Enabling Microfluidics: From Clean Rooms to Makerspaces

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TRENDS BOX

- The use of simple tools and materials to manufacture microfluidic devices provides an opportunity for makerspaces to serve as a hot bed for microfluidic device development.
- Materials such as plastic, adhesive, and paper along with tools such as plotter/laser cutters and 3D printers enable the building of integrated microfluidic systems that are more easily translated to large-scale manufacturing.
- Makerspaces provide low-cost access to prototyping tools, access to technically diverse human capital, and enable those without advanced skills to participate in microfluidic device development.

ABSTRACT

Fabrication of microfluidic devices has been traditionally focused on photolithographic methods requiring a clean room facility and specialized training. The lack of devices commercially available from these methods leads us to believe that this approach has reached a point of diminishing returns. Makerspaces are a growing alternative to clean rooms, as they provide low-cost access to fabrication equipment such as laser cutters, plotter cutters, and 3D printers, use commonly available materials, and attract a diverse community of product designers. This opinion discusses the introduction of microfluidics into these spaces and the advantages of maker microfluidics improving the accessibility and scalability of microfluidic device fabrication.

MICROFLUIDICS AND THE MARKET

Over the past few decades, thousands of novel microfluidic point-of-care (POC) diagnostic platforms and applications have been published in peer-reviewed journals; however, few have reached market [1]. Even with large investments from government and industry in both Europe and North America, surprisingly few “lab-on-a-chip” (LOC) microfluidic diagnostic tests have translated to commercial products [2]. This discrepancy somewhat restrains market growth for these devices, which are expected to grow from $1.6 billion in 2013 to $3.6 - $5.7 billion by 2018 to meet the rising incidence of lifestyle diseases within a growing geriatric population [3,4].

Thus far, the field of POC microfluidic diagnostics has been predominantly addressed in academia with polydimethylsiloxane (PDMS) devices manufactured using soft lithography techniques, originally popularized by the Whitesides group [5,6]. Soft lithography methods create ‘master’ molds from photolithography techniques followed by curing of a pre-polymer (PDMS) on top of the mold master, where after curing, a PDMS negative stamp of the mold is created and bonded irreversibly to glass (Figure 1). Soft lithography techniques have proven useful in microfluidics under a wide range of applications from channel fabrication to pattern generation [7]. The key benefit of soft lithography methods is the ability to rapid prototype [8]. The technique is ideal as feature resolution can match the micrometer and even nanometer feature sizes often found in biology. The PDMS polymer provides an ideal candidate for microfluidic devices as it is nontoxic, widely available, transparent, hydrophobic, gas permeable, and elastomeric [6,9]. Oxidized PDMS surfaces can be irreversibly bonded together by a spontaneous dehydration of SiOH groups and PDMS can be passivated and functionalized through various chemistries for high efficiency molecular assays. The flexibility of the PDMS polymer enables a wide variety of geometries, layering, and unit operations applicable to a plethora of unique microfluidic manipulations [6].

On the other hand, the photo- and soft lithography methods used to create these devices suffer from the nature of artisanal and resource-consuming process (pour, cure, cut, punch and bond) as opposed to traditional industry-standard injection molding process where a mold is filled, the polymer is rapidly cured, and the part is ejected. Soft lithography prototyping can also be done using contract manufacturers, such as FlowJEM (Ontario, Canada) and SIMTech Microfluidics Foundry (Singapore), who provide custom low-cost molds for a fee; however, the design process is slowed down waiting for molds to be manufactured and shipped. While PDMS devices may be well-suited for the research setting, the lack of scalability in soft lithography and the high-cost of PDMS (relative to cost-efficient thermoplastics) has limited commercial potential [10]. A technology map developed by Chin et al. shows how virtually none of the major players in the microfluidic *in vitro* diagnostics market use PDMS in their products, leaning towards plastic, glass, or paper materials instead which can be more easily mass-manufactured through processes such as injection molding, casting, and die cutting respectively [11]. These common manufacturing materials and methods offer additional benefits such as standardization of fabrication, improving quality control, and better integration with other parts made of similar material [11,12]. A wide variety of advances in microfluidics manufacturing, materials, functions, and operations has yielded a powerful toolkit to enable plastic microfluidic development for a plethora of applications [13–15].

