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Cell Aggregate Dissociation and Filtration Through the Use of Nylon Woven Mesh Membranes

THESIS

submitted in partial satisfaction of the requirements
for the degree of

MASTER OF SCIENCE

in Biomedical Engineering

by
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DEDICATION

To

My parents for their constant love, support, and instilling
the personal motivation, dedication, confidence, and positive attitude
to prepare me for life's journey and achievement of dreams.

Family and friends for their endless love and support,
and for inspiring me to always test my limits

Haun Lab for teaching me what it really means to be
a researcher and an engineer.

My second family away from home, Summer Session! You helped teach
me the lessons of life - beyond the classroom which strengthened my skills
and abilities in teamwork and leadership. Your guidance helped me build
upon a level of passion, desire, creativity, team attitude, work ethic,
and support that I will continue to bring to programs,
projects, and experiences in the future.

Mr. and Mrs. Redding, thank you for making me feel a part of your family
and Matt, thank you for being a symbol hope. Hope of fulfilling dreams,
hope of making a difference, hope of becoming the person I want to be
I hope to continue making you proud

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ABSTRACT OF THE THESIS

Cell Aggregate Dissociation and Filtration
Through the Use of Nylon Woven Mesh Membranes

By

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Master of Science in Biomedical Engineering

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Advances in the field of microfluidics show great promise for enhancing the fight against cancer. This technology enables the medical field to obtain more detailed information– specifically molecular characteristics of tumor cells. These molecular characteristics can provide additional information to make more accurate, earlier diagnoses and provide additional treatment options. The Haun Lab's dissociation device provided the first stride towards reaching those targets. It mechanically disrupts aggregates and produces a sample containing majority singlets, enabling more accurate analysis results. The addition of a filtering mechanism to the outlet of this device will improve upon this concentration of singlets, while also providing a connection between sample preparation and analysis resulting in an increase in tumor cell yield.

A filter device was proposed to provide different processing tasks – 1.) partial disruption of lingering aggregates into singlets and 2.) concentration of singlets to undergo molecular analysis. Characterization of the filter device was conducted through a series of experiments that narrowed down pore size performance, filtration efficiency, and aggregate disruption. The direct-flow experiments showed an increase in singlet count with decreasing pore size, until a size limit

was reached. There was also an observed 5% decrease in aggregates when incorporating the SEFAR 15μm filter. Due to the membrane's well-define aperture structure, it was more than ideal for Filter 1. To improve filter efficiency, a tangential-filter device model was created. When this device was set to withdraw 80% of the effluent from the top outlet and 20% from the bottom, it became more efficient at generating singlets at higher flow rates.

Background: The Importance of Diagnostics

Cancer is the uncontrolled growth of abnormal cells in the human body and today, it is considered the second leading cause of death in the United States. The American Cancer Society estimated 1,685,210 new cases and 595,690 cancer related deaths to occur in the United States this year alone.[1] This universal disease can develop at different rates in different individuals resulting from a variety of direct and indirect factors. Understanding these factors (genetic, environmental, etc.) can help the medical field better understand the root causes and thus work towards better identification, treatments, preventions and cures. Tumor heterogeneity has been recognized as an underlying factor for tumor progression, metastasis, and drug resistance.[2] Physicians and pathologists believe that tumor heterogeneity can describe the genetic and epigenetic differences in tumors.[3] However, a more effective cell-based analysis method is required to characterize these diverse cell populations in the tumor tissues.[4]

The transformation of oncology by molecular diagnostics has enabled the field to strive towards predictive and personalized medicine - a medicine and regiment tailored to the individual patient. Cell based technologies today (flow cytometry, mass cytometry, single cell gene sequencing, etc.), designed to quantitate cancer, are mostly qualitative, involve large sample sizes and many reagents.[2, 5] However, these conventional platforms have the potential to result in false positives, negatives, and cause damage to certain biomarkers or targets of interest. The molecular knowledge collected by these platforms grows increasingly and reiterates the need for new tools capable of single cell purification, smaller sample size, little reagent consumption, and quicker molecular analysis. The Haun Lab at the University of California, Irvine (UCI) has contributed to this effort by successfully designing and demonstrating the benefits of a

microfluidic dissociation device to further dis-aggregate biopsied tumor tissue into single cells for subsequent quantitative molecular and mechanical analysis. It's technology like this that helps bridge the gap between conceptual theory, bench work and clinical reality and bring forth the next generation platforms of precision medicine.

The microfluidic dissociation device is a critical focus of the research completed in the Haun Lab. The device is composed of five sections; each with their own unique shear force and fluid handling capabilities (Figure 1). The shear forces induced by the expanding and constricting layout of these micro-channels are the driving force to carry out the main goal: to dissociate (or separate) large clumps of tumor tissue into single cells with minimum enzymatic assistance. With the dissociation device, the Haun Lab has proven the advantage of utilizing microfluidics for mechanical dissociation of various cancer cell lines; and has greatly reduced pre-ezymatic treatment and processing time. An in-vitro three-dimensional model, known as spheroids, was also introduced into the dissociation device to gather preliminary results on the disruption of a more complex tumor model. These spheroids were created in a microwell tray by the hanging-drop method. When the tray is inverted, the suspended HCT 116 colorectal cancer cells collect and form a solid aggregate containing approximately 1000 cells. The following results display the total cell

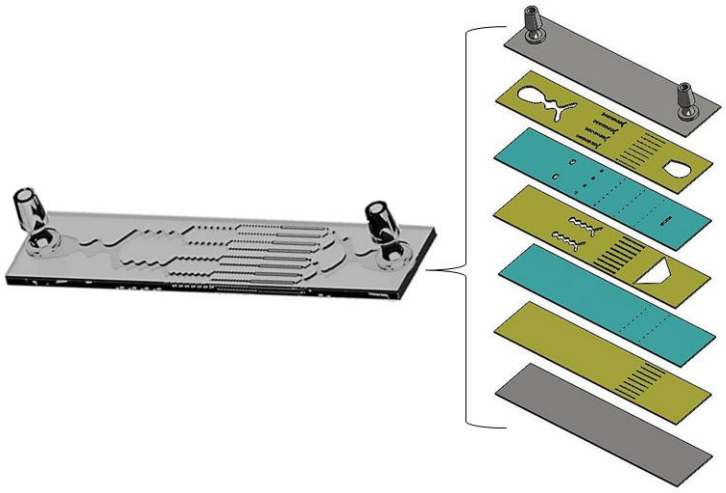


Figure 1: A 3D schematic and exploded view of the Haun Lab dissociation device, designed to mechanically disrupt tumor tissue into single cells.

counts when 12 HCT116 spheroids were processed using the dissociation device at a rate of 12.5 mL/min (Figure 2).[2]

