OSTEMER Microfluidic Device Fabrication Method

Maya Sultan

TABLE OF CONTENTS

ABSTRACT

The primary goal of this research project is to develop a cost-effective fabrication method for rigid microfluidic devices in order to enable high-pressure flow. Research activities include the testing of various chemical treatments to improve device biocompatibility, alongside the conducting of a microfluidic experiment with cell lines to test cell-channel interaction and assay post treatment cell viability. If assay development on the OSTEMER-devices determines that cells are not biocompatible, chemical passivation methods and buffer additives can be applied to reduce adhesion within the devices. Assay development on jurkat and human peripheral blood mononuclear cells (PBMC) produced cell viability results that proved OSTEMER is a viable material for microfluidic device fabrication, with injected cells maintaining high viability under various levels of high-pressure flow.

CHAPTER 1. Introduction

1.1 Microfluidics Background

Microfluidics entails the manipulation of fluids at a very small scale, specifically in the range of microliters, to simulate a lab-on-a-chip and organ-on-a-chip. This technology encompasses various applications in drug delivery, cell microanalysis, miniaturization (organ-on-a-chip and lab-on-a-chip), wearable biosensors, and disease diagnostics [Fallahi]. Lab-on-a-chip is a growing area of microfluidic research, allowing the miniaturization of processes like polymerase chain reactions (PCR); the PCR method can be applied to a variety of medical issues, including the diagnosis of viral diseases [Becker]. The most beneficial aspect of working with microfluidic devices is that the amount of sample, reagent, and cells needed to conduct experiments is relatively small. This minimizes waste, which is optimal when working with biologically hazardous materials. In addition, these systems can be used to carry out specialized forms of biochemical synthesis; one example of this is the multistep synthesis process of an F-labeled probe for PET scanning, which is an imaging test that is utilized to detect cancer, heart, or brain conditions [Weibel].

The devices that will be referenced and utilized for data collection are microfluidic transfection devices. Transfection devices are designed to introduce foreign nucleic acids into eukaryotic cells [Chong]. To summarize the process, the transfection devices compress the inserted cells to stretch them out; as the cell membrane becomes more permeable, the cells can relax and take up the substances that are passed through the buffer. The cells must be slightly larger than the channel's gap size for the device to effectively compress the cells; for the purposes of this study, the cells are about double the size of the device's gap size. By modifying the host cell's genetic code, microfluidic transfection devices can be utilized in ex vivo gene therapy.

In the ex vivo gene therapy process, T-cells are extracted from the patient's blood and manipulated in the transfection devices to produce chimeric antigen receptors (CARs). These modified CAR T-cells are then administered back to the patient, typically through intravenous (IV) infusion. In terms of cancer treatment, once the modified cells are administered back into the bloodstream, they can detect and destroy the cancer cells that express the corresponding antigen. Therefore, this microfluidic technology provides a unique opportunity for use in cancer treatment.

1.2 Research Problem

Although microfluidic technology presents a revolutionary concept, some of the primary issues include the lack of sustainability and the added costs of disposing fouled devices [Li]. In addition, cell adhesion due to biofouling and accumulation at channel ridges hinders the possibility of high-pressure flow and eliminates the possibility of reuse. As a result, the previously mentioned issues endorse the search for a new fabrication material that promotes long-term usage and high-pressure flow through the microfluidic device.

1.3 Research Solution and Goals

Off-stoichiometry thiol-ene-epoxy resin (OSTEMER 322 crystal clear) is a commercially available, two-step curable polymer that can be utilized in the biocompatible bonding of polymer microfluidic cartridges to a biofunctionalized silicon biosensor. Compared to the traditional polydimethylsiloxane (PDMS) fabrication method, OSTEMER is slightly hydrophilic and has low water vapor permeability. Utilizing a UV-initiated bonding method, the final reverse cured package can withstand up to 2500 millibars of fluidic pressure [Sønstevold]. These properties allow for the long-term utilization of the OSTEMER-devices, which demonstrates a more sustainable approach to device fabrication. To maximize longevity of the devices, low concentrations of Pluronic additives can be used to limit protein adsorption. Pluronic passivation will reduce accumulation at the ridges and can, therefore, enhance the desired effect of high-pressure flow through the OSTEMER-device. After device fabrication and protocol optimization, cell biocompatibility testing can be conducted through assay development. The goal is to test if the OSTEMER-devices can efficiently deliver genes into the cell nucleus, and to continue to optimize the device by testing the long- and short-term impacts on cell physiology.

