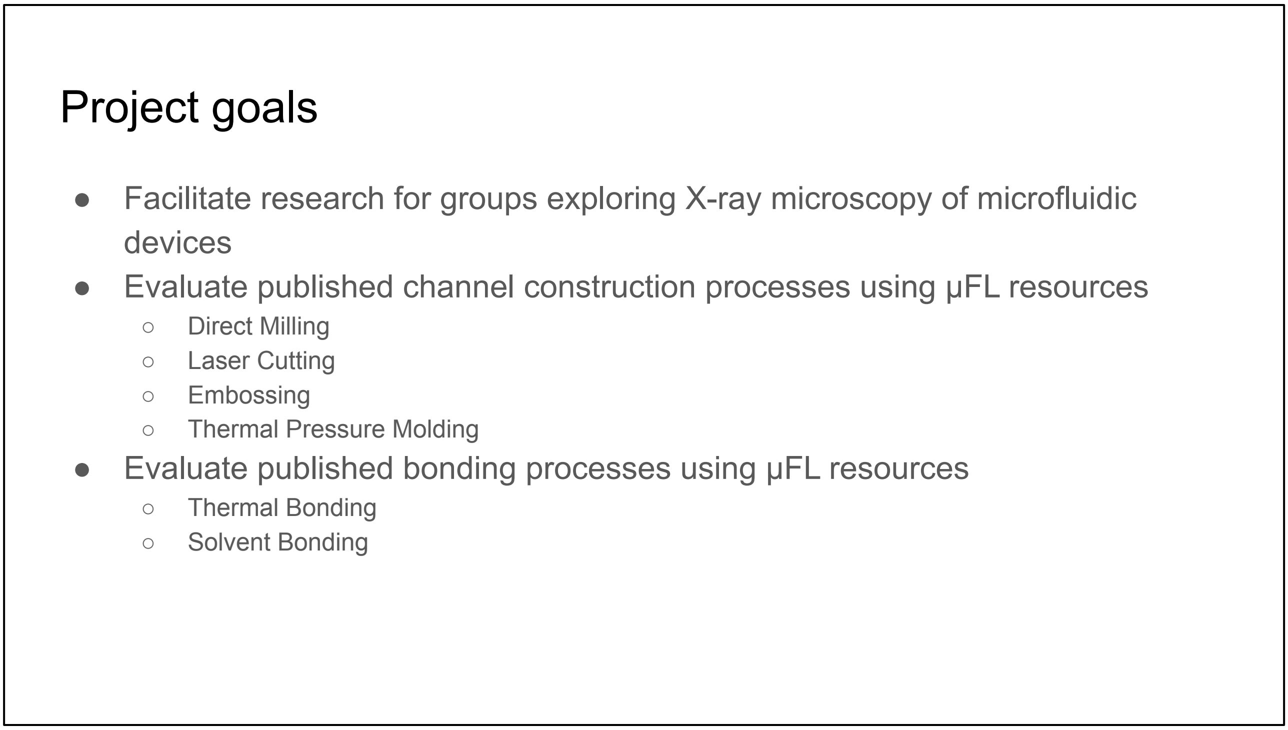
Review of Fabrication Techniques of X-ray Transparent Microfluidic Chips

Carried out in the CNSI µFL by Andrew Furst with
funding from BioPACIFIC MIP; oversight provided by
Dave Bothman.



Project goals

- Facilitate research for groups exploring X-ray microscopy of microfluidic devices
- Evaluate published channel construction processes using μFL resources
    - Direct Milling
    - Laser Cutting
    - Embossing
    - Thermal Pressure Molding
- Evaluate published bonding processes using μFL resources
    - Thermal Bonding
    - Solvent Bonding

Our main goal in this project is to support the Hegelson group and other research groups in observing chemical reactions under X-ray microscopy within microfluidic chips. What this mainly entailed was the the development of methods for channel construction and consistent bonding in an X-ray transparent substrate. We explored many different methods of channel construction and bonding based on different research papers with the hope of providing numerous options of chip construction depending on the desired geometry or limitations outlined by the researchers.

Constraints

1. Material
    a. X-ray transparent
    b. Bondable with available resources
2. Geometric
    a. Y-mixer
    b. 2:1 or 3:1 depth to width ratio specified by Prof. Helgeson
    c. 500 micron to 2mm channel width
    d. Approx microscope slide dimensions
    e. Convenient connection to pumps
3. Scientific
    a. Minimize top/bottom channel thickness to reduce X-ray scattering/absorption
4. Manufacturing
    a. Maintain optical clarity of COC

The scientific constraints given to us originally were that the substrate material had to be X-ray transparent with a Y mixer leading to 1mm square channels. This was later changed to a 2:1 or 3:1 depth to width ratio so some information is provided on that as well. After talking with Youli we also added an additional constraint of minimizing the COC thickness above and below the channel plate to reduce scattering. All experimentation was done with the goal of maintaining optical clarity of the COC chip

