2015 article by Holstein et al. described the process of implementing a heavier writing emphasis in an

introductory course in the field of neurobiology (Holstein et al., 2015). The writing emphasis included more writing assignments and more student interaction with scientific literature. Researchers found that this increased exposure improved the students' knowledge of genre writing but did not improve their elemental writing skills such as grammar. While this did not improve their elemental writing skills, it is important for students to develop genre specific knowledge through the WID style of instruction to be able to communication effectively as professionals.

2.2 Teaching Scientific Writing: Implicit vs. Explicit Instruction

WAC and WID are two different methods of teaching writing within or outside of subject areas. Within each subject area, however, there are two different methods of teaching and learning writing - implicit and explicit. Explicit learning has been defined as knowledge that learners are consciously aware of (Aral et al., 2016). Explicit learning is taught through the deductive style of teaching. Deductive teaching has students learn principles before they are taught real world applications. Felder et. al claims that deductive teaching is how teachers naturally teach as it is easier to explain concepts as a whole before explaining why they are important (Felder, 1988).

Implicit learning is when students learn without formal explanation. In general terms, students are not explicitly told what to do and instead learn for themselves based off of experience. Implicit learning is taught through the process of inductive teaching. Through inductive teaching, students draw conclusions from observing processes. This has been thought to be the natural style of human learning (Aral et al., 2016). Felder et. al found through surveys

that most engineering students viewed themselves as inductive learners and their professors viewed themselves as almost completely deductive teachers.

This distinction is important when considering the use of writing intensive courses. One of the qualifiers for a writing intensive course at WPI is class time devoted to the teaching of writing. This is an example of explicit teaching. In contrast, the use of annotated examples, which may be provided in non-writing intensive lab courses, could be considered implicit teaching as it encourages students to use examples on their own to learn. Although faculty may view themselves as teaching explicitly, they may in fact be teaching implicitly. If explicit learning is more beneficial for students, then there may be changes that could be implemented to encourage faculty to teach writing more explicitly, such as meeting the requirements of a writing intensive course. In order to assess if students and faculty had the same perceptions of the teaching of writing in the BME curriculum as the perceptions found in literature, we conducted both student and faculty surveys to gather information about the teaching of writing in the department.

III. Methods

3.1 Informational Interviews with WPI Faculty

In order to assess the wants, needs, and practices of writing within the Biomedical Engineering department we conducted a series of preliminary informational interviews with WPI faculty.The goal of these interviews was to gather information about the current state of writing within the department.

We began this investigation by interviewing the head of the BME department, Professor Kristen Billiar. He shared that he had completed prior research on where writing and communication skills were being developed within the BME curriculum. He focused on the department's desire to meet the Accreditation Board for Engineering and Technology (ABET) requirements, as the department is up for re-accreditation in the coming years. From him, we obtained a survey that he had conducted almost ten years prior. This survey investigated where certain writing "competencies" were occurring in classes and can be seen in Figure 3.1 below.

Core Competency	BME 1001	BME 2101 Measurements	BME 2102 Biomechanics	BME 2103 Biomaterials	BME 2104 Bioelectronics	BME 3111 Physiology	BME 3300 Design	BME 3011	BME 3504a,b	BME 4011	BME 4023	BME 4025	BME 4201	BME 4504	BME 4541	BME 4606	BME 4814	BME 4828	IQP	MQP	New Course	Total
Concise writing		X	X	X	X		X		X									X				0
Grammar and mechanics		X	X	X	X				X									X				0
Style and professional tone		X	X	X	X		X		X													0
Proper citations and crediting		X	X	X	X	X	X		X									X				0
Graphical communication (tables, charts)		X	X	X	X	X			X													0
INTERMEDIATE:																						
Preparing an Executive Summary			X		X		X		X													0
Lab report (organization and formatting)		X				X			X													0
Lab notebook		X				X	X		X													0
Power-Point presentations				X	X		X		X									X				0
Public speaking				X	X	X	X											X				0
Elevator pitch				X	X		X															0
Unbiased presentations /critique of work				X		X																0
Team dynamics			X				X		X									X				0
Electronic media (emails, voice mails)		X			X		X															0
ADVANCED:																						
Technical report organization and formatting						X			X											X		0
scientific paper organization and formatting						X																0
Abstract writing						X			X											X		0
Proposal writing									X													0
Synthesizing ideas from multiple sources (not									X									X		X		0
Concise summaries of literature																		X		X		

Figure 3.1: Survey of where forms of communication are occurring in BME department

After interviewing Professor Billiar and establishing that the department saw a need for our project, we set out to create surveys for faculty to update Billiar's findings and to determine where the current faculty thought writing was happening in their classes. To design the survey, we followed the survey-design state as described by Weisberg et al. in "The Survey Process" (Weisberg et al., 26-27). For this survey, our population was BME faculty at WPI. It was therefore reasonable to survey all of our population because our target population was so small (only 16 faculty members). We determined the best way to collect data would be handing out the survey to faculty members at department meeting and follow-up surveys in the form of face-to- face interviews.

In addition to collecting preliminary information from BME department faculty, we utilized resources outside of the department to gather more information writing in the curriculum at WPI. To better understand how teaching assistants (TA) and rubrics fit into the teaching of writing, we interviewed Natalie Farney, Associate Director of the Morgan Teaching and Learning Center. This center hosts mandatory TA training for newly hired TAs and reviews with TAs how to create rubrics. Professor Billiar had also mentioned that certain classes in BME were at one time designated as writing intensive (WI), so in order to learn more about the criteria for writing intensive classes we met with Lorraine Higgins who is the Director of the Summer Institute of Teaching Writing, a program for faculty to better learn the importance of and how to incorporate the teaching of writing within their classes.

3.2 Faculty Survey and Follow Up Interviews

After assessing core competencies listed on the original chart Billiar had used, we decided to edit the list of competencies to update it to meet the current needs of the BME department. As ABET was Billiar's main focus, we researched what ABET requirements involved in communication and writing. The student outcomes for engineering programs had little to do with writing, except for expected student outcome (g), which required a student outcome to be "an ability to communicate effectively." (ABET).

ABET provided little direction for our research, so we decided to draw on the types of writing students may encounter in the professional world after graduation to determine if the core competencies listed on the survey were still relevant to the department. We adjusted the competencies slightly to become more writing-specific, for example, we removed "team

dynamics" and "public speaking".

The original survey given to us only evaluated if the core competency was present in the class or not, with no further information into how or to what extent. In order to try to gain more information from the survey we decided to break down how these competencies could be expressed. We did this by asking professors to specify if the competencies were being assigned, taught, evaluated for, or if feedback was given on them in their classes. *Assigned* indicated that students were explicitly told to do that competency in assignments, and *evaluated* indicated that students were graded on that competency. *Teaching* was defined broadly – discussions in class or providing annotated examples were both considered teaching. *Feedback* was limited to formative feedback, which was defined as allowing students to make corrections based on feedback and resubmit or perform a similar assignment later in the class. We also eliminated the course titles from the survey so faculty could fill it out only for their classes. The finalized survey can be seen in Figure 3.2 below. We gave out the survey at the BME faculty meeting in B term.