1.4 Results and Conclusions

Off-stoichiometry thiol-ene-epoxy resin (OSTEMER 322 crystal clear) proved to be a viable material for the purposes of this experiment. After device fabrication and protocol optimization, cell biocompatibility testing was conducted through assay development on jurkat and human peripheral blood mononuclear cells (PBMC). Cell viability and high-speed video results proved that, in the OSTEMER-fabricated devices, the cells maintained high viability under various levels of high-pressure flow.

CHAPTER 2. Methods and Materials

2.1 Physical Characterization

As mentioned previously, off-stoichiometry thiol-ene epoxy resin (OSTEMER 322 crystal clear) is a two-step curable polymer. OSTEMER 322 is a product sold and developed by Mercene Labs AB, a Swedish company specializing in high performance surface coatings; the OSTEMER products were curated to meet the requirements of microfluidic, lab-on-chip (LOC) and micro-electromechanical systems (MEMS). The resin is a transparent, rigid, and non-reactive substance that is compatible with soft PDMS molds and hard molds, including Si/SU8 and aluminum (after being passivated) [OSTEMER 322]. This biocompatible resin can be used in the bonding of polymer microfluidic cartridges to a biofunctionalized silicon biosensor. OSTEMER 322 provides the physical properties of being slightly hydrophilic and having a low water vapor permeability [Sønstevold]. This hydrophilic property enhances adhesion by making it easy to bond to other materials, while low water permeability improves material stability and prevents moisture-related issues such as swelling or degradation. Furthermore, the process is highly replicable because the material's low shrinkage guarantees precision and reliability.

Utilizing a UV-initiated bonding method, the final reverse cured package can withstand high levels of pressured flow [Sønstevold]. These properties allow for the long-term utilization of the OSTEMER-devices, which demonstrates a more sustainable approach to device fabrication. Au proposes the use of amphiphilic droplet additives – pluronic coblock polymers of poly(propylene oxide) (PPO) and poly(ethylene oxide) (PEO) – as a solution to improve sustainability and remove the added costs of disposing fouled devices. Pluronic is a triblock copolymer that is popularly used as a surfactant to bring hydrophilic coatings onto microfluidic device surfaces [Li]. As a result, Pluronic passivation can be applied to OSTEMER-devices to further reduce adhesion and improve flow.

2.2 OSTEMER Device Fabrication Procedure

2.2.1 Pre-bonding Procedure

Prior to OSTEMER bonding, ensure the mold wafer is silanized and free of debris by cleaning it with tape or compressed air (nitrogen gas). The silanization process entails surface treating a material (typically glass or silicon) with a thin layer of silane solution. This treatment creates a hydrophobic and non-stick barrier between the mold and the cast material, facilitating the ability to remove the cured material from the mold without damaging it.

The OSTEMER resin is typically delivered in two components, with Component A having a more fluid consistency, and Component B being more viscous. If Component B has not been utilized in some time, shake the bottle vigorously to recombine the substance. In preparation for UVA initiated bonding, it is suggested to run the UV program equipment 2-3 times to heat up the UV light.

2.2.2 OSTEMER Preparation

All work should be conducted inside of a chemical fume hood. Begin the process by combining a 1:09:1 ratio of Component A to Component B, respectively. For the purposes of this experiment, a ratio of 11 grams of Component A to 10 grams of Component B was measured using a high precision digital weight scale. After measuring the components in the correct proportions, mix asymmetrical (up-down, left-right) using a plastic lab stirring rod for about 5 minutes. The mixture is light-sensitive and can harden quickly if exposed to large amounts of light. To minimize the presence of bubbles, place the mixture in a desiccator for 10 minutes to degas.

2.2.3 Bonding Procedure

Pour the OSTEMER mixture on the mold wafer and degas in a plasma cleaning system for 30-60 seconds, or until bubbles are no longer present. Immediately place the mixture in a UV chamber and cure at 800 mJ/cm². Remove the cured layer from the mold wafer with sharp tweezers and cut the desired channels in addition to punching any inlet and outlet holes (if applicable, depending on mold wafer layout.)

Prepare the bonding substrate, such as a glass slide, by cleaning with tape or compressed air (nitrogen gas). Place the device and glass slide in the machine and plasma treat with the channel side up for about 1 minute. The plasma treating step is necessary to remove any air bubbles within the OSTEMER mixture. Failure to remove air bubbles prior to UV treatment could result in defects within the final cured structure that comprise the microfluidic device's function. Remove from the plasma cleaning system and firmly press the device (channel side up) and glass slide together. Insert a syringe needle, such as a blunt end luer lock syringe needle, into the inlet hole. Place the final product into a lab oven at 80 degrees celsius for at least 60 minutes to complete the second phase of the curing process.