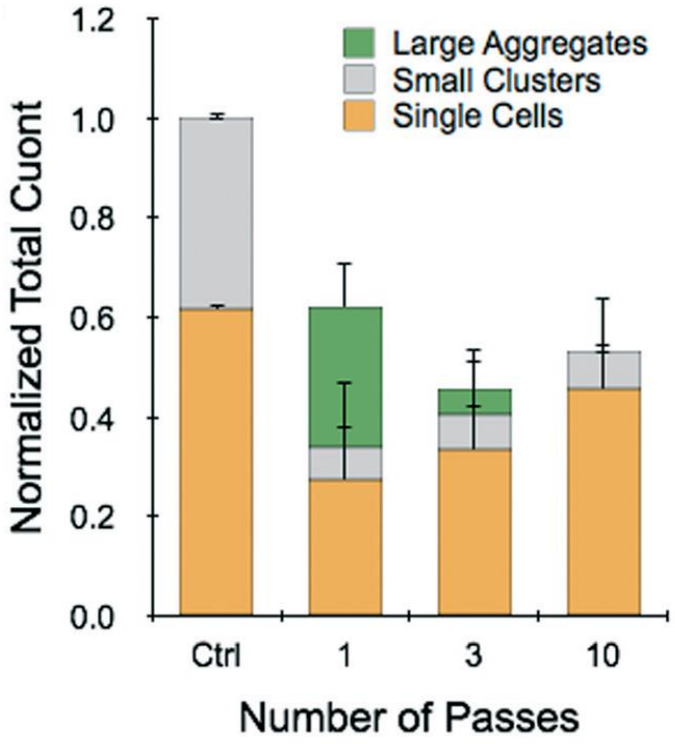


Figure 2: Tumor Spheroid Dissociation

HCT116 spheroids passed through the Haun Lab dissociation device at a rate of 12.5 mL/min for a total of 1, 3, and 10 passes. As the number of passes increased, there was a decrease in total cell recovery; but an increase in single cell percentage.[2]

After one pass through the device, the total count recovered was 60% of the trypsinized control. Additional passes (back and forth) through the dissociation device didn't improve total cell recovery, but did show an increase in single cell yield. The low recovery percentage suggests that the device was not able to produce the high shear forces required to effectively disrupt tumor spheroids without compromising cell viability.[2] To improve this low recovery yield, a filter device integrated with the dissociation device has the potential to improve this current sample processing component. The filter device will more adequately disrupt lingering tumor aggregates into singlets as the sample is selectively forced through a filtration barrier; and provide single cell enrichment within the sample for molecular analysis downstream.

As a member of the Haun Lab, I've participated in this endeavor by manufacturing, utilizing, and evaluating new filtering devices. The concept objective of the filter device is to

selectively allow single cell passage and retain device-treated aggregates within the dissociation device for further dissociation; additionally, it can also act as a bridge between two independent platforms. This filter will also present our lab with the opportunity to connect the sample preparation segment of the dissociation device with a cell-based analysis segment downstream, as seen in Figure 3.[6] It will interface different components under a single-housed microfluidic platform. By applying this additional separation method, "filtration", the medical and biomedical engineering community can study specific characteristics of cancer in an enriched population of single cells.

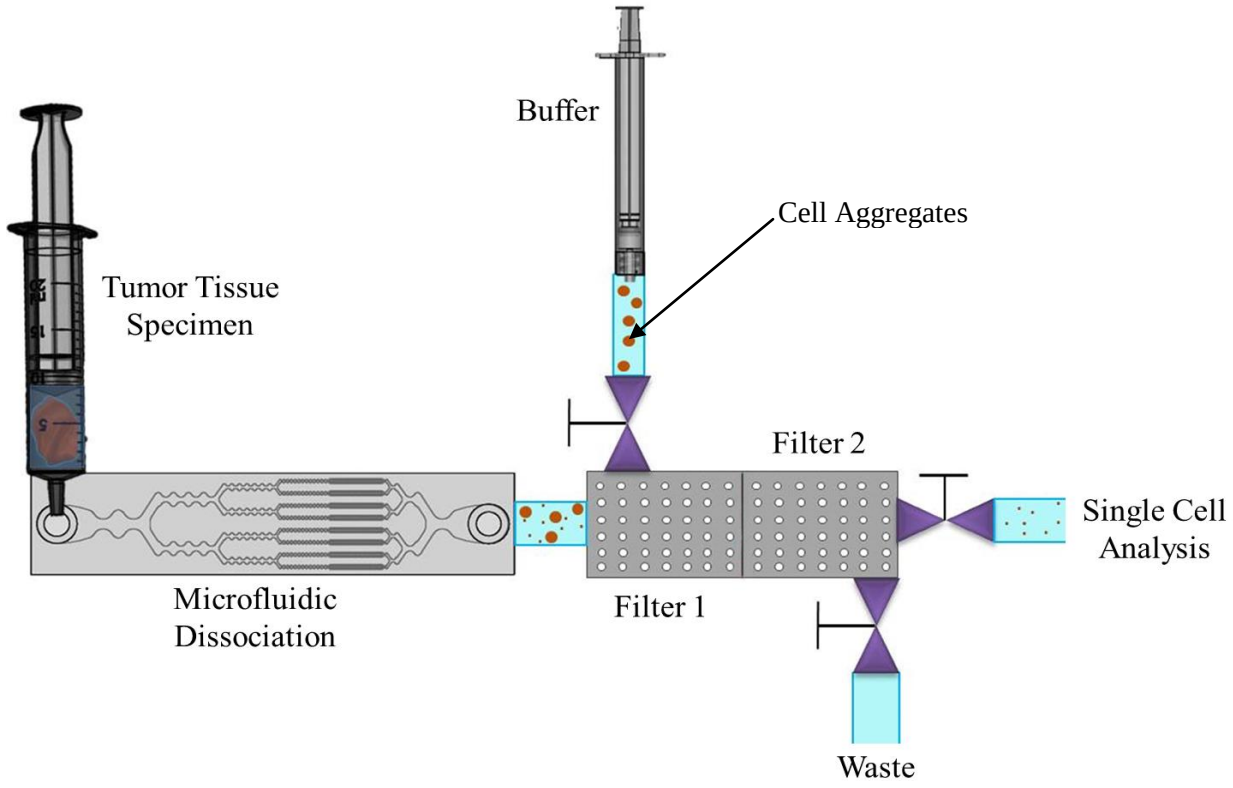


Figure 3: A fully integrated diagram of the processing platform for tumor tissue specimen. Tumor tissue is introduced into the dissociation device to be mechanically broken up into single cells. Single cells and un-disrupted tumor aggregates continue to Filter 1 & 2: lingering aggregates are recycled into the dissociation device to be further broken down, and single cells are concentrated for single cell analysis downstream.

Chapter 1: Microfluidic Separation

Microfluidics has proven to have a huge influence on the biomedical engineering field in the past few decades and has itself become a field of study. Microfluidics is the manipulation and control of fluids at the micron scale.[7] It can create a foundation for new innovative and upcoming technologies, each offering advantageous opportunities in high-throughput, high sensitivity, cost efficiency, minimal sample/reagent consumption, and decrease sample processing durations and precision control all within a microscale environment.[8, 9] The adoption of intricate and geometric designs have also provided microfluidic systems with the ability to interface with other downstream analysis tools (biochemical, image, molecular, etc.), thus assisting in the development of the lab-on-a-chip concept. Lab-on-a-chip technology integrates different lab functions onto a single device circuit. This concept allows users to integrate various enhanced biomedical applications including diagnostics, therapeutics, and cell biology.

