White Paper on common microfluidic components materials: properties and fabrication

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1. Introduction

This document provides an overview of commonly used materials in microfluidics, their properties and fabrication processes. Furthermore, the connectivity between the microfluidic and the external equipment is discussed in detail.

2. Materials in Microfluidics

The reliability of microfluidic devices is defined by the interplay of all materials involved. The choice of materials depends mostly on the specific use and function of the final microfluidic device, but also the availability of fabrication technology and production cost can play an important role in it. Furthermore, the chosen materials must withstand the operation conditions (pressure, temperature), reliability demands and its compatibility with the liquids to be handled.

PDMS (polydimethylsiloxane) is very popular in academic circles, due to its ease of fabrication microfluidic structures, but not acceptable for the industry. There is a strong preference in the industry for COC/COP (cyclic olefin copolymer), glass, glass/Si (silicon) and PMMA (poly(methyl methacrylate)). Glass is especially used for demanding applications: for devices that are reused frequently and for long periods of time; and/or operate at higher pressures and temperatures. However, it appears that glass is also used in applications where the microfluidic parts are used only once and for a very short time, for instance Point of Care. This might be related to the higher accuracy by which structures in glass can be made compared to polymers. COC/COP is mainly used for disposables for Point-of-Care, but on average for those systems that have a longer “time to result” than glass. It may be that miniaturization is easier with glass and therefore the time to measure is shorter. The difficulty of combining polymers with electronics could encourage the use of glass. PC and PS are popular choices for those that are using cell cultures / organ on chip for experiments and tests. Often combinations of materials are used.

In general, the three most important material groups are used in microfluidics:

- polymers,
- inorganic materials (glass, silicon, oxides), and
- metals.

2.1 Polymers

There are three main classes of polymers resp. plastics:

Thermosetting

Thermosetting polymers have a high capacity to resist higher temperature and retain their strength and shape when heated. They have a little bit of flexibility and are impermeable. They cannot be molded more than once. For instance: epoxies.

Thermoplastics

Thermoplastics are a class of synthetic polymers. Thermoplastic polymers have a good resistance to creep and are electrically isolated. They can be melted and solidified many times. Due to the long polymer backbone, it exhibits softening behaviour as it returns to its original state after cooling. This property distinguishes it from thermosetting polymers and elastomers.

Examples: PMMA (poly(methylmethacrylate)), PEEK (polyether ether ketone), PP (polypropylene), PS (polystyrene), PTFE (polytetrafluoroethylene), FEP (fluorinated ethylene propylene), PC (polycarbonate) and COC (cyclic olefin copolymer)/COP (cyclic olefin polymers).

Elastomers

Elastomers are flexible and have a low creep resistance. There are two types:

a. Thermoplastic elastomers (they can melt).
b. Thermosetting elastomers (cannot melt).

Examples of elastomers / synthetic rubber: EPDM (ethylene propylene diene monomer rubber), FFKM (perfluorinated elastomer) and silicone.

2.2 Glass

The most often used glass types for microfluidic components are D263® T eco, D263® bio and MEMpax® (all three from the supplier SCHOTT). Their properties are described by the supplier's data sheets, available online or received directly from the supplier upon request. Material properties, which are relevant for the selection of the base material before processing it to microfluidic parts and components, are listed in Table 1.

In general, all three glass types are suitable for microfluidic applications, but their use may differ depending on specific requirements. D263® bio exhibits a very low intrinsic autofluorescence across the UV to NIR spectrum compared to D263® T eco.

If glass is combined with silicon, MEMpax® should be chosen to avoid stresses due to thermal expansion (CTE of silicon ~ 2.6 µ/K). Such induced stresses can, in the worst case, lead to fracture of the entire component.

Table 1: Properties and their metrology for three glass types.