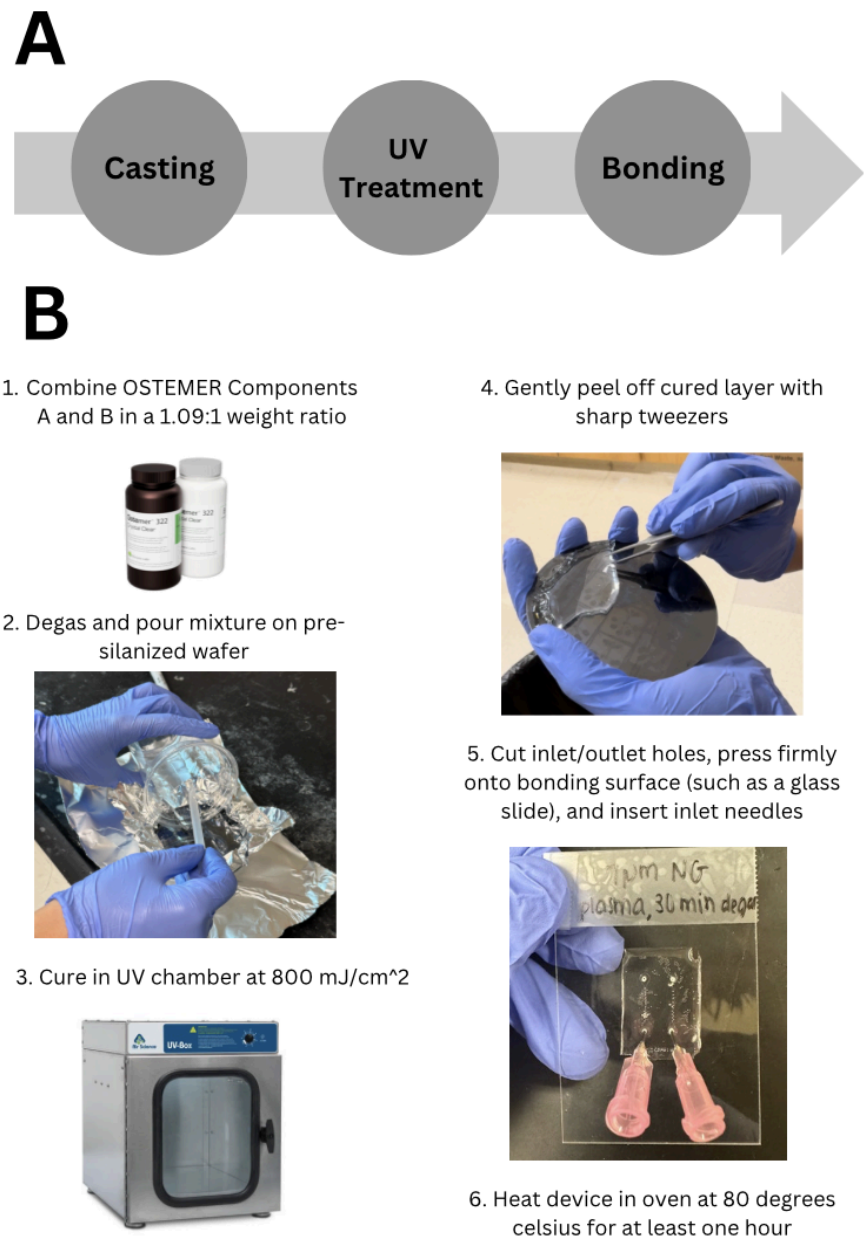

Figure 1. Fabrication process of OSTEMER microfluidic device. *(A) Major microfabrication steps of OSTEMER, following the process from casting to UV exposure to final bonding. (B) Detailed operation process of OSTEMER activation, casting, and bonding. Utilizing a silanized wafer, pour the degassed OSTEMER mixture onto the wafer and place it in the UV chamber. After UV treatment, the cured layer should be soft and elastic, making it easy to peel off the wafer with a sharp tool (such as tweezers). Since the layer is soft and elastic, the desired channel can easily be cut out and the inlet/outlet can be punched.*

In this two-step polymerization process, the initial liquid prepolymer mixture solidified to an elastic intermediate state through UVA light exposure at 800 mJ/cm². OSTEMER 322 contains three different monomers consisting of thiol, allyl or epoxy functional groups. During this first curing step, the UVA light allows the thiol and allyl function groups to react [Sønstevold]. Once the OSTEMER 322 is demolded and set on the substrate, the second curing step is initiated through heat exposure at 80 to 110 degrees Celsius; this step causes the thiol and epoxy

functional groups to react. After completing both steps, the polymer becomes rigid and covalently bonds to the substrate (which, for the purposes of this experiment, is a glass slide). This covalent bonding of the OSTEMER device to the substrate occurs as the epoxy groups form covalent bonds to the hydroxyl groups on the surface of the substrate.

