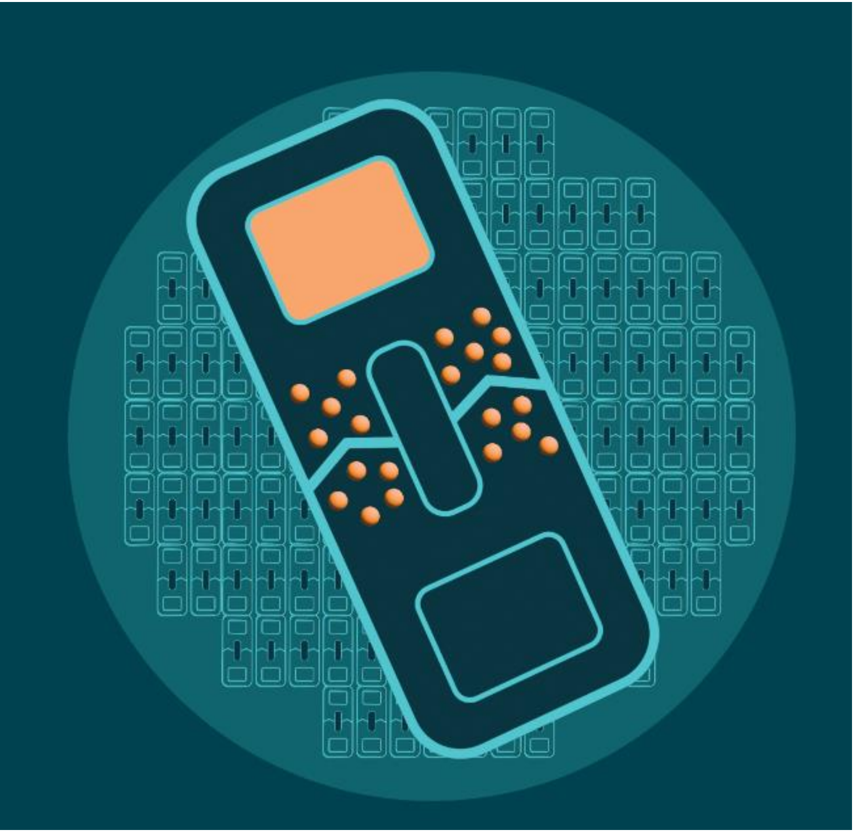
HV Device

Jacky Chen

Quick Overview: Jacky

- Graduated from the University of Waterloo in 2022 with a bachelors engineering degree in mechatronics
    - Did 6 internships over the course of my undergrad 4 months a-piece for a total of 2 years of internship experience
        - Mostly mechanical I experienced manufacturing, prototyping, program management, quality assurance, design etc
- Worked at Lithos Energy as a mechanical design engineer after graduating
    - Lithos energy made custom design battery packs for various applications, did design work from specifications to manufacturing
    - I worked on the low voltage side
- Most Recent work was at CellFE, this slide deck will focus on a project I did at CellFE

Background:Overview



CellFE is a biotech company that creates biomedical devices

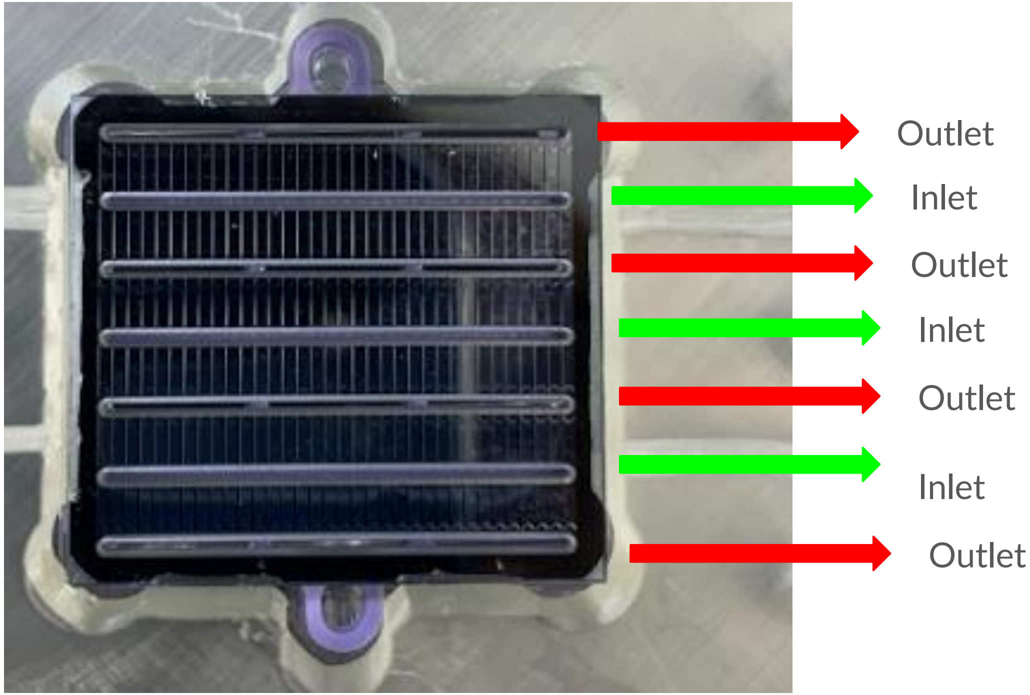
- It is in the space of creating tools to help enable cell therapies and gene editing
- Traditionally electroporation is the most widely used tool to enable biological cell editing
  - Cells are zapped with an electrical field to open up their pores and a payload is induced changing their biological makeup
  - This is used by larger companies such as lonza, thermo fisher etc
- CellFE uses a technology called mechanoporation which uses a microfluidic chip to squeeze cells under a tiny ridge
  - A payload with a type of cell is placed at the inlet of a consumable with a microfluidic chip inside
  - The consumable is then pressurized to send the cell at a certain velocity through a tiny gap inside the microfluidic chip
  - This squeezing motion tears tiny holes open in the cell membrane and when induced with a payload will enable cell transfection

Background: Existing Technology

At the time I had joined CellFE there was a low throughput version of this technology and a low volume consumable

- There were small chips with only two channels per chip and the ability to send a limited volume of cells through at a time
    - The reason throughput was limited was because not all cells pass under the ridge and get transfected
    - Some cells die upon impact and eventually the channel becomes clogged
    - Currently per chip throughput was limited to around 500k-1 million cells per chip.
- I was put in charge of scaling this technology to a higher throughput number of cells
    - I lead the high throughput consumable design and had a large impact on the instrument as well since the consumable characteristics informed how the instrument would be built

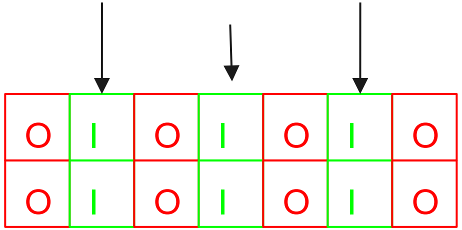
New High Volume Chip



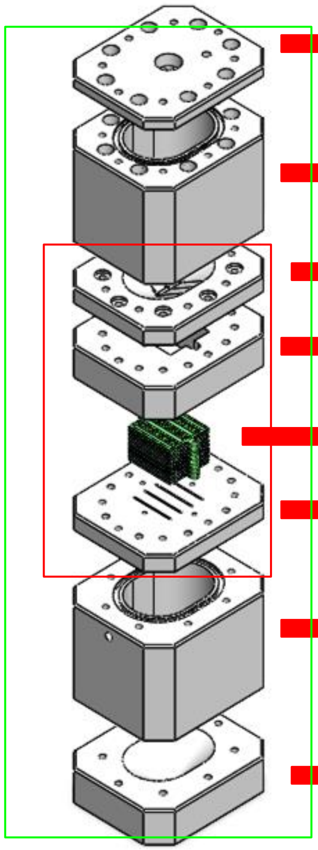
To enable high volume processing we essentially needed to stack and seal chips with a greater number of microfluidic channels

- There were many candidates for design but we wanted to preserve the geometry of the low volume channel while maximizing the number of chips we could make per wafer. This is what the chip ended up looking like
- New Chip had 206 Channels on it

Side View



Components of the High Volume consumable



Lid with pressure sensor port fill port and pressurization Port

Inlet Reservoir

Chip Stack Top Plate, has funnel feature and liquid distribution geometry

Chip Spacer dependent on number of chips used

Chip Stack with gaskets

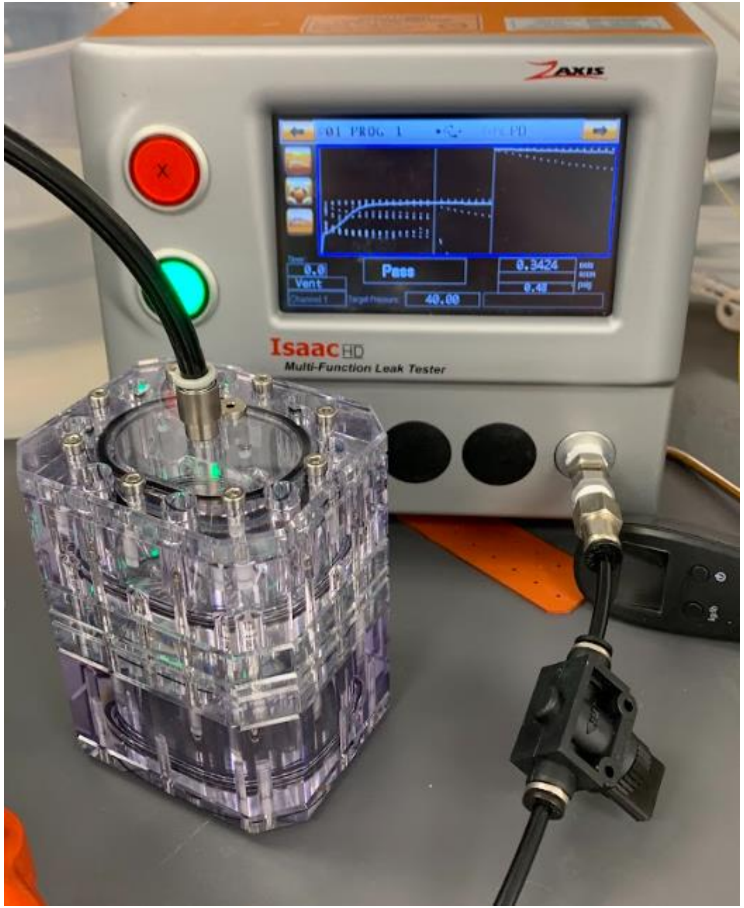
Bottom plate

Outlet reservoir with flow sensor port

Outlet funnel

*This is close to what the final design of the High Volume Consumable was looking like I will talk about earlier iterations and problems look like later

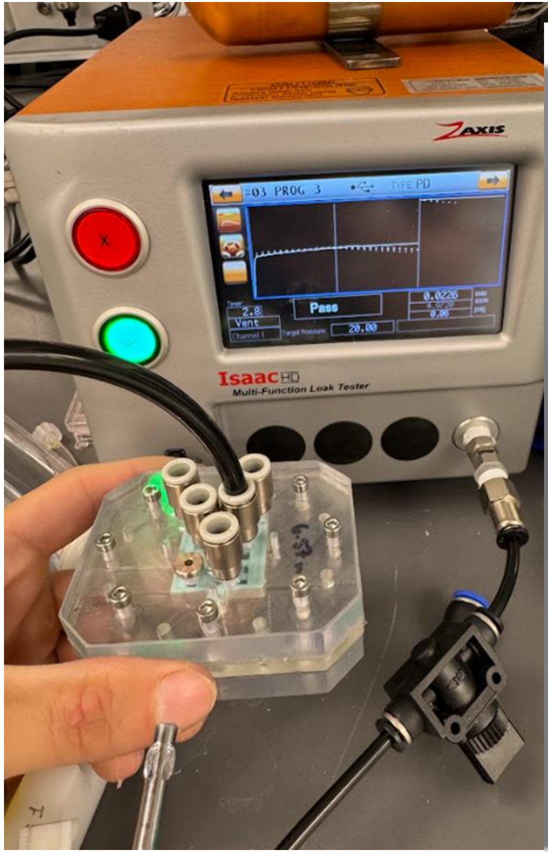
Testing the Consumable

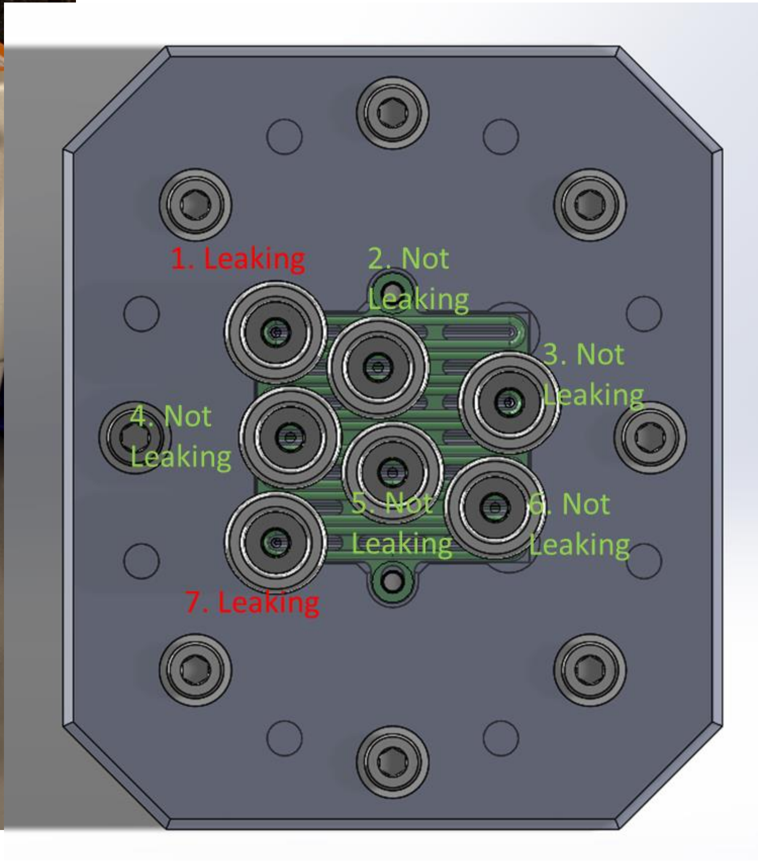


First test of the consumable would be to see if it held pressure

- You could hook up the overall consumable to a pressure leak tester and plug the open holes to see if it held
    - Consumable leaked dramatically the first time
- This worked for an overall test but it didn't tell you where the leak was occurring or what the mode of failure was
    - Could do soapy water test etc if it was an outward leak where one of the o-ring gaskets were but if it was a problem with the chip gaskets themselves then it was a much more difficult problem to diagnose
    - Machined polished parts themselves also created a pretty tight seal so I had to make an intentional score on the spacer to properly analyze if it was leaking

Fixture to Pressure Test the Consumable Gaskets



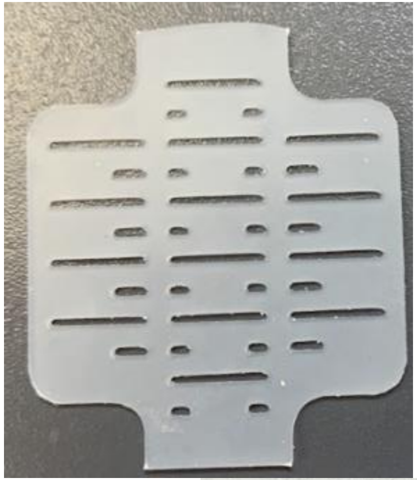


Designed a separate fixture to diagnose exact points on the chip and the chip gasket that were leaking

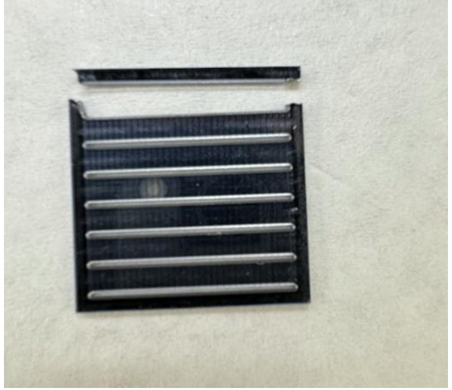
- Had push to connect fittings at several major leakage joints to diagnose where the problem was coming from
- Could also troubleshoot multiple stack of chips

Gasket Problems

Mcmaster



Molded

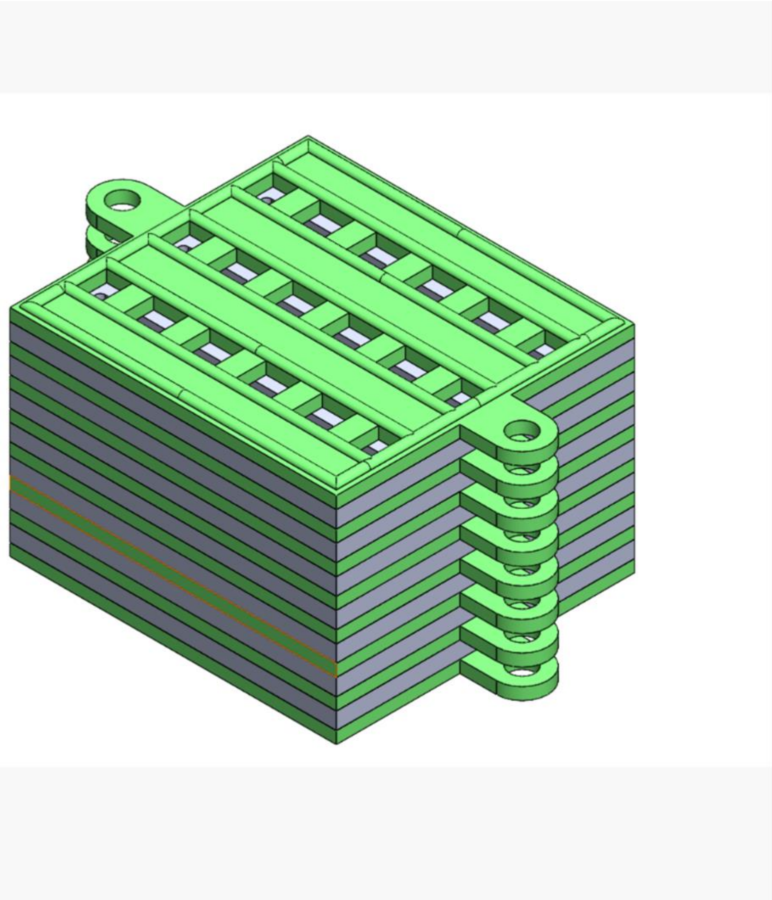


Chips Cracking and gaskets leaking were the biggest challenges with this design.

SIlicon chips are extremely fragile.

- Started with off the shelf 2D gaskets
  - Took silicon rubber off of mcmaster-carr and cut holes in the rubber
  - Problem was there was too much variation with off of the shelf sheet rubber when stacking chips there would eventually be an unacceptable tolerance stack up
- Then molded inhouse 2D gaskets to address the tolerance concern
  - 3D printed the top and bottom portions of the gasket mold and then injected a silicon rubber mixture manually and clamped it together, heat treated and let it set overnight
  - These gaskets didn’t work either, was applying too much force onto the gaskets and would crack the chip when trying to seal

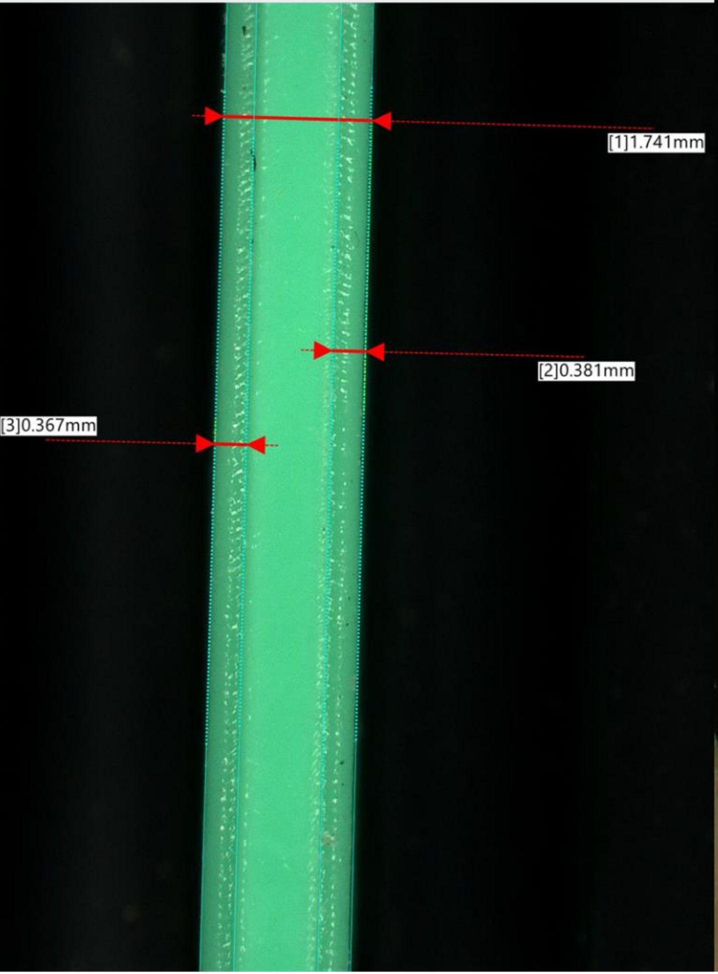
3 Dimensional Gaskets

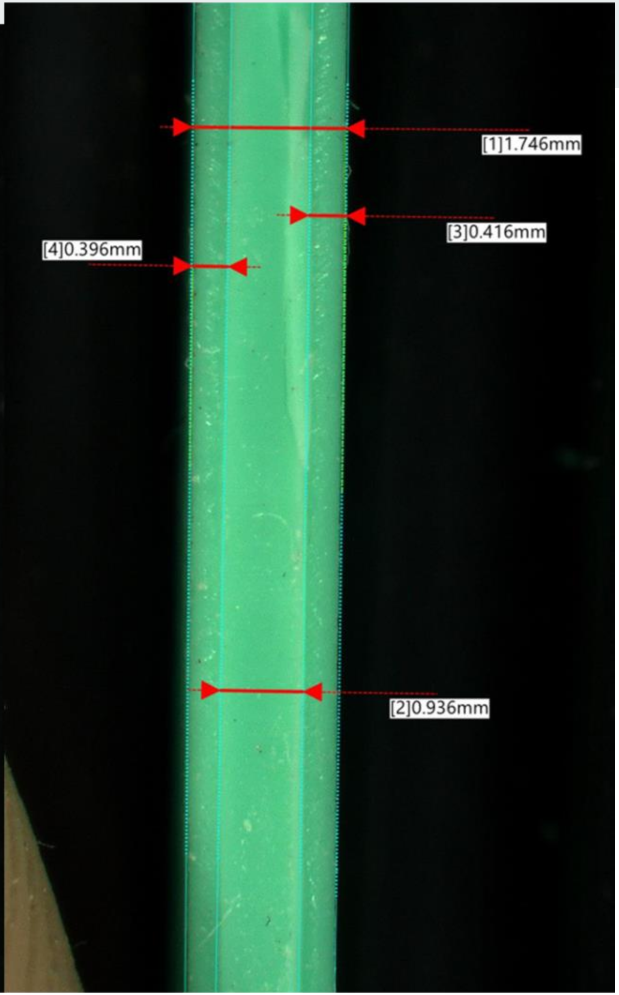


Moved onto the idea of 3 Dimensional Gaskets

These gaskets would have ridges that would seal around the fluid flow paths and only compress the gasket bumps applying much less force onto the chips overall and introducing less unwanted bending moments and torsion etc

Adapts better to minor surface unevenness/tolerance misalignments, less overall force required to seal a smaller surface area etc

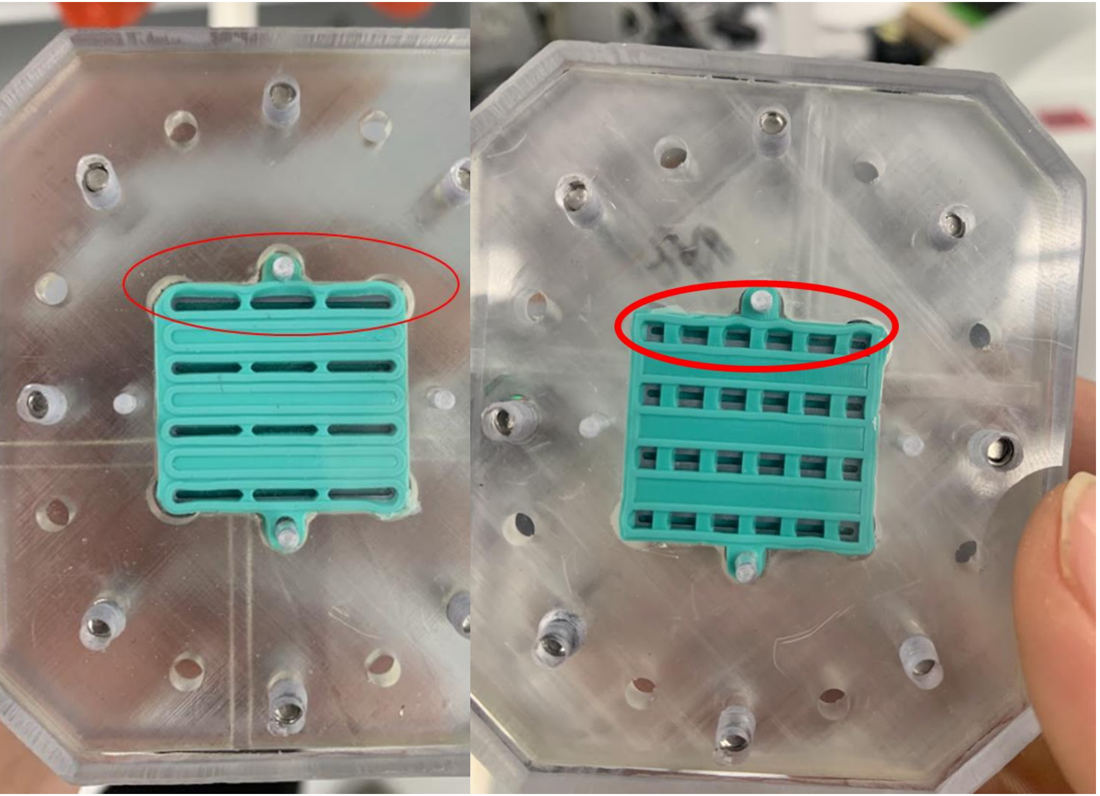




Measured the molded gaskets under a microscope to get a sense of the tolerance level I was seeing

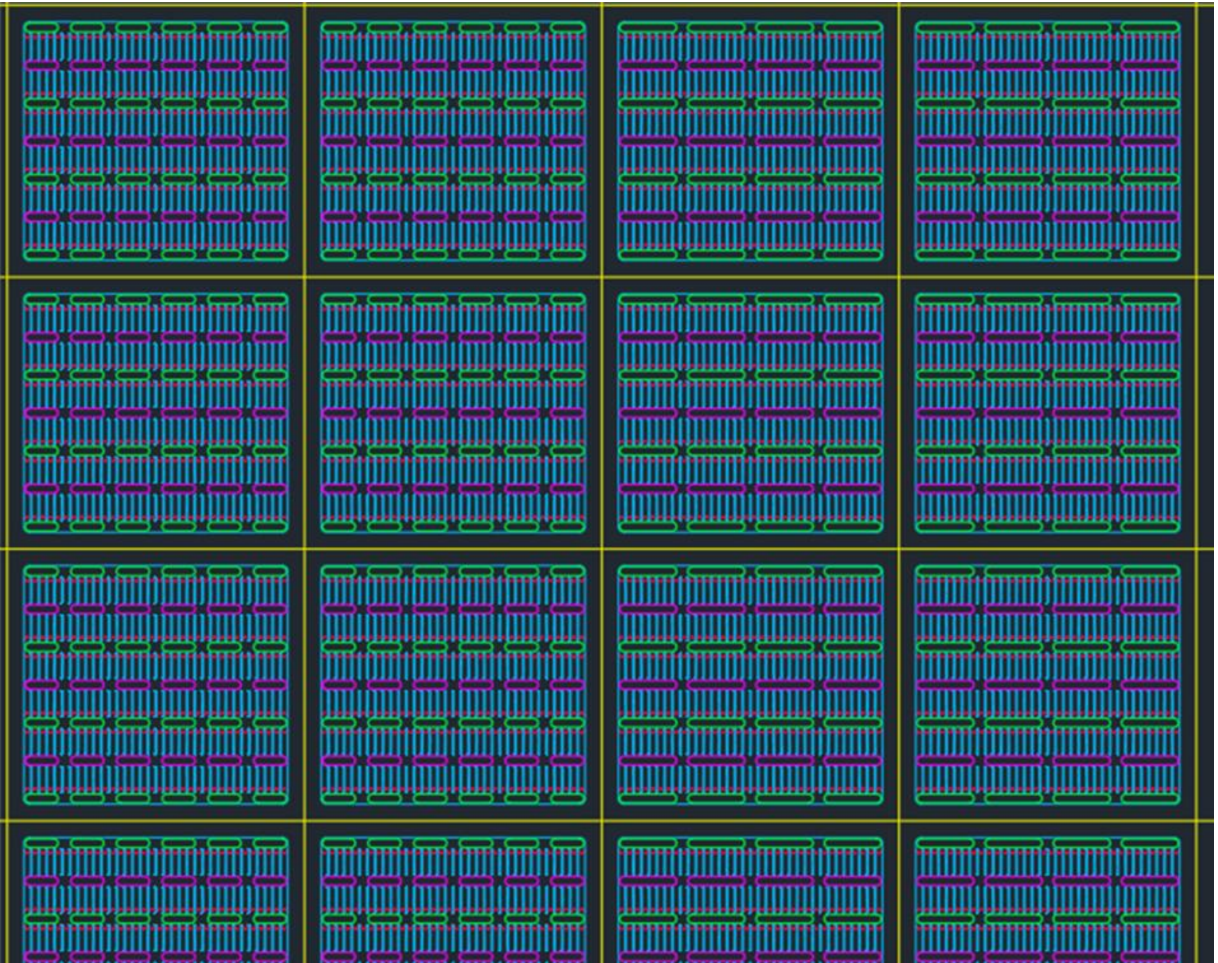
- Was difficult to measure by caliper since gaskets compress

3D Gaskets Problems



- Gasket blowout inside of the testing jig
  - Ridges were being blown out of place
    - Made the ridges slightly thicker to add stiffness
    - Added thicker and stiffer bridges in the gasket to prevent blowout
    - Made the radius of the corner fillets smaller (.4 mm)
    - And moved the locator holes horizontal to create material to push back against the blowout
    - Evaluated multiple different shore hardnesses to pick the optimal material
    - Tried different compression percentages to find the acceptable range a consumable could work in
- Chips were still cracking

Addressing the Cracking chips



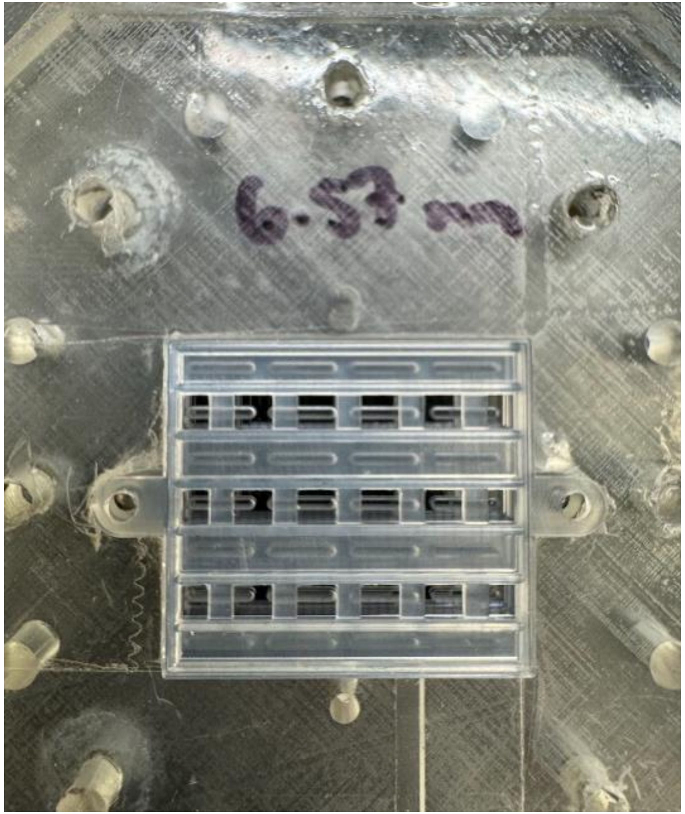
- Added Bridges to stiffen the chip to resist bending moments
  - Made 3 Bridge stack and 5 bridge to test which would work
  - 3 Bridge performed well

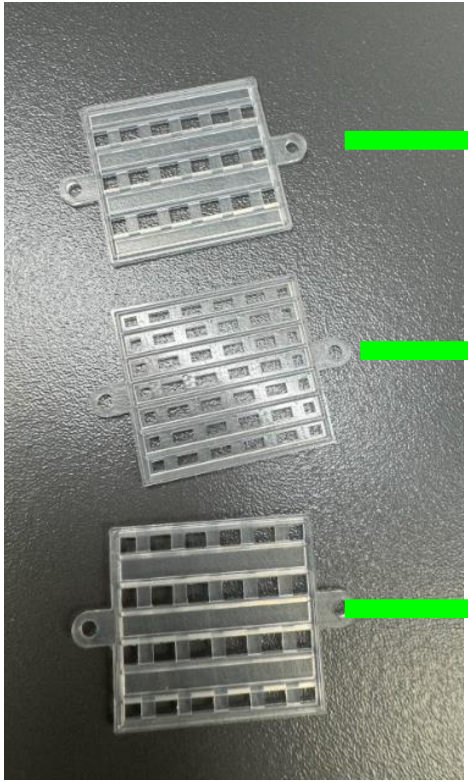
Example of Test Data

	Nominal Gasket Dimension	Nominal Chip Thickness (3 Bridges)	Nominal Spacer Size	Actual Spacer Size	Actual Compression Percentage (Shooting for 19 %)	Shore 60A Results	Shore 70A Results
1 Stack	1.75 mm (2 Gaskets)	1.39 mm (1 Chip)	4.05 mm	[4.00, 4.05]	[19.9 % ,18.4 %]	**Similar to 70A**	**0.020 psi leakage**
2 Stack	1.75 mm (3 Gaskets)	1.39 mm (2 Chip)	6.70 mm	[6.67, 6.73]	[20.0 % ,19.4 %]	**0.0226 psi leakage**	**0.0284 psi leakage**
3 Stack	1.75 mm (4 Gaskets)	1.39 mm (3 Chip)	9.45 mm	[9.45, 9.50]	[19.0 % ,18.25 %]	**0.1106 psi leakage**	**0.0677 psi leakage**
4 Stack	1.75 mm (5 Gaskets)	1.39 mm (4 Chip)	12.16 mm	[12.24, 12.30]	[19.5 % ,19.0 %]	**2.382 psi leakage**	**0.0390 psi leakage**

Injection Molded Gaskets

Injection molded final iteration of the microfluidic chip gaskets after getting a 5 stack to seal consistently without breaking or leakage





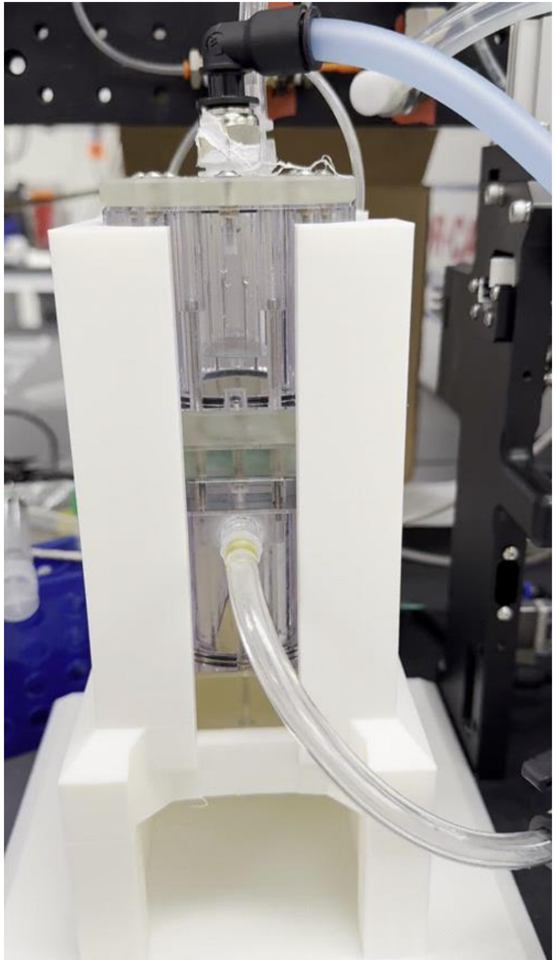
Top Gasket

Middle Gasket

Bottom Gasket

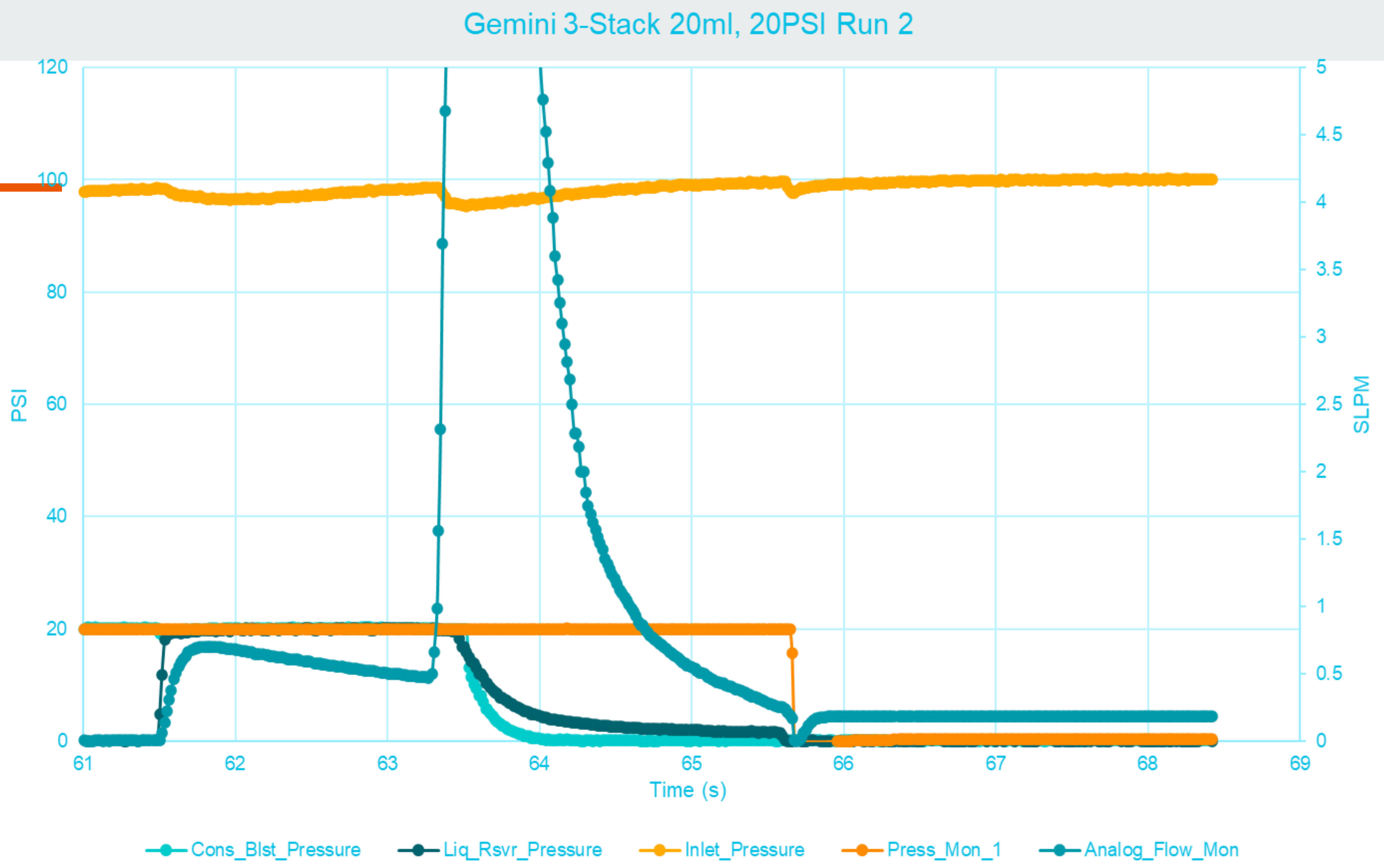
Did Comsol fluid flow simulation to see if there was a pressure drop across the inlet of the chip from the center of the chip to the outside and through a large stack of chips

Testing Flow Rate



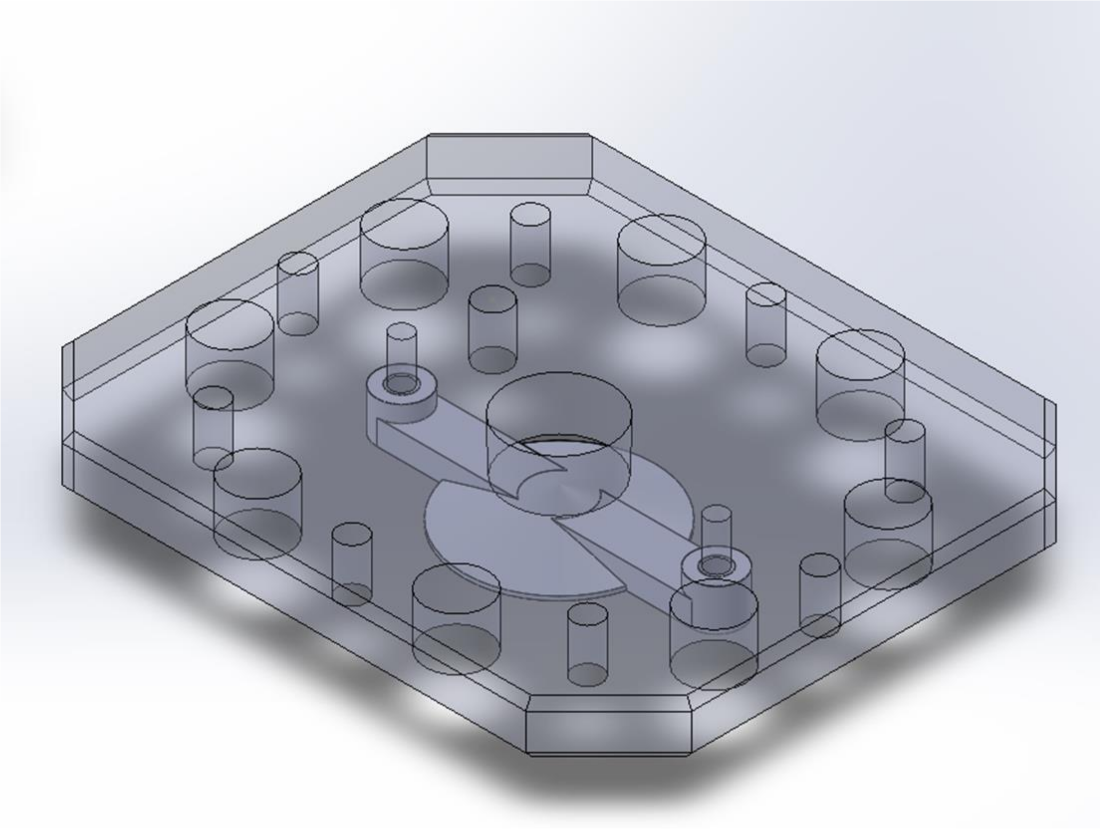
Once the gaskets were sealing I wanted to test if the liquid was actually passing through the chip channels before running a cell test

- Did this by flowing water through a multi stack of chips and observing the expected flow rate
- Can extrapolate flow from the low volume chip design and scaling it
- Flow was as expected but there were some small problems
    - Huge flow spike at the start of the run
        - Was a spike of air at the start pushing away the water and going through the chips first
        - Also created splashing
    - Water got inside of the flow sensor port
    - Hydrostatic pressure of the water in the bottom of the reservoir prevented me from retrieving all of the sample



- 703 SCCM/612
- 1.14 SCCM per channel

Addressing Water Flow Problems



Designed a diffuser to prevent a spike of air at the beginning of the run

Extruded the bottom plate to have a small shield for the flow sensor prot to prevent sample from going in

Lofted the bottom funnel to let sample flow out of the bottom reservoir

Other Issues

First Time I ran a cell test the results were horrible

- I had used a biocompatible 3D material (bioclear) and soaked it in ethanol to sterilize it
- I think this process infused the bioclear with ethanol and killed the cells during the transfection process
    - Clues were that the bioclear had a foul odour afterwards
    - There were flakes of material coming off if you applied the slightest amount of pressure to it
- Although a lot more expensive I had the parts machined the second time and autoclave which is the more standard process of sterilization
    - This worked and I achieved transfection parity with the low volume throughput device

3D printing wasn't super precise and I had a lot of issues with surface finishes

Ended up shimming or sanding parts manually to get them to fit

Did a lot of systems level testing to source the correct fittings and tubings

Questions/Comments

What the breadboard looked like

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