Material property	Metrology, ISO or SEMI	Measured value/s		
		D263® T eco	**D263® bio**	**MEMpax®**
thickness tolerance	SEMI MF 1530 GBIR	± … µm*		
total thickness variation (TTV)	SEMI MF 1530 GBIR	≤ … µm*		
flatness	SEMI M1 GBINFER	≤ … µm*		
coefficient of thermal expansion (CTE)	length measurements at different temperatures, ISO 7991 for glass	7.20 [µ/K]	7.20 [µ/K]	3.26 [µ/K]
spectral transmittance	spectrophotometric measurements	% over λ-range in [nm]; spectrum plot		

**depends on substrate thickness*

Processes similar to those used in the semiconductor industry are used to convert a glass substrate into a microfluidic part/device. The following properties are monitored and/or measured during and after processing:

- Mechanical interfacing: dimensions of device, hole dimensions and positions, channel depth and width, surface roughness in channels.
- Chemical properties: wettability.
- Optical interfacing: reflectance, transmittance, autofluorescence.
- Electrical interfacing: electrical resistivity.

Channels in glass are isotropically wet etched using hydrofluoric acid-based etching solutions. Monitoring the dimensions in width and depth of the channels are crucial for the microfluidic application. The roughness of the channel plays an important role in the flow behaviour and should be as smooth as possible. Functionalization of surfaces is used for biosensing applications and is also strongly influenced by roughness and wettability of the surface. The wettability of a surface can be determined by the contact angle between the droplet of a liquid/medium and the surface. It gives also further information on hydrophobicity respectively hydrophilicity of a surface. Procedures for measuring wettability in microfluidics are given in the MFA whitepaper: Whitepaper on the measurement of hydrophobicity, hydrophilicity, and wettability.[1] This can be downloaded from: https://microfluidics-association.org/downloads/

Structuring of glass substrates for microfluidics also includes optical and electrical coatings, such as gratings, anti-reflectance coatings or electrode structures.

Table 2: Material properties and their metrology, which are relevant during and after processing for glass substrates.

Property in/on glass	Metrology, ISO or SEMI	Measured value/s
channel dimensions like depth or width	optical microscope; white light interferometer, confocal laser microscopy, profilometer	dimension [µm]
dimensions of coating structures	optical microscope; white light interferometer, confocal laser microscopy, profilometer	dimension [µm]
surface roughness of the channel, coating or device surface	AFM, white light interferometer, confocal laser microscopy; ISO 4287; ISO 21920 (new, draft)	R_a value [nm] = arithmetic mean height
wettability	contact angle measurements, described by ISO 19403-2:2017: determination of the surface free energy of solid surfaces by measuring the contact angle	angle [°], depends on medium
optical property of a coating	spectrophotometric measurements for reflectance, transmittance over a specific wavelength range	ISO 9211-1: Optical coatings vocabulary
electrical property of a coating	four-point probe; ASTM F390-98	electrical resistivity [Ω cm]

2.3 Summarized list of materials used in microfluid chips and components.

- Tubing: PEEK, FEP, Silicone, PTFE (stainless steel, Teflon, Fused Silicon, Tygon)
- Fittings/ ferrules: PEEK, PTFE, FFKM[5]
- Reservoirs, blisters: PEEK, PP, COC, PC
- Connectors: PEEK (PP, Ultem[6])
- Electrodes: Pt and Au
- Pumps and valves: COC, EPDM, PEEK, FFKM, PP, PTFE, (PCTFE, FPM, Vespel®[7], PPS and PMMA, Silicone)
- Flow sensors: Monel or Hastelloy, FFKM, PEEK, fused silica, glass
- O-rings: mostly synthetic rubber
- Gasket: synthetic rubber (epoxy/PMMA or epoxy/PEEK)

[1] Henne van Heeren. (2022). Whitepaper on the measurement of hydrophobicity, hydrophilicity, and wettability (1.0). Zenodo. https://doi.org/10.5281/zenodo.7181091

- Glass (base material): D236® T eco, D236® bio and MEMpax®

3. Microfluidic Manufacturing

Microfluidic structures are produced on flat substrates or chips (except in the case of 3D manufacturing). The manufacturing of microfluidics is mainly based on:

1) Injection moulding (polymers).
2) Etching of glass.
3) Hot embossing (polymers).

Each of these technologies has its own merits and result in products with specific characteristics:

(These values are an indication only, exact specifications can vary between suppliers.)

Table 3: Technical aspects of the different microfluidic fabrication technologies.