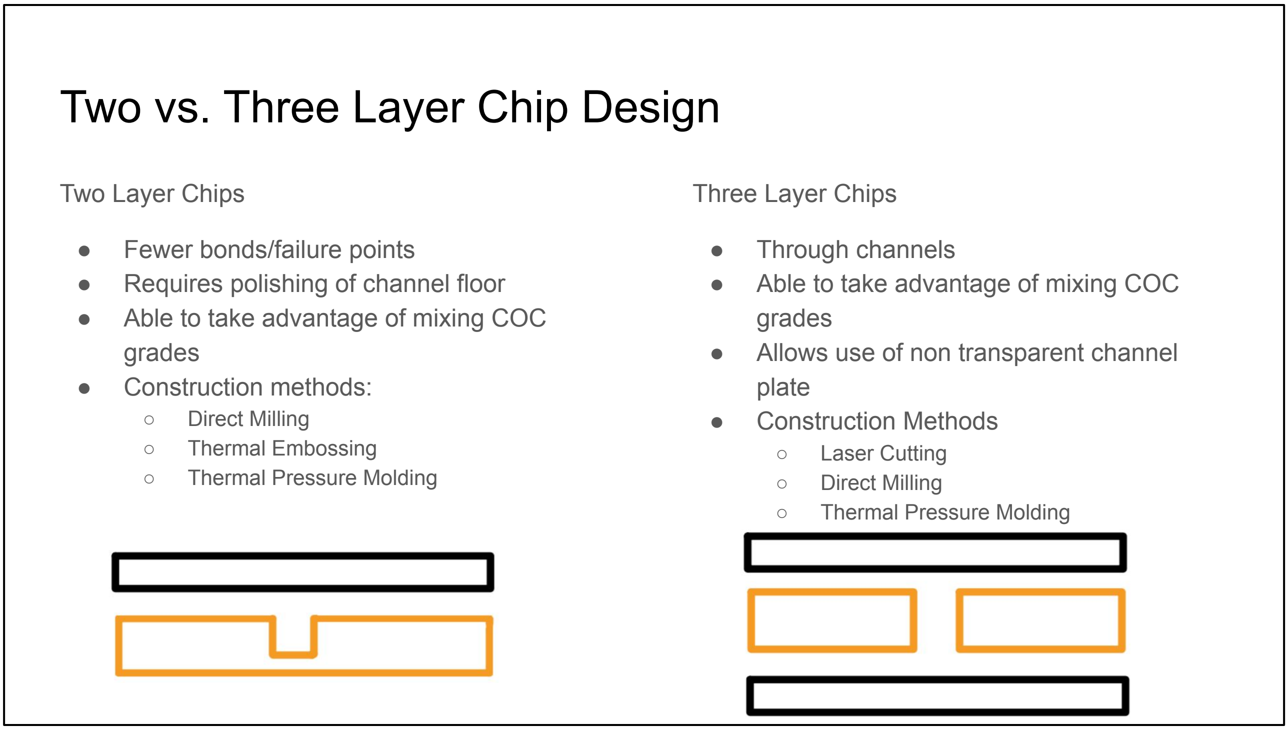
Material Selection

- Typical Microfluidic devices Constructed from of glass and PDMS
  - Allows for casting at room temperature as PDMS is thermoset polymer
  - Not X-ray transparent
  - Relatively easy to bond utilizing ozone or plasma surface activation
- COC
  - X-ray transparent
  - 2 grades tested
    - 6013M-07 w/ Tg of 142 °C
    - 8007X-04 w/ Tg of 78 °C
  - Difficult to bond
  - Thermoplastic

Traditional microfluidic devices are usually constructed from glass and PDMS, which allows for channels to be cast from a resin at room temperature and images, however, neither glass nor PDMS are conducive to X-ray microscopy.

COC was chosen due to its UV and X-ray transparency, as well as its solvent resistance and rather inert nature. Two main grades were tested, 8007X-04 and 6013M-07 with the main difference being that 8000 series has a Tg of 78 °C and 6000 series has a Tg of 142 °C. We did explore mixing grades, which we used to our advantage later when making chips.

Unless otherwise stated, the COC grade is the 8000 series with a Tg of 78 degrees.



Two vs. Three Layer Chip Design

Two Layer Chips

- Fewer bonds/failure points
- Requires polishing of channel floor
- Able to take advantage of mixing COC grades
- Construction methods:
  - Direct Milling
  - Thermal Embossing
  - Thermal Pressure Molding

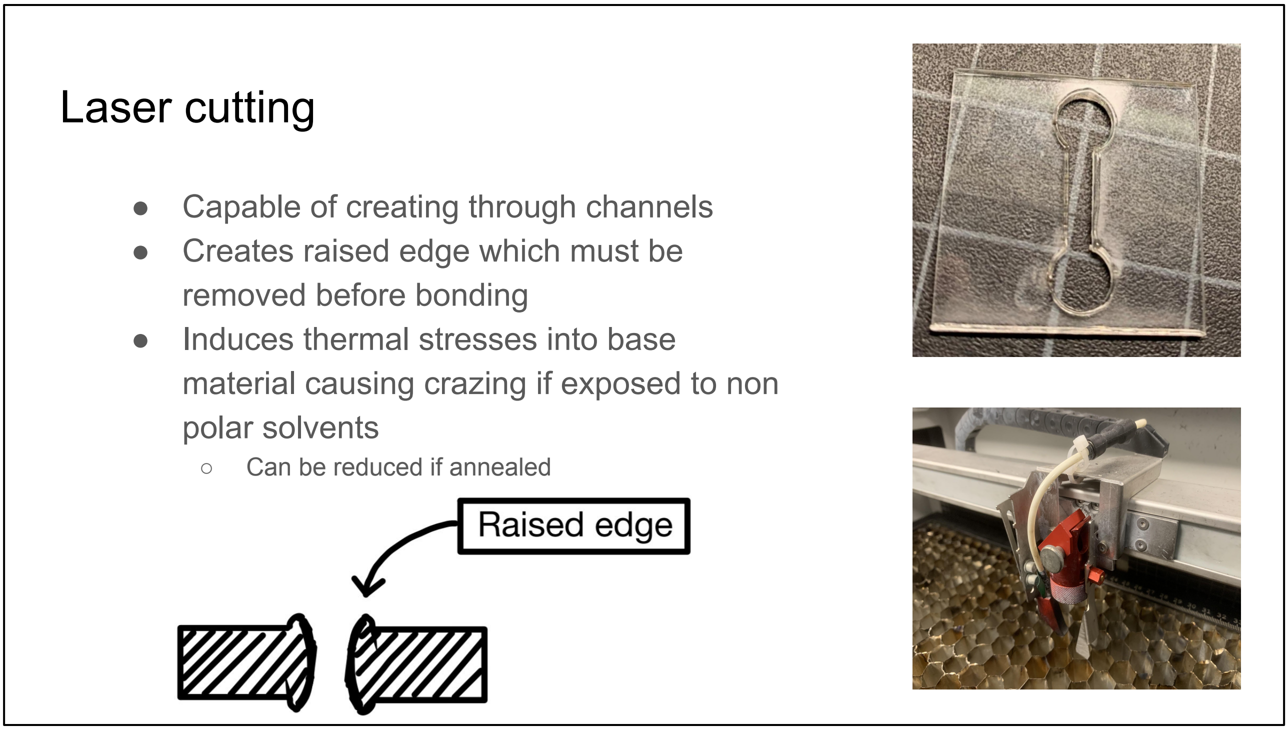
Three Layer Chips

- Through channels
- Able to take advantage of mixing COC grades
- Allows use of non transparent channel plate
- Construction Methods
  - Laser Cutting
  - Direct Milling
  - Thermal Pressure Molding

There are two main styles of chips, two layer chips which incorporate a cover slip and a plaque with partial channels, and three layer chips which consist of two coverslips and a channel plate with through channels. The main advantage of two layer chips is that they only require one bond, with the main disadvantage of them being that partial channels often require polishing to avoid scattering electromagnetic waves.