2.3 Data Analysis

To examine the efficiency and repeatability of OSTEMER 322 device fabrication, human peripheral blood mononuclear cell (PBMC) and naive T-cell viability can be analyzed at various gap sizes and pressure flows. The cells were prepared with OPTIMEM 1% Pluronic cell flow buffer, and cell viability was examined in 4µm and 6µm gap-sized OSTEMER-devices at levels of high-pressure flow (4-6 bar). The chosen gap sizes of 4µm and 6µm are significant because they mimic typical dimensions of biological microchannels and can therefore provide realistic results on cell viability. High pressure flows were selected to demonstrate the durability of the OSTEMER material under extreme conditions.

The analysis of PBMC and naive T-cell viability under different conditions and parameters can provide a substantial assessment of the structural integrity of OSTEMER 322 devices. If the cells maintain high viability under high-pressure stresses and various device designs, the fabrication process can be considered consistent and repeatable.

CHAPTER 3. Results

To determine the optimal gap size for further testing, a comparative study was performed with both 4µm and 6µm OSTEMER devices under high-pressure conditions. The primary goal was to assess the efficacy of the material and gap size in preserving cell viability. The gap size with the highest cell viability was then selected for additional testing to confirm that the OSTEMER material can withstand pressures of up to 6 bar, while also maintaining a relatively high cell viability.

3.1 PBMC Cell Viability in 4µm and 6µm OSTEMER Devices

Two gap sizes, 4µm and 6µm, were tested at pressure levels of 4 bar and 5 bar to evaluate the effects of gap size on human peripheral blood mononuclear cell (PBMC) viability. The cells were diluted in OPTIMEM with 1% Pluronic, and all devices were passivated to reduce the risk of cell adhesion within the microfluidic channels.

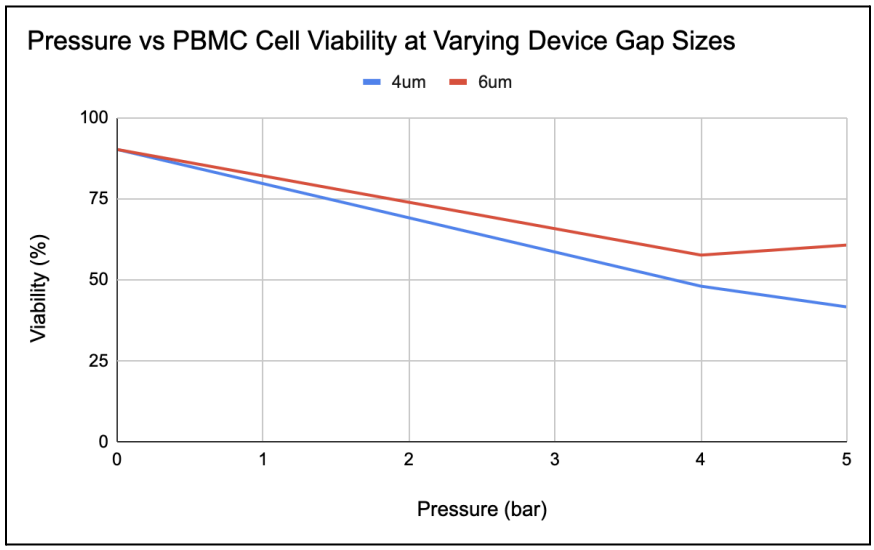

Figure 2. Pressure vs PBMC Cell Viability in Varying Gap Sized OSTEMER-Devices. *PBMC viability was analyzed at high pressure flows of 4 and 5 bar in 4µm and 6µm gap-sized OSTEMER devices. The naive T-cells were diluted in 2mL of OPTIMEM with 1% Pluronic, and all devices were passivated with a cell flow buffer.*

Figure 2 displays the relationship between pressure and cell viability for both 4µm and 6µm gap sizes. The data demonstrates that in comparison to the 4µm, the 6µm gap size possessed a higher PBMC viability across both pressure conditions. From this initial analysis and basic biomechanical properties, we can confirm that the larger gap size offers a more suitable environment for cell survival due to the increased surface area and the reduced shear stress. Furthermore, the larger gap size provides stable flow by minimizing cell clogging and breakage. As a result of these viability trends, the 6µm gap size was chosen for further testing of OSTEMER-device durability under higher pressure conditions of up to 6 bar.