Geometric feature	Glass: planar processing	Polymer: injection molding	Polymer: embossing
typical chip sizes [mm]	15 x 15; 15 x 22.5; 15 x 30; 15 x 45	credit card format or microscope slide (25 x 75)	credit card format or microscope slide (25 x 75)
chip thickness [mm]	thinnest: 0.03 standard: 0.7; 1.1; 2.0	standard: 0.6 thickest: 5 (preferably < 1.5)	
channel width [µm]	Mask width + 2* channel depth; min. mask width at 10, 1 on special request	> 50	
etch depth [µm]	0.01 - 500	< 70	
min. spacing between structures [µm]	5 + 2.6* etch depth	Same as structure height, but at least 50	
aspect ratio for the channels	< 1:2	< 1:2	
electrodes [µm]	min. width = 2 standard width = 5 thickness = 0.05 - 1		

These technologies are described in the White Paper "Design for Microfluidic Device Manufacture Guidelines", which dates from 2014, but are still valid (see http://www.microfluidicsinfo.com/wp-content/uploads/2017/08/DesignforManufacture-1.pdf).

Most microfluidic products are made from transparent materials, which includes the top five materials for microfluidic devices. The main fabrication methods for devices made from

- cyclic olefin copolymer (COC),
- cyclic olefin polymers (COP),
- polycarbonate (PC)
- polymethylmethacrylate (PMMA) and
- glass

are described in the following.

3.1 Glass chips

The glass and glass/silicon-based devices are created by wafer-scale processing in a similar way to the semiconductor industry: through lithographic processes, a structure is generated onto a glass or silicon wafer. Follow-up processes, such as wet etching, dry etching, and/or powder blasting result in microfluidic channels and other structures (e.g., access holes). For glass/metal hybrid devices, additional processes, such as metallisation, are included between these steps.

Bonding glass wafers to form closed microfluidic devices can be done by fusion-, adhesive- or low-temperature bonding methods. Separating the whole wafer into individual devices is done via dicing. The advantages of glass processing are the accuracy of structuring, the durability of the devices and the transparency.

3.2 Polymeric chips

Polymeric microfluidic devices offer great flexibility and low cost compared to glass devices. They are often produced in one single step taking advantage of the thermal deformation or melting of the polymer. The most used fabrication processes are injection moulding, hot embossing or thermoforming. In injection moulding, powder or granular polymer is heated until it melts or becomes plastically formable. The polymeric mass is injected and compacted by a rotating screw under high pressure into a closed mould. The mould determines the shape and surface structure of the finished part. Injection moulding is regarded as the most cost-efficient method for fabricating microfluidic parts in large quantities.

Embossing and thermoforming rely on the deformation of the polymer when submitted to heat and pressure. In these processes, a solid plate or sheet of the polymer is forced into an open cavity containing the mould and is pressed against the mould at a given temperature.

To form an enclosed microfluidic chip, the microfluidic substrate must be bonded to a cover plate. Thermal bonding of polymeric microchips has been the dominant method. However, driven by the need of adding biomaterials for functionalizing surfaces onto the chip before bonding, other low-temperature methods, including adhesive, solvent bonding and surface modifications, have been explored to allow for the preservation of functional surfaces. Bonding polymers with very small channels is difficult since the channel integrity and structure should not be altered during the process.

4. Connectors to microfluidic chips

Although several chip sizes are used for microfluidics, substantial numbers of commercial microfluidic devices have the exact outer dimensions of standard microscope slides or microtiter plates. As glass based microfluidic chips are often much smaller. The pro-forma standards for glass chip dimensions are based on multiples of 15 mm. Apart from gluing connectors on a chip, glass chips often use clamping systems to connect to tubing. In contrast, moulded plastic devices often use mini-Luers. This section introduces commonly used connectors, their size and location on commercial devices.