Alternative rapid prototyping methods that take advantage of these materials for microfluidics have been reviewed previously [16]. For example, laser cutting can be used to cut microfluidic channels in double-sided pressure sensitive adhesive (PSA) [17], to directly ablate microfluidic channels in polymer materials [18], and even to create molds for PDMS from laser cut adhesive [19]. Plotter cutting, also known as xurography, uses a drag knife printer to cut microfluidic designs from laminate and masking films [20–22]. Xurography has even been employed to directly cut microfluidic channels in PDMS and cyclic olefin copolymer films [23,24]. 3D printing technologies have also begun to show promise for microfluidic device fabrication [25–27]. While these methods do not provide the superior resolution of photolithographic methods, the use of plastic, paper, and laminate substrates are more translatable to scalable manufacturing methods–such as die cutting, hot embossing and injecting molding–to translate a finished prototype into a commercial product. An example of a rapid prototyping method amenable to scaled-up manufacturing is laser cutting. Figure 1 shows a comparison device prototyping using of soft lithography methods versus laser cutting of plastics, laminates, and paper.

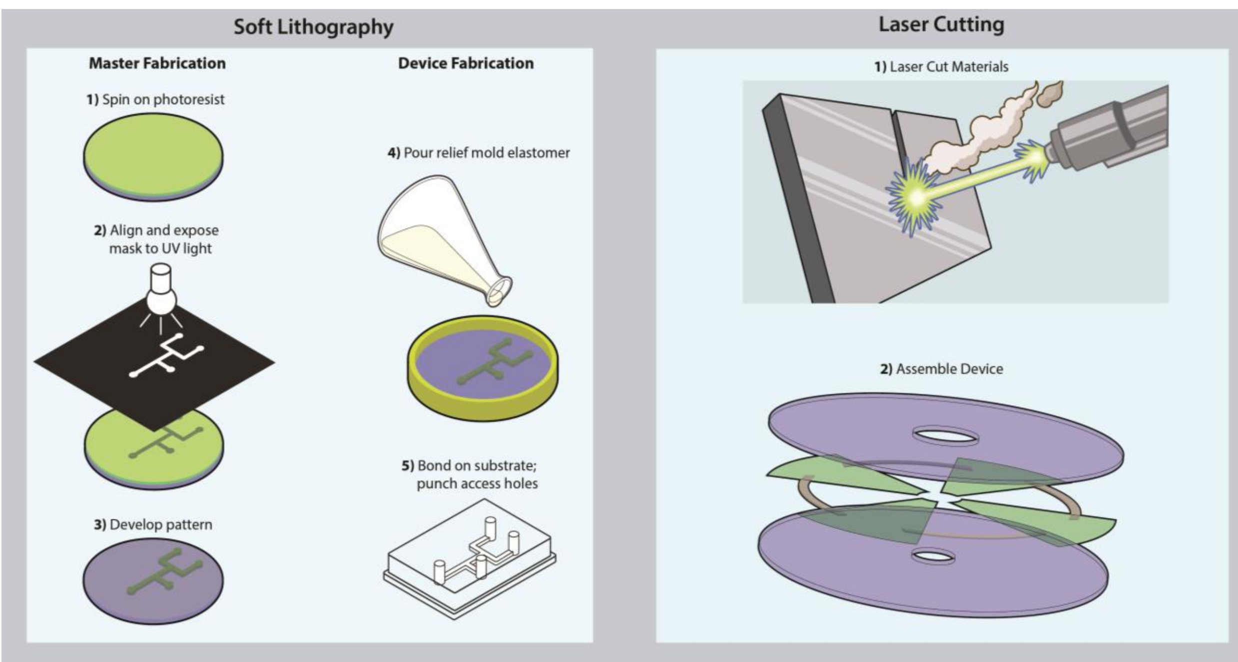

Figure 1. Rapid prototyping using soft lithography vs. laser cutting. (Left) The multi-step process of soft lithography, wherein first a 'master mold' is developed followed by curing a pre-

polymer substrate above, peeling off, bonding to a substrate, and punching access holes. (Right) The more straightforward process of laser-cutting all device parts followed by lamination or thermal bonding to assemble a device.

MAKERSPACES, DIYBIO, AND INTEGRATED THINKING

The investigation of these 'alternative' materials is well-suited for exploration in the emerging ecosystem of community 'makerspaces' [28]. In the broadest sense, makerspaces are physical spaces, usually accessible to the public, where communities are able to access tools—spanning additive and subtractive techniques—for fabricating "almost anything" [29]. Such spaces can be formalized as part of an organization like the Fab Lab network (www.fabfoundation.org), or more informally organized. With over one thousand active spaces around the world, makerspaces have lowered the barrier to accessing fabrication technologies, enabling the exploration of microfluidic rapid prototyping techniques reviewed in this work.

In the past several years, there has also been a growing movement of "Do-It-Yourself" (DIY) biology and similar emergence of "bio-makerspaces" [30] which typically feature tools and basic infrastructure for conducting molecular biology and microbiology projects. As the majority of applications for microfluidics have involved biological systems, we believe the reviewed techniques will also be of interest, and accessible, to DIYBio communities as well.

A key factor in the shift of microfluidic manufacturing from traditional photolithographic methods to 'maker manufacturing' is the push for fully-integrated microfluidic systems that can be readily translated to industry. A major roadblock for lab-on-a-chip devices is plugging and sealing the device to all the interfaces needed (e.g. detection, electric manipulation, inlets/outlets) [31]. For example, Lafluer *et al.* used 3D-printed and paper substrates to develop an entirely integrated sample-to-result nucleic acid amplification test [32]. Kinahan *et al.* used laser-cut acrylic and double-sided pressure sensitive adhesive (PSA) to develop an integrated bi-plex liver assay [33]. These technologies show off the power of 'simple' devices that anyone can make and rapidly scale to bulk manufacturing. To enable others to take part in this type of product design and development, we review the materials and tools used by current researchers to develop these platforms.

MAKER MICROFLUIDICS MANUFACTURING

The below section reviews development of microfluidic platforms using simple materials and manufacturing equipment often found in makerspaces. While microfluidics can be made from of a wide variety of materials and methods, this Opinion focuses on plastics, adhesives, and paper substrates with a brief discussion of the promise of 3D-printed microfluidics.

MATERIALS

Plastics are a popular material choice for microfluidics as they collectively offer a wide variety of properties such as optical clarity, solvent resistance, and scalable manufacturing methods, which have been reviewed previously [34]. Studies have shown promise for polymeric materials with regard to biocompatibility [35], surface modification and integration of functional materials [36], and material autofluorescence [37,38]. Acrylic is one of the simplest and most useful plastics for the makerspace as it has low cost, high optical clarity, wide availability and compatibility with a wide variety of manufacturing tools such as laser cutters. Similar plastics, such as polycarbonate, may be desired for even greater optical clarity and standardization in large-scale manufacturing; however, this material cannot be cut on a conventional laser cutter and specialty contract manufacturers, such as Axxicon (http://axxicon.com), often require large bulk orders to make a profit. For spaces without a laser cutter, materials can be shipped pre-cut by laser cutting services such as Ponoko (www.ponoko.com) at a low cost with no minimum order.