A critical and primary step in diagnostics is the separation of different populations in heterogeneous samples. Purifying or enriching these samples into well-defined groupings greatly increases the efficiency of cell handling, processing, and analysis. The conventional technologies today, used to isolate rare species of cells, can be divided into two systems: active and passive. Active systems utilize external fields like magnetic, optical, and electric forces to fractionate or meticulously pick out targets of interest from the general background population, while the passive systems incorporate the intrinsic physical properties of the cells for separation.[10] Fluorescence-activated cell sorting (FACS), an active separation system, relies on fluorophore-conjugated antibodies to identify certain cells or gene expressions.[6] This platform can distinguish between types by analyzing the discrete scattering of fluorescents each cell produces at a rate of 50,000 cells/sec.[11] Another active sorting platform, known as magnetic-activated cell sorting

(MACS), operates in congruent to FACS by immuno-magnetic separation. The external fields produced by the electromagnetic coils or magnets embedded in the platform diverts bead-labeled cells towards a collector.[6] The high efficiency and real-time classification these conventional technologies present are valuable for diagnostics; however, like many systems come limitations. Researchers are developing new generations of cell separation platforms that interface with microfluidic principles to improve upon those persistent deficiencies which prevent the translation to clinical settings.

Passive Cell Separation

Great strides have been made towards "label-free" techniques in microfluidic devices, especially those that focus on passive systems of separation. In order to manipulate samples, the separation forces derived in these devices are tied to the physical characteristics of the cell: size, shape, density, deformability, impedance, and hydrodynamic properties.[11] Size-based particle/cell separation is considered the most basic "label-free" method to employ in microfluidic devices. Obstacles fabricated in the microchannels are used to induce cell size cutoff filtration and alter fluid flow directing cell targets trajectories towards certain areas. There are four types of microfabricated filter designs: weir, pillar, cross-flow, and membrane.[10]

Weir-type – Implements the simplest form of filtration by obstructing flow path within the microchannel using a single barrier. This barrier extends a certain distance across the width of the channel creating a narrow passage; restricting particles or cells of a certain size to pass through (Figure 4). Weir-type filters are usually employed for blood

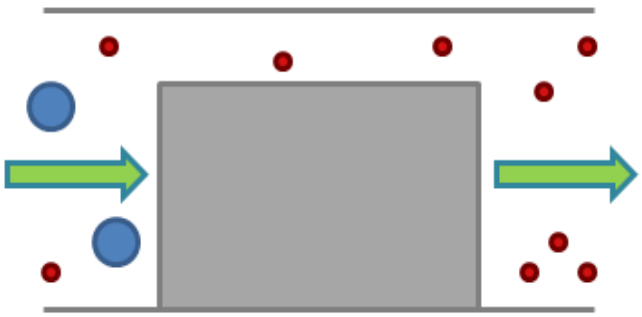


Figure 4: Weir-type filter size exclusion.[11]

processing. Blood is driven through the planar slit or passage by capillary action and only the cellular components of blood are prevented from continuing further down the microchannel, thus producing cell-free plasma. These filters are also used to trap circulating tumor cells (CTCs) within blood, allowing only white (WBC) and red blood cells (RBC) through.[6, 11]

Pillar or Post Array – A matrix array of pillars/posts meticulously placed within a microchannel has accomplished size separation. The placement of these columns helps shift cells or particles to particular streamlines depending on the size of the particle itself. This principle is known as deterministic lateral displacement (DLD). The pattern or layout of the posts helps determine the displacement of cells or particles (Figure 5). Small particles follow the general flow path while larger particles are deflected sideways towards neighboring streams. Researchers have applied pillar-type filtration towards the fractionation of E. coli cells and later extracted DNA by directing the cells toward a streamline containing cell-lysing solution.[10, 11]

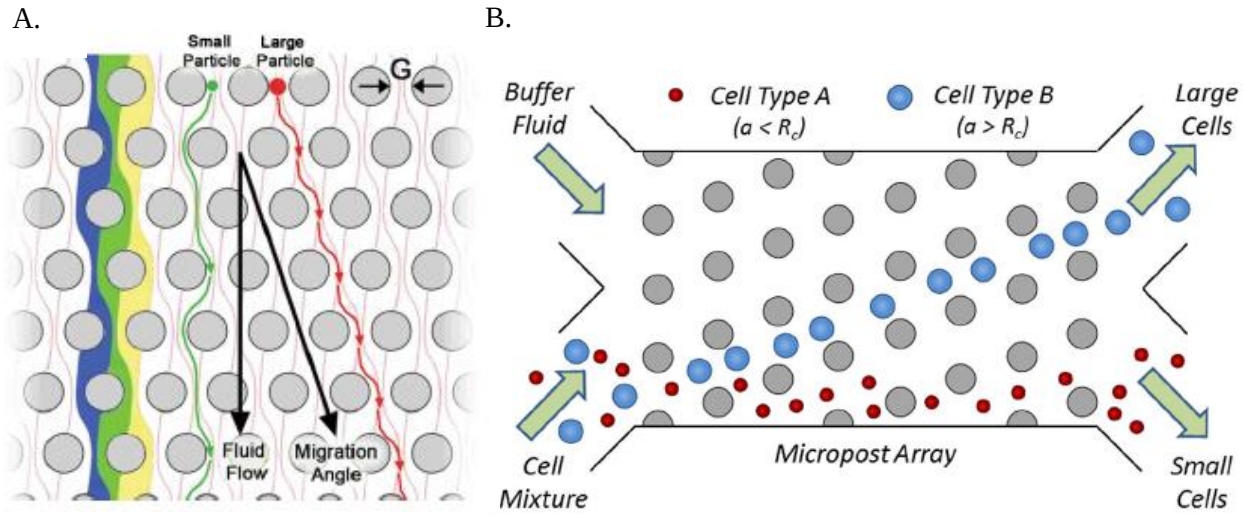


Figure 5: A.) Streamline profiles caused by deterministic lateral displacement. B.) A microfluidic model that contains an array of pillars intricately placed to sort large cells towards one outlet and small cells towards the other outlet.[10, 11]

Cross-flow filtration – Cross-flow filtration incorporates an array of capillary-like branches perpendicular to the direction of the main flow channel, as seen in Figure 6. The gap size of these branches is narrow enough to prevent any particle from entering or large to allow a particle or cell of a specific size to continue through the capillaries. This method has been extremely useful in the fractionation of cell populations in a heterogeneous mixture. The extraction of plasma from blood is one of the common applications that utilize these passive filters. The capillary branches allow the carrier fluid (plasma) to continue on while the RBCs and WBCs are held within the main channel. [6, 11]

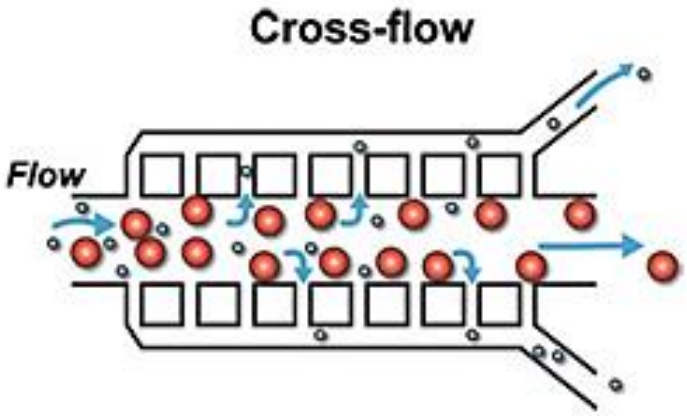


Figure 6: A schematic of cross-flow filtration. The flow of separation is perpendicular to the main stream.[10]

Microscale membranes – Membrane-based filtration has also been known to be integrated to microfluidic chips. The semipermeable membranes consist of precisely designed pores that restrict the filtering of cells below a critical size. Since membrane microfilters are an uncomplicated separation tool, these cost-effective filters are commercially available in a range of pore sizes and give rise to the purification of various cell targets. There are several different fabrication techniques that provide well-defined pore sizes to membrane microfilters: 1.) photolithography, 2.) track-etching, and 3.) nylon woven.[12] Photolithography, also known as optical lithography, is a microfabrication method of transferring an array of geometric patterns from a photomask to a photoresist (light-sensitive) substrate.[13] The photoresist used can produce membrane films as thin as 5 µm, ideal for integration; although considered too fragile to

implement within the dissociation device which operates at high flow rates. Track-etched membranes are barriers with uniform cylindrical pores. These commercially available microfilters are fabricated by exposing the polymer (polycarbonate) film to energetic ions that etch away at the matrix of the film. However, track-etching provides a low porosity and tends to generate a random control over the distribution of non-uniform pore sizes throughout the membrane.[12] Nylon woven filters are another commercially made membrane commonly used in the research and medical fields. These synthetic fibers are woven to ensure tightly controlled apertures held within high tolerances; resulting in consistent, even, clean, and repeatable structures. Nylon woven membranes are also highly durable, hydrophilic, and compatible with most solvents.