Three layer chips utilize through channels which means less, or much easier polishing and a broader range of manufacturing techniques, however there are two bonds which increases the possibility of having a leak. Another unexplored advantage of three layer chips is that the channel plate does not necessarily need to be X-ray transparent which increases the material choices and manufacturing techniques.

There are several construction methods available, two layer chips can be made by milling, embossing, and molding of partial channels, whereas three layer chips can be created by laser cutting, milling of through channels, and thermal pressure molding of through channels.



Laser cutting

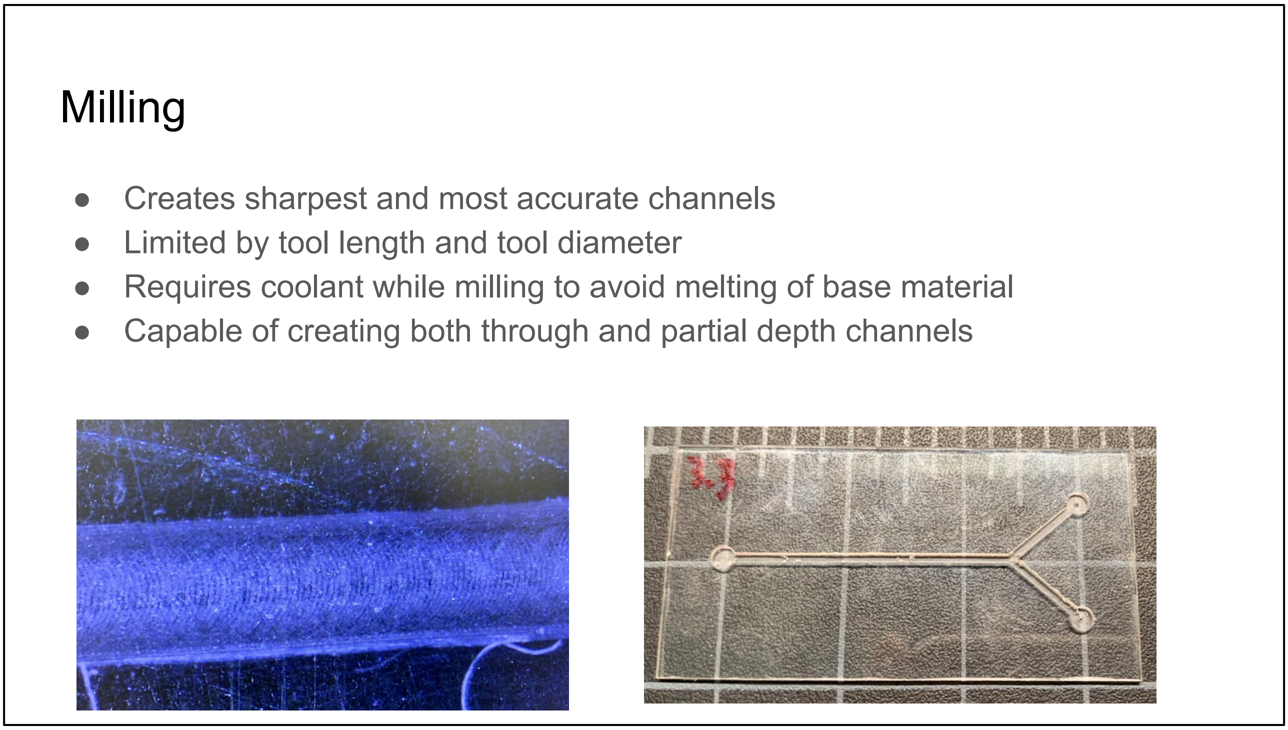
- Capable of creating through channels
- Creates raised edge which must be removed before bonding
- Induces thermal stresses into base material causing crazing if exposed to non polar solvents
    - Can be reduced if annealed

Raised edge

Laser cutting is the easiest and more traditional means of creating through channels for 3 layer chips. The benefits are that the design can be easily altered and recut with minimal setup time. Typically channels can be accurately cut down to around 250 micron however thicker material requires more power resulting in a wider minimum channel width. With COC however laser cutting presents several challenges:

1. The main challenge is that COC is a thermoplastic with a relatively low melting point, this means that it is challenging to laser cut without melting the base material and wall surface is likely to be rounded over with a raised edge, especially with thicker materials. These problems can be reduced with some post processing, but the minimum channel width is likely going to be greater than 500 micron due to the melting of the material.
2. The second problem is that laser cutting COC it induces stresses into the material, and when exposed to solvents these stresses tend to form microcracks resulting in whats known as “crazing”. This can be mitigated by annealing the material slightly below Tg overnight. However this does kinda negate the speed advantage of laser cutting channels.

Overall laser cutting can be a relatively simple means of creating through channels, but there are some considerations and quite a bit of post processing that needs to be done to create useful microfluidic chips.



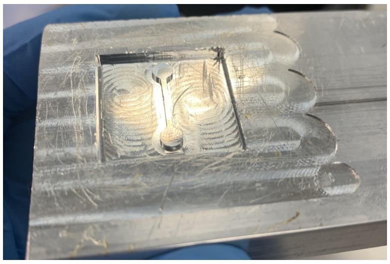
Milling

- Creates sharpest and most accurate channels
- Limited by tool length and tool diameter
- Requires coolant while milling to avoid melting of base material
- Capable of creating both through and partial depth channels