Cut double-sided adhesive tapes are ideal materials for bonding microfluidic architecture to substrates. Selecting a tape adhesive can be a daunting task considering the expansive selection from companies such as 3M (www.3m.com) and Adhesives Research (www.adhesivesresearch.com). The key considerations for selecting a tape are 1) fabrication considerations, 2) tape thickness, and 3) cost/availability. For fabricating a plastic device held together by double-sided thin-film adhesive, cutting microfluidic channels into the adhesive can be challenging if the product is not 'double lintered', meaning both sides of the adhesive have a removable liner. While tape converter companies such as Converters Inc. (www.converters.com) offer to add a second liner, large minimum orders can be cost prohibitive. Converters can be avoided by purchasing tapes that already come with liner on both sides. Another adhesive selection consideration is choosing between a transfer tape and a double-sided tape. Transfer tapes are entirely composed of adhesive material whereas traditional double-sided adhesive have a carrier layer coated on both sides with adhesive. Thus, transfer tapes are typically better suited for thinner applications (<50 µm); whereas, double-sided adhesives are suited for thicker applications (50 – 200 µm). A final consideration is cost and availability of the desired adhesive as the minimum order direct from 3M or Adhesives Research are typically on the range of 1500 foot rolls and can cost upwards of $10,000. Oftentimes, free samples of certain products are available or their products can be purchased in smaller amounts from distributors such as Grainger (www.grainger.com) and Amazon.com (www.amazon.com) depending on availability. Table S1 contains a list of adhesives appropriate for microfluidics.

Paper substrates have gained renewed popularity in 2004 when the World Health Organization (WHO) declared specific performance criteria for developing POC, ultra-low cost diagnostics in low resource settings [39]. Selecting a paper substrate is entirely dependent on the context for its use in applications that include nucleic acid and protein separation, immunoassays and even cell culture [40–43]. GE Heathcare Life Sciences's Whatman line (www.gelifesciences.com) offers a wide variety of paper substrates with thicknesses appropriate for integration into plastic/tape microfluidics and stand-alone devices. Table S2 contains a list of all of the paper substrates used by the authors along with comments to best help guide paper selection.

TOOLS

Laser and plotter cutting are two simple methods for cutting microfluidic channels in plastic, paper, and tape. Both of these methods are similar in workflow–feeding in a substrate to be cut by either a laser or knife. Laser cutters have the benefit of non-contact cutting and higher resolution. These benefits come at the expense of higher capital equipment costs, requirement for a vacuum pump to clear out debris and fumes, and potential burn residue created during the cutting [44]. Plotter cutters (also commonly referred to as vinyl cutters or cutting plotters) are significantly cheaper, require no pumping system, and leave no burn residues. With the growing popularity of makerspaces in both academia and industry, many facilities now have these capabilities already available in a shared space. Table S3 highlights the key differences between laser and plotter cutting.

METHODOLOGY

A simple and enabling methodology for maker microfluidics is Design-Cut-Assemble, shown schematically in Figure 2. This method streamlines rapid prototyping of microfluidic devices using plastics, paper, and adhesive substrates and can be appropriately edited to incorporate different materials and technologies [45]. While more traditional material combinations such as a plastic-adhesive device may seem an easy first step, more creative solutions may also be more efficient such as a paper-adhesive microfluidic origami device [46]. Once the materials are chosen, a computer-aided design (CAD) file must be designed to guide the cutting process. Next, the substrates need to be cut using methods such as laser and plotter cutting. While this report focuses on laser and plotter cutting, 3D printing and CNC-micromilling machines are viable alternatives [26,47]. Finally, once all parts are cut, assembly is typically completed by a manual process such as lamination, thermal bonding or folding. A set of considerations for each step of this process is shown in Box 1.