Since passive filters have no functional dependence on external fields, there are some that don't rely on channel obstructions but channel geometries to provide size-based filtering. The microfluidic phenomena produced by the curved microchannels is enough to actively divide complex mixtures into concentrated populations.[6] Guan *et al*. demonstrated the focusing and entrapment of particles of different sizes towards distinct streamlines by changing the channel geometries. As the sample flows through the spiral channel, the inertial lift forces induce the movement of different sized particles in suspension to different positions within the trapezoidal cross-section of the channel. These particles are then caught within two distinct Dean vortices causing a size separation between large and small particles. The large particles are focused along the inner wall of the channel, while the smaller particles positioned towards the outer channel wall.[14] This model generates a high-throughput size-based separation; but since the dissociation device operates on multiple passes, the ease of integration with the dissociation device is limited.

These many passive filtration methods have the potential to be implemented in our filter component, but three concerns were taken into consideration when narrowing down the options: ease of integration, design simplicity, and readily available fabrication processes. The membrane microfilters provide that simplicity of fabrication, automation, and are readily available.[15] For our filter device design, it was decided to utilize the nylon woven mesh membranes because of their precision, durability, ease of integration, and the chance of serial filtration. Since the dissociation device was manufactured by a microfabrication company, ALine Inc., the ability for the filter device to interface with ALine Inc.'s laminate process was also taken into design consideration. The thinness of the nylon membranes is ideal, providing the filter component with the opportunity to be incorporated within the plastic layers of the dissociation device. In the end, the filter device will act as an additional processing step resulting in an increase in single tumor cell yield. In the following chapters, eight nylon membranes and five polycarbonate track-etched filters from various bioscience companies were evaluated and tested using the different filter device models (Figure 7). The different size nylon membranes were provided from GE Healthcare (1µm), Azzota (3 µm), Millipore (11 µm), and SEFAR (6 µm and 15 µm). The track-etched membranes were all from GE Healthcare (1 µm, 3 µm, 5 µm, and 8 µm).

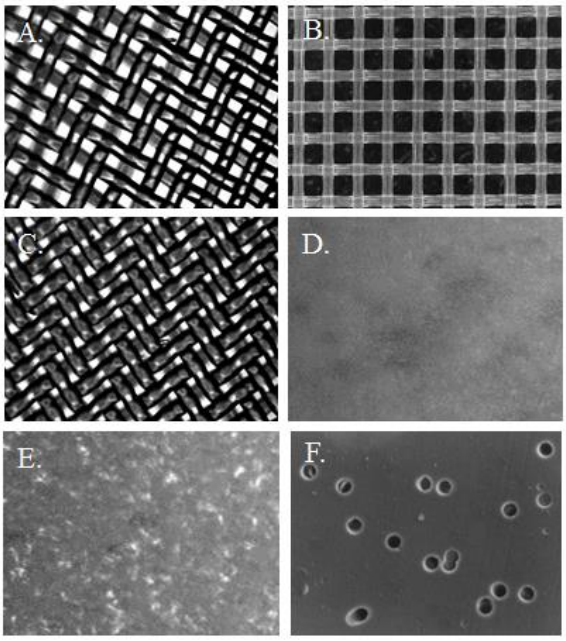


Figure 7: Nylon filters – A.) SEFAR 15µm, B.) Millipore 11µm, C.) SEFAR 6µm, D.) Azzota 3µm, E.) GE Healthcare 1µm. F.) Track-etched filter

Chapter 2: Design and Fabrication of Filter Device

2.1 Overview:

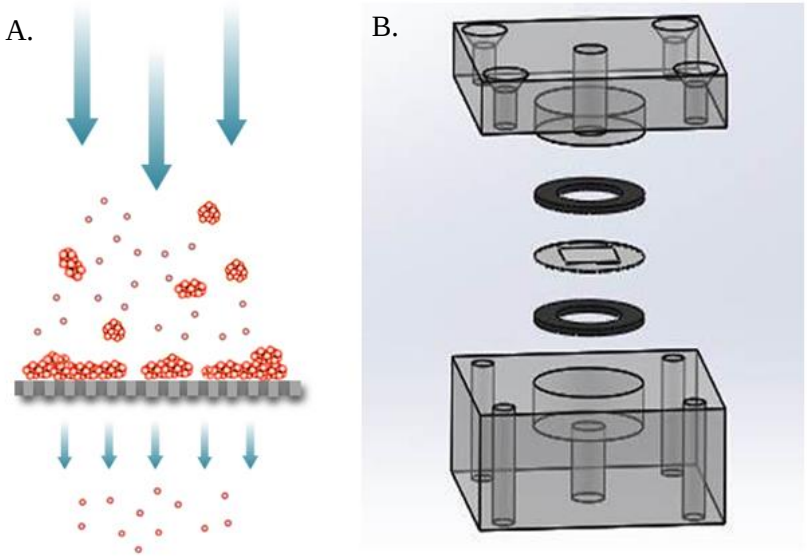
In this study, the main objective was to improve the sample processing capability the Haun Lab designed implementing microfluidics by creating and evaluating a new adjunct filtering device. The filtering device will be used immediately after the dissociation process and help further break down the tumor tissue samples. Two models (direct-flow and tangential-flow) were created to characterize this filtering device and are discussed in further detail in this chapter. The design of the filter section will contain two filters. Filter 1 will consist of a large pore size nylon woven mesh to allow partially dissociated tumor aggregates to be further disrupted into single cells. Filter 2 will selectively retain single tumor cells to undergo additional analysis downstream.

2.2 Engineering Design Process for the Filter Device:

The first version, known as the direct-flow filter, was created to operate in a "dead-end mode". While the heterogeneous mixture of tumor tissue feeds into the filter device, a batch processing occurs. The tumor aggregates are rejected by the nylon woven membrane causing a retentate to form on the membrane surface; and single tumor cells selectively pass through.[11] The direct-flow filter was modeled in SolidWorks, a 3D CAD design software. As seen in Figure 8, the nylon membrane is housed between two clear acrylic pieces. The top acrylic piece firmly seals the membrane in the cavity of the bottom piece. The silicone gaskets also provide an additional seal preventing any fluid from escaping around the circumference of the membrane. Nylon flat head screws will be utilized to help fasten the top of the filter device to the bottom creating a single housing unit for the membrane filter. This filter device model retains the tumor

aggregates within the dissociation device to undergo further processing, while allowing single cells to continue on to the analysis component of the microfluidic platform.