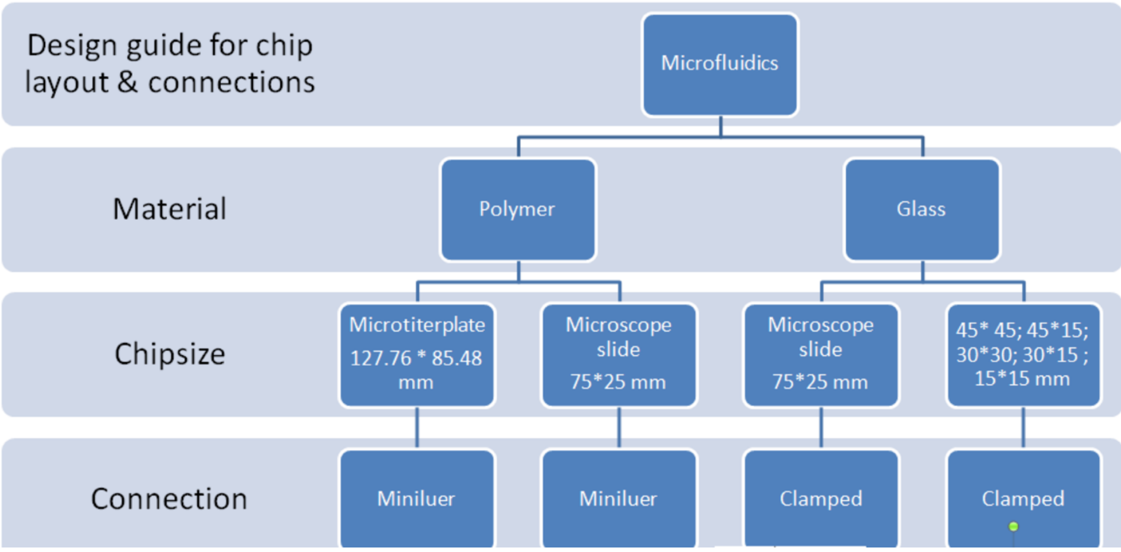

Figure 1: Overview of chip layout and connections for polymeric and glass devices. (MNT white paper)

An overview of the most used microfluidic connectors is given below (Figure 2):

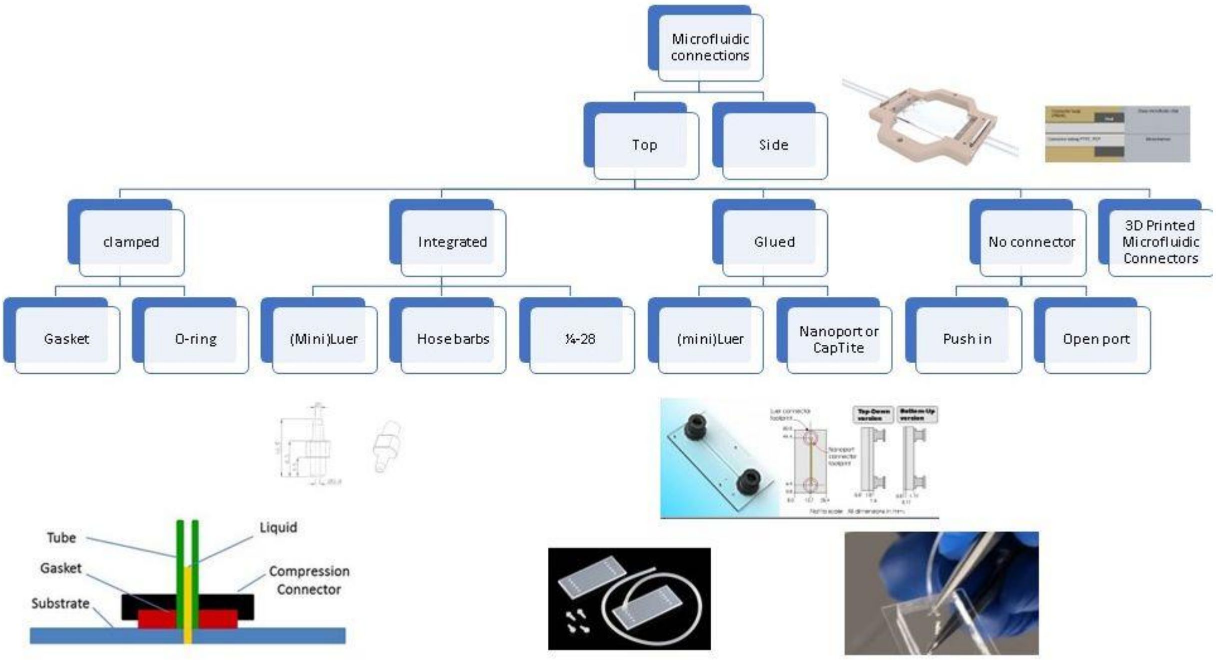

Figure 2: Overview of microfluidic connectors to chips or substrates.