3.2 PBMC Cell Viability at High Pressure Flow

Utilizing the initial comparison between gap sizes, the next stage of experimentation involves testing 6µm OSTEMER-devices at increasing pressure levels. The objective was to establish whether or not PBMC viability can be maintained at pressure levels of up to 6 bar, which is uncommon in typical microfluidic devices.

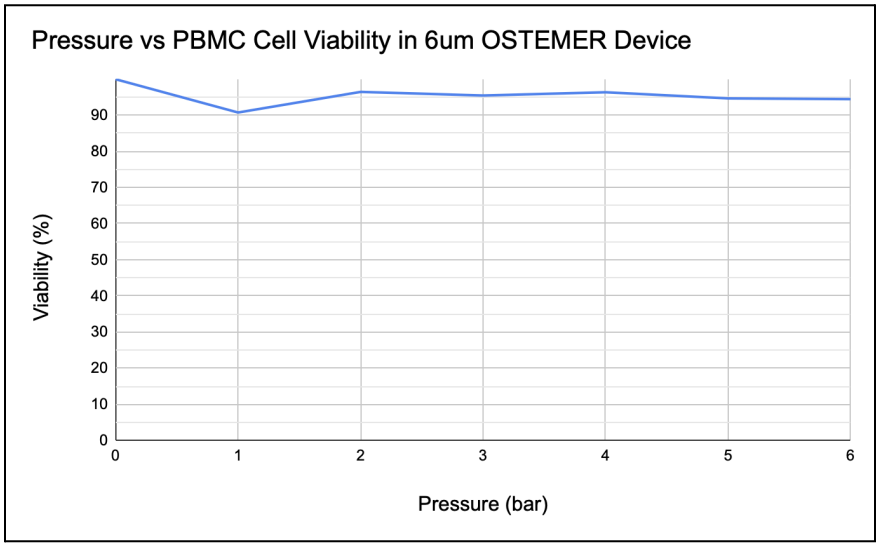

Figure 3. Pressure vs PBMC Cell Viability in a 6µm OSTEMER Device. *The figure displays PBMC viability as a percentage at various levels of pressured flow. The cells were prepared with OPTIMEM 1% Pluronic cell flow buffer. The cells were then injected through the inlet of the microfluidic device at varying pressures between 0 to 6 bar. After cell flow through the microfluidic channels, a sample of cells was collected at the outlet and cell viability was assessed with the NucleoCounter NC-200, an automated and consistent cell counter.*

Figure 3 presents cell viability (%) at various pressure levels (bar) in 6µm OSTEMER devices. Although there is a definite reduction in viability in comparison to the control group at 0 bar, the results show relatively consistent viability at all pressure levels. These findings demonstrate the device's efficacy and ability to function well under extreme pressure conditions, proving that OSTEMER is a viable and promising material to be used in further microfluidic applications.

CHAPTER 4. Discussion

4.1 Problem Statement

The primary goal of this research project is to develop a cost-effective fabrication method for rigid microfluidic devices in order to enable high-pressure flow. In advanced microfluidic applications, such as gene therapy, high-pressure flows are essential in ensuring effective cell manipulation in addition to high cell viability. Research activities include the testing of various chemical treatments to improve device biocompatibility, alongside the conducting of a microfluidic experiment with cell lines to test cell-channel interaction and assay post treatment cell viability. If assay development on the OSTEMER-devices determines that cells are not biocompatible, chemical passivation methods and buffer additives can be incorporated to reduce adhesion in the devices. The material being investigated within this study is off-stoichiometry thiol-ene-epoxy resin (OSTEMER 322), a hydrophilic substance with low water vapor permeability. The results of this study confirm that OSTEMER 322 is a viable microfluidic device fabrication material.

4.2 PBMC Cell Viability in terms of Gap Size

The first experiment involved a comparative analysis between OSTEMER-devices of 4µm and 6µm gap sizes, proving that the 6µm gap size is the best configuration for optimal cell viability. The devices being utilized are all transfection devices, which are designed to compress eukaryotic cells; as the cell membranes are stretched, they become permeable to substances that are passed through the buffer. As a result of the compression aspect of the transfection process, the cells must be slightly larger than the channel's gap size in order to compress properly.
In terms of cell size, human peripheral blood mononuclear cells (PBMC) include two subsets: lymphocytes and monocytes. Lymphocytes generally range from 7-10µm in diameter, while monocytes range from 12-20µm in diameter. The cells utilized in this study were on the smaller end of the 7–20µm range, making the 6µm gap size more effective than the 4µm. The larger gap size offered a greater surface area, which closely matched the cell size and allowed for better compression of the cells as they passed through the transfection device.