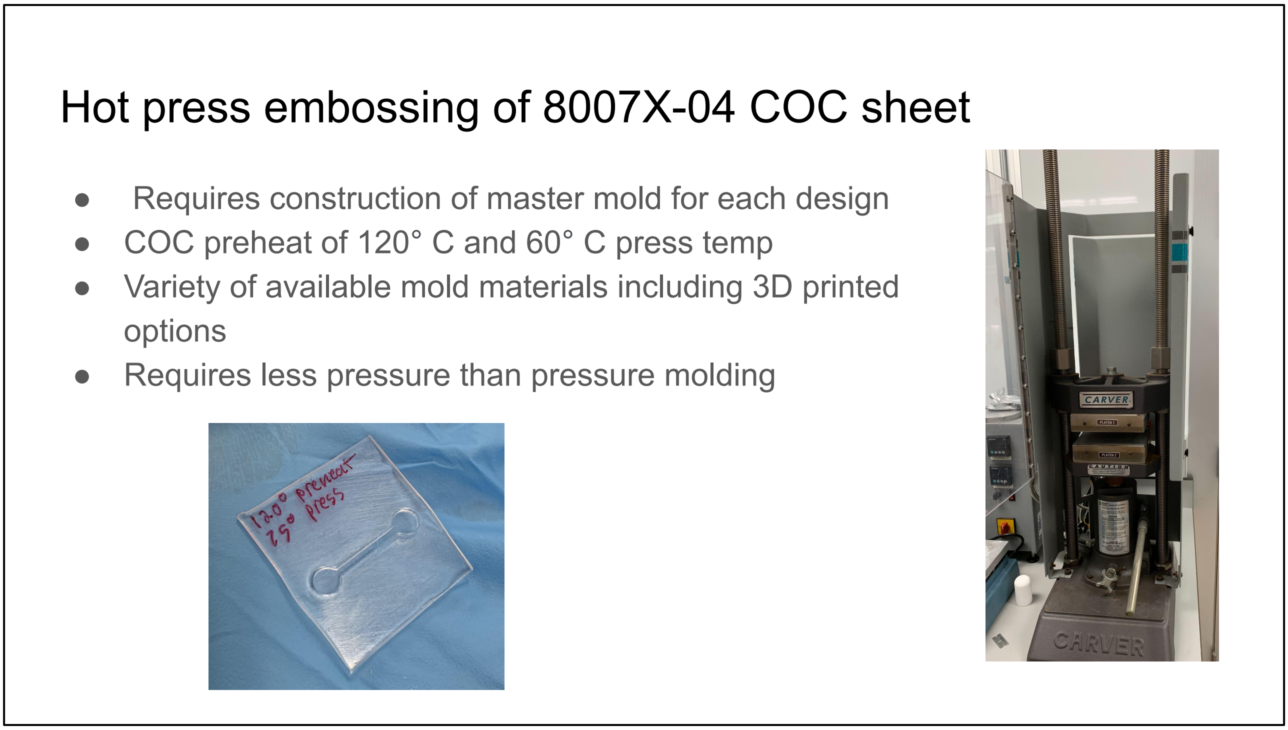
CNC milling of the channels is my personal favorite method of creating channels because of the consistency and relatively small amount of post processing necessary. It does require some set up time (aprox 15 min) and a somewhat skilled operator, however it allows for designs to be modified quickly and gives the ability to go from design to finished chip in under an hour. Milling the channels also allows for the construction of partial, as well as through channels. Partial channels do require some polishing post processing, but that is a relatively short and consistent process. Because of the low Tg of COC, careful selection of feeds and speeds, as well as coolant is used to avoid melting the base material during milling. The main limitation of milling channels is the geometry of the cutter. Endmills are available down to 200 micron in diameter, however the depth to width ratio is typically 2:1 max, which means if we mill from both sides of the chip that gives us a 4:1 depth to width, but I wouldn’t guarantee that.

Hot press pressure molding of 8007X-04 COC pellets

- Mimicking injection molding commonly used in mass manufacturing
- Requires construction of metal master mold for each design
- COC preheat of 210° C and 60° C press temp
- Requires pressure exceeding 2 tons
- Increased risk of trapping bubbles



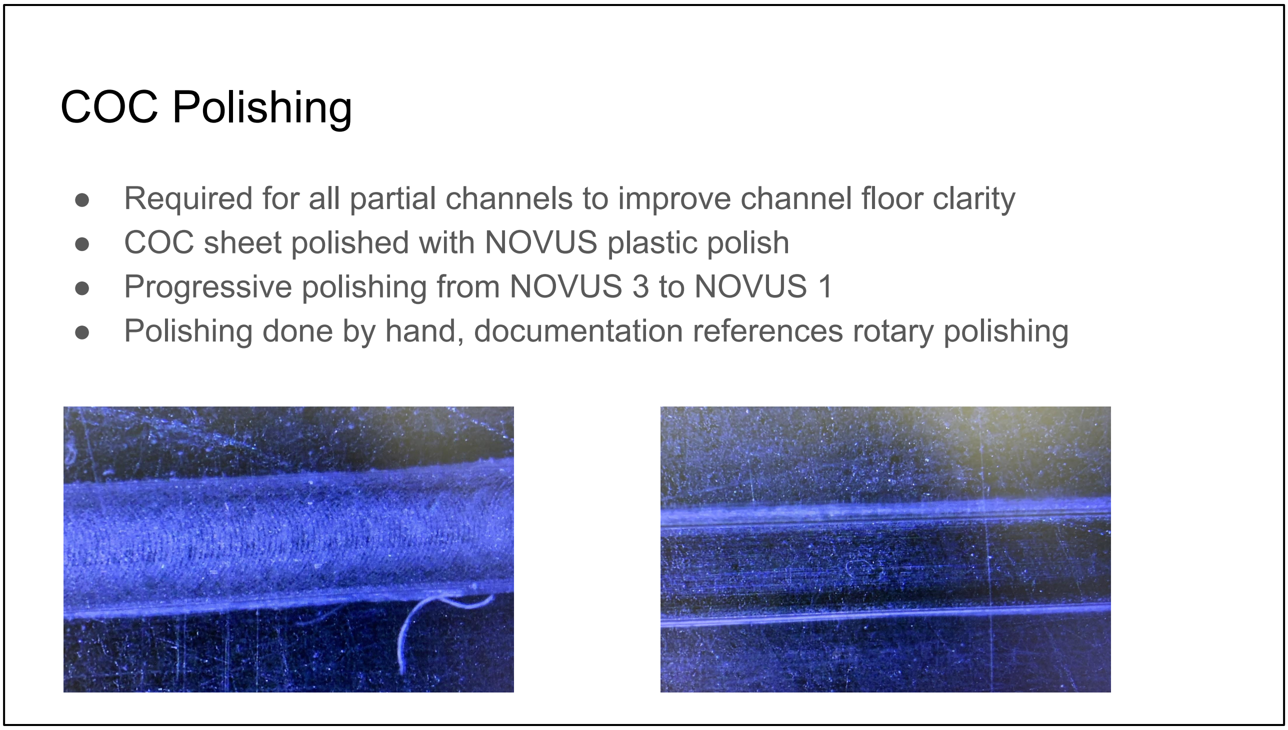
For pressure molding I started out with some of the raw COC resin, heated it up above its melting point to injection molding temperatures, and compressed it between two preheated aluminum dies. This method has a bad habit of trapping bubbles, but I found that overfilling the mold and allowing for a lot of squeeze out typically eliminated that problem. It does require quite a bit of pressure, but I believe better designed dies would drastically pressure necessary to mold the heated resin. The main problem with this and the next method of creating channels is that they require specific aluminum molds for each design. The dies are relatively quick to make and are cheap, however it is an intermediary step that needs to be taken between each design iteration and the creation of the chip. Channels with a 3 or 4 to 1 depth to width ratio can be constructed with a minimum channel width of likely 200 micron.



