3D printing: an emerging tool for novel microfluidics and lab-on-a-chip applications

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Abstract

In the past few years, 3D printing technology has witnessed an explosive growth, penetrating various aspects of our lives. Current best-in-class 3D printers can fabricate micrometer scale objects, which has made fabrication of microfluidics devices possible. The smallest achievable resolution is already at nanometer scale, which is continuing to drop. Since geometric complexity is not a concern for 3D printing, novel 3D microfluidics and lab-on-a-chip systems that are otherwise impossible to produce with traditional 2D microfabrication technology have started to emerge in recent years. In this review, we first introduce the basics of 3D printing technology for the microfluidic community and then summarize its emerging applications in creating novel microfluidic devices (MFDs). We foresee widespread utilization of 3D printing for future developments in microfluidic engineering and lab-on-a-chip technology.

Keywords: 3D printing, Additive manufacturing, Microfluidics, Lab-on-a-chip

1. Introduction

With the goal of shrinking bulky and costly laboratory equipment onto small, user-friendly, easily replicable chips, lab-on-a-chip (LOC) technologies have been revolutionizing many fields such as medicine, chemistry, and biotechnologies (Barry and Ivanov 2004; Beebe et al. 2002; Dittrich and Manz 2006; Hong and Quake 2003; Stone et al. 2004; Thorsen et al. 2002; Wang et al. 2009; Weibel and Whitesides 2006; Whitesides 2006; Xu 2014; Guo et al. 2015). Fluid flow at the characteristic dimensions of microfluidic devices (MFDs) exhibits unique behavior not otherwise replicable at macroscopic scales (Karniadakis et al. 2006; Squires and Quake 2005).

Currently, most microfluidic fabrication techniques are largely constrained by the complexity of real 3D structures, and limit researchers' ability to produce complex 3D flow paths with nonstandard cross-sections and of differing sizes and directions. Fortunately, the rapidly advancing 3D printing technology, also known as additive manufacturing (AM) technology, has brought us a possible route to overcome this problem. In this technology the structure of interest is sliced into numerous 2D cross-sections, and hence, the production becomes a straightforward layer-by-layer fabrication process. Such direct approach for microfabrication is rapidly becoming

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established as an attractive field of interest to microfluidic engineers. The intent of this article is to introduce the state-of-the-art 3D printing technologies and its applications in fabricating microfluidics for novel experiments. We will focus on emerging applications in the past two years, and provide our perspectives on possible future directions.

1.1. 3D microfluidics

Complicated 3D microproducts such as medical devices, micro-optical systems, integrated microsensors, etc., significantly contribute to the evolution of various fields in MEMS, microfluidics, and lab-on-a-chip technologies. However, fabrication of 3D microstructures with arbitrary geometries has been a great challenge in micromechanics/microfabrication field (Vaezi et al. 2013).

Over the years, various studies have demonstrated or predicted that microfluidics may exhibit unique phenomena in complex three-dimensional structures that can be harnessed for novel applications (Fan et al. 2015). Another reason to construct 3D microstructures is to provide a virtual environment that replicates, to a large extent, the physical condition appropriate for the growth of cells or microorganisms (Hou et al. 2014). In biological assays, for instance, 3D cell culture can be implemented in microfluidic devices by taking advantage of all the properties provided in an artificial microenvironment. However, cellular responses can be substantially modified in 3D instead of 2D microfluidic structures. In fact, 3D culture mimics physiological conditions *in vivo*, allowing experiments to be conducted with a more clinical or biological relevance (Justice et al. 2009; Chan et al. 2013) compared to culture in a Petri dish or flask. Moreover, in bio-micro-systems, 3D microfluidic environment enables an efficient observation and analysis of the microorganism dynamics and functions; an imperative task in studying biological cells (Hanada et al. 2008). Similarly, in the separation of biological cells, 3D geometries enable attractive control over the separation efficiency of circulating tumor cells (CTCs) (Zhang et al. 2014; Aghaamoo et al. 2015).