Generally, microfluidic connectors are on the top of the substrate or chip. With injection moulding, it is relatively easy to integrate structures that enable microfluidic connections like tubulars or male (mini)-Luer parts. There are many variations and sizes used, not yet standardised.

Figure 3: Example of integrated microfluidic connectors.

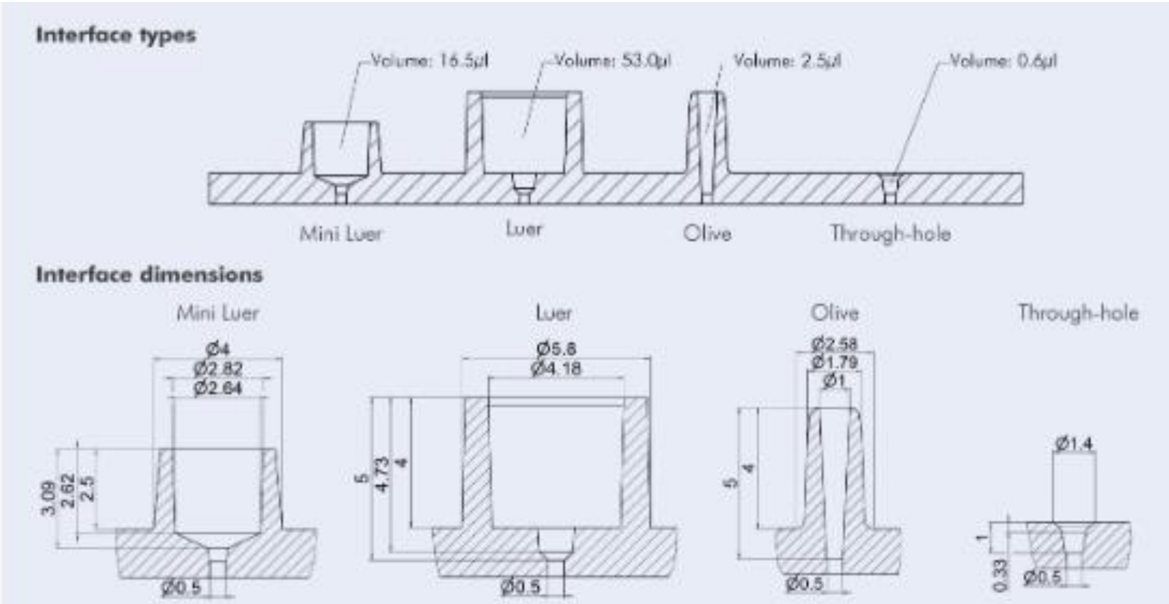

Figure 4: Examples of integrated microfluidic connectors (courtesy of microfluidic chip shop).

4.1 (Mini-)Luer

Luer connectors were developed to connect the needles to the syringe body, but are now found in many other microfluidic accessories. There are two types, slip- and lock, but only lock is used in microfluidics. A male lock can be attached to its counterpart by screwing it. This type of connection is safer for higher pressures (i.e. above 2 bar), but has a high thread, meaning that components must be bigger and have a higher internal volume.

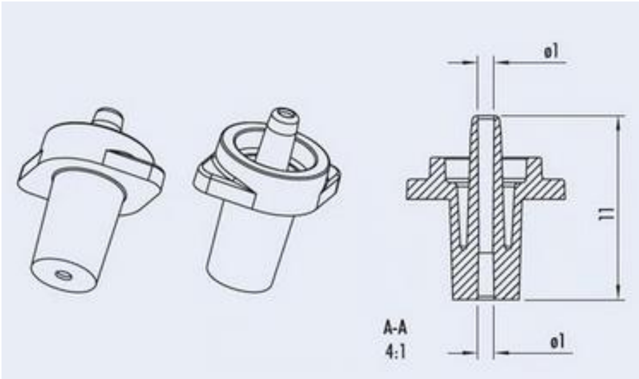

Figure 5: Technical details Luer interface.