Core Competency	Course Title: Biomedical Example Class, BME XXXX	Course Title:	Course Title:	Course Title:	Course Title:	Course Title:	Course Title:
BASIC:							
Concise writing	A/T/E/F	A/T/E/F	A/T/E/F	A/T/E/F	A/T/E/F	A/T/E/F	A/T/E/F
Grammar	A/T/E/F	A/T/E/F	A/T/E/F	A/T/E/F	A/T/E/F	A/T/E/F	A/T/E/F
Style and professional tone	A/T/E/F	A/T/E/F	A/T/E/F	A/T/E/F	A/T/E/F	A/T/E/F	A/T/E/F
Proper citations and crediting	A/T/E/F	A/T/E/F	A/T/E/F	A/T/E/F	A/T/E/F	A/T/E/F	A/T/E/F
Graphical communication (tables, charts)	A/T/E/F	A/T/E/F	A/T/E/F	A/T/E/F	A/T/E/F	A/T/E/F	A/T/E/F
Other: ____________	A/T/E/F	A/T/E/F	A/T/E/F	A/T/E/F	A/T/E/F	A/T/E/F	A/T/E/F
INTERMEDIATE:							
Preparing an Executive Summary	A/T/E/F	A/T/E/F	A/T/E/F	A/T/E/F	A/T/E/F	A/T/E/F	A/T/E/F
Lab Report Organization	A/T/E/F	A/T/E/F	A/T/E/F	A/T/E/F	A/T/E/F	A/T/E/F	A/T/E/F
Lab Report Format	A/T/E/F	A/T/E/F	A/T/E/F	A/T/E/F	A/T/E/F	A/T/E/F	A/T/E/F
Poster Writing	A/T/E/F	A/T/E/F	A/T/E/F	A/T/E/F	A/T/E/F	A/T/E/F	A/T/E/F
Concise summaries of literature	A/T/E/F	A/T/E/F	A/T/E/F	A/T/E/F	A/T/E/F	A/T/E/F	A/T/E/F
Other: ____________	A/T/E/F	A/T/E/F	A/T/E/F	A/T/E/F	A/T/E/F	A/T/E/F	A/T/E/F
ADVANCED:							
Technical Report Writing	A/T/E/F	A/T/E/F	A/T/E/F	A/T/E/F	A/T/E/F	A/T/E/F	A/T/E/F
Scientific Paper Writing	A/T/E/F	A/T/E/F	A/T/E/F	A/T/E/F	A/T/E/F	A/T/E/F	A/T/E/F
Abstract Writing	A/T/E/F	A/T/E/F	A/T/E/F	A/T/E/F	A/T/E/F	A/T/E/F	A/T/E/F
Proposal Writing	A/T/E/F	A/T/E/F	A/T/E/F	A/T/E/F	A/T/E/F	A/T/E/F	A/T/E/F
Other: ____________	A/T/E/F	A/T/E/F	A/T/E/F	A/T/E/F	A/T/E/F	A/T/E/F	A/T/E/F
			A: Assign, T: Teach, E: Evaluate, F: Feedback				

Figure 3.2: Finalized survey for BME faculty

3.3 Student Perspectives

3.3.1 Investigating Lab Courses - Student Surveys

We hypothesized that one reason there may be a gap in between faculty and student perceptions of writing is the difference between implicit teaching of writing and explicit teaching of writing. Through casual conversations with students and faculty within the department, the results of the faculty surveys, and our own prior experience, we determined that analyzing lab classes and the writing within lab classes would be the best subject for an in-depth investigation

on student and faculty perception of implicit compared to explicit in-class teaching.

To obtain an undergraduate degree in Biomedical Engineering from WPI, you must take four lab courses. There are nine total lab courses offered, three in each of the three focus areas of BME. Students are recommended to take the three labs within their focus area and one outside of it, however, nothing mandates that those be the classes taken by the student. There is also no enforced order for students to take lab courses in, so any lab could be a student's first lab.

With the knowledge that all BME undergraduates must take lab courses but the content and order of these lab courses is variable by student, we decided to use BME labs offered in C term as a case study. Three lab courses were offered in C term, and we obtained permission to study the student perspectives in two of them.

We conducted a student survey to determine where they believe explicit class instruction is occurring and what materials they are using to complete class assignments. We asked questions about class materials and in-class instruction. These questions can be found in Appendix A. This survey was completely volunteer based and was conducted in the form of an online questionnaire through Qualtrics. The participation in the survey was incentivized with random participants being selected to win a Dunkin Donuts gift card.

From this survey, we had several questions we attempted to answer. We were trying to determine whether students felt that the writing intensive course better improved their writing skills and if the way students approached writing assignments was different between the two courses. For example, asking them to indicate their perceived writing ability on a scale of 1-10 both before and after the course would help us to determine if students perceived more improvement in the writing intensive or the non writing intensive course. These include

understanding the effectiveness of the prompt, in-class teaching, and what outside materials were used. Each question was worded carefully in order to not lead respondents to answer in a certain way. This was especially important because it not only affected the results of our survey, but also had the potential to affect student course evaluations at the end of the term. By asking students questions that force them to further reflect on their assignments, there was the potential that we could make them question the course as a whole, which could lead to more criticism of the class. In order to avoid this, we had to make sure questions were as unbiased as possible. All survey questions were approved by both our advisor and the instructor of the course. The instructor of the course also had access to all responses.

3.3.2 Focus groups - BME Undergraduates

To collect undergraduate perceptions of the writing happening in the BME curriculum beyond lab courses, we decided to conduct a semi-structured focus group. This method has proven effective for generating discussion and allowing the participants a space to express their opinions (Villard, 2001). The purpose of our focus group was to gather the opinions and perceptions of students in their last year of study in the curriculum. We decided to perform a focus group rather than a survey to allow the students we were questioning to have more of an ability to guide the conversation and get more interactive feedback about writing in the BME curriculum. We chose to do the focus group with senior students because they had already taken most if not all of their required BME classes and had the most experience with courses in the curriculum. We used this specific population to ensure that the participants had been exposed to a majority of the classes offered in the curriculum.

The questions were organized in order from general to specific; however, we allowed time for follow up questions if the conversation dictated it. The exact questions asked in the focus group can be found in Appendix B. Participation for this focus group was voluntary from start to finish. During the focus group, responses were noted and recorded.

3.4 Research on other school's curriculums

While analyzing writing within the BME department would hopefully provide some useful information, there was no guarantee we would find instances of implicit learning compared to in-class teaching let alone be able to provide any recommendations based on our results. Our analysis of the WPI BME lab writing was also a small case study that was not statistically significant and did not evaluate the teaching of writing on the scale necessary to make an immediate and large change in the department.

In order to provide further recommendations to the department, we researched what other universities were doing in terms of writing in engineering and STEM. Similar universities have already been touched on in the background section, but we examined more universities similar in size and scope to WPI. With this information, we planned to provide the BME department with an overview of the practices of other universities. The BME department will be able to utilize this review for future work on writing within their department, which will likely continue after the conclusion of our project.