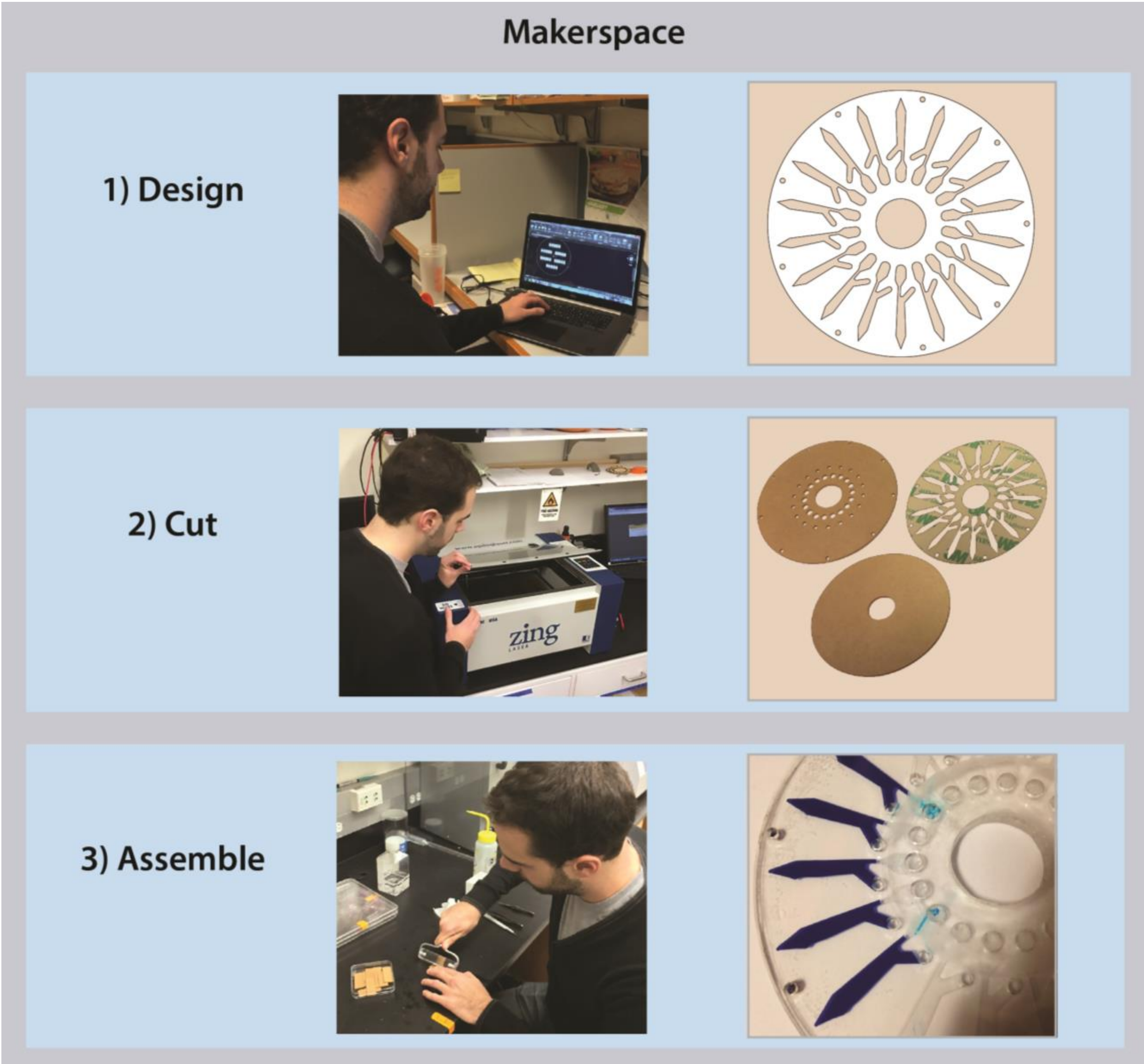

Figure 2. Design-Cut-Assemble methodology: designing device parts in CAD, cutting them out using a laser or plotter cutter, and assembling them using lamination.

3D PRINTING

While Design-Cut-Assemble is a powerful process for maker microfluidics, makerspaces offer other enabling technologies for microfluidic manufacturing. One of the most ubiquitous technologies in makerspaces is 3D printing which has been referred to as the start of a ‘revolution’ in microfluidics [27]. While many devices have been developed, there are still inherent challenges faced by makerspace-available systems such as low optical clarity and material leaching [48]. These challenges are being rapidly overcome by new 3D-printing technologies such as Dolomite’s Fluidic Factory, which can rapidly (20 minutes) produce leak-proof devices out of clear, biocompatible cyclic olefin copolymer instead of traditional resins. While these printing technologies further develop to produce fully integrated microfluidic platforms, current technologies provide another use by fabricating complementary microfluidic components–such as 3D-printed spinners for centrifugal devices, alignment rigs for multi-layered device building, and even common laboratory equipment [49]. These tools are just as important as the microfluidic themselves to produce a complete system that replaces expensive engineering equipment such as syringe pumps and custom fluidic locking connectors. Additionally, the design files for such complementary hardware can be easily shared via repositories such as Thingiverse (www.thingiverse.com) and specifically for microfluidics, Metafluidics (www.metafluidics.org), which is accessible to both technical experts and amateur makers alike.

ACCESSIBILITY AND SCALABILITY OF MICROFLUIDICS

Along with enabling integrated microfluidic system development, maker microfluidics addresses another key limitation in microfluidics–accessibility. The use of simple materials and tools to fabricate microfluidic devices obviates the need for clean room facilities and specialized training in photo- and soft lithography methods. And the application of makerspace principles further allows non-experts in microfluidics to participate. Lesson plans have been developed for students as young as 12 years old to engage in microfluidics, which can be expanded through further makerspace involvement [50]. In contrast to clean room facilities, makerspaces, which include 'biological making' or 'DIYBio,' grant low cost access to capital intensive manufacturing tools, access to a diverse community of individuals from varying backgrounds spanning technical and even non-technical fields, and promote product development through collaboration and innovation [28]. In addition, the cost of makerspace memberships are comparable to monthly gym memberships at $40 - $75 per month, while monthly clean room memberships can cost an academic around $1500 - $3500 and a non-academic almost $10000 per month. Material costs are also considerably different, as soft lithography methods use silicon wafer masters ($6-20 ea., University Wafer), UV masks ($84 mylar mask, Fine Line Imaging), and polymer ($92/kg PDMS kit, Krayden); whereas makerspaces use low cost plastics ($5/sqft [or $13/kg] cast 1/16" acrylic, McMaster-Carr) and adhesives ($2/sqft Double Lintered Adhesive Tape, Amazon.com). The drastic difference in accessibility is underscored in Figure 3 showing a technician at work in a clean room in contrast to a high school group learning in a makerspace.

Figure 3. Contrasting clean rooms and makerspaces (A) A technician working in the George J. Kostas Nanoscale Technology and Manufacturing Research Center at Northeastern University, photo is taken outside the clean room where an orange glass window prevents particular light wavelengths from polymerizing materials inside (Reprinted with permission courtesy of Matthew Modoono and Northeastern University, Boston, Massachusetts). (B) The Technology Office Innovation Laboratory (TOIL) at MIT-Lincoln Laboratory, as an instructor teaches a group of high schoolers how to 3D-print prosthetic hands (Reprinted with permission courtesy of MIT Lincoln Laboratory, Lexington, Massachusetts).

Another key limitation addressed by maker microfluidics is the poor scalability of research-developed platforms to develop into commercial products. In addition to greater compatibility of makerspace materials with large-scale manufacturing methods, makerspaces allow more seamless device integration with upstream and downstream processing. For example, on-chip sample preparation, sample analysis, and optical detection methods can be designed synonymously in the same space for a potentially instrument-free sample-to-result microfluidic system. These advantages come with the loss of the superior feature resolution granted by photolithography methods used in clean rooms (hundreds of nanometers) compared to laser and plotter cutters (tens to hundreds of micrometers). However, innovation of new microfluidic methods, such as inertial and centrifugal microfluidics, has allowed some users to bypass the need for small features, which may be typically required in applications such as cell separations. [51,52]. These methods leverage various inherent physical properties of fluids and particles such as density and size to perform a wide variety of microscale fluid manipulations and processing typically not possible in classic convective flow.

CONCLUDING REMARKS

The benefits afforded by makerspaces, specifically increased participation and the use of low-cost materials and prototyping methods, overcome major barriers to microfluidic device commercialization–accessibility and scalability. And while clean room manufacturing may still provide powerful research-scale solutions to massively multiplexed testing and screening (e.g. drug screening, sepsis diagnostics, and ultra-rare cell types), new innovations in microfluidics have obviated some of the need for the ultra-fine resolution of photolithographic techniques for many clinical applications. Makerspace prototyping promises to increase the success of microfluidics broadly by providing a thriving innovation space for a diverse population to create simple and robust POC microfluidic solutions for current clinical problems.

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OUTSTANDING QUESTIONS BOX

- Can high resolution features be fabricated in makerspaces in a high-throughput manner?
- Can the clean room be moved into makerspaces—similar to the SoftLithoBox by BlackHoleLab?
- Will pipelines be produced to enable microfluidic product development in makerspaces for inventors to rapidly reach the market?
- Will manufacturing standards be developed to easily translate devices between different spaces?
- How will the advancement of 3D printing materials and techniques influence the development of microfluidic devices?
- What novel materials, such as TPX 'breathable' plastic, can be applied to 'maker' microfluidics?
- As makerspaces further penetrate into academic instructions, can 'maker' microfluidic training become a standard for future bioengineers?
- World-to-chip interfaces: how rapidly will the integration of standard parts (e.g. connectors) occur with the simpler fabrication techniques described herein?

Table S1. Recommended adhesives for microfluidics from 3M and Adhesives Research.

Adhesive	Pros	Cons
ARseal 8026 – Clear Silicon Transfer Tape (25 micron)	-Cuts well -Minimal burn residue	-Very difficult to peel and place (too thin, no carrier layer)
ARcare 90445 – Clear Polyester Double-Sided Adhesive Tape (81 micron)	-Popular in microfluidics -Authors' second top choice	-Burn residue may effect PCR and similar reactions
ARcare 92848 – While Polyester Double-Sided Heat Sealing Tape (97 micron)	-Tape seal improves with heat instead of pressure	-Not translucent
ARcare 92712 – Clear Polyester Double-Sided Adhesive Tape (48 micron)	-Cuts well	-Difficult to peel and place (too thin, very sticky) -Burn products
ARcare 90106 – Clear Polyester Double-Sided Adhesive Tape (142 micron)	-Serves well as a single-sided tape	-Opaque liner cuts oddly on laser cutter (burn residue)
ARseal 90880 – Polypropylene Double-Sided Adhesive Tape (142 micron)	-Easiest to cut -Easiest to peel and place -Most forgiving -Pressure activated -Authors' top choice	-Material only available in one thickness
3M 9964 – Clear Polyester Diagnostic Microfluidic Medical Tape (60 micron)	-Easy to cut -Easy to peel and place -Bioassay compatible	-Single-sided adhesive
3M 9965 – White Polyester Double-Sided Tape (90 micron)	-Bioassay compatible	-White (not translucent)
3M 9969 – Adhesive Transfer Tape (60 micron)	-Easy to cut	-Can be difficult to place
3M 468MP – Adhesive Transfer Tape (130 micron)	-Easy to cut -Widely available from distributors (Amazon) -Provides initial repositionability on plastics	-Not targeted for microfluidic platforms

Table S2. Recommended Whatman paper substrates available from GE Healthcare Life Sciences.

Paper	Good for:	Bad for:
Standard 14 and 17 – Glass Fiber	-Holding large volumes of fluid	-Fluorescence microscopy (high background)
Fusion5 – Proprietary Single-Membrane Matrix	-Fluorescence microscopy (low and uniform background)	-Holding large volumes of fluid
CF1, CF3, CF4, CF5, CF6, CF7 – Cotton Linter	-When you need a specific thickness -Fluid transfer	-Fluorescence microscopy (non-uniform background)
CF2 – Cellulose Fiber	-Applications that require sturdy paper	-Does not excel in any particular area
Grade 470	-Blotting paper and gelatinous samples	-Fluid transfer

Table S3. Key differences between laser and plotter cutting for microfluidics. (supplemental)

Laser Cutter (Universal VLS 4.60)	Plotter Cutter (Graphtec CE6000-40)
Easy-to-use	Requires some optimization
Expensive ($22,500)	Low Cost ($1,195)
50 micron resolution	200 micron resolution
Tight Corners	Overcut Corners
Produces burn residue	No burn residue
Cuts plastic, tape, paper	Cuts tape and paper only

Box 1. Design-Cut-Assemble Considerations.

Design Considerations	
Gas Permeability	While some plastic and adhesive materials such as polymethylpentene are gas permeable, most materials are not and may require venting ports
Inputs/Outputs	Connecting tubing to plastic microfluidics can prove challenging, consider a 3D printed connector, using ring magnets as gravity fed wells, or a PDMS block on top
Channel Volume	Designing microfluidic channels based on volume enables simpler protocols
Fiducial Marks	The addition of fiducial or registration marks play a vital role downstream in alignment for device assembly, imaging, and automation. Consideration should be made as to locations, accessibility, and orientation of fiducial markings at an early stage.
Fluidic Considerations	Consider the path of fluids through your device, for example sharp corners and rapid expansions can often hinder fluidic movement and lead to bubbles; also, gas permeable devices may lose fluid due to evaporation
Cut Considerations	
CAD Software Selection	Most CAD software can produce acceptable file formats for cutters (*.dxf, *.dwg), oftentimes cutters are directly compatible to select CAD software
Cutting Lines	Ensure no repeated lines are in the drawing to prevent redundant cuts
Cutting Resolution	Best resolution can be achieved by keeping the material as flat as possible when cutting, use painter’s tape on edges of thin substrates to prevent blowing away on laser cutters or an adhesive backing to prevent unwanted skewing and bowing on plotter cutters
Cutting Force	Trial-and-error of laser power/speed and plotter knife force/speed/cut-style is important to get the best cut, an ideal cut for double-sided adhesive would only cut through the first liner and adhesive layer while keeping the bottom liner intact (which will prevent feature ‘droop’ during the assembly process)
Design vs. Cutting	While a design may look perfect on CAD, the order of cuts may cause a feature to blow

	away or skew during cutting, consider redundant or incomplete cuts that can be manually completed afterwards to overcome these issues
Assembly Considerations	
Cleanliness	Dust removal is important for microfluidics, a simple cleaning protocol is using a mild detergent and a sonic toothbrush to directly clean plastic surfaces, followed by a wash and dry with pressurized gas or a microfiber cloth, be wary of harsh organics which may damage substrates
Feature Removal	Use tweezers to remove all unwanted features cut out from adhesive before assembly, it is best to only remove the top liner and adhesive to prevent feature ‘droop’ during assembly
Peeling Off First Liner	Peeling off the top liner from cut adhesive is best done in one continuous motion if possible, tweezers are useful in complicated areas
Alignment	Using a simple alignment rig (such as a dowel for disc devices) is recommended for aligning adhesive on substrates
Lamination	A laminator or even a smooth laminating roller (McMaster-Carr #7533A12) to apply heavy pressure is important to activate most adhesives to set devices together
Adhesive-Paper Integration	When a paper substrate is integrated into a thin-film adhesive layer, apply additional lamination pressure at the boundary between adhesive and paper to best seal the device

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