A smaller variant (mini-Luer) is often used.

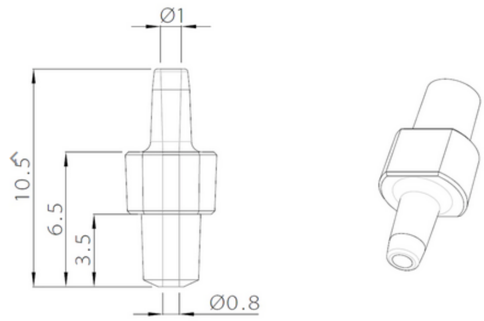

Figure 6: Technical details of mini-Luer connector.

Mini-Luer interfaces have been regarded as a proforma standard, but experts in this field have stated that mini-Luer from different suppliers are not always compatible. Other disadvantages are the large internal volume and the limited pressure they can sustain.

4.2 Hose barbs

Hose barbs are also often used, but these can only be used with flexible tubing. The pressure limitations are similar as with mini-Luer.

4.3 Clamped connectors

Integrating tubulars or male (mini-)Luer parts is not easy to be used for glass based products and products made with hot embossing. These products are combined with either clamped or glued connectors.

Clamped connectors are described in two White Papers that are available for download from the MFA website:

- Design Guideline for Microfluidic Device and Component Interfaces (part 1 and part 2)[2]

[2] https://microfluidics-association.org/downloads/

The essentials of such connectors are described in a recently published ISO document:

- ISO/CD 22916 Microfluidic devices – Interoperability requirements for dimensions, connections and initial device classification[3]

It is also possible to make a microfluidic connection to the side of the chip resp. substrate. The design rules for such connectors are described in the following White Paper (also downloadable from the MFA website):

- Design Guideline for Microfluidic Side Connect

A big advantage of clamped connectors is the ability to make several microfluidic connections at the same time. Clamped connectors can withstand much higher pressures (up to 30 bar has been reported).

4.4 Glued connectors

In principle, any structure can be glued onto a chip to enable microfluidic connection. For instance, male parts for mini-Luer connectors. There are also companies with their own connection systems, such as IDEX NanoPorts™:

A threaded port is glued to the substrate surface. If done properly, this approach does isolate the adhesive from the liquid, by using an O-ring to make the actual seal to the substrate surface. However, the presence of this O-ring creates a large dead volume gap in the process. This dead volume gap is unlikely to be completely filled with liquid, creating an air pocket in the system. The presence of such an air pocket could cause the system integrity to be compromised. It is possible that bubbles would be released from the air pocket into the system. It is also possible that the compressed gas in the system's flow path could create an uncontrollable fluid pulsing. If live cells were used that came into contact with the air pocket, it is possible that cell death would occur. In addition to dead volume introduction, other drawbacks of the technique include its large size which eliminates the possibility of tight port densities, only one-time use, and significant cure wait-times.

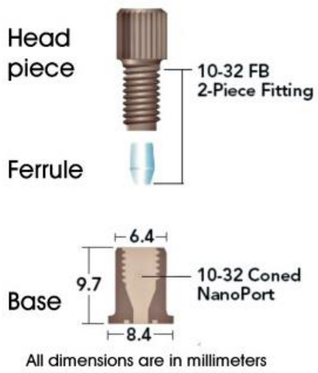

Figure 7: IDEX NanoPorts™ connection system.

There are several other options, for instance products from Parallel Fluidics:

[3] https://www.iso.org/standard/74157.html

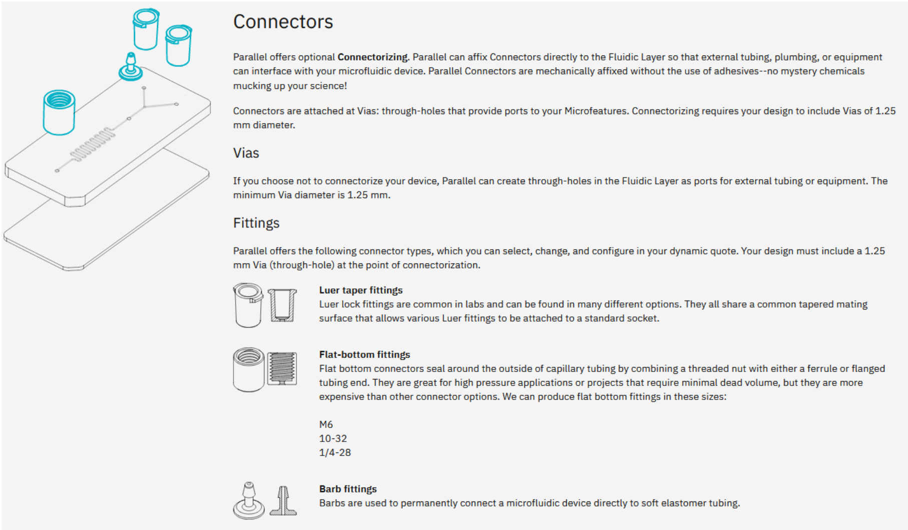

Figure 8: Examples of glued connectors from Parallel Fluidics (https://www.parallelfluidics.com).

With treaded ports the connection can withstand similar high pressures as clamped connectors. The fact that the connections are made one by one by hand is seen as disadvantage. Another disadvantage is the fact that the glue used will be in contact with the fluid during use of the connector.

An overview has been created to link the different connector types and chip dimensions with the typical characteristics of microfluidic devices.

4.5 Summary of connector characteristics

Table 4: Overview of microfluidic connectors to chips resp. substrates.

Connector / Characteristic	Mini-Luer	Hose barbs	Clamped connectors	Glued threaded connectors	Glued other connectors
Dead volume	--		++		
Pressure resistance (typical values)	< 2 bar	< 2 bar	up to 10 bar or more	up to 10 bar or more	< 2 bar
Easy of making multiple connections	-	-	++	--	--
Use of glue				--	--
Only for flexible tubing		yes			
Suitability of glass-based devices	--	--	++	++	++
Suitability for hot embossing-based devices	--	--	++	++	++
Suitability for injection molding-based devices	++	++	?		

5. Connection to microfluidics instruments and components

Microfluidic connections to chips resp. substrates have only been considered so far. The microfluidic connection of instruments and components is another area of interest for the community. The diversity is huge. This is particularly challenging when combining several microfluidic components or instruments, for example:

- Flow sensor from Sensirion: 1/4-28 flat bottom port.
- Micropump from Bartels: barbed tube clip.
- Dolomite: clamped top and side connectors.
- Cellix: company proprietary plug & play connection system.

Table 5: Overview of the status of microfluidic connector types.

Connection type	Chips		Pumps				Other				Comments
	glass	*polymer*	*pressure regulated*	*peristaltic*	*membrane*	*syringe*	*flow sensor*	*other sensors*	*valves*	*cells or organs*	
Hose		(x)			x				x	x	
(Mini-)Luer		x	x			x		x		x	mostly Luer
Glued	(x)										
¼ -28 flat bottom port or similar			x			x	x	x	x	x	mostly ¼-28
Clamped	X										
Manifold or docking station	x								x	(x)	
Other		(x)	(x)			(x)					company unique, push in etc
Non-existing connector					x					x	

Beside the variation in connection technologies, the industry is also hampered by the fact that there are no agreements about operational classes (based on pressure, temperature and flow ranges) that help to select components that are compatible.

One part of that problem has been solved by the work of the Microfluidics Association that defined operational classes based on temperature and pressure. The results are translated into an ISO document: ISO/CD 22916 Microfluidic devices – Interoperability requirements for dimensions, connections and initial device classification.

Table 6: Operational classes (adapted from ISO 22916:2022).