Nevertheless, in recent years we have seen explosive growth in the fabrication of 3D microstructures, using various techniques and materials. Fabrication of such devices is an integral part in many industries comprising optics, electronics, and biotechnology (Alting et al. 2003) in the development of highly functional applications. Continuing advancements and developments of various manufacturing processes will inevitably influence the field of MFD fabrication.

1.2. Traditional fabrication limits for 3D MFDs

An overview of the most cited techniques reported as an attempt to fabricate 3D microfluidic structures shows the widespread use of glass, silicon, and polymer manufacturing. Polymer-based microfabrication includes replication and direct micromachining, while glass- and silicon-based methods involve surface and bulk micromachining (Mitra and Chakraborty 2011).

Bulk micromachining is the oldest MEMS technology (Ziaie et al. 2004). Traditional bulk micromachining simply applies a chemical etchant to the substrate to form the desired structures (Fang et al. 2015; French and Sarro 1998). However, the possible 3D structures that can be made are very limited. On the other hand, *surface micromachining* techniques use a sequential process of thin-film deposition, patterning, and selective etching on the surface of a

substrate, and can obtain certain 3D structure for microfluidics applications (French and Sarro 1998).

Micromachining technology has continued to advance, and many researchers have reported complex micromechanical devices fabricated by taking advantage of surface micromachining (Galambos et al. 2004; Lin et al. 2000; Okandan et al. 2001). For instance, Okandan and co-workers produced monolithic chips with tightly-integrated valves, pumps, and micromechanical cell manipulators by incorporating a layer of silicon nitride into the device blank (Okandan et al. 2001). They also fabricated flow channels with integrated electrodes useful for electroporation, vesicle fusion, or any other bioMEMS application requiring the manipulation of electromagnetic fields. However, neither of these two techniques became widespread for 3D microfluidics fabrication due to their intrinsic limitations. For instance, bulk micromachining cannot offer precise control of channel height because of spatial etch-rate variations (Fang et al. 2015). Moreover, surface micromachining is largely constrained by the availability of an etchant required to etch a sacrificial layer without imposing any damage to the mechanical layers or the substrate.

Over the years, polymers have been introduced as an alternative to silicon and glass mainly because of their lower cost of manufacture, increased biocompatibility, and resistance to chemical attack. On this basis numerous techniques for polymer-based manufacturing have been proposed. These techniques can be divided into two major groups; *direct micromachining* and *replication* (Mitra and Chakraborty 2011).

Direct Micromachining techniques are a set of more evolved procedures for the microfabrication of 3D objects. *Laser-assisted subtractive micromachining* such as laser ablation (Papakonstantinou et al. 1999; Rötting et al. 2002; Wang et al. 1999) and laser cutting (Schuettler et al. 2005) are examples of direct micromachining technology. Rapid fabrication of polymeric microfluidic devices in *laser micromachining* may be accomplished by direct patterning for single prototypes or laser-assisted indirect procedure to produce larger volumes (Malek 2006). In either case, a high-intensity laser beam focused onto the material evaporates the polymer at the focal point, leaving a void in it.

Replication micromachining techniques, as the name suggests, are composed of a series of steps which result in the replication of mold or master, using a polymer-based material. The process may include the compression of a polymer onto a heated mold (*hot embossing*) (Heckele and Schomburg 2004), or the injection of polymer into a pre-heated chamber inside which the mold is located (*injection molding*) (Becker and Locascio 2002; Do and Ahn 2008; Liou and Chen 2006). Sometimes the material used for fabrication might be a mixture of two compounds in its liquid state, or the so-called pre-polymer, which finally acquires the shape of the mold after the hardening process triggered by exposing the liquid to heat or ultraviolet (UV) light (Mitra and Chakraborty 2011). *Soft lithography*, one of the most widely used replication methods (Xia and Whitesides 1998) belongs to this type.

Soft lithography refers to a range of microfabrication techniques sharing a common feature: the use of a patterned elastomer, typically polydimethylsiloxane (PDMS), as a stamp or replica mold to transfer features to a desired substrate (Xia and Whitesides 1998; Zhao et al. 1997). Soft lithography is an attractive alternative to photolithographic etching techniques due to its much-reduced materials costs and mild reaction conditions: often, assembly can be carried out on the benchtop. Since its inception, soft lithography has quickly become prevalent in producing all kinds of microfluidic devices, and accordingly, a robust library of engineering solutions exists in

the literature for soft lithographic microfluidic devices.

Despite its advantages compared to earlier methods, soft lithography is only capable of producing 2.5D microparts, and microfluidic structures with high aspect ratios are difficult to produce reliably (Folch 2012). New innovations in fabrication techniques are necessary to provide flexibility in design and fabrication of arbitrary 3D microparts.

Other manufacturing methods such as Lithography Galvanoforming Abforming (LIGA) (Bertsch et al. 1998; Ehrfeld and Schmidt 1998), electroplating (Beuret et al. 1994), microextrusion (Saotome and Iwazaki 2001), micromechanical cutting (Ruprecht et al. 1997), micromilling (Guckenberger et al. 2015; Zhang et al. 2011; Hashmi et al. 2013), paper stacking (Li and Liu 2014), etc., have also been used in the past but are not very practical for the fabrication of complex microfluidic structures. Moreover, these techniques typically require costly machinery and expensive materials, and are often not compatible with microfluidics requirements.

To summarize, many techniques have been explored for the microfabrication of 3D objects. However, traditional microfabrication methods are hindered by major setbacks to fabricate complicated 3D structures necessary for more compact or highly-multiplexed lab-on-a-chip devices. Nowadays, more and more approaches of microfabrication techniques to produce 3D microfluidic structures are based on a layer-by-layer additive process.

2. Additive manufacturing

As defined by American Society for Testing and Materials (ASTM International), additive manufacturing (AM), is a process of making objects from three-dimensional solid model data by joining materials layer-by-layer. It is also known as rapid manufacturing, rapid prototyping, 3D printing, solid freeform fabrication or direct digital manufacturing (Kruth 1991; Kruth et al. 1998). Additive manufacturing technology was first developed in the 1980s (Gibson et al. 2010; Huang et al. 2013; Kruth 1991). Since then, significant progress has been made in improving feature resolution, manufacturing speed, and reliability. While additive manufacturing technology was mainly suitable for rapid prototyping in its infancy, the technology is growing as a reliable method to design and manufacture functional products (Bourell et al. 2009; Wong and Hernandez 2012). Today, additive manufacturing processes offer widely attainable, effective methods for producing complicated microstructures at comparatively low cost and with reduced investment in manufacturing infrastructure.

3D printing began with stereolithography (SL) technology, first introduced by the US company 3D Systems. In this laser based technology a pre-polymeric resin is cured with UV light in the shape of a 2D cross-section of one layer of the finished device. Following a similar procedure, other layers are built up successively to form the desired 3D structure, and the uncured resin can be washed away, leaving the final structure (Zhang et al. 1999). Compared to tediously slow soft lithography and other traditional fabrication techniques, stereolithography offers an automated fabrication technique of 3D geometries which is more convenient, faster and more cost-efficient (Au et al. 2014). Many approaches of stereolithography can be found in the literature (Zhou et al. 2015; Pan et al. 2012a; Lee et al. 2015; Park et al. 2009), including two-photon approaches which substantially increase the resolution and curing depth achievable with this technique. Fig. 1 represents briefly three typical configurations of stereolithography.

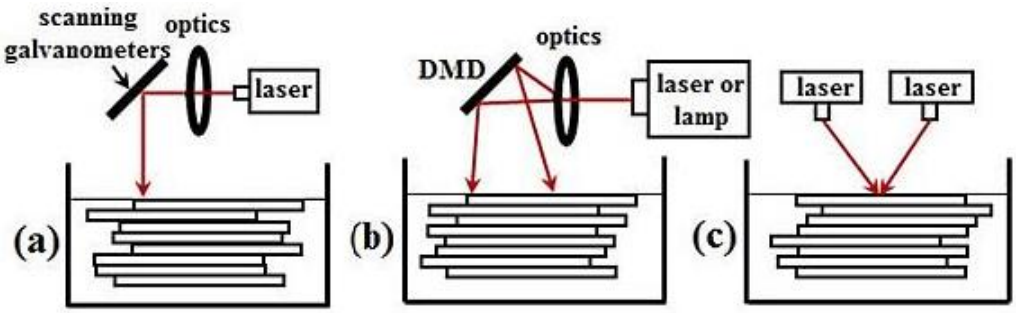

Fig. 1. The principle of stereolithography (SL); photopolymerization of curable-resins to build layers of the finished part. (a) Vector scan SL or point-wise approaches widely used in commercial SL machines; this method guides a laser beam to scan the resin surface and cures the resin point-by-point within each layer. (b) Mask projection SL or layer-wise approach; a variation of SL in which a digital micromirror device (DMD) projects 2D mask images on the resin surface and cures the resin layer-by-layer. (c) High resolution two-photon SL approach in which femtosecond laser energy is used for curing the resin point-by-point.

2.1. Fundamentals

Unlike traditional manufacturing methods, AM technology came about as a result of the development of computer-aided-design (CAD) technologies.

The process of 3D printing begins with the designer producing a CAD file for the specified geometry of the part to be made, which then need to be converted to a surface tessellation (STL) file describing a polygonal representation of the part's surface geometry. The file is transferred to a computerized system, where the digital representation is "sliced" into virtual horizontal layers of varying thicknesses by computer software. The manufacturing system then builds each layer individually, with each successive layer added to the previous one. This bottom-up build process is repeated until the part is completed.

Due to the layer-based nature of additive processes, a key aspect of additive manufacturing is its ability to quickly produce components which would require extensive processing through subtractive techniques. Furthermore, the layer-based process allows for the design of almost any geometry, a drastic expansion of the previously constrained design space (Berman 2012; Crump 1991; Gershenfeld 2012). The elimination of tooling, the ability to manufacture complex geometries, and the selective placement of material only where necessary, contribute to an expansion in design freedom and an increase in process efficiency (Hopkinson et al. 2006; Pan et al. 2012b; Sreenivasan et al. 2010). In 3D printing technology, product design is not constrained by principles of design for manufacturing and assembly (DFM/DFA); hence such design freedom greatly enables product innovation.

2.2. Classification

Numerous additive manufacturing processes have been developed by using different energy sources and material accumulation mechanisms. Mask image projection stereolithography (MIP-SL) (Zhou et al. 2013), electrochemical fabrication (EFAB) (Cohen et al. 1999), fused deposition modeling (FDM) (Tsang and Bhatia 2004), inkjet printing processes (Abe et al. 2008), selective laser sintering (SLS) (Dickens Jr et al. 1999), electron

beam melting (EBM) (Murr et al. 2012), etc., are only a few examples of additive techniques.

There are a handful of potential classification systems for additive manufacturing processes. A straightforward approach is to classify AM processes according to its baseline technology (Burns 1993; Kruth et al. 1998), for example, inkjet printing, extrusion, electrophotography or photopolymerization, etc. A more general approach is to classify AM processes according to the type of feedstock material used (Kai et al. 2003; Kruth 1991). By using this approach, AM processes could be classified into liquid-based processes, solid-based processes, and powder-based processes (Kruth 1991). More comprehensive classification methods have also been proposed. For example, a two dimensional method was proposed by Pham (Pham and Gault 1998) to classify AM processes according to both the type of materials and also the processing dimensions. Among all those classification approaches, a family tree classification developed by Kruth (Kruth 1991) and modified by Wong and Hernandez (Wong and Hernandez 2012) is very clear and inclusive. Fig. 2 is a representation of such categorization adapted from the classification scheme of these authors.

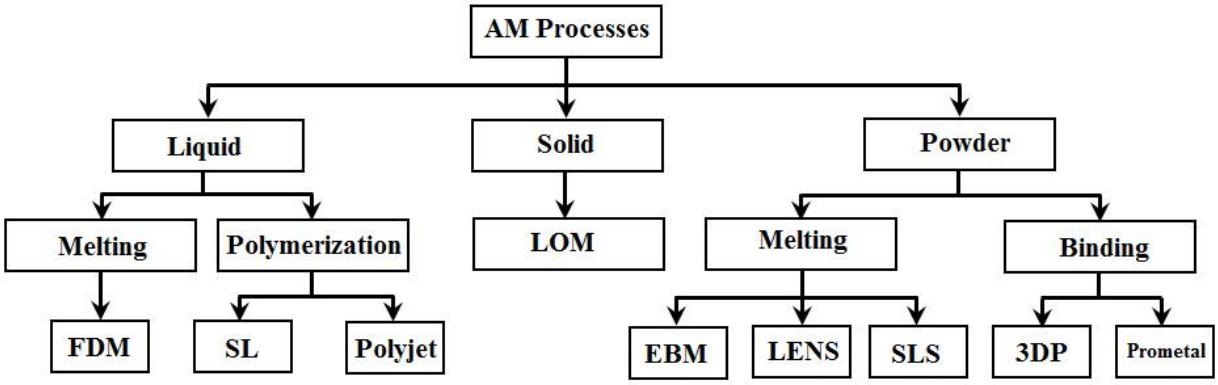

Fig. 2. Additive Manufacturing processes classification. Readers are referred to appendix containing a glossary of terms used in this classification. Reproduced with permission from Elsevier (Kruth 1991; Wong and Hernandez 2012).

2.3. Materials

Polymeric material, waxes, and paper laminates were the first materials used for AM processes. Subsequently, attempts have been made in improving mechanical properties by introducing composites, metals, and ceramics. For instance, various lightweight structures (Hutchinson and Fleck 2006; Hutmacher et al. 2001; Moon et al. 2014; Ning and Pellegrino 2012; Williams et al. 2011; Zheng et al. 2014) and composites have been developed to fabricate functional products using AM technologies (Campbell et al. 2012).

Selection of materials for AM highly depends on the fabrication technique. As a few examples, powder based techniques are dependent upon powder particle size, and thus a wide range of materials including metals, ceramics, and polymers can be used. In these techniques accurate production and smoother parts are often produced using finer particles, while larger particle size facilitates powder delivery and process. On the other hand, however, finer particles are difficult to spread and handle, and larger particle size hinders surface finish, minimum feature size and minimum layer thickness. Moreover, in liquid based techniques radiation-curable resins, also known as

photopolymers, are the primary materials for the fabrication. In solid based AM processes, however, a wide variety of materials including polymers, metals, paper, and ceramic are used. In these techniques the object is formed by simply trimming a sheet of material as one layer of the finished device (Gibson et al. 2010).

2.3.1. Polymers

Most polymers used in AM technologies are divided into two main categories: photosensitive polymers, and thermoplastic polymers. The first group is widely used in photopolymerization based 3D printing like SL, and includes acrylate, epoxy, or hybrid resins. Hybrid resins, such as epoxides with acrylate content, are broadly used in SL processes so as to increase the integrity of layers during the fabrication and the strength of the finished parts. More importantly, acrylate/epoxy resins enable the fabrication of transparent and biocompatible MFDs with high resolution.

Second group, thermoplastic polymers, includes acrylonitrile butadiene styrene (ABS) and polylactic acid (PLA) that are widely applied for extrusion based AM such as FDM. In FDM process, for instance, fabrication materials comprised of polymers are extruded in a viscous paste rather than in a lower viscosity form, as this technique is based on melting of polymer filaments. Unfortunately, PLA and ABS are usually non-transparent and non-biocompatible. Therefore, for MFD design and fabrication, attempts have been made to print masters that are made of PLA and ABS to be used for PDMS soft lithography. This will be discussed in detail later in this review.

Table 1 lists more examples of polymers with some of their properties used in additive manufacturing of MFD. For example, MED610, from Stratasys, Ltd. (Eden Prairie, MN), is a biocompatible Polyjet photopolymer. It is a rigid medical rapid prototyping material, featuring high dimensional stability and colorless transparency. The material is ideal for applications requiring prolonged skin contact of more than 30 days and short-term mucosal-membrane contact of up to 24 hours (Stratasys).

Table 1. Polymers used in additive manufacturing of MFDs (MakerBot ; 3DSystems ; Stratasys).

Type	Glass transition temperature T_g (℃)	Transparent?	Biocompatible?	Example
ABS	~105	No	No	MakerBot ABS filament
Acrylate/epoxy	45~200	Choice of transparent or non-transparent	Choice of biocompatible or non-biocompatible	-
E-Glass	NA	Yes	NA	EnvisionTEC
E-Guard	~109	Yes	Yes	EnvisionTEC
E-Shell 300, 600 Series	86~160	Choice of water clear, rose clear, red, and blue	Class-IIa Biocompatible	EnvisionTEC
MED610	52~54	Yes	Yes	Stratasys
PLA	45~60	No	No	MakerBot PLA filament
RGD720	48~50	Yes	No	Stratasys

Veroclear RGD810	52~54	Yes	No	Stratasys

2.3.2. Composites

In terms of composite materials, tremendous progress has been achieved during the past decade to expand the material choice. Composites made of multiple photopolymers (Khalil et al. 2005), multi-metallic materials (Santosa et al. 2002; Jackson et al. 2000; Liew et al. 2001; Liew et al. 2002), metal/polymer particles and liquid (Wurm et al. 2004; Kumar and Kruth 2010; Bartolo and Gaspar 2008), were developed during this time. As a few examples, Zhou and co-workers developed a multi-material mask image projection stereolithography (MIP-SL) system, using composites of photopolymers (Zhou et al. 2011). Also, Pan and co-workers developed an electro-stereolithography process for composite printing by combining electrostatic deposition of metal powders and photo-curing of polymers (Pan et al. 2015). Pascall et al. developed a metal/polymer composite printing process by using light-directed electrophoretic deposition (Pascall et al. 2014), and Hwang et al. investigated a composite filament made of ABS polymer mixed with copper and iron powders for extrusion based 3D printing processes (Hwang et al. 2015).

Although these newly developed composite additive manufacturing technologies have not been adopted for microfluidic device fabrications yet, the recent advancement demonstrates a potential of printing more functional microfluidic devices in the future.

2.4. Applications

Besides the widespread usage of 3D printing in MFD design discussed in this review, this technology contributes significantly to the fabrication of medically related products with a broad range of biological and physical properties (Michalski and Ross 2014). It is expected that AM technologies will rapidly revolutionize healthcare by combining the 3D medical imaging and manufacturing technologies.

More specifically, researchers have become interested in aspects of AM technology that can be incorporated in tissue engineering, medical implants, anatomical models, customized prosthetics, and pharmaceutical research (Ventola 2014; Klein et al. 2013). For instance, the incorporation of 3D imaging such as Computerized Tomography (CT) into AM techniques will lead to an exponential growth of implants fabrication and anatomic models (Mitsouras et al. 2015).

3D printing have also exhibited interesting applications for aerospace and automotive engineering (Gibson et al. 2010). AM offers flexible and cost-effective designs of very complex geometries that would otherwise take many steps to fabricate by using non-AM methods. Recently, