IMPLEMENTATION OF PULSATILE FLOW ON MICROFILTERS
FOR EFFICIENT CELL SORTING

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IMPLEMENTATION OF PULSATILE FLOW ON MICROFILTERS
FOR EFFICIENT CELL SORTING

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To my mother and father, Sang Deuk and Myung Shin Lee
ACKNOWLEDGEMENTS

I dedicate this thesis to my parents Sang Deuk and Myung Shin Lee, as I would not be where I am now without their love and support. I want to thank Dr. Sulchek, for giving an inexperienced, unproven freshman a remarkable opportunity to work with him in his lab. I want to thank Dr. Qiu for being a great professor and mentor, and for helping me grow professionally and personally. I want to thank Aaron Enten for guiding me through five semesters of research, and for his continuous insight and wisdom.

Thank you for helping me grow into who I am today.
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SUMMARY

Cell sorting has been an important process in both clinical testing and medical diagnostics, and is used in many applications ranging from emergency trauma evaluation to cystic fibrosis checkups\textsuperscript{1-4}. Currently, efficient blood cell sorting needs to be done in a laboratory setting with dedicated machinery, and the process is expensive\textsuperscript{2, 5}, labor intensive\textsuperscript{6} and time consuming\textsuperscript{3}. Multiple microfluidic solutions have been proposed to address these issues, including separation through hydrodynamics\textsuperscript{7}, magnetism\textsuperscript{8, 9}, and dielectrophoresis\textsuperscript{10, 11}. Although these devices alleviate some of the problems surrounding laboratory blood sorting, the chips remain complex, costly, and lacks the specificity needed to be used directly in a clinical setting\textsuperscript{12, 13}.

The purpose of this study is to investigate the effectiveness of a new cell sorting method: pulse width modulated periodic backflush in a dead-end filtration system. Dead-end filtration is a cheaper, simpler approach to cell sorting; particles are passed directly through a membrane filter which blocks larger particles while passing smaller ones.

Dead-end filtration is easy to implement, and often has higher sorting efficiencies\textsuperscript{12}. The problem of particle throughput reduction due to clogging is alleviated by a periodic backflush mechanism that maintains high sorting efficiency while retaining particle throughput. We examine the impact of superimposing periodic backflush in a large fluidic system and create a prototype to validate this effect in a microfluidic environment. We use a mixture of staph epidermidis and lung epithelial cells to validate the application of backflush filtration in a clinical environment.
CHAPTER 1

INTRODUCTION

Cell Sorting

With many diverse applications ranging from evaluating the severity of traumatic injury to monitoring organ health, efficient cell isolation has become an important requirement for multiple areas in medical diagnostics and clinical experimentation\textsuperscript{1-4}. In diagnostics, blood cell isolation may be performed for clinical evaluation of trauma patients. In clinical testing, the separation of cystic fibrosis patients’ lung epithelial cells from infectious bacteria may be required for further investigation about the disease, leading to disease diagnostics.

Traditional cell sorting requires manual labor in multiple steps in a laboratory including centrifugation, sorting, and analysis\textsuperscript{2,5}. However, this process is costly\textsuperscript{2-5}, cumbersome\textsuperscript{6}, and time consuming\textsuperscript{3}, making it inconvenient or non-viable in many situations. Many different approaches have been taken to address these issues, taking advantage of distinguishable properties such as hydrodynamics\textsuperscript{7}, magnetism\textsuperscript{8,9}, and dielectrophoresis\textsuperscript{10,11}. While miniaturization of these platforms also allows for quick filtration and smaller sample quantity, many of these devices remain complex and expensive, and often also lack the specificity to be used in clinical settings\textsuperscript{12,13}.

Current Filtration Methods

A method of quick and precise cell sorting has been sought after due to its applications in clinical and diagnostic settings\textsuperscript{2}. Initial attempts to separate blood through filters were done in macrosystems, where microfilters were fabricated and tested outside of a microfluidics platform. One novel iteration was fabricating filter membranes out of Parylene-C, a biocompatible, rigid polymer that could be etched to have uniform micropores\textsuperscript{14}. Two of these microfilters could be stacked to create a bilayer microfilter,
and separable filters were prototyped to gather circulating tumor cells (CTCs) in blood\textsuperscript{15}. Various microfilters like Parylene-C were created from different materials such as silicon or silicon nitride, but these filters had complex manufacturing methods that were not scalable for use in rigorous clinical testing\textsuperscript{16}. Similar micro-patterned membrane filters were made from photolithography to alleviate this issue.

As these microfilter pores were round, the cells that clog the pore membrane may deform or lyse due to the increased stress around the cell\textsuperscript{17}. To lessen the impact of stress on the cells at the pore membrane, a ‘flexible micro spring array’ (FMSA) membrane was created from parylene. FMSA membranes have a comb like structure, which allows fluid and smaller cells to pass through the filter while a large cell is trapped in the filter\textsuperscript{13}. These FMSA devices allow greater throughput, maintain the same levels of cell viability, but also have complexities regarding manufacturing and scaling.

In macrosystems, the filter is bonded to polydimethylsiloxane (PDMS) or clamped with pressure, with one inlet and one outlet fitted with tubing. A syringe is at the end of the inlet, and is able to push through the filter system to effectively separate the desired components of the blood. The transition from macrosystems to microfluidic devices came when additional components were needed to increase the sorting efficiency, throughput, filtering time, and longevity from previous devices. One major problem with macrosystems was they did not address membrane fouling, or the decrease in filter efficacy due to eventual pore clogging\textsuperscript{12,18}. As a result, a new filter had to be used or the filter had to be manually cleared – both undesirable, labor-consuming tasks.

To address the issue of clogging, the Chen lab initially integrated a cross-flow system with their microfilter\textsuperscript{19}. Using Polyethylene (glycol) Diacrylate (PEGDA) microfilters fabricated by photolithography, the lab ‘sandwiched’ the filter to PDMS layers to create a three-layer chip that included a main channel as well as a cross flow channel to clear the membrane. While managing to get a high capture efficiency of CTCs,
the device had poor capture purity, as high amounts of blood cells accumulated on the filter.

By implementing multiple PDMS microfilters within one chip, the Zare group achieved higher capture specificity while isolating multiple components within one device\textsuperscript{12}. The Zare lab also incorporated cross flow channels to their system, but added another layer to their microfluidic device for pneumatic valves. This automated the collection and membrane clearance process as the open channels could now be controlled. Through this system, a sorting efficiency of \(~99.7\%\) could be achieved to isolate red blood cells and platelets from leukocytes.

As seen in the Zare lab, microfluidic devices can have a variety of implementations which make it very desirable for cell sorting. Pneumatic valves will allow complex channels and more precise liquid handling within the chip. Sensors can be added to detect and regulate different variables such as temperature and pH, which can be useful when processing cells for long periods of time. Actuators can be used in lieu of syringes to control and divert liquid flow. For example, a piezoelectric actuator was used to oscillate the flow within a microfluidic chip to eliminate particle clogging within the device\textsuperscript{19}.

However, a major drawback of current microfluidics is that the intricacy of the devices increases as additional features are added onto the system. Almost all the current blood sorting microfluidics chips require clean room facilities for fabrication, as they use photolithography to pattern their microfilters and channels. The addition of more layers such as the Zare chip also increase the difficulty in chip bonding and alignment. Aside from the increase in manufacturing labor, there is also an increase in cost – both for access to a clean room facility and the photomasks required to create a mold. This may be detrimental in the transition to having widely available chips used for sorting. Commercially available membranes such as polycarbonate were previously undesirable for blood sorting due to poor sorting efficiencies compared to uniform, microfabricated
filters. However, now that cross flow and backflush systems can be implemented to clear the fouling that occurs within polycarbonate membranes, they may be viable alternatives to reduce the costs of creating an effective sorting chip. Adding these polycarbonate membranes in parallel may also be the solution for the poor throughput the filters normally have due to their smaller pore density.

**Dead-End Filtration with Periodic Backflush**

One promising method of blood cell sorting is dead end filtration, which is cost effective, easy to implement, relatively quick, and user friendly. The biggest drawback to dead-end filtration is membrane fouling, or the loss of filter efficiency due to pore clogging by incoming particles. Over time, the filter’s throughput will decay exponentially due to membrane fouling. This problem is further complicated by the mechanical sensitivity of cells: any localized pressure on the cell membrane can lead to unwanted deformation or cell lysis.

The Sulchek lab has proposed a new method of clearing the fouling layer in dead-end filtration systems through a fluid control mechanism called pulse width modulated (PWM) periodic backflush. In PWM periodic backflush, the liquid that traverses a membrane filter periodically changes to flow in the opposing direction. While the time of forward flushing is still greater than the time of backflush for a fixed fluid velocity, the periodic change in direction allows the system to maintain high throughput levels along with high sorting efficiency.

While PWM periodic backflush has been performed with microbeads on a macrofluidic level, it has not been implemented with living biological components or on a microfluidic level. The objective of this thesis is to explore both the clinical applicability of this system through the separation of epithelial cells and lung bacteria, and its potential to be scaled down to a microfluidic level.
CHAPTER 2

MATERIALS AND METHODS

A549 Lung Epithelial Cell Culture

Lung epithelial cancer cells (A549 cell line) were cultured in media consisting of 89% Roswell Park Memorial Institute (RPMI) base, 10% Fetal Bovine Serum (FBS), and 1% Penicillin-Streptomycin (Pen-Strep). Cells were kept in a CO2 incubator at 37 degrees Celsius, and passaged every four days when the cells reached approximately 75% confluency. To passage the cells, the RPMI media was aspirated from the flask, and PBS was added to wash the cells. The PBS was then aspirated, and trypsin was added to detach the cells. Whole media was added to the detached cells to neutralize the trypsin, and centrifuged at 200xG for five minutes. After centrifugation, the cell pellet was isolated and resuspended in new whole media. The cells were then seeded at a lower confluency and incubated.

Staphylococcus epidermidis Bacteria

Staphylococcus epidermidis was cultivated in a conical tube with LB broth as an analog for S. Aureus, a common infectious agent in Cystic Fibrosis. When the staph colony reached high turbidity, the bacteria solution was inoculated and seeded on an agar plated petri dish. After twelve hours, a new grown colony was reinoculated into a new conical tube. To determine the colony forming units (CFU) per volume, 0.1 mL of new bacterial broth was added to three different conical tubes diluted by 1:100, 1:10,000, and 1:1,000,000 respectively. The tubes were vortexed until the bacteria were uniformly distributed, and 0.01 mL of the fluid from the tubes were inoculated and evenly spread onto agar coated plates. The plates were incubated for 24 hours, and the individual formed colonies were counted from the plate that had distinct, individual colonies.
original number of CFU per mL of solution derived by multiplying the CFU by the given
dilution factor and averaged between plates.

**Spiking Cell Culture with Staph Epidermidis**

A sterile, neutral buoyancy, suspension media consisting of PBS, tween, percoll, and EDTA was created. The A549 cells were detached using the same procedure above and centrifuged to isolate the cells. 10uL of the cell suspension were transferred to a hemocytometer to count the number of cells per volume. The calculate volume of staph broth was also centrifuged, and both pellets were diluted in the suspension media. After mixing both tubes until the solution was uniform, the staph and cells were combined in the suspension media in a ratio of 50:1 respectively.

**Macrofluidic Filtration with Periodic Backflush**

Two filters – a Whatman Nuclepore Track-Etch Membrane and a Whatman Cyclopore Track Etched Membrane were used to separate the cell-bacteria mixture. The filter was encased in tight housing and connected to a customized syringe pump. This system was set up vertically, with the syringe pump on the bottom, driving fluid flow with negative pressure differentials, and a magnetic mixer on the top chamber such that settling was minimized but would happen in the direction of the filter.

Prior to introducing the mixture, the system was primed with the suspension media to remove any air bubbles from interacting with the filtration process. After priming, the cell-bacteria mixture was added to the top chamber of the system. The syringe pump was used to experimentally test two scenarios – dead-end filtration without oscillation and dead-end filtration with PWM periodic backflush. For the periodic backflush, the ratio of forward flush to backward flush volume used was 1.22 (Duty cycle of 55%). This ratio was previously determined with microbeads to be the most effective
in terms of sorting efficiency without sacrificing a significant amount of throughput time. After the samples were processed completely, the permeate and retentate were collected.

**Microfluidic Filtration with Periodic Backflush**

**Evaluation of polycarbonate filters for sorting**

Polycarbonate (PC) has been used in previous research for blood component filtration\(^{18}\). While these track-etched filters suffer in capture efficiency due to pore fusion and scattering\(^{17}\), they are much more cost effective than microfabricated membranes which require photolithography. As APTES-functionalized polycarbonate also bonds with PDMS, the polycarbonate membrane can be ‘sandwiched’ in-between two PDMS layers to create an effective filter chamber. Moreover, the proposed polycarbonate chip design allows for easy filter scalability in series or in parallel, which allows for high throughput and specific particle separation. Polycarbonate filters were determined to be suitable for use and testing for the backflush mechanism in a microfluidics setting.

**Creation of a one-chamber, two-channel prototype**

An aluminum 6061 plate was milled to contain the mold negative of a channel and a chamber (Fig. 1A). PDMS and its curing agent were mixed, poured on the mold, vacuumed to remove bubbles, and cured at 60°C for two hours. The cured PDMS was cut and fluidic access holes were punched at the ends of the channels. The pieces were then taped to ensure no dust contacted the surface.

**Polycarbonate membrane functionalization**

To bond polycarbonate to PDMS, surface modification had to be done on both substrates to create silanol groups, which will irreversibly bond to each other on contact\(^{20}\). After oxygen plasma treatment, the polycarbonate membranes are submerged
in a 5% (3-Aminopropyl) triethoxysilane (APTES) solution at 80°C. The functionalized polycarbonate surface molecules react with APTES transferring the silicon-carbon-amine chain of the APTES molecule to the polycarbonate surface. When treated with oxygen plasma a second time, the –NH₂ end will be replaced with an –OH group, which can now bond with functionalized PDMS to form siloxane groups keeping the two materials together.

PDMS chambers made from the aluminum molds were used to test the bond strength of the PDMS-PC-PDMS chip. As the bonding process required the two silanol groups to be in direct contact, all cured PDMS was kept taped and sealed to prevent contamination. Additionally, the APTES solution was heated under a fume hood after plasma treatment as hazardous vapors were created from the heating process. The polycarbonate filters were plasma treated and submerged into the APTES solution. After treatment, the filters were dried and plasma treated a second time with one PDMS slab. These two pieces were bonded, and immediately plasma treated again with the second PDMS slab. The second PDMS slab was bonded to finish fabrication. The detailed APTES functionalization procedure can be found in Appendix A.

After ensuring that the microfluidic device had proper bonding between the three layers, surgical tubing was inserted at each end. The devices were tested to ensure that there were no leaks within the system.
Figure 1: Aluminum Mold and Microfluidic Chip. An aluminum 6061 plate was milled with the negative shape of the microfluidic chip (A). After pouring the PDMS and curing agent mixture, the mold was put under a vacuum to remove any air bubbles, and placed in an oven at 60°C for two hours. Two PDMS pieces, combined with a polycarbonate membrane, were used to create one microfluidic chip (B).

Creation of sorting system using microfluidic chips

To facilitate precise backflush motion in the microfluidic chips, a two-syringe actuating system was built (Fig 2). The actuating system is able to translate the stepper motor’s rotation to a linear motion, moving the actuating plate to push and pull the syringe plunger. The stepper motors are controlled by an Arduino Mega with the Adafruit Stepper Motor Driver v2, which is able to send move commands to two stepper motors simultaneously. The system is comprised of laser cut acrylic plates, and a tapped, milled spacer that moves the plates as the stepper motor runs. The system is compatible with Hamilton gastight high precision syringes which will be able to actuate fluids more precisely microfluidic liquid handling.
Figure 2: CAD Model and Prototype of a Two-syringe Actuating System Compatible with Microfluidic Chip. A two syringe actuating system was designed and manufactured such that efficient oscillation can be performed on the polycarbonate filter microfluidic chip. The chip can be inserted onto a slot on the bottom of the system, and can connect to the syringes with surgical tubing. The syringes can actuate in opposing directions to create pulsatile flow within the chip.

Data Analysis

Several methods were used to analyze the samples post-filtration. For the first three trials, the permeate and control fluids were taken and processed with flow cytometer BD Accuri to analyze the composition of the mixtures. The forward scatter detection was used to distinguish the size of the particles. For trial three, the Nuclopore permeate samples were diluted, plated, and incubated such that the bacterial CFU counts could be analyzed. The resulting histograms were used to examine if improvements were made in particle throughput, purity, enrichment factor, and recovery percentage.
CHAPTER 3

RESULTS

Macrofluidic System – Flow Cytometry Analysis

Figure 3: Trial 1 - Forward Scatter Histograms for Filtration Modes of 50:1 Mixture of Staph Epidermidis to A549 Cells. Staph Epidermidis and A549 cells were sorted with Cyclopore and Nuclepore filters at $\alpha$ of 100 and 55. Counts of the stock solution indicate the cell-bacteria ratio is around 19:1 instead of the calculated 50:1; this may indicate cell shearing or degradation. There is still a small improvement seen in filtration with periodic backflush, especially with Cyclopore filters. Furthermore, the Nuclepore filters exhibit a high enrichment factor (7.84) compared to that of the Cyclopore filters (1.25).

Table 1: Trial 1 – Flow Cytometry Counts for Filtration Modes of 50:1 Mixture of Staph Epidermidis to A549 Cells. The number of particles found per subset. The counts below are in the same arrangement as the figure above.

<table>
<thead>
<tr>
<th>Stock</th>
<th>Cyclopore</th>
<th>Nuclepore</th>
</tr>
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<tbody>
<tr>
<td>Subset 1</td>
<td>Subset 2</td>
<td>Subset 1</td>
</tr>
<tr>
<td>900,485</td>
<td>52,841</td>
<td>941,253</td>
</tr>
<tr>
<td></td>
<td></td>
<td>950,072</td>
</tr>
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Figure 4: Trial 2 - Forward Scatter Histograms for Filtration Modes of 50:1 Mixture of Staph Epidermidis to A549 Cells. Similar to the first trial, the flow cytometer counts do not reflect the calculated ratio of bacteria to cells.

Table 2: Trial 2 – Flow Cytometry Counts for Filtration Modes of 50:1 Mixture of Staph Epidermidis to A549 Cells. The number of particles found per subset. The counts below are in the same arrangement as the figure above.

<table>
<thead>
<tr>
<th>Stock</th>
<th>Cyclopore</th>
<th>Nuclepore</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Subset 1  Subset 2</td>
<td>Subset 1  Subset 2</td>
</tr>
<tr>
<td>Stock solution</td>
<td>136,099       4,602</td>
<td>75,094  265</td>
</tr>
<tr>
<td></td>
<td>71,262       831</td>
<td>74,802    350</td>
</tr>
</tbody>
</table>
Figure 5: Trial 3 - Forward Scatter Histograms for Filtration Modes of 50:1 Mixture of Staph Epidermidis to A549 Cells. Similar to the first and second trials, the flow cytometer counts do not reflect the calculated ratio of bacteria to cells.

Table 3: Trial 3 – Flow Cytometry Counts for Filtration Modes of 50:1 Mixture of Staph Epidermidis to A549 Cells. The number of particles found per subset. The counts below are in the same arrangement as the figure above.

<table>
<thead>
<tr>
<th>Stock</th>
<th>Cyclopore</th>
<th>Nuclepore</th>
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<tbody>
<tr>
<td></td>
<td>Subset 1</td>
<td>Subset 1</td>
</tr>
<tr>
<td></td>
<td>790,970</td>
<td>999,536</td>
</tr>
<tr>
<td></td>
<td>1,144</td>
<td>164</td>
</tr>
<tr>
<td></td>
<td>999,441</td>
<td>163</td>
</tr>
</tbody>
</table>
Figure 6: Flow Cytometry Counts for Samples Containing Only A549 Samples. Flow cytometry counts for a suspension mixture including only A549 samples during trial 1 (left) and trial 3 (right). There is a wide distribution in the size of the particles seen in the sample.

Macrofluidic System – Permeate Plating

Figure 7: Trial 3 – CFU Counts Comparing $\alpha = 55$ and $\alpha = 100$ Filtration for Nuclepore filters. After filtration, the permeate sample was placed on a petri dish and grown. The $\alpha = 100$ sample (left) had a count that was approximately 2,000 CFU per plate, while the $\alpha = 55$ sample (right) was too numerous to count. This result shows the periodic backflush increases the bacterial count in the permeate, and implies that the flow cytometry counts include A549 cell debris in a size range approximately equal to the staph epidermidis.
CHAPTER 4
DISCUSSION

Macrofluidic System – Analysis of Initial Test Runs

Based on the initial test runs, the effect of periodic backflush on a mixture of Staphylococcus epidermidis and A549 cells is unclear. However, based on the disparity seen in the CFU counts and the flow cytometry data, not all particles in the low forward scatter range may be bacterial particles. The CFU counts show a clear increase when periodic backflush is applied, which aligns with the expected results based on previous microbead sorting analyses. This, along with the absence of A549 cell sized particles in the flow cytometry data, shows that cell shearing and disintegration may be occurring during filtration, causing smaller, unwanted particles in the permeate. This may also be the reason why the flow cytometry data of the sample with only A549 cells has high variance in the size range of the cells.

A few factors may be in play leading to cell disintegration during filtration. One possibility is that the processing time of the cells after they are detached is too long. As A549 cells are adherent cells, they are not in their natural state after trypsinization and transfer to suspension media. The cells were in suspension for over one hour prior to flow cytometry analysis for the first set of trials. Taking flow cytometry data for an A549 cell sample at various time points will show if suspension of A549 cells over long periods is detrimental.
A second factor that needs to be analyzed is the effect of cell shearing during filtration. Cell shearing and lysing was an issue previously identified in porous membranes\textsuperscript{17}, and may occur on both Cyclopore and Nuclopore membranes. In future experiments, the bacteria may need to be tagged with fluorescence or dye to differentiate the flow cytometry datum. If cell fragments are confirmed to be present, the syringe actuator may need to run at a slower RPM, or new filter types such as the FMSA may need to be considered.

Additionally, future experiments may need a more precise bacterial count when creating the cell-bacteria mixture. As plating for CFU counts only gives an estimate on the amount of bacteria present, experiments may have high variance from the 50:1 bacteria-epithelia ratio. Flow cytometer readings may show high numbers of smaller particles because a higher bacteria-cell ratio was introduced in the suspension media. To eliminate this possibility, a spectrophotometer can be used in tandem with plating to determine bacterial counts based on the optical density of the bacterial suspension.

**Microfluidic System - Exploring Effects of Periodic Backflush**

With successful bonding of the three-layer microfluidic chip and creation of a dual-syringe actuation system, experimentation can begin to start testing the backflush mechanism on a microfluidic level. Two mold materials were tested for the manufacturing process – acrylic and aluminum. One observation during leak-testing of these devices was that higher actuation pressures will cause chip leakage at the insertion point of the surgical tubing. This can be prevented by adding uncured PDMS on that surface and allowing the PDMS to seal the chip around the tubing.
Moving forward, the produced polycarbonate filter chips need to be tested for microbead entrapment and unwanted flow within the device. If the polycarbonate membrane is not fully bonded or bonded with folds, microparticles entering the device may become irreversibly clogged within the membrane. Flow tests need to be performed to observe if any major losses are present with the bonded membrane. These tests may also be helpful in further optimizing the dimensions of the device if flow and throughput data can be gathered during experimentation.

After these tests are completed, the chips can be connected to the high precision syringes. External stopcocks can be added to control the flow within the device and remove air bubbles within the system. With stopcock implementation, the device can be primed, and backflush can be performed with a 50/50 microbead solution ratio for two and seven micrometer diameters. The concentration of beads before and after the filter can be analyzed to determine the efficiency of backflush in increasing the throughput of dead end filtration while maintaining high levels of sorting efficiency. After validating chip success in separation of microbeads, the device can be tested with cells and bacteria.
CHAPTER 5

CONCLUSION

While initial macrofluidic test runs have been inconclusive in validating the effects of periodic backflush on cell-bacteria sorting, several improvement considerations have been identified for future experimental runs. By analyzing the effect of A549 cells in suspension media, examining the effect of cell shear during filtration, and collecting experimental data with different sources to eliminate variance, future test runs will provide more accurate indications on how periodic backflush affects cell sorting.

On a microfluidic level, the polycarbonate filter membrane was successfully integrated onto PDMS, creating a leak-free chip. Furthermore, a dual-syringe actuating system was prototyped which will be able to interface with the chip to facilitate periodic backflush filtration. Testing can begin with microbeads to examine if the microfluidic chip is capable of filtering at a similar capacity to its macrofluidic counterpart.

After validation of the periodic backflush mechanism on biological organisms and on a microfluidic level, a sorting device that facilitates this filtration mode can be commercialized. Ranging from blood tests to cystic fibrosis diagnostics, these chips may provide clinicians and lab personnel a quick, cost-effective, reliable method of cell isolation.
APPENDIX A

Procedure for bonding PDMS to polycarbonate

1. Clean 50 mL beaker with Alconox, use deionized water to rinse and dry with Kimwipes.
2. Create a solution of 5% APTES in water (by volume) using a pipette or syringe.
3. Use a hotplate to heat APTES solution to 80°C.
4. Put polycarbonate membrane inside plasma cleaner with the shiny side facing up. The membranes may need to be on a petri dish as they may flip when the vacuum to the chamber is turned on. Set RF level to high and wait one minute.
5. Remove membrane with cleaned tweezers and place in 80°C APTES solution. Soak membrane for 20 minutes.
6. Use tweezers to take membrane out of solution. Let the membranes dry on Kimwipes for twenty minutes.
7. Put first PDMS slab as well as dried polycarbonate membrane in the plasma cleaner. After turning on the vacuum, set RF to high and wait one minute.
8. Remove the PDMS slab and membrane from plasma cleaner. Gently place the membrane on the desired location on the PDMS slab and poke with tweezers. The polycarbonate-PDMS bonding should be visible in a similar manner to PDMS-PDMS bonding.
9. Repeat steps 7 and 8 with the second slab of PDMS and the PDMS-polycarbonate slab.

AFTER BONDING, APPLY SOME PRESSURE TO THE COMPLETED DEVICE AND PUT IN THE OVEN AT 60°C FOR THIRTY MINUTES.
REFERENCES


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