Figure 8: A.) Dead-end mode filtration. As the fluid flows directly into the filter, smaller cells selectively pass while larger cluster are excluded. B.) A 3D exploded view of the direct -flow filter. Top and bottom pieces are made of acrylic and help hold the filter membrane in place. The filter membrane is sealed in between two silicone gaskets to prevent leaking around the circumference of the filter.



The second filter model was created in collaboration with Dr. Hui's Lab and revolved around the idea of tangential-flow filtration. Tangential-flow or cross-flow filtration occurs when the stream flow runs parallel to the membrane surface, it exploits size differences among the biomolecules in a heterogeneous mixture.[16] Tangential-flow filtration is more efficient in size separation than direct-flow and the flow prevents the buildup of molecules on the membrane surface. Our new filter component will contain two membranes each with a different processing task: 1.) single cells will selectively pass through the membrane barrier, while tumor aggregates are recirculated back into the dissociation device for further processing, and 2.) the single cells that continued will be retained by the second membrane. Figure 9A illustrates the filtration mechanism presented. In addition to single cell purification, the incorporation of the second filter will help prevent excess dilution to the sample during processing.

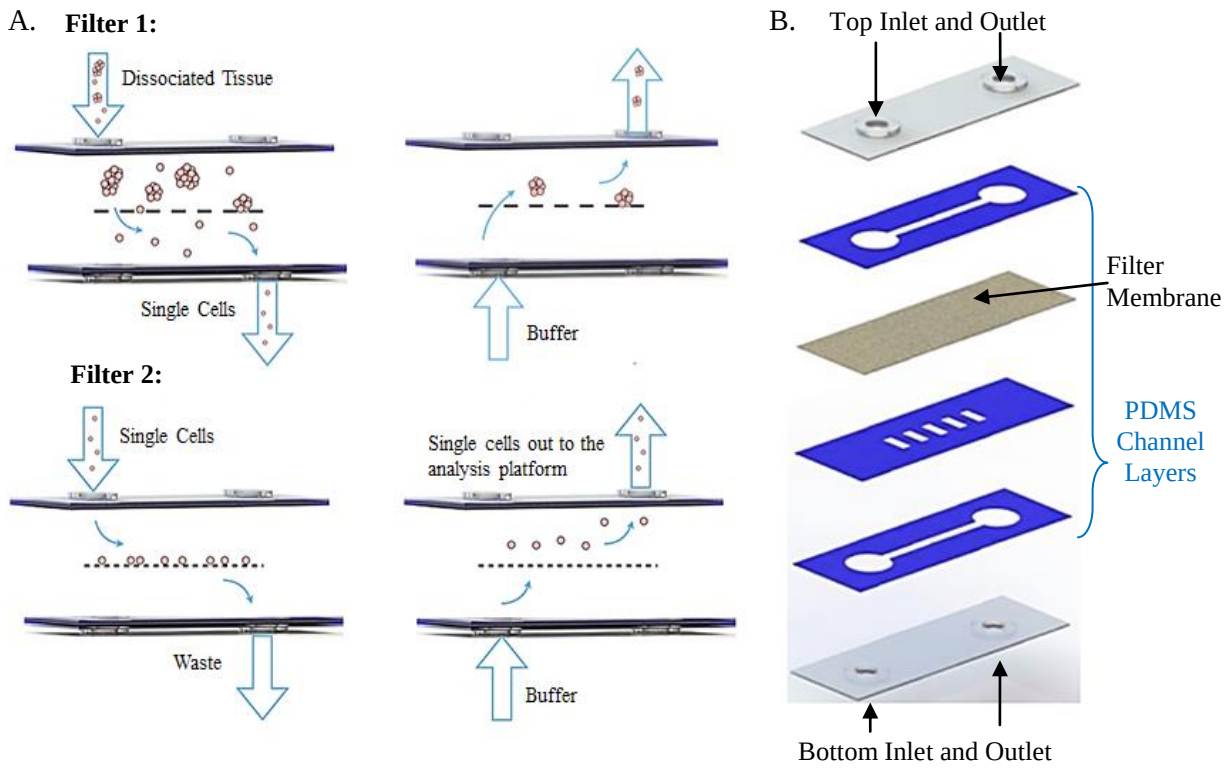


Figure 9: A.) Filter mechanism that occurs in the tangential filter. Filter 1 – Small tumor aggregate dissociation. Filter 2 – Single cell concentration. B.) 3D exploded view of the tangential filter.

This device has several layers (see Figure 9B). The nylon membrane is inserted between three polydimethylsiloxane (PDMS) sheets each fabricated using a vinyl cutter machine. The first and third PDMS layers contain the channels where the tumor aggregates and single cells flow through. The second PDMS layer acts as a support structure, it prevents the nylon membrane from concaving into the channel of the third PDMS layer. This layer also contains square cutouts to allow single tumor cells or excess fluid to pass through. The manufacturing of both filter devices was conducted by the Henry Samueli School of Engineering machine shop here at UCI.

Chapter 3: Characterization of the Filter Device using the Direct-Flow Model

3.1 Overview:

The two filter designs mentioned in the previous chapter underwent several experiments to test performance efficiency. The direct-flow filter helped determine which of the nylon membranes would participate in Filter 1. Filter 2 was eliminated from the overall filter device due to observed significant cell loss. Cancer cell lines grown in our lab, including the colorectal cancer cell line used in the preliminary dissociation device results, were used to produce consistent, reliable, and reproducible cell yields. In addition, these cell lines provide a level of complexity seen in tumor heterogeneity.

3.2 Experimental Work:

In order to determine which membrane pore size will provide the highest yield of single cells, a series of experiments were conducted using the direct-flow filter device. The direct filter was prepared by filling the device interior with Superblock (Thermo Fisher Scientific) and required to settle within the device for 15 minutes at room temperature. Superblock is a blocking buffer commonly used within microfluidic devices to help prevent cells or target molecules from adhering to the walls of the devices. It was then washed away with a phosphate buffer solution (PBS) containing 1% bovine serum albumen (BSA). The decision to use this wash (PBS+) revolved around the need to submerge the interior of the channels; in addition, it's the same buffer solution used to resuspend all cell samples.

To help determine cell yield, "before counts" or baseline counts were taken of the cell sample suspensions prior to device loading. The cell concentration was quantified using an automated commercial cell counter, Orflo Moxi Z. This Control value provides an insight on the starting concentration and reinforces concentration consistency before filtration. Device treated

counts were also normalized to this Control value. The HCT116 cells from the cultured flasks were loaded into the filter device and forced through the membranes at a rate of 1 mL/min using a syringe pump (Harvard Apparatus). A volume of 1 mL PBS+ was then flushed through the filter at the same rate to gather any remaining cells that might have collected on the retentate side of the membrane. The HCT116 cells and PBS+ wash were collected in a 15 mL conical and measured to provide a final concentration count. The following results display how much of each cell classification were collected from the outlet after selectively being filtered through the different membranes (nylon and track-etched) over three independent experiments (n = 3) (Figure 10A-D).

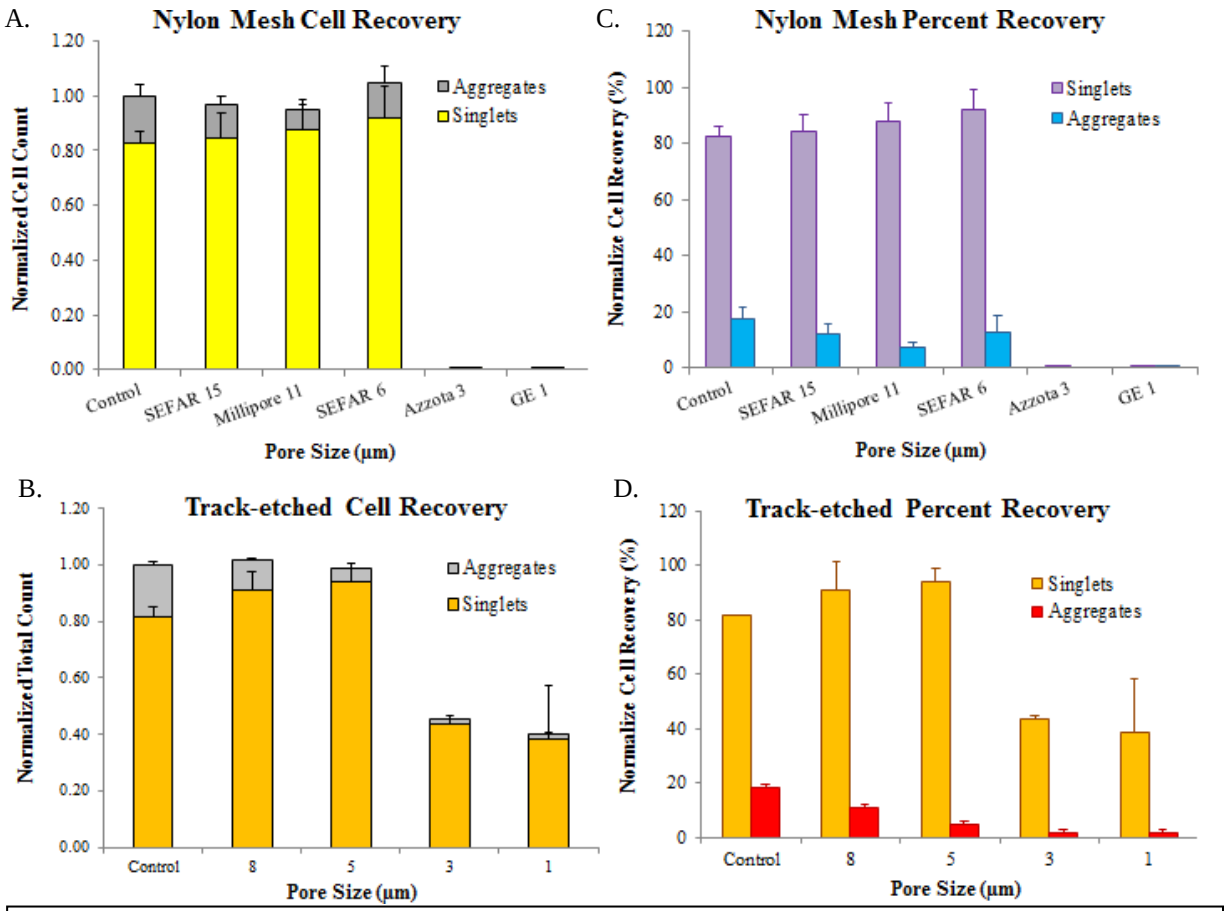


Figure 10A-D: Direct-Flow Filtration using Nylon Mesh and Track-etched membranes. Single cell count increased with decreasing pore size, until a size limit was reached. All counts were normalized to "Control" (before count). A.) Nylon membrane. B.)Track-etched filter. C.) SEFAR 15µm and 6µm filters decreased aggregate population by 5%, Azzota 3µm and GE 1µm had a significant cell hold-up amount. D.) 8µm track-etched filter produced 7% less aggregates than the control, little cell hold up in smaller filter sizes. (n = 3)

As the pores of the membranes decrease in size for both the nylon and track-etched filters, the number of single cells collected increased until a cutoff size was reached. Pore sizes below 3 µm in diameter restrict any tumor cell from continuing further downstream. These smaller filters are ideal for Filter 2. The cell recovery percentages calculated also imply the breakdown of aggregates from large clusters to possibly triplets, doublets, or singlets.

Another experiment was conducted to determine the cell percentage being retained by the smaller filters. Similar to the direct-flow filter experiment, a back flow (BF) was performed by reversing the flow of PBS+ through the filter membrane after the HCT116 cells plus wash were collected. The back flow results revealed about 35% of the total sample population was prevented from filtering through the Azzota 3 µm membrane and 19% from the GE Healthcare 1 µm filter (Figure 11).

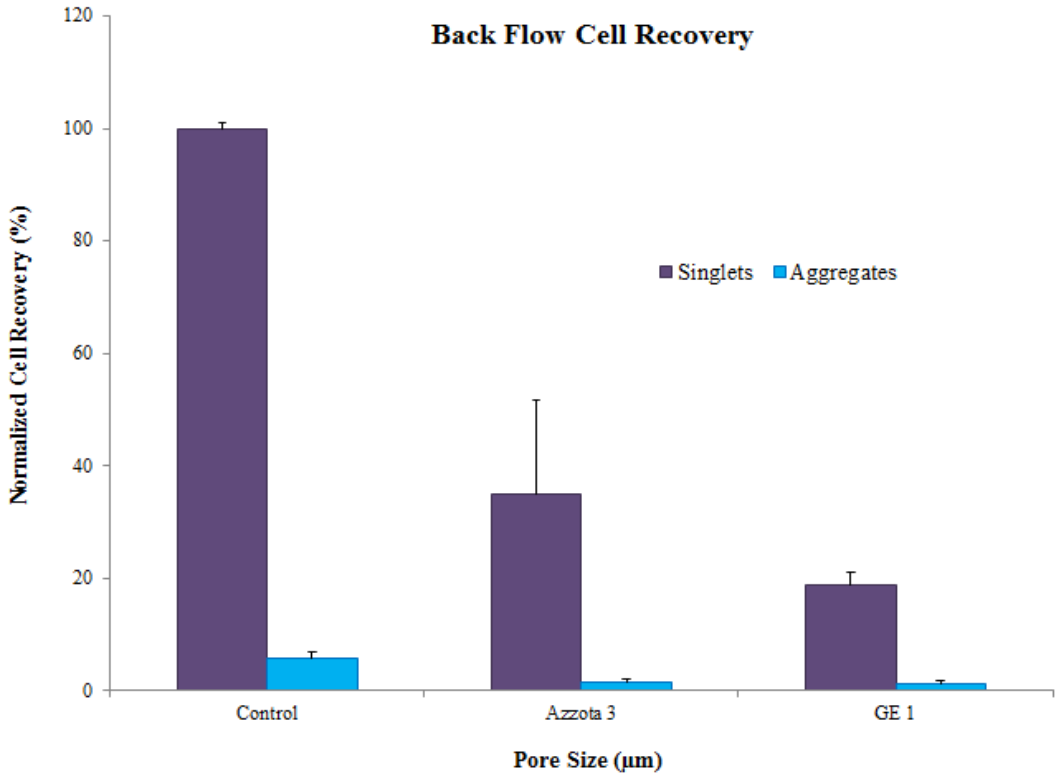


Figure 11: Percent Cell Recovery for the Back Flow Experiment. Azzota 3µm BF condition had 34.8% singlets and 1.66% aggregates. GE1 µm BF had 18.7% and 1.29% aggregates. Still significant cell recovery loss in direct-flow filter.