4.3 OSTEMER Device Performance under High-Pressure Flow

The 6µm was then tested under a variety of high-pressure flows, ranging from 4 bar to 6 bar. The results demonstrated that the OSTEMER 322 material could maintain PBMC viability under high-pressure flows of up to 6 bar without compromising cell integrity. This finding provides significance as it suggests that this material can meet the extreme conditions required for advanced biological applications.

The most commonly used material for microfluidic device fabrication is polydimethylsiloxane (PDMS). PDMS can generally withstand pressures up to 3 bar, which can vary depending on the material thickness and device design. As a result, materials like OSTEMER are preferred for applications requiring higher pressures. The high-pressure thresholds of OSTEMER provide a

durable and reusable platform for continuous drug systems and biological applications. This device longevity can be further increased through Pluronic additives, which minimize biofouling affects and protein adsorption. Biofouling refers to the accumulation of biological materials at the channel's ridges. Reduced biofouling can prevent flow disruptions and maintain surface properties, thereby extending the device's lifespan and improving its reliability.

4.4 Limitations and Future Applications

Microfluidic transfection devices provide widespread applications in ex vivo gene therapy. Within this process, T-cells are extracted from a patient and modified biomechanically through microfluidic transfection to produce chimeric antigen receptors (CARs). These modified CAR T-cells can then be intravenously infused back into the patient's bloodstream to detect and destroy cancer cells that express the corresponding antigen. As a result, this microfluidic technology provides a unique opportunity for use in cancer treatment.

In general, microfluidic device fabrication is a costly endeavor that lacks sustainability. Within this study, OSTEMER 322 has proved to be a viable and cost-effective alternative that provides the possibility of reuse and withstands high-pressure flow. While there are limitations to the current work, specifically in terms of the devices and cell types utilized, the research and results provide a strong foundation for future research of the OSTEMER material and its potential applications in the biomedical field. Future work should focus on expanding the scope of cell viability through testing on various cell types and a larger range of pressures to ensure comparable results can be produced. Similarly, clinical trials must be conducted in order to assess and confirm the applications of OSTEMER transfection devices as an ex vivo cancer treatment.

APPENDIX

Appendix A.1 contains raw viability data tables produced by the NucleoCounter NC-200.

A.1 PBMC Viability in 6µm OSTEMER-Device

Prepared cells with OPTIMEM 1% Pluronic cell flow buffer.

Viability (%)	99.9
Live (cells/ml)	1.29E6
Dead (cells/ml)	1.32E3
Total (cells/ml)	1.29E6
Estimated cell diameter (um)	9.6
Cell diameter standard deviation (um)	4.4
(%) of cells in aggregates with five or more cells	1

Table 1. Initial PBMC Viability

Viability (%)	90.7
Live (cells/ml)	1.39E5
Dead (cells/ml)	1.41E4
Total (cells/ml)	1.53E5
Estimated cell diameter (um)	9.3
Cell diameter standard deviation (um)	3.9
(%) of cells in aggregates with five or more cells	0

Table 2. PBMC Viability through 6µm OSTEMER-Device at 1 bar

Viability (%)	96.4
Live (cells/ml)	2.79E5
Dead (cells/ml)	1.06E4
Total (cells/ml)	2.90E5
Estimated cell diameter (um)	9.2
Cell diameter standard deviation (um)	4.1
(%) of cells in aggregates with five or more cells	11

Table 3. PBMC Viability through 6µm OSTEMER-Device at 2 bar

Viability (%)	95.4
Live (cells/ml)	7.00E5
Dead (cells/ml)	3.37E4
Total (cells/ml)	7.33E5
Estimated cell diameter (um)	9.3
Cell diameter standard deviation (um)	3.9
(%) of cells in aggregates with five or more cells	0

Table 4. PBMC Viability through 6µm OSTEMER-Device at 3 bar

A.2 PBMC Viability in 6µm OSTEMER-Device at High Pressure Flow

Diluted naive T-cells in 3mL of OPTIMEM 1% Pluronic cell flow buffer.

Viability (%)	96.3
Live (cells/ml)	5.95E5
Dead (cells/ml)	2.29E4
Total (cells/ml)	6.18E5
Estimated cell diameter (um)	9.4
Cell diameter standard deviation (um)	3.8
(%) of cells in aggregates with five or more cells	0

Table 5. PBMC Viability at 4 bar

Viability (%)	94.6
Live (cells/ml)	1.08E6
Dead (cells/ml)	6.19E4
Total (cells/ml)	1.14E6
Estimated cell diameter (um)	9.5
Cell diameter standard deviation (um)	3.7
(%) of cells in aggregates with five or more cells	0

Table 6. PBMC Viability at 5 bar

Viability (%)	94.4
Live (cells/ml)	1.33E6
Dead (cells/ml)	7.95E4
Total (cells/ml)	1.41E6
Estimated cell diameter (um)	9.6
Cell diameter standard deviation (um)	3.8
(%) of cells in aggregates with five or more cells	0

Table 7. PBMC Viability at 6 bar

A.3 PBMC Viability in Varying Gap Sized OSTEMER-Devices (4µm and 6µm)

Diluted naive T-cells in 2mL of OPTIMEM 1% Pluronic cell flow buffer. Passivated all devices with a cell flow buffer.

Viability (%)	90.2
Live (cells/ml)	2.93E5
Dead (cells/ml)	3.17E4
Total (cells/ml)	3.25E5
Estimated cell diameter (um)	9.1
Cell diameter standard deviation (um)	5.7
(%) of cells in aggregates with five or more cells	1

Table 8. Initial PBMC Viability

Viability (%)	48.0
Live (cells/ml)	6.53E4
Dead (cells/ml)	7.07E4
Total (cells/ml)	1.36E5
Estimated cell diameter (um)	8.9
Cell diameter standard deviation (um)	4.5
(%) of cells in aggregates with five or more cells	0

Table 9. PBMC Viability in 4µm OSTEMER-Device at 4 bar

Viability (%)	41.6
Live (cells/ml)	8.01E4
Dead (cells/ml)	1.12E5
Total (cells/ml)	1.93E5
Estimated cell diameter (um)	8.8
Cell diameter standard deviation (um)	4.3
(%) of cells in aggregates with five or more cells	0

Table 10. PBMC Viability in 4µm OSTEMER-Device at 5 bar

Viability (%)	57.6
Live (cells/ml)	5.86E4
Dead (cells/ml)	4.31E4
Total (cells/ml)	1.02E5
Estimated cell diameter (um)	9.1
Cell diameter standard deviation (um)	5.3
(%) of cells in aggregates with five or more cells	0

Table 11. PBMC Viability in 6µm OSTEMER-Device at 4 bar

Viability (%)	60.7
Live (cells/ml)	8.78E4
Dead (cells/ml)	5.68E4
Total (cells/ml)	1.45E5
Estimated cell diameter (um)	9.1
Cell diameter standard deviation (um)	4.5
(%) of cells in aggregates with five or more cells	0

Table 12. PBMC Viability in 6µm OSTEMER-Device at 5 bar

REFERENCES

Alhmoud, H., Alkhaled, M., Kaynak, B. E., & Hanay, M. S. (2022). Leveraging the elastic deformability of polydimethylsiloxane microfluidic channels for efficient intracellular delivery. *Lab on a Chip*, *23*(4), 714–726. https://doi.org/10.1039/d2lc00692h

Au, S. H., Kumar, P., & Wheeler, A. R. (2011). A new angle on pluronic additives: Advancing droplets and understanding in digital microfluidics. *Langmuir*, *27*(13), 8586–8594. https://doi.org/10.1021/la201185c

Becker, H. (2002). Polymer microfluidic devices. *Talanta*, *56*(2), 267–287. https://doi.org/10.1016/s0039-9140(01)00594-x

Cheng, Z., Gu, Y., Li, S., Wang, Y., Chen, H., Cheng, J., & Liu, P. (2017). Enclosed casting of epoxy resin for rapid fabrication of rigid microfluidic chips. *Sensors and Actuators B: Chemical*, *252*, 785–793. https://doi.org/10.1016/j.snb.2017.06.053

Chong, Z. X., Yeap, S. K., & Ho, W. Y. (2021). Transfection types, methods and strategies: A technical review. *PeerJ*, *9*. https://doi.org/10.7717/peerj.11165

Cox, J. D., Curry, M. S., Skirboll, S. K., Gourley, P. L., & Sasaki, D. Y. (2002). Surface passivation of a microfluidic device to glial cell adhesion: A comparison of hydrophobic and hydrophilic Sam Coatings. *Biomaterials*, *23*(3), 929–935. https://doi.org/10.1016/s0142-9612(01)00205-8

Fallahi, H., Zhang, J., Phan, H.-P., & Nguyen, N.-T. (2019). Flexible microfluidics: Fundamentals, recent developments, and applications. *Micromachines*, *10*(12), 830. https://doi.org/10.3390/mi10120830

Garibyan, L., & Avashia, N. (2013). Polymerase chain reaction. *Journal of Investigative Dermatology*, *133*(3), 1–4. https://doi.org/10.1038/jid.2013.1

Huang, S., He, Q., Hu, X., & Chen, H. (2012). Fabrication of micro pneumatic valves with double-layer elastic poly(dimethylsiloxane) membranes in rigid poly(methyl methacrylate) microfluidic chips. *Journal of Micromechanics and Microengineering*, *22*(8), 085008. https://doi.org/10.1088/0960-1317/22/8/085008

Li, Y., Keefe, A. J., Giarmarco, M., Brault, N. D., & Jiang, S. (2012). Simple and robust approach for passivating and functionalizing surfaces for use in complex media. *Langmuir*, *28*(25), 9707–9713. https://doi.org/10.1021/la301691d

Liu, J., Sun, X., & Lee, M. L. (2007). Adsorption-resistant acrylic copolymer for prototyping of microfluidic devices for proteins and peptides. *Analytical Chemistry*, *79*(5), 1926–1931. https://doi.org/10.1021/ac0617621

Luk, V. N., Mo, G. CH., & Wheeler, A. R. (2008). Pluronic additives: A solution to sticky problems in digital microfluidics. *Langmuir*, *24*(12), 6382–6389. https://doi.org/10.1021/la7039509

Mukhopadhyay, R. (2005). When microfluidic devices go bad. *Analytical Chemistry*, *77*(21). https://doi.org/10.1021/ac053496h

Murray, C., McCoul, D., Sollier, E., Ruggiero, T., Niu, X., Pei, Q., & Carlo, D. D. (2012).

Electro-adaptive microfluidics for active tuning of channel geometry using polymer actuators. *Microfluidics and Nanofluidics*, *14*(1–2), 345–358. https://doi.org/10.1007/s10404-012-1055-y

OSTEMER 322 crystal clear. OSTEMERS. (n.d.). https://www.ostemers.com/products/ostemer-crystal-clear/

Oyama, T. G., Oyama, K., & Taguchi, M. (2020). A simple method for production of hydrophilic, rigid, and sterilized multi-layer 3D integrated polydimethylsiloxane microfluidic chips. *Lab on a Chip*, *20*(13), 2354–2363. https://doi.org/10.1039/d0lc00316f

Padash, M., Enz, C., & Carrara, S. (2020). Microfluidics by additive manufacturing for Wearable Biosensors: A Review. *Sensors*, *20*(15), 4236. https://doi.org/10.3390/s20154236

Persson, F., Fritzsche, J., Mir, K. U., Modesti, M., Westerlund, F., & Tegenfeldt, J. O. (2012). Lipid-based passivation in Nanofluidics. *Nano Letters*, *12*(5), 2260–2265. https://doi.org/10.1021/nl204535h

Ren, Y., Ray, S., & Liu, Y. (2019). Reconfigurable acrylic-tape hybrid microfluidics. *Scientific Reports*, *9*(1). https://doi.org/10.1038/s41598-019-41208-y

Siddiqui, S., Chandrasekaran, A., Lin, N., Tufenkji, N., & Moraes, C. (2019). Microfluidic shear assay to distinguish between bacterial adhesion and attachment strength on stiffness-tunable silicone substrates. *Langmuir*, *35*(26), 8840–8849. https://doi.org/10.1021/acs.langmuir.9b00803

Sønstevold, L., Yadav, M., Arnfinnsdottir, N. B., Herbjørnrød, A. K., Jensen, G. U., Aksnes, A., & Mielnik, M. M. (2022). Biocompatible bonding of a rigid off-stoichiometry thiol-ene-epoxy polymer microfluidic cartridge to a biofunctionalized silicon biosensor. *Journal of Micromechanics and Microengineering*, *32*(7), 075008. https://doi.org/10.1088/1361-6439/ac6ebf

Weibel, D., & Whitesides, G. (2006a). Applications of microfluidics in Chemical Biology. *Current Opinion in Chemical Biology*, *10*(6), 584–591. https://doi.org/10.1016/j.cbpa.2006.10.016