Hot press embossing of 8007X-04 COC sheet

- Requires construction of master mold for each design
- COC preheat of 120° C and 60° C press temp
- Variety of available mold materials including 3D printed options
- Requires less pressure than pressure molding

Embossing is relatively similar to pressure molding but instead of starting with raw resin, it starts with 1mm plaque, and instead of heating up to molding temperatures, its simply heated a bit above Tg. This method does not run the risk of trapping bubbles and requires far less pressure to create the channels, however a master mold must still be created for every design iteration, and if your press temp isn't high enough has the tendency to create rounded rather than sharp corners. Because of the lower temperature of embossing over molding there is a possibility that 3D printed molds could be used to emboss the channels.



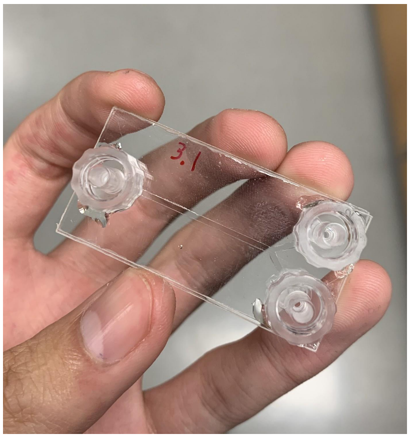
COC Polishing

- Required for all partial channels to improve channel floor clarity
- COC sheet polished with NOVUS plastic polish
- Progressive polishing from NOVUS 3 to NOVUS 1
- Polishing done by hand, documentation references rotary polishing

Polishing of partial channels was done using NOVUS 3 part plastic polish and was done by hand using the included rags and several wooden implements. The finish on the floor of the channel on the left was right off the machine. The right hand photo is the same channel after around 5 min of polishing. Overall this is a relatively simple and quick process and can greatly improve optical clarity and reduce scattering of electromagnetic waves. Although this process is demonstrated on milled channels, there is no reason this could not work for molded or embossed channels \.

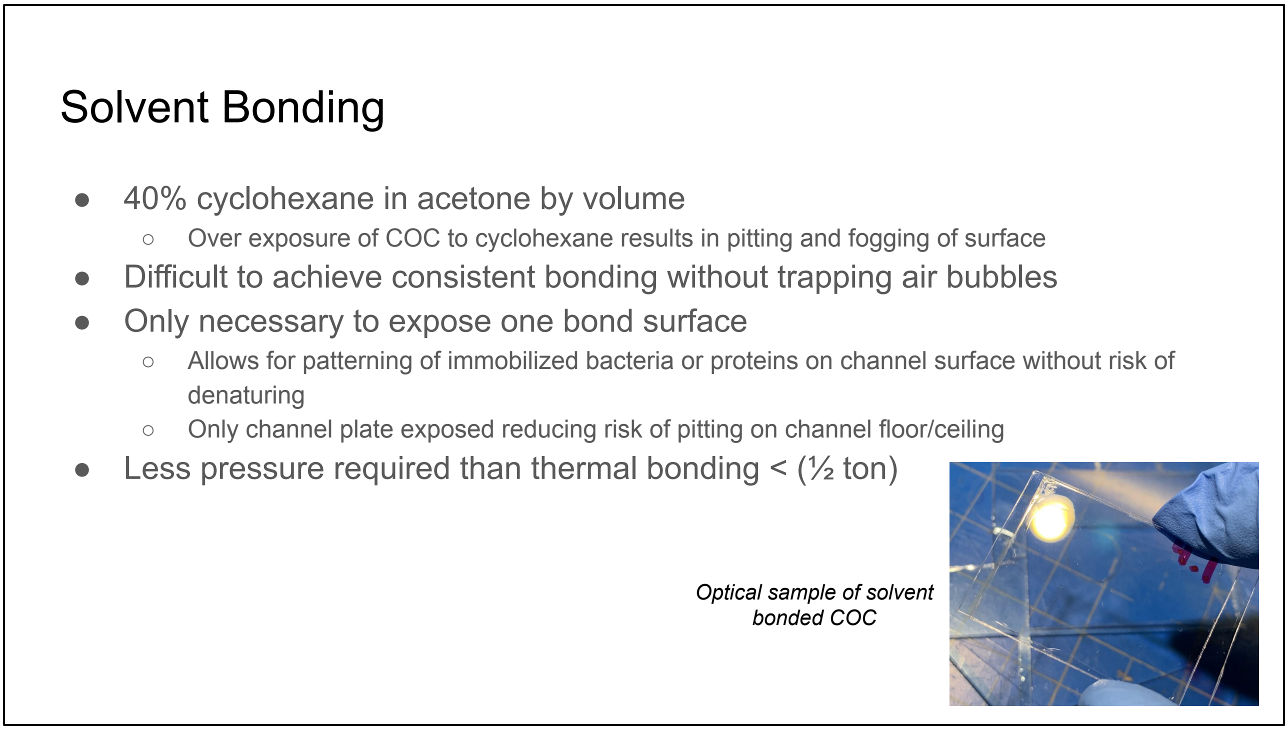
Thermal Bonding

- Preferred method of bonding due to consistency
- Occurs below the Tg for minimal distortion of channels
- Required ozone treatment before bonding
- Able to bond both sides of a three sided chip simultaneously
- Can take advantage of different Tg during bonding
    - Higher Tg for top/bottom plate resulting in less warping of thin sheet



Moving onto bonding processes, Thermal bonding of COC chips is the preferred method as I have found it to be more consistent than solvent bonding. To bond, both substrates are thoroughly cleaned and exposed to ozone for 10 min before being pressed between two heated and polished aluminum plates. The plates are preheated just below the glass transition temperature of the COC material, and is allowed to cool around 10 degrees celsius while in the press and slightly above ½ metric ton of pressure. Because bonding occurs below Tg of both the channel plate and the cover plates, channel distortion is fairly minimal.

In an attempt to keep the cover plates as thin as possible, 240 micron film was used but found to distort into the channel during bonding. To prevent this, 6000 series was used for the cover slips which reduced sag into the channel due to its higher Tg. This method could also be used to create channels of small depth to avoid the chance of bonding the top and bottom cover plates together.



Solvent Bonding

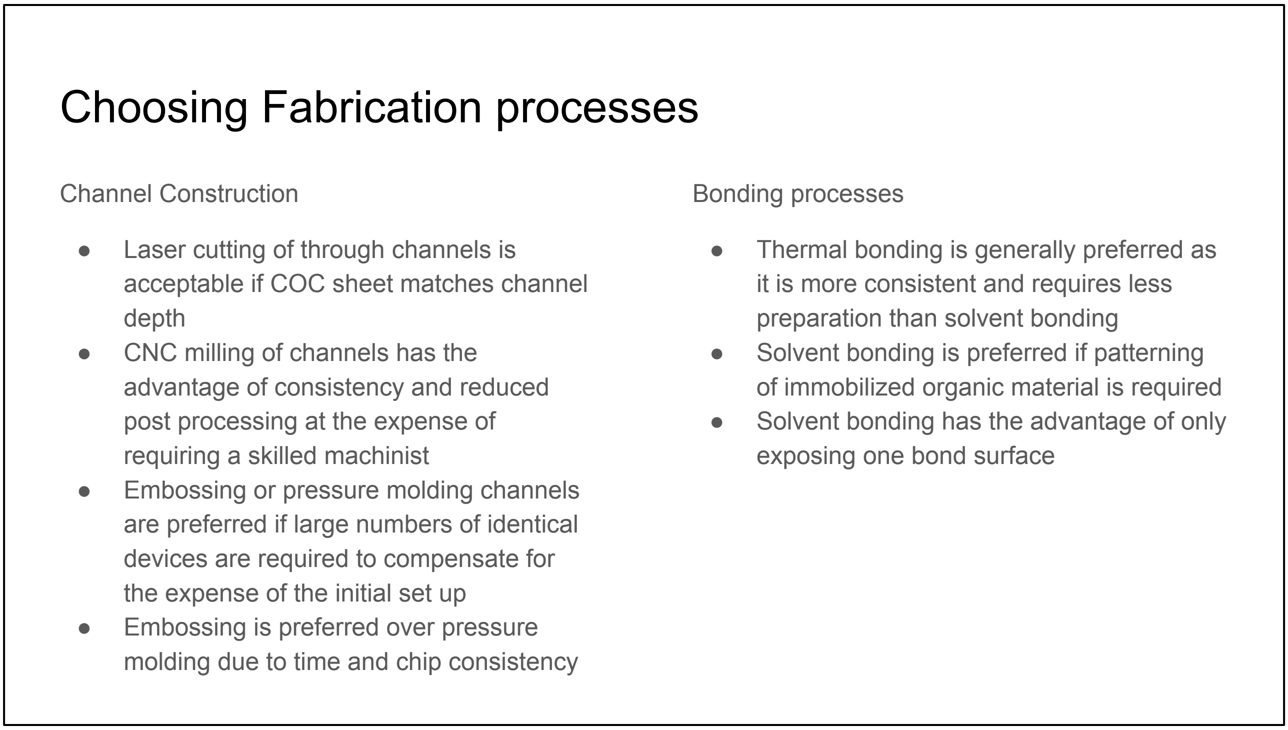
- 40% cyclohexane in acetone by volume
    - Over exposure of COC to cyclohexane results in pitting and fogging of surface
- Difficult to achieve consistent bonding without trapping air bubbles
- Only necessary to expose one bond surface
    - Allows for patterning of immobilized bacteria or proteins on channel surface without risk of denaturing
    - Only channel plate exposed reducing risk of pitting on channel floor/ceiling
- Less pressure required than thermal bonding < (½ ton)

Optical sample of solvent bonded COC

Solvent bonding was the second method of bonding COC chips, and utilizes a non polar solvent cyclohexane diluted in acetone. COC has very good resistance to polar solvents which is why acetone is used to dilute the nonpolar organic solvent and prevent the cyclohexane from overpenetration the COC which can cause fogging and pitting of the surface.

The advantages of solvent bonding is only one surface must be exposed to the solvent, so it is possible to pattern immobilized bacteria or proteins on the channel floor, expose just the channel plate to cyclohexane, and then bond the two together without risk of denaturing proteins. This also keeps any pitting of fogging of the COC to the channel plate which does not affect the clarity of the channel itself.

The main difficulty with solvent bonding is getting a consistent bond across the entire surface. Cleanliness is incredibly important, and exposure time must be perfect which is difficult as the solvent solution concentration changes over the course of the day due to solvent evaporation.



Choosing Fabrication processes

Channel Construction

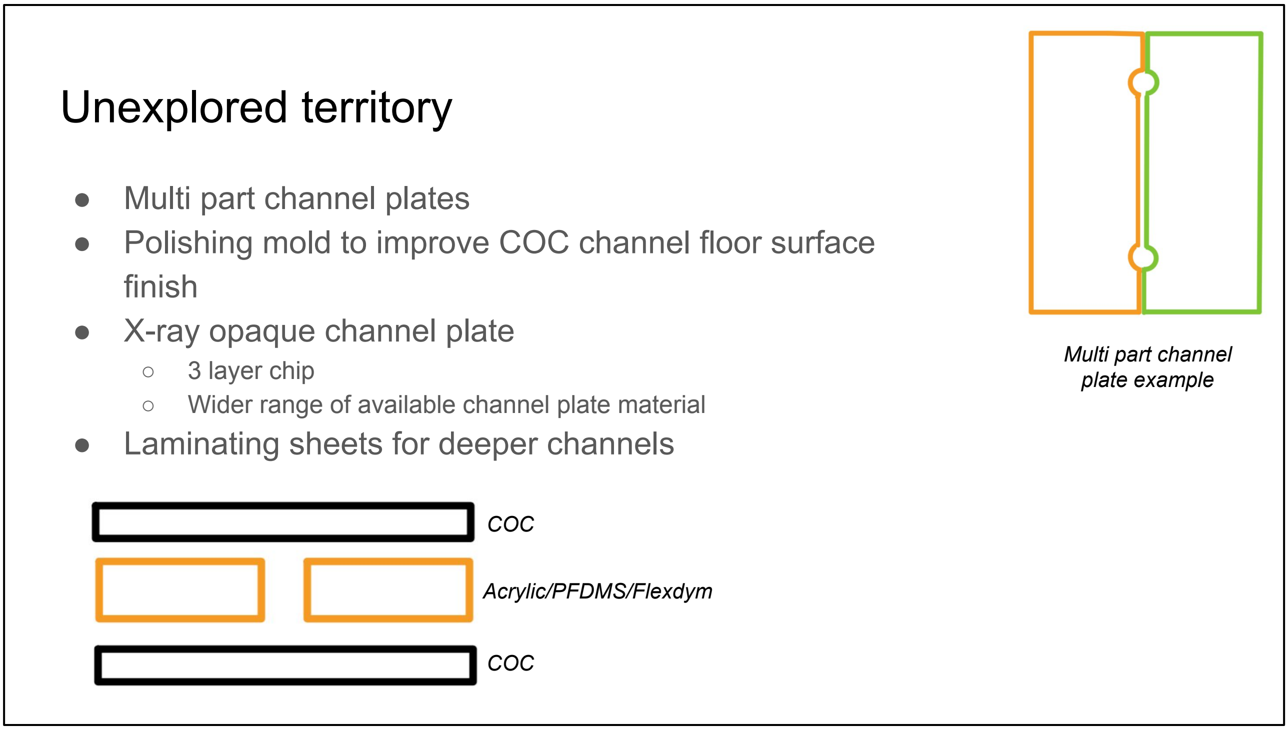
- Laser cutting of through channels is acceptable if COC sheet matches channel depth
- CNC milling of channels has the advantage of consistency and reduced post processing at the expense of requiring a skilled machinist
- Embossing or pressure molding channels are preferred if large numbers of identical devices are required to compensate for the expense of the initial set up
- Embossing is preferred over pressure molding due to time and chip consistency