3.3 Discussion:

The direct-flow filter experiments helped narrow down the optimal membranes to achieve the different tasks of the filter device. It was decided to employ the nylon membranes over the track-etched because of the observed deformation the track-etched filters experienced. The track-etched filters underwent stretching of the membrane when fluids traveled at a rate of 1 mL/min through the device. If the filter component is to be integrated with the dissociation device, this device needs to be able to operate at a maximum flow rate of 12.5 mL/min. This deformation could also lead to a biasing of cell recovery due to the stretching of the individual pores. Nylon membranes are presented to be more durable and contain controlled aperture structures. The nylon filter with 15 μm pores (SEFAR) was selected because of its well-defined pore opening, increased single cell filtration and decreased amount of aggregates collected from the outlet. This membrane size has the potential to benefit Filter 1 by allowing the device treated aggregates to be disrupted into single cells as it is forced through the constrained openings of the filter. The SEFAR 15 μm decreased the aggregated population by 5%. Azzota and GE HealthCare's smaller nylon membranes (3 μm & 1 μm) were initially thought to be incorporated in Filter 2 to help selectively prevent the filtration of single tumor cells from Filter 1. However, the preliminary filter data collected from the back-flow experiments reveal the potential for sample accumulation on the membrane surface and significant sample cell loss. Only 34% (Azzota 3 μm) and 19% (GE 1 μm) of the total population was returned in the back-flow effluents when using these filter membranes. Since more than half of the tissue sample was entrapped within the direct-flow filter device, it was decided to remove the Filter 2 component from the filter device entirely.

Chapter 4: Tangential-Flow Filtration

4.1 Overview:

These nylon microfilter membranes were housed within the tangential-flow filter model set up and displayed a greater filtration efficiency compared to the direct-flow model. ALine's 15 µm filter set to withdraw 80% of the total flow rate 12.5 mL/min from the top outlet and 20% from the bottom, was capable of generating more single tumor cells – thus reinforcing the potential of integration with the dissociation device. This tangential-flow filter device has the ability to produce enriched samples of single cells for molecular analysis downstream.

4.2 Benefits of the Tangential-Flow Filter Model:

Cell samples are placed in a 15 mL conical, open to the lab environment to help prevent pressure build up and processed through the filter by means of withdrawal. Once the cells load into the filter, the cells are pulled across or through the nylon membranes due to split fluid flows exiting the device at different flow rates. The two outlets (top and bottom) are set to withdraw a certain amount of fluid; for example, 50% from both outlets or 20% from the top outlet and 80% from the bottom. Tangential-flow filtration will help concentrate the cell population of interest (single cells) in the permeate and the cross-flow current allow the self-cleaning of the membrane surface. As fluid velocity increases, the lingering particles on the filter, which would be considered part of the retentate population (aggregates), will be swept off the membrane back into the stream line and recirculated into the dissociation device for further processing. This tangential filter device model will extract and concentrate the single tumor cells from the sample as they continue downstream. The fluid flow interaction with the nylon membranes will not only increased single cell purification; but provided a new unintended method of breaking apart lingering device-treated aggregates. To demonstrate this, the HCT116 cells were cultured into spheroids, pre-treated with trypsin-EDTA to break down these complex tumor tissues into

medium sized clusters, and passed through the filter device. This tangential filter device model experienced leaking at higher flow rates because the PDMS layers didn't provide an efficient seal around the nylon membranes. Our lab had ALine fabricate and demonstrated the implementation of the 15 µm membrane within their laminate layer process. This design improvement provided a more durable filter.

A cell suspension of MCF7 human breast cancer was withdrawn through the ALine filter at a total rate of 12.5 mL/min, the same flow rate the dissociation device performs at. When trypsinized, this cell line produced a sufficient amount of large aggregates around the same size as the trypsinized spheroids without the needed culture time (Figure 12). During these experiments, the syringe pump was set to withdraw certain effluent ratios to determine performance efficiency (direct, 80/20, 60/40, 40/60). Figure 13 displays the normalized cell yields from the direct and 80/20 tangential outlet conditions. The direct condition still yielded more single cells; however, the tangential-flow was more efficient at breaking apart lingering aggregates into single cells within a single population run.

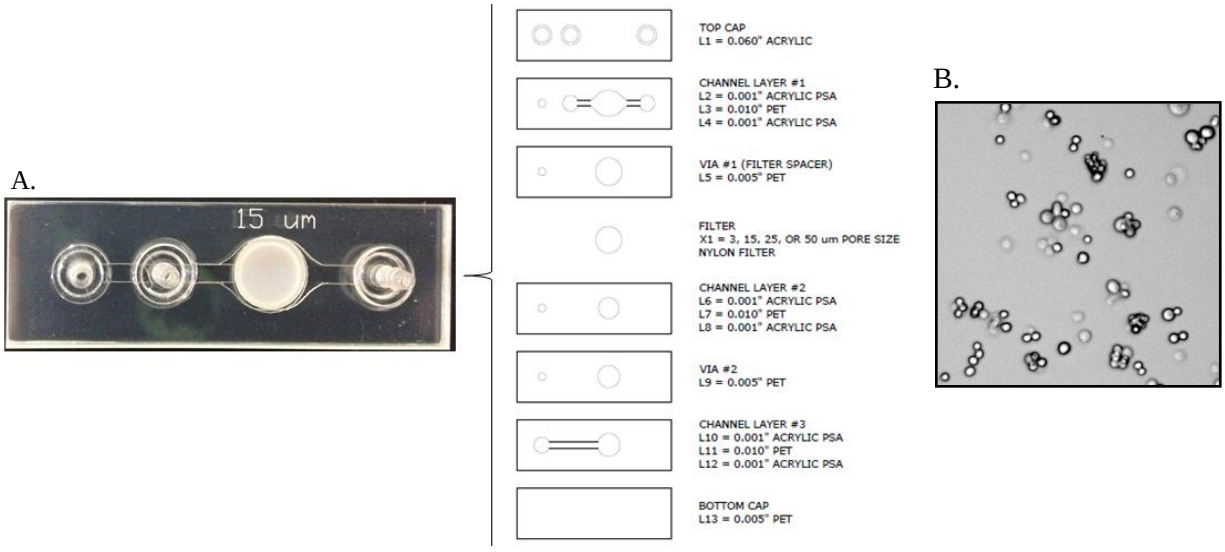


Figure 12: A.) ALine's fabricated 15µm filter and the laminate layers. B.) MCF-7 tumor clusters after a brief trypsinization.