Class	Maximum pressure [kPa]	Maximum temperature [°C]	Minimum temperature [°C]
Capillary devices	-	50	4
PT 200/50	200	50	4
PT 200/75	200	75	4
PT 200/100	200	100	4
PT 700/50	700	50	4
PT 700/100	700	100	4
PT 3000/50	3000	50	4

For the connector issue the feeling was that this was not enough and extensive discussions[4] were held with leading microfluidic suppliers during which flow range classes were proposed:

After several interviews and group discussions we agreed on the three main classes:

- Low flow rate: 1 nl/min to 1 µl/min → calibration by optical methods preferably, or low-flow volumetric methods in general. National metrology standards (best calibration uncertainty) would be around 1 % in general.
- Middle flow rate: 1 µl/min to 100 µl/min (hot spot) → calibration by gravimetric or volumetric methods, national metrology standards (best calibration uncertainty) would be around 0.5 % in general.
- High flow rate: 100 µl/min to 10 ml/min → calibration by gravimetric methods, national metrology standards (best calibration uncertainty) would be around 0.1 % in general.

Of course, there is also diversity in terms of the medium used: water based, alcohol, oil, gas etc.

The experts also agreed that there is a particular hotspot in term of people using devices operating on the following operational conditions:

- flow rate: 1 µl/min to 100 µl/min,
- water based fluids containing biomolecular matter,
- temperature: 4 ºC to 50 ºC, and
- pressure: below 2 bar.

The main applications in this hot spot are shown in Table 7.

The companies involved in the discussions want to work towards one preferred microfluidic connector type with low internal volume which is easy to connect (plug and play) and provides a seamless route to further integration.

[4] Co-organised by the Microfluidics Association and MFMET

Table 7: Market segments for devices that operate in the hotspot.

<table>
<tr><th>Market</th><th>Specific requirements resp. needs</th></tr>
<tr><td>Research</td><td>Flexibility and easy to connect</td></tr>
<tr><td>Analytical instruments</td><td rowspan="2">Reliability, low dead volume, ordered layout (separation between electrical and fluidic lines), roadmap to further integration</td></tr>
<tr><td>Medical diagnostics</td></tr>
<tr><td>Bioreactors, “physical” reactors</td><td>Pull resistant connectors, limited biofilm formation</td></tr>
</table>

Demands on a microfluidic connector:

- Withstanding a pressure of 2 bar and a temperature of 4-50 °C
- Suitable for flows of 1µl/min to 100 µl/min and suitable for water-based fluids containing biomolecular matter
- Low internal volume, low flow resistivity, limiting risk of biofilm formation
- “Biocompatible” materials as wetted materials, materials used should be affordable and the supply chain sufficiently covered
- Reusable / cleanable / (preferable sterilisable)
- Leak tightness
- Suitable for rigid tubing 1/16” / 1.6 mm OD tube (preferable flexible for ID size, preferable for soft wall tubing too)
- “One click fastening”, (preferable pull resistance)
- Lowest “footprint” possible / not bulky
- Design is freely available, but fixed in a standard

Based on this discussion a proposal was made. Sample products made with this technology are expected to become available fall 2023.

6. Conclusion

Based on older survey results, investigation of suppliers of microfluidic components and discussions with experts, we concluded that although there is a huge range of materials used, the top five materials that are in contact with the medium and most often used in microfluidics are:

- COC/COP for microfluidic chips/substrates and several other applications,
- glass for microfluidic chips/substrates,
- PEEK and PFTE for connectors, tubes, pumps etc., and
- PC for cell cultures / organ on chip.

For the fabrication of glass microfluidic devices, photolithography coupled with wet isotropic etching is the most widely used method. It has a distinct advantage in generating a smooth surface (< 5 nm) but is unsuitable for making submicron features and vertical sidewalls. Thermal bonding is the method of choice for making enclosed chips for high-pressure applications. Attention should be paid to the adhesive bonding method, which may affect the optical properties of the substrate.

For fabricating devices made from COC and COP, injection moulding is the method for high throughput manufacturing of devices at a low cost per device. However, the process parameters could alter microstructures and the flatness of the substrate.

In terms of standardized chip dimensions, guidelines are now giving clear direction for glass chips. Several types of connectors were described. There are many variants and sizes in use and only one standardization effort yet, led by the Microfluidics Association and supported by MFMET.
Regarding compatibility between different microfluidic components, there is some pro-forma standardisation for chip dimensions, but that might not be much of a barrier for the industry. More problematic is the situation with microfluidic connections. There the diversity and lack of compatibility is seriously hindering the industry.

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