Bonding processes

- Thermal bonding is generally preferred as it is more consistent and requires less preparation than solvent bonding
- Solvent bonding is preferred if patterning of immobilized organic material is required
- Solvent bonding has the advantage of only exposing one bond surface

Of the methods of chip fabrication that we have covered, laser cutting is by far the easiest method of creating channels as long as the COC sheet matches the depth of the channel as only through channels can be created. Laser cutting must be followed up with some post processing of removing burrs/raised edges, and annealing if solvent bonding is used. CNC milling is my personal favorite way of creating partial or though channels due to how clean the channel comes out right off the machine, however a skilled operator is needed to run and program the machine. Embossing and Pressure molding are both great options if many chips are necessary, however they do both necessitate an intermediary step of making a master mold.

As far as bonding is concerned thermal bonding is more consistent and forgiving than solvent bonding, however solvent bonding does have the advantage of being able to pattern the channel floor with immobilized organic material and expose just the channel plate without risk of denaturation any organic material due to heat.



Unexplored territory

- Multi part channel plates
- Polishing mold to improve COC channel floor surface finish
- X-ray opaque channel plate
  - 3 layer chip
  - Wider range of available channel plate material
- Laminating sheets for deeper channels

Multi part channel plate example

COC

Acrylic/PFDMS/Flexdym

COC

There is still room for further research and improvement, the first being the construction of channels from a multi part channel plate. This method should allow for the construction of channels with a much higher depth to width ratios at the expense of a more risky assembly and bonding process. It is also not known how polishing the mold will affect the raw surface finish of embossed or pressure molded chips; because we are not making large numbers of chips polishing chips by hand is not a significant bottleneck.

A final process that may be worth exploring is using a completely different polymer for the channel plate such as PDMS or Flexdym which can supposedly bond to glass, PDMS, and COC but we have been unable to secure a sample or even a reply from the company. This process of using a different polymer for the channel plate would allow for a wider range of available geometries due to the more favorable properties of the material.

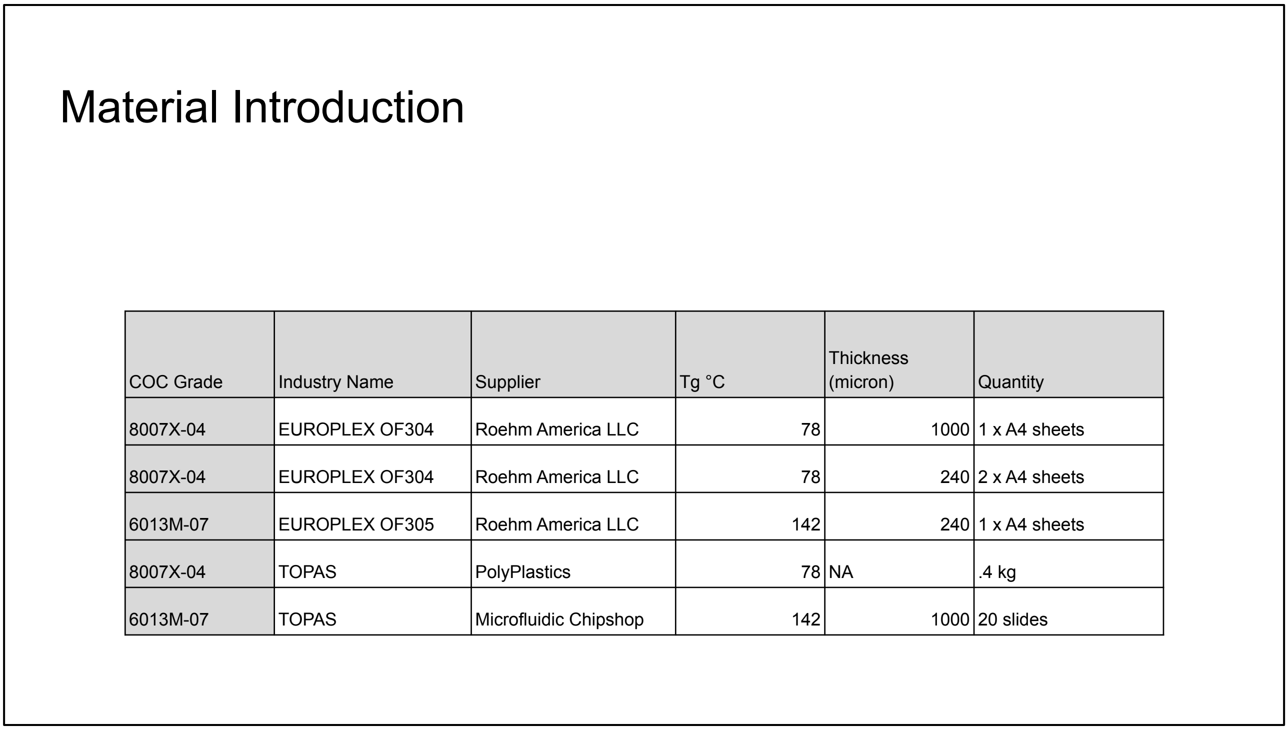
Works cited

- Gleichweit, E.; Baumgartner, C.; Diethardt, R.; Murer, A.; Sallegger, W.; Werkl, D.; Köstler, S. UV/Ozone Surface Treatment for Bonding of Elastomeric COC-Based Microfluidic Devices. *Proceedings* **2018**, *2*, 943. https://doi.org/10.3390/proceedings2130943
- Keller, N,; Tobias M. Tacky cyclic olefin copolymer: a biocompatible bonding technique for the fabrication of microfluidic channels in COC, *Proceedings* **2016**, DOI: 0.1039/C5LC01498K. http://dx.doi.org/10.1039/C5LC01498K
- GDenz, M.; Brehm, Gerrit and Hémonnot. Cyclic olefin copolymer as an X-ray compatible material for microfluidic devices. *Proceedings* **2018**, http://dx.doi.org/10.1039/C7LC00824D