IV. Results

4.1 Faculty Survey and Follow Up Surveys

After conducting the faculty surveys, we first analyzed the perceptions of faculty within the department. Figure 4.1 below shows the number of faculty that report they are either assigning, evaluating, teaching, or providing feedback on the writing core competencies.

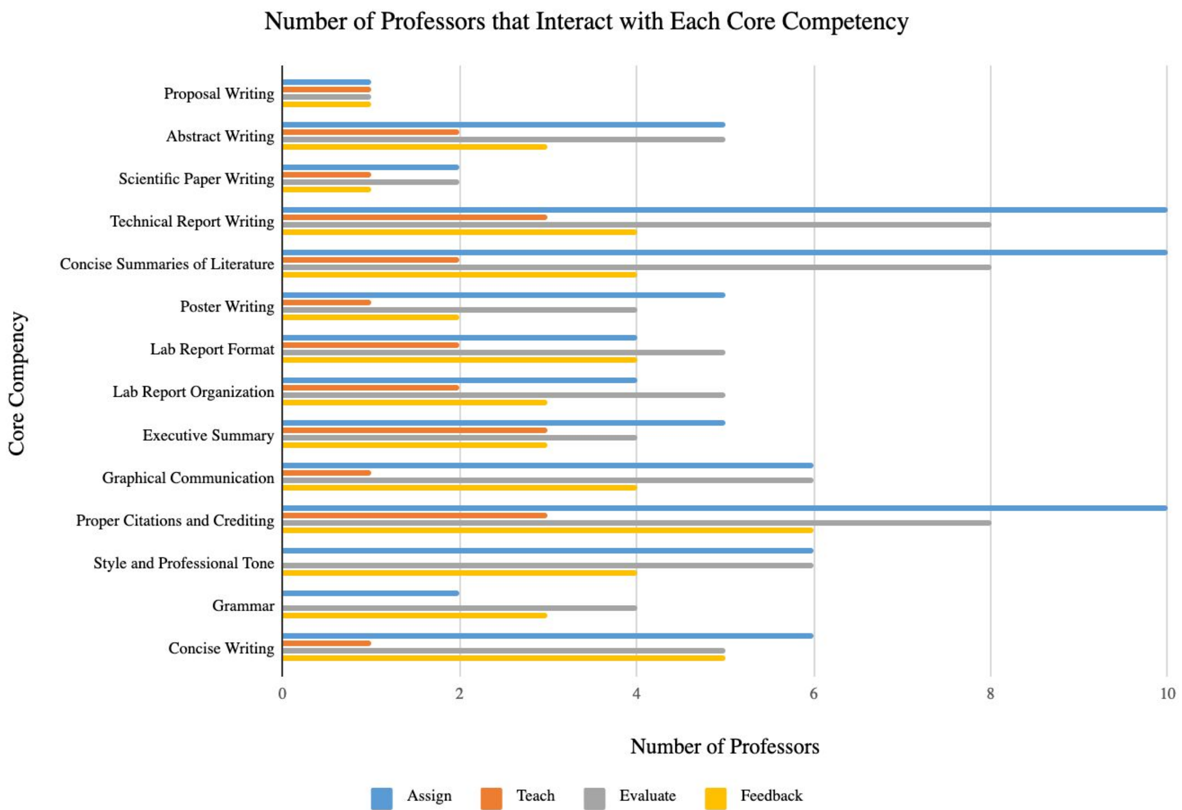

Figure 4.1: Number of Professors who believe they provide feedback on, evaluate, teach, or assign each core competency.

We then analyzed how many faculty believed they were either assigning, evaluating, teaching, or providing feedback, or some combination of, in regards to the writing core competencies. This was to frame this section of the results in terms of how many faculty members overall, rather than split up by core competencies as shown in 4.1 above, to take a closer look at faculty's individual perceptions of their interactions with the writing core competencies. Figure 4.2 below shows faculty perceptions of their interaction with the core competencies.

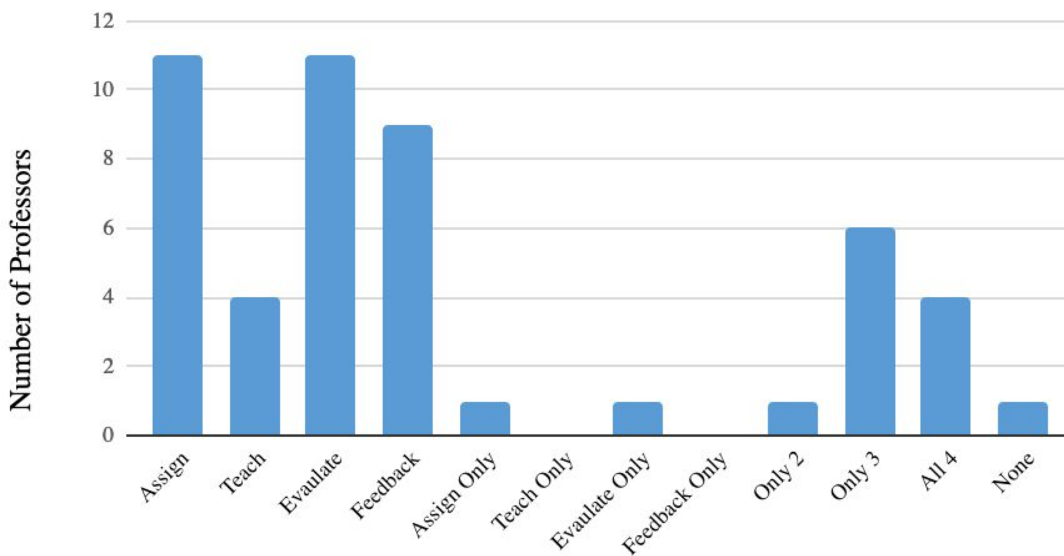

Figure 4.2: Number of professors who assign, teach, evaluate, give feedback on, or some combination of those regarding the writing core competencies

As shown in Figure 4.2 above, the teaching of writing (through methods such as lectures or covering annotated examples in class) is one of the least often perceived interactions with the

writing core competencies. After analyzing faculty perceptions, we proceeded to analyze where the core competencies were occurring in BME lab courses. Figure 4.3 below shows how many of the core competencies were being addressed in BME courses overall and in what form they are being addressed.

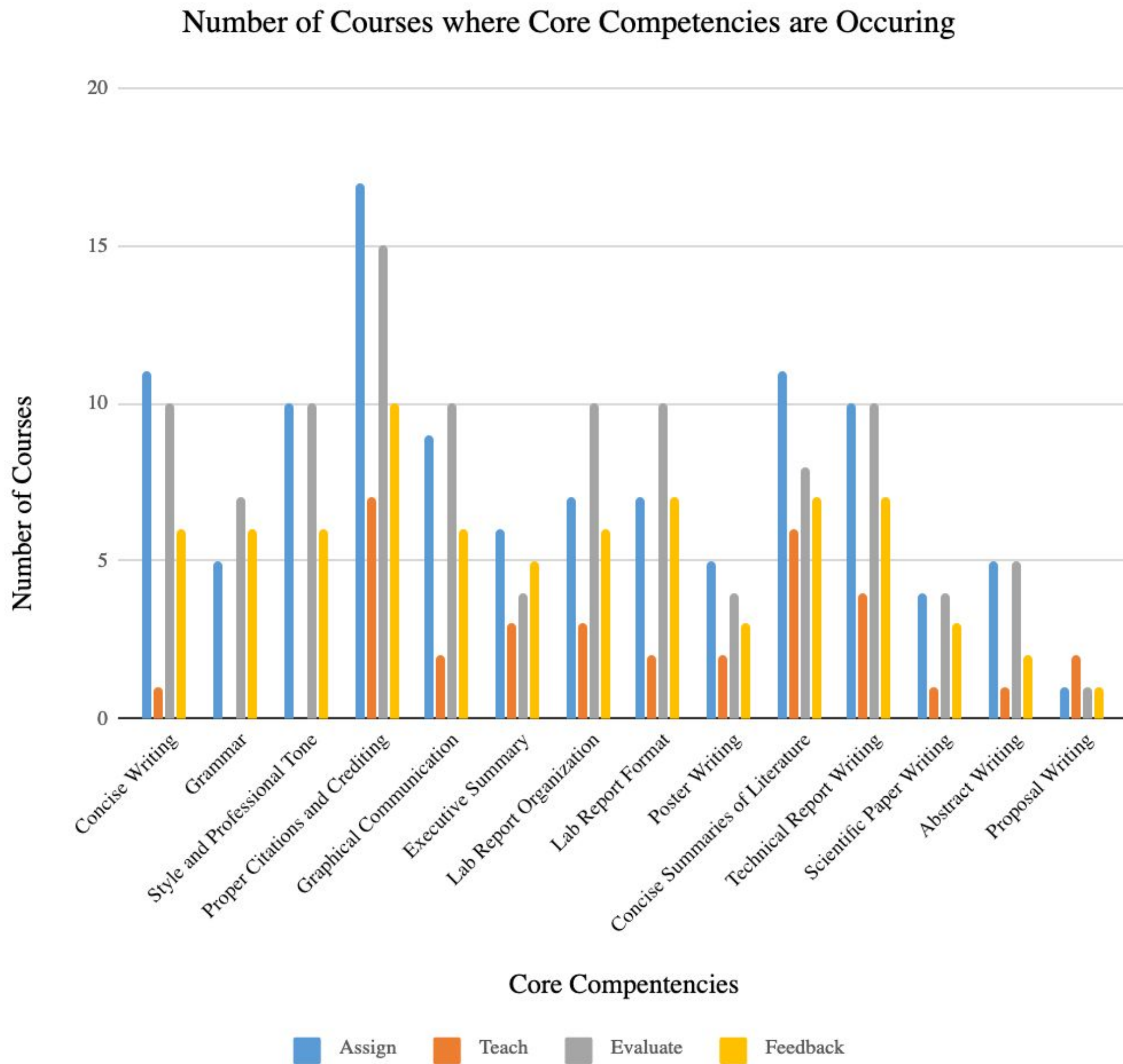

Figure 4.3: How often the core competencies are being addressed in BME courses

After determining which core competencies were occurring, we decided to determine where the core competencies were occurring based on course level to determine at one point in the curriculum and students' education the core competencies were occurring. Figures 4.4 - 4.6 below shows how frequently the writing core competencies are occurring in each course level .

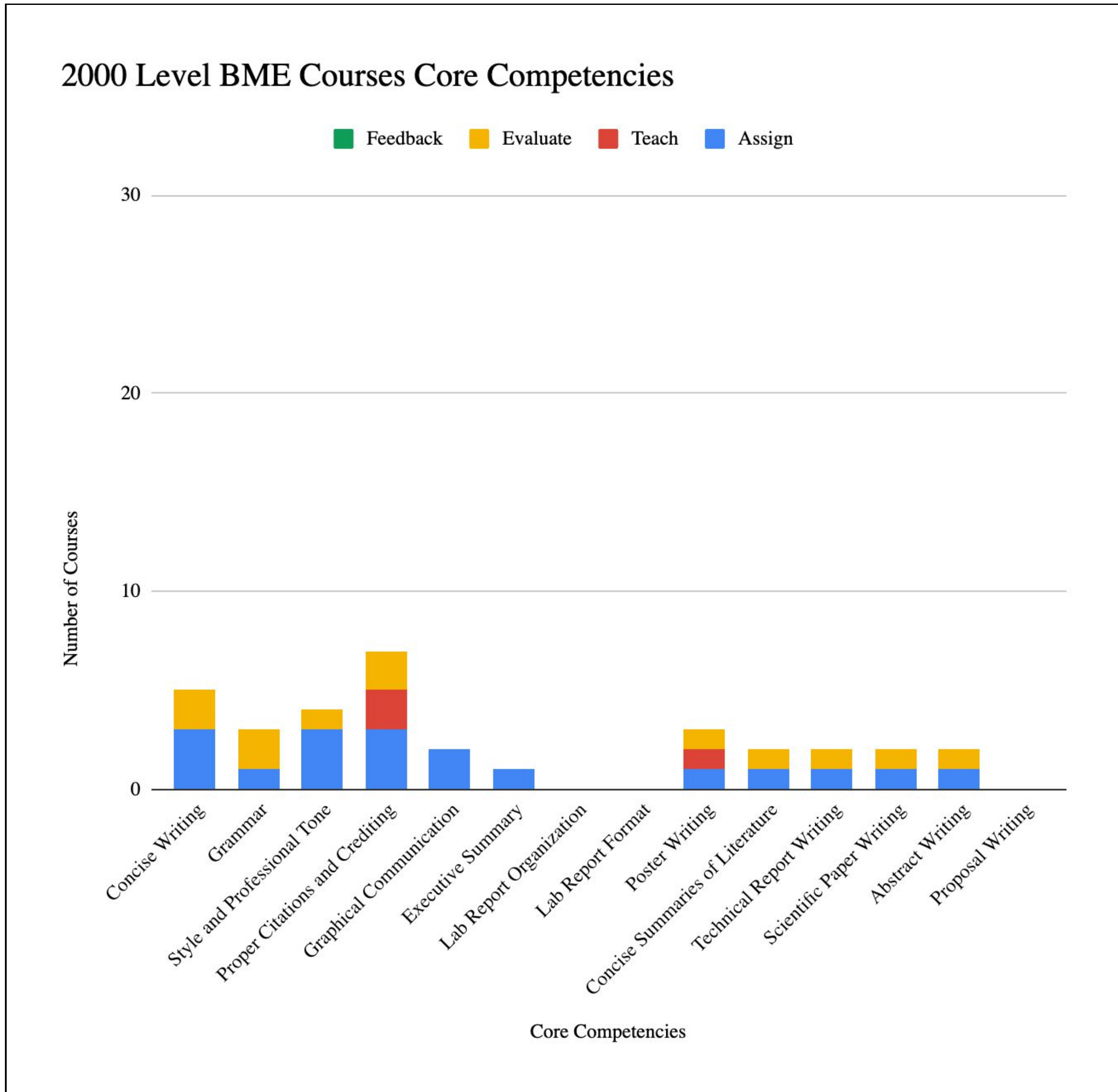

Figure 4.4: Where the core competencies in 2000 level BME courses

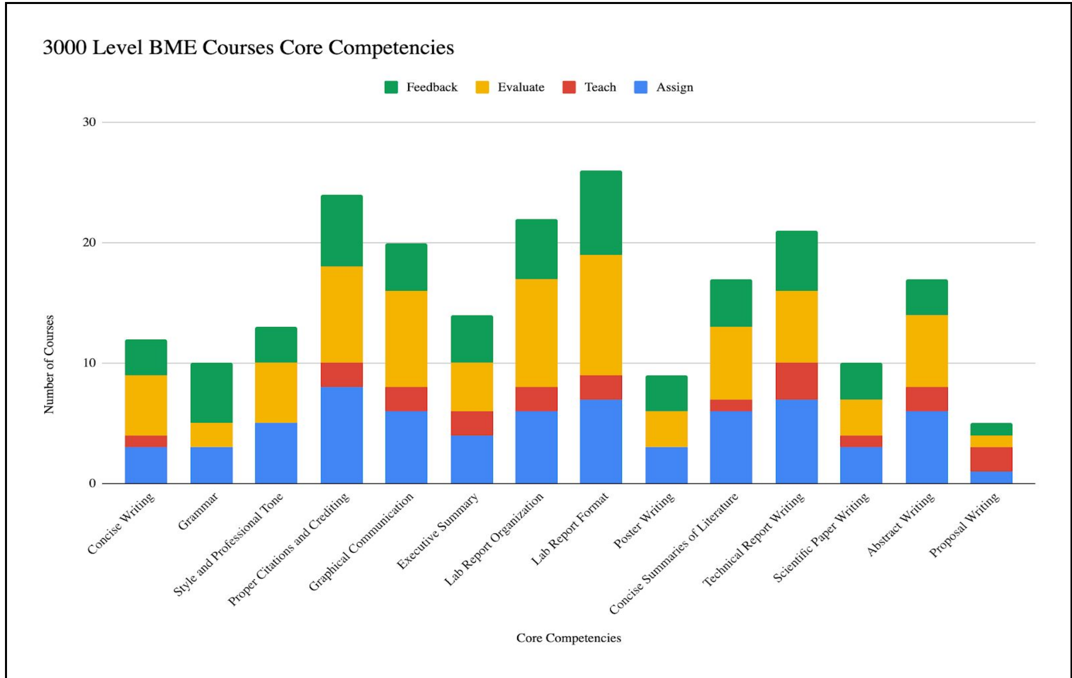

Figure 4.5: Where the core competencies in 3000 level BME courses

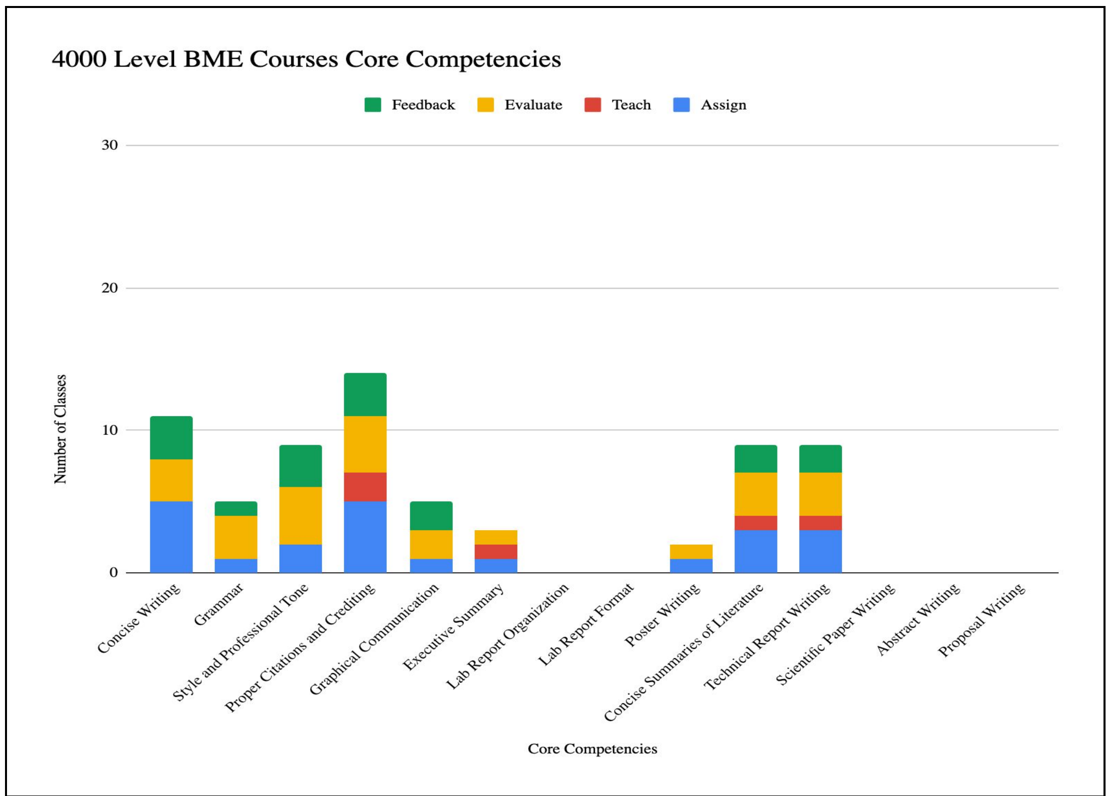

Figure 4.6: Where the core competencies in 4000 level BME courses

As shown in Figures 4.4 - 4.6 above, the writing core competencies are most frequently occurring in the 3000 level courses. Eight out of the eleven 3000 level courses are lab courses, and with that in mind we decided to compare how frequently the writing core competencies were occurring in lab courses when compared to all other BME courses. The results of this comparison are shown in Figure 4.7 below.

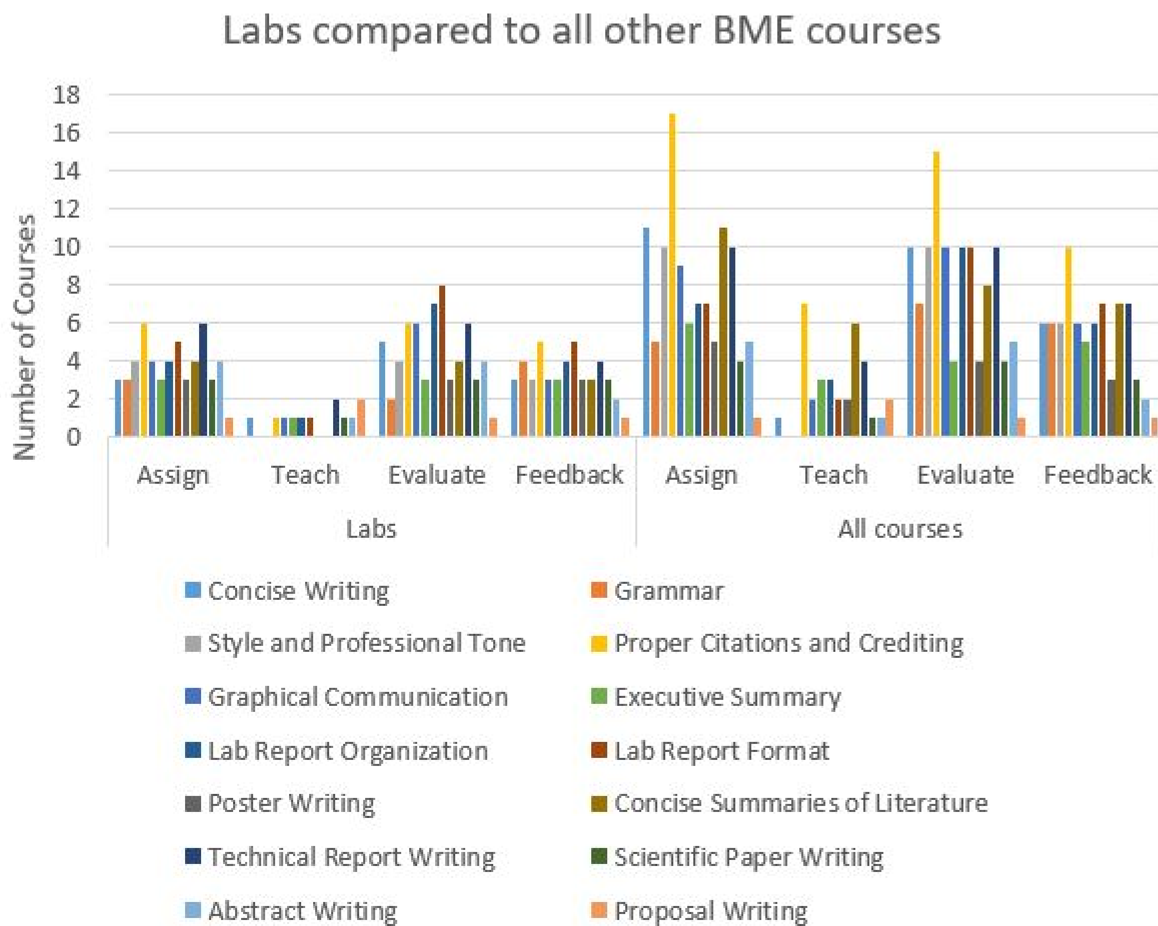

Figure 4.7: Where the core competencies are occurring in lab courses compared to other courses

As shown in Figure 4.7 above, a significant amount of the writing core competencies are occurring in BME lab courses. Because of this analysis, we decided to conduct a study on student perceptions of writing in lab courses. We compared student perceptions of writing in a writing intensive BME lab course compared to a non writing intensive BME lab course. Both occurred concurrently in the same term and were taught by the same instructor.

4.2 Student Perspectives Results

4.2.1 Investigating Lab Courses - Student Surveys

We sent out a survey to students in two lab courses occurring concurrently in the same term taught by the same professor; one course was writing intensive, the other was not. We first analyzed students' perception of their writing ability in the beginning of the course compared to the end of the course, categorized by which class they were in. These results are shown in Table 4.1 below.

Table 4.1: Student Perceptions of Writing Ability Based on WI or non-WI course

Lab Course	Perception of Writing Ability Before Class (1-10)	Perception of Writing Ability After Class (1-10)	Average Improvement
non-WI	8	9	1
non-WI	7	7	0
non-WI	9	9	0
non-WI	5	6	1
non-WI	5	8	3
WI	1	3	2
WI	10	10	0
WI	8	8	0
WI	6	8	2
Average non-WI	**6.8**	**7.8**	**1**
Average WI	**6.25**	**7.25**	**1**

As shown in Table 4.1 above, students in the writing intensive course believed that their writing improved around the same amount when compared to students in the non-writing intensive course. It is worth noting that two of the students currently enrolled in the non-writing intensive course had taken the writing intensive course previously, and rated their writing ability as 7 and 9 at the beginning of the non-writing intensive class. They also indicated their perceived writing ability did not improve over the course of the non-writing intensive class.

Upon further analysis of data, we determined that a larger indicator of student perception of writing was based on whether or not the course taken was the student's first lab course. The results of this analysis are presented in Table 4.2 below.

Table 4.2: Student Perceptions of Writing Ability based on number of lab courses taken

Number Lab Course Taken	Perception of Writing Ability Before Class (1-10)	Perception of Writing Ability After Class (1-10)	Average Improvement
One	1	3	2
One	8	8	0
One	5	8	3
Two	10	10	0
Two	6	8	2
Three	8	8	0
Three	9	9	0
Three	5	6	1
Four	8	9	1
Four	7	7	0
Average One	**4.67**	**6.33**	**1.66**
Average Two	**8**	**9**	**1**
Average Three	**7.33**	**7.67**	**0.34**
Average Four	**7.5**	**8**	**0.5**

4.2.2 Focus groups - BME Undergraduates

After performing the faculty surveys, we determined that we wanted to gain a broad student perspective on the BME curriculum as a whole to compare their views to faculty perception. A total of six undergraduate seniors in the BME department participated in our focus group. Each student had taken all four lab courses required for the lab course requirement.

When asked about writing they have done in their BME courses and the most impactful writing assignment they have completed the conversation was focused on lack of feedback and guidance given. “The most impactful writing I’ve done wasn’t in the BME department, I don’t think BME actually teaches writing,” one student said reflecting on this. “No guidance or meaningful feedback was ever given to me...” another student shared.

Participants specifically mentioned BME 3300 Biomedical Design, BME3505: Solid Biomechanics Laboratory: Techniques, and BME 4300: MQP Capstone Design as BME courses with the impactful writing assignments. At this point, the conversation began to focus on BME 3300 and 4000 level BME courses. BME 3300 is a design course almost every BME student has to take that revolves around the biomedical engineering design process and culminates in a lengthy report similar to the MQP. All students present said they wished more writing instruction was worked into this class and that there could be more of a focus on instruction for writing International Organization for Standardization (ISO) and American Society for Testing and Materials (ASTM) standards. This brought up discussion of writing students have completed while working industry internships and co-ops. Four students said that they wished writing assignments they were asked to do in their BME classes better aligned with more “business style” writing such as validation and verification reports and they wished more writing of this nature could be incorporated into BME 3300 and their 4000 level BME courses to better prepare them for industry. “I would rather know the importance of the paper rather than just what to write to get an A,” a student shared after talking about the difference between writing they have completed in their internship and writing done in their BME courses.

This conversation continued in a similar direction after asking about thoughts on how writing instruction in BME had prepared students for their post graduation plans. “Test method development, writing up summaries and protocols have been glazed over in all my classes,” one student shared. “I have a good background on theory but no ASTM or ISO standards awareness and I’m going to need that in industry,” another student continued. Several students shared that they wanted more of a background on industry specific writing and did not feel adequately prepared in this area. The discussion moved toward the topic of areas within the BME curriculum where gaps in writing exist. Students said they felt feedback on assignments before grading is lacking and they wished there was more of an incentive for revising assignments they had already received a grade on. Students also commented on consistency between courses. There was a consensus that students wished there was more consistency across lab courses and expectations for format and structure of reports. There was also a consensus that the curriculum was lacking in terms of instructing students on how to interpret and write about statistics and data.

The next notable topic of discussion from the focus group came from asking if there should be a writing class required for students. Almost all students agreed that there should be more instruction on writing and that a background in technical writing was missing from their educations, but they disagreed about whether a requirement would be best. One person said, “It’s so disappointing that WPI does not have a required writing class. In at least each project there is one person who cannot write well, and it is more work for teammates to go back and decipher what they have written.”

While all of the students felt there was more writing instruction needed, not all of the students agreed this should be done in the form of adding another course to the curriculum. "I think there is enough writing happening, I just don't think it's focusing on the right stuff," one student said. "If there was one writing class everyone had to take, people would dread it. Adding writing into the existing classes and consistency between classes would be better," another student said reflecting on this idea. Students suggested that writing should be incorporated more into the 2000 level BME courses rather than lab courses because labs are already very "loaded".

4.3 Review of Other Colleges' Curriculum in Respect to WPI

To further put the WPI BME curriculum into perspective, a review of other university's writing requirements was conducted. Six universities that WPI names as either peer or aspirant universities were chosen and analyzed. There were four categories reviewed: a writing requirement within the department, a writing requirement outside of the department, whether a discipline specific writing class was offered, and whether a writing intensive class in the department was offered. The results from this review are shown in Table 4.3. Most of the schools reviewed had at least a school writing requirement. MIT and RIT had a specific writing requirements within the Biomedical Engineering Department. MIT's curriculum had two laboratory classes that fulfilled this requirement. RIT had a technical writing class within the department that was required for every student. Although RPI did not have a department specific writing requirement, the BME design course was considered to be Writing Intensive and was required for every student. The (#) behind a requirement indicates how many classes are required for that category.

Table 4.3: Comparison of BME Curriculum and Writing Requirements for WPI Benchmark Universities

University	Requirement within the Department	Requirement outside the Department	Discipline Writing Offered	Writing Intensive Offered
WPI	No	No	No	Yes
MIT	Yes (1-WI)	Yes (1-W)	No	Yes
RIT	Yes (2-W)	Yes (1-W)	Yes	Yes
RPI	No	Yes (2-W)	No	Yes
IIT	Yes (2-WI)	Yes*	No	Yes
SIT	No	Yes (2-W)	No	No
Clarkson	Yes (2-WI)	Yes (1-WI)	No	Yes

*Requires students to meet basic proficiency levels, and a class is only required if levels are not met
W= General Writing or Communication Class; WI= Writing Intensive Class in Specific Major

V. Discussion and Future Recommendations

5.1 Discussion of Results

Based on the results of our faculty surveys, we discovered that the 3000 level courses house most of the core competencies. We also determined that the most "teaching", based on faculty perception, was occurring in 3000 level courses. Lab courses make up a majority of the 3000 level course level, and so we decided to further investigate lab courses.

From a small sampling of student surveys of students enrolled in lab courses, we determined that students' perceptions of the improvement of their writing ability from the beginning to the end of the course were similar in the writing intensive lab course and the non-writing intensive lab course. These results were not what we expected, and we have several theories as to why. One of the most likely causes is the small sample size of our data. We only had four and five survey responses for the WI and non-WI courses each. While our data may be indicative of certain trends, it is still not statistically significant. Students' perception of their writing ability could be affected not just by one factor but the interaction of several factors – if we had more data, we could potentially determine what these factors are and how they interact. Another potential source of the lack of a difference in student perception of writing ability in the two courses is that both labs are taught by the same professor who has been through the Summer Institute of Writing with Professor Higgins and acknowledges the importance of writing in the curriculum. Because of this, even the non-writing intensive class might include more writing than other labs. This data, although not statistically significant, suggests that writing intensive

courses alone are not the solution to improving students' perception of writing ability, but may be a contributing factor.

Interestingly, two students who had previously taken the writing intensive lab course rated their writing ability relatively highly at the start of the non-writing intensive lab course and indicated they did not believe that their writing ability had improved at all over the course of the term. This may indicate taking the writing intensive lab course early helped better prepare them for subsequent lab courses.

The larger indicator of student's perception of the improvement of their writing ability was whether or not it was the student's first lab course. Those who had taken fewer lab courses believed that their writing improved more over the course of the term, whether or not the first course was the writing intensive course or the non-writing intensive course.

With the information that the number lab course a student was taking was more important than whether or not it was writing intensive, we decided to consult other resources than our survey data alone. In our focus groups, the group that included more students who had worked in industry indicated that they wished they had been taught more writing, and called out the writing intensive lab course that we had been studying specifically as significantly contributing to their writing ability. They expressed that they believed more classes like that should be included in the BME curriculum. Through conversations with Professor Higgins, we also believe in the significance and importance of writing intensive courses, as they have to incorporate the teaching of writing, They also require students to complete writing assignments and offer the opportunity for revision, both of which can help students become more confident writers.

Even though our survey results don't necessarily alone indicate that writing intensive courses significantly improve students' perceptions of their writing ability, though conversations with Professor Higgins and conversations within our focus groups we believe that taking a writing intensive course early in a student's academic curriculum may better prepare them for future writing within the curriculum and their discipline.

While the results of the student surveys were too small to be statistically significant, they do show the importance of lab courses in students' writing ability, as a majority of students believed that their writing ability improved over the course of the term-long 1/6 lab. Our focus groups also indicated the importance of lab courses in the BME curriculum, as many students indicated that these courses had a positive impact on their writing ability.

One of the most interesting takeaways from the focus group happened completely by chance. One of the groups happened to be a majority of students who had done research in academic labs over summers and the other group happened to have a majority of students who had had internships in industry. Interestingly, the group that had more industry experience specifically mentioned the writing intensive lab we studied as beneficial to their experience writing in the BME curriculum, and mentioned another non-writing intensive lab, that provided detailed templates, as more "plug and chug" that did not improve their writing ability. The other group without as much industry experience thought the exact opposite. They believed the writing intensive course had prompts that were "vague" and "unguided" and preferred the clear templates of the non-writing intensive course. This observation is interesting to note when determining how well writing in the BME curriculum prepares students for industry.

5.2 Recommendations

Our first recommendation for the Biomedical Engineering department is to continue to gather data through student surveys, potentially spanning all four years of students' time at WPI. Being able to track students perception of their writing ability over a longer span of time would allow the BME department to see where significant changes occur in the curriculum. This could show if certain courses or course levels have a more significant impact than others. It could also potentially show if a students' perception of the significance of classes changes after industry internships or other similar experience. The BME department should therefore ask questions in these surveys such as perceived writing ability, significance of BME classes, and other outside experiences. If a long-term study with repeat surveys of the same sample group is not feasible, then it could be potentially beneficial to repeat our study of surveying students in writing intensive compared to non-writing intensive lab courses on a larger scale to see if results are consistent with the observations we made from our small sample size.

The second recommendation we have for the BME department is to introduce more writing intensive lab courses, and recommend they be taken as the first lab in students' lab course sequence. While our limited data did not show that the writing intensive course significantly raised students' perceptions of their writing ability over the non-writing intensive course, the data we did obtain combined with the focus group results which said that students' would like to see more writing in the curriculum led us to believe that a writing intensive course could be beneficial for students' perceived writing ability. Every BME student is required to take four lab courses, and offering more writing intensive sections would give more students the chance to practice and further develop writing skills. We also recommend that the BME department recommend (though not require) that students' enroll in a writing intensive lab

course as the first in their sequence, as our data suggests that students' perception of writing ability improved more after their first lab course than later course. A writing intensive lab course as a first lab course may help better prepare students for future lab courses and other writing assignments.

Our third recommendation is to offer writing intensive sections of BME 3300 Biomedical Engineering Design. The class was mentioned multiple times in our focus groups, and students believed that there was an opportunity to teach more writing in this class. The class already incorporates a lot of writing, as shown by our faculty surveys, which indicate that the course explores more of the core competencies than any other course listed. Members of our focus group even called the course a "mini MQP". We only received data from one of the two professors that teach the course, but the survey results we did receive indicated that while there was some teaching occurring we believe based off focus group feedback that the teaching of more of the core competencies would be beneficial. Offering writing intensive sections would likely give students the opportunity to learn more about writing, because the requirements for a writing intensive course at WPI require at least some in-class instruction on writing, and have to offer opportunity for revision. This is also based off of our review of BME curriculum at other schools - several peer institutions had similar BME design courses that were writing intensive. For example, writing intensive courses at RIT must have classroom -based discussions about writing and have at least one writing-based learning outcome (WI Course Objectives). It is likely that it would not be feasible to make all sections of this course writing intensive due to limitations in resources, but beginning with opening some writing intensive sections would start the transition of this course into a more writing-intensive class.

Our final recommendation involves increasing the amount of core competencies addressed and the number of core competencies taught in the 4000-level classes. From the data we gathered, some of these courses teach writing, some assign writing, and some even assess writing, but very few actually provide feedback, which is fundamental in the learning process. We believe that if writing can be implemented in real-world scenarios in the 4000-level courses, it would be more beneficial and prepare students for post-graduation better than in the 2000-level sequence. Based on asking our focus group participants how prepared they felt writing in industry, students indicated they felt slightly unprepared to write technical documents for industry purposes, which is something that can be addressed in a concentration-specific 4000-level elective course.

While we do believe all of our recommendations will improve the writing experience of students in the department, we recognize that our study had several limitations. The first of these is the minimal data we were able to collect over the short window that our project took place. We were only able to collect data for our student surveys from a small group of students over one term, and this data was not statistically significant. Our focus groups also took place with a small group of students, and the views expressed in that focus group may not necessarily represent the views of students in the BME department as a whole.

We would also like to acknowledge that we as researchers may have had some bias in our investigations as we are double majors in Biomedical Engineering and Professional Writing, so we are inclined to believe additional writing is beneficial. However, while our bias may be viewed in some ways as a limitation, it also gave us the opportunity to research this topic from a unique perspective. As students in the department ourselves, we have taken the lab courses we

were studying, and have taken courses we are making recommendations on. While we may have not gotten the exact results we expected (some students prefer "plug and chug" templates for lab reports as indicated from some focus group participants, for example) we believe our recommendations would have improved our experience with writing in the BME curriculum.

The BME department may not currently have the resources to carry out our recommendations, as they require the investment of both time and resources. Making labs writing intensive requires time investment for restructuring the class and creating additional material. It also requires faculty to recognize the importance of writing intensive courses and want to put in the time to create that change. The same is true for increasing the amount of core competencies in the 4000 level courses – they require both faculty and time investment.

Even if the BME department invested the time and resources necessary to follow our recommendations, there is another barrier in encouraging students to take writing intensive courses. Students may feel like writing intensive courses are more work and not want to sign up for them, and to counter this the importance of writing intensive courses will have to be communicated and believed by students, which will also require faculty commitment. The BME department may also limited by WPI policies on what courses they can require.

Through our study, we researched writing across the curriculum and writing in the disciplines, investigated the implications of implicit compared to explicit writing instruction, and researched writing intensive courses at WPI and BME curriculums at peer institutions. Our study did have limitations, and we recommend that the BME department complete further surveys to gather more student perspectives before moving forward with changes to writing within their curriculum.

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Appendix A - Student Survey

1.Is this your first BME lab course at WPI?

Yes

No, I have taken these lab courses: (fill in the blank)

2. To what degree did **previous lab courses** prepare you to write the assignment(s) in this course? (rate from 1 to 10, 1 is not prepared at all, 10 is extremely prepared)

3. To what degree did **this lab course** prepare you to write the assignment(s) in this course?(rate from 1 to 10, 1 is not prepared at all, 10 is extremely prepared)

4. To what degree did **other WPI courses or experiences** prepare you to write the assignment(s) in this course?(rate from 1 to 10, 1 is not prepared at all, 10 is extremely prepared)

5. To what degree did writing the assignment(s) **in this course** improve your understanding professional/technical writing?(rate from 1 to 10, 1 is not prepared at all, 10 is extremely prepared)

6. While you were writing the assignment(s) in this course, did you refer to(new word for this maybe) any **in-class materials** (eg: material from lectures, annotated examples, notes, handouts)?

Yes, I used the following in-class materials: (fill in the blank)

No

7. If yes, of these which was the most helpful? (fill in the blank)

8. While you were writing the assignment(s) in this course, did you refer to any outside-of-class resources (eg: TA hours, past assignments, writing center etc...)?

Yes, I used the following outside-of-class resources: (fill in the blank)

No

9. If yes, which was the most helpful? (fill in the blank)

10. If yes, which was the most helpful?(fill in the blank)

11. Were there any resources you wish you had access to?(fill in the blank)

12. Were there any instructions on how to write the assignment(s) that you had trouble understanding?(fill in the blank)

13. Do you have any other comments/thoughts about writing in all BME lab courses you would like to express? (fill in the blank)

14. How would you rate your writing ability before this class? (rate from 1 to 10, 1 is weak, 10 is strong)

15. How would you rate your writing ability after this class? (rate from 1 to 10, 1 is weak, 10 is strong)

Appendix B - Student Focus Group Questions

Disclaimer: Participation in this focus group is completely voluntary and participants have the option to decline to answer any of the question. This session will be recorded. These responses will only be used for the purposes of our report and will not be shared with anyone else. Participants in this focus group will be kept completely anonymous.
While anonymity will be maintained throughout the session, the team may quote some responses in our MQP report, are you comfortable with the group quoting your responses for our report?
The team may want to quote direct language from your responses. Are you comfortable with this? We will not identify you.
yes/no
Are you comfortable with us recording this session?
Yes/no
Initial demographic questions:
What is your year?
How many bme classes (labs) have you taken?
What is/was your humanities?

Do you feel there is anything lacking in terms of the types of writing assignments you have experienced in your BME courses? Is there anything else writing related would you like to see in the BME curriculum?
What writing have you completed in you BME classes so far?

What was the most helpful/impactful writing assignment that you have done in BME

Do you think that the writing instruction in bme is satisfactory? Do you feel like it has/will prepare you for any technical/professional writing you'll need to do post grad? (teaching, assignments, feedback)