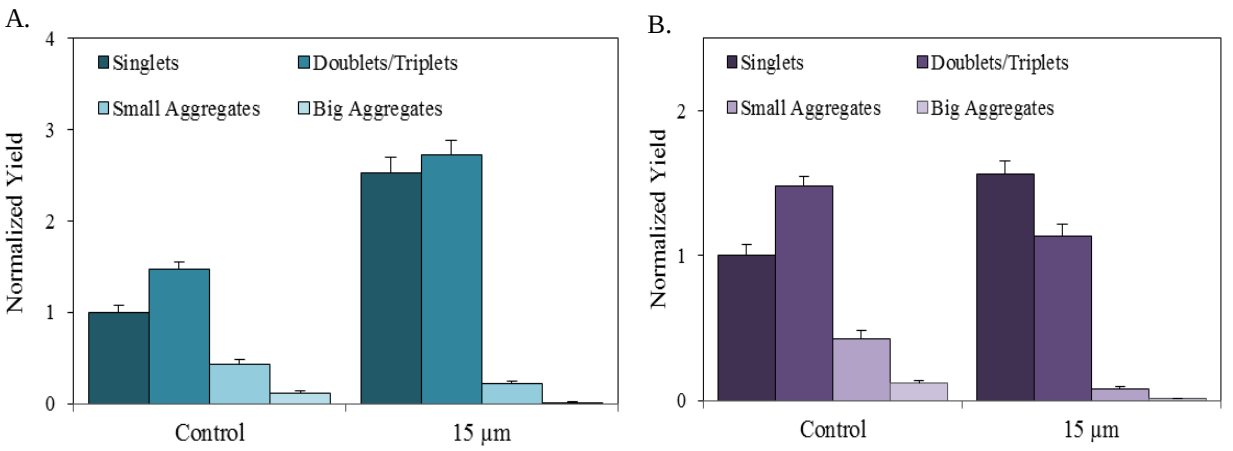


Figure 13A-B: MCF-7 Normalized Cell Yields.
A.) Direct-flow cell yield. B.) 80/20 withdrawal cell yield. This withdrawal condition was more efficient at generating single tumor cells.

4.3 Discussion:

Based on the tangential filter findings, this filter has proven to be efficient at concentrating single tumor cells. It is believed that the uneven membrane surface of the woven nylon fibers can act as a tool to help further break down tumor aggregates flowing across the membrane and recirculating to the dissociation device. As the main fluid stream travels in parallel to the membrane surface, the large aggregates incapable of selective filtering and entrapped at the inlet of the pores, are forced to return to the fluid flow of the channel due to the cross-flow currents. The nylon microfilters provide a fast and unforced mechanism of purifying the effluent sample with single tumor cells at high flow rates. With the selective barrier and controlled effluent ratios, the filter component has the potential to integrate with the dissociation device.

Chapter 5: Conclusion

To see any strives in the battle against cancer, the real victory lies within molecular diagnostics. As a front runner, molecular diagnostics has evolved precision medicine and help design healthcare plans (treatments, preventions, and cures) that take into account the specifics of the individual patient. The Haun Lab has adopted the promising fluid handling capabilities of microfluidics and created a device to process tumor biopsies into single cells. This dissociation device mechanically disrupts and converts the tumor sample into single cells. The proposed filter component can improve upon this sample processing by incorporating tangential flows to remove remaining aggregates to undergo further dissociation (Filter 1) and increase single tumor cell yield. When the tangential-flow filter is set to configured to distribute 80% effluent from the top outlet and 20% from the bottom, the filter becomes increasingly efficient at generating more and more single cells at higher flow rates. The woven mesh structure and defined pores of the 15 μm nylon membrane allow un-disrupted aggregates being recycled back into the dissociation device to be further broken down into single cells. This filter component in a tangential mode will provide an additional processing step to produce a more superior sample for the analysis component of the microfluidic platform. Since the filter device's initial designs were to also incorporate a second filter to help retain single tumor cells for sample enrichment, future work will focus on improving this aspect by incorporating other means of passive filtration. Inertial focusing can provide this opportunity by forcing single cells towards specific streamlines for sample purification. Once the effort to redesign Filter 2 is complete, the focus of future work will then move to the integration of the dissociation device and the filter component. A series of experiments will be conducted with this configuration to help determine single cell yield.

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APPENDIX: STANDARD OPERATING PROCEDURES

Direct-Flow Filtration Protocol:

Materials

Superblock (Thermo Scientific)
PBS+ (1% BSA): 500mL of Dulbecco's Phosphate Buffered Solution (Thermo Scientific), 5g of Bovine Serum Albumin (Sigma Aldrich)
DI water
Orflo Moxi Z counter and S cassettes

Sample Preparation

1. Prepare cell culture sample using standard cell culture methods provided by American Type Culture Collection (ATCC).
2. Resuspend cell sample in PBS+.
3. Using a 200µL pipette, transfer 75µL of each sample conical to the automated Moxi Z cell counter and take a "Before" Count. Record this value.

Methods

1. Set up the syringe pumps at the desired flow rate and place the specific membrane type into the direct-flow filter device.
2. Run 1mL of DI water to wet the membrane.
3. Run 1mL of Superblock through the entire device. Let it incubate for 15 minutes at room temperature.
4. Run a final device prep run using 1mL of PBS+ to wash the device channels of Superblock.
5. *For device runs:*
    a. Briefly vortex cell samples.
    b. Run trypsinized cells through the filter device at a rate of 1mL/min.
    c. Collect effluent in a 15mL conical.
    d. Run an additional 1mL of PBS+ through the filter to gather any remain cells.
    e. Collect the PBS+ wash in the same conical with the device treated effluent.
6. Briefly vortex the 15mL conical (device treated cells + PBS wash).
7. Count cell recovery with the Moxi Z cell counter and record this value.
8. Wash entire filter device with DI water (5x) back and forth at the same flow rate as the device runs.
9. Run 1mL of PBS+ to fill the device channels.
10. Repeat Step 5 with all other sample conicals and filter membranes.
11. Clean up bench.

Tangential-Flow Filtration Protocol:

Materials

Superblock (Thermo Scientific)
PBS+ (1% BSA): 500mL of Dulbecco's Phosphate Buffered Solution (Thermo Scientific), 5g of Bovine Serum Albumin (Sigma Aldrich)
DI water
Orflo Moxi Z counter and S cassettes

Sample Preparation

See Direct-Flow Filtration Protocol

Methods

Experimental Conditions:

- 0/100 – Top Outlet is completely closed. Bottom Outlet is withdrawing 100% of the flow rate (direct-flow).
- 50/50 – Top and Bottom Outlet withdraw 50% of the flow rate.
- 20/80 – Top Outlet withdraw 20% of the flow rate. Bottom Outlet withdraw 80% of the flow rate.
- 40/60 – Top Outlet withdraw 40% of the flow rate. Bottom Outlet withdraw 60% of the flow rate.
- 60/40 – Top Outlet withdraw 60% of the flow rate. Bottom Outlet withdraw 40% of the flow rate.
- 80/20 – Top Outlet withdraw80% of the flow rate. Bottom Outlet withdraw 20% of the flow rate.

Flow Rate: 1mL/min, 12.5mL/min

1. Set up the syringe pumps at the desired withdrawal flow rate and place the specific nylon membrane type into the tangential-flow filter device.
2. Repeat steps 2-4 found under Methods in the Direct-Flow Filtration Protocol.
3. *For device runs:*
    a. Briefly vortex cell samples.
    b. Run trypsinized cells through the filter device at a rate of 1 or 12.5mL/min.
    c. Collect all effluents (top and bottom) in separate 15mL conicals.
    d. Run an additional 2mL of PBS+ through the filter to gather any remain cells.
    e. Collect the PBS+ wash in the conicals with the device treated effluents (top and bottom)
4. Briefly vortex the "top" and "bottom" labeled 15mL conicals (device treated cells + PBS wash)
5. Count all cell effluents with the Moxi Z cell counter and record these values
6. Wash entire filter device with DI water (3x) back and forth manually
7. Run 2 mL of PBS+ to fill the device channels
8. Repeat Step 3 with all other sample conicals, experimental conditions, and filter membranes.
9. Clean up bench