The journal "Lab on a Chip" had a great paper by Nico Keller and many others regarding solvent bonding of COC with immobilized bacteria

Eva Gleichweit also had an incredibly helpful paper on UV treatment of COC before thermal bonding

There was also a helpful paper on thermal embossing of COCs done by Manuela Denz which provided the starting temperatures



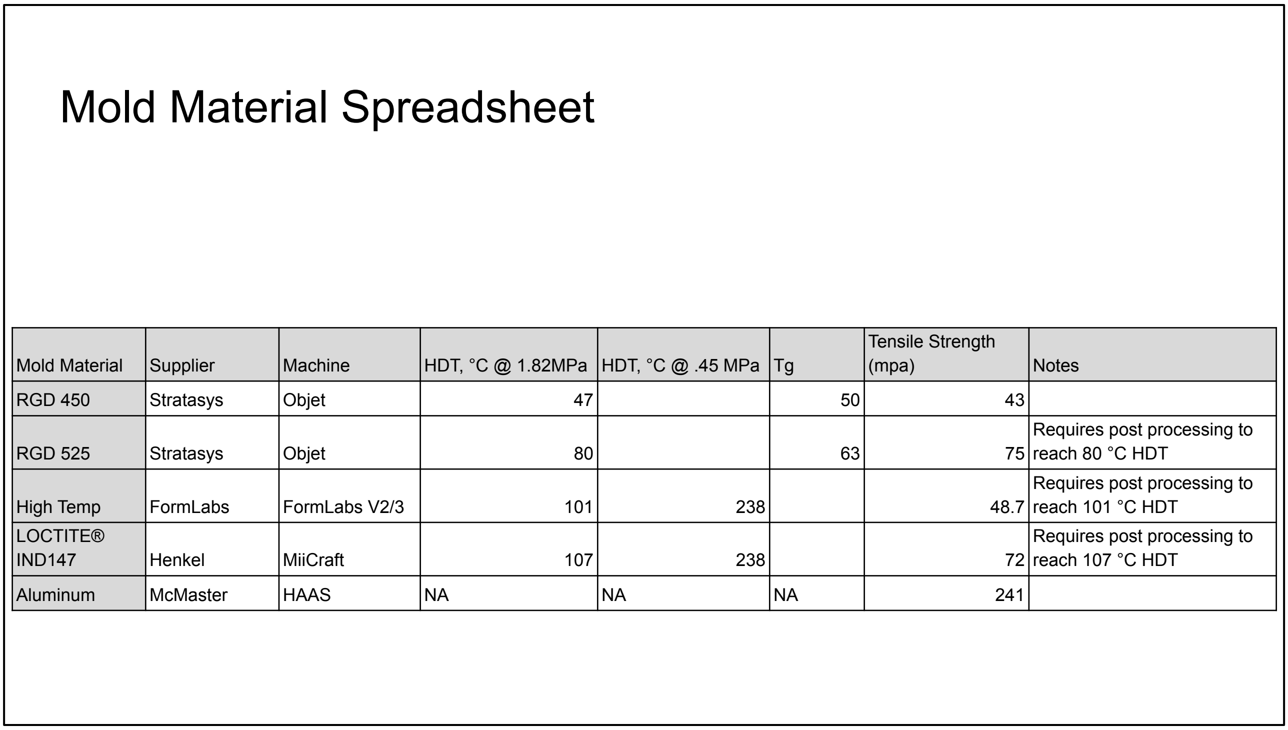
Material Introduction

COC Grade	Industry Name	Supplier	Tg °C	Thickness (micron)	Quantity
8007X-04	EUROPLEX OF304	Roehm America LLC	78	1000	1 x A4 sheets
8007X-04	EUROPLEX OF304	Roehm America LLC	78	240	2 x A4 sheets
6013M-07	EUROPLEX OF305	Roehm America LLC	142	240	1 x A4 sheets
8007X-04	TOPAS	PolyPlastics	78	NA	.4 kg
6013M-07	TOPAS	Microfluidic Chipshop	142	1000	20 slides

COC stands for cyclic olefin copolymer, and it is basically all produced by Polyplastic under the brand name TOPAS. There are several resellers such as ROHM that are mainly geared towards industrial applications, and microfluidic ChipShop is the only reseller i have found focused on selling small quantities to labs. Typically Polyplastic and ROHM deal in the 25 kg minimum order quantity, which is a bit much for what we need.

The experimentation done focused on two grades with their main difference being Tg. COC is a thermoplastic so it has a relatively low melting point and Tg with 8007X-04 having a tg of 78 degrees celsius and 6013M-07 having a Tg of 142 degrees celsius.

The samples we acquired are 240 micron film and 1 mm plaque/sheet in both 8007x and 6013M, as well as 8007X raw COC resin (pellets)



Mold Material Spreadsheet

Mold Material	Supplier	Machine	HDT, °C @ 1.82MPa	HDT, °C @ .45 MPa	Tg	Tensile Strength (mpa)	Notes
RGD 450	Stratasys	Objet	47		50	43	
RGD 525	Stratasys	Objet	80		63	75	Requires post processing to reach 80 °C HDT
High Temp	FormLabs	FormLabs V2/3	101	238		48.7	Requires post processing to reach 101 °C HDT
LOCTITE® IND147	Henkel	MiiCraft	107	238		72	Requires post processing to reach 107 °C HDT
Aluminum	McMaster	HAAS	NA	NA	NA	241	

This is a quick compiling of resins that work in some of our printers. The only one we have on hand is FormLabs High temp, and our resin was expired and had considerable cracking on the cured mold. None of these would be suitable for pressure molding, however FormLabs HighTemp and loctite ind 147 could possibly be used for embossing channels, however I am worried about the strength of a 200 micron fin at a 4:1 or even a 2:1 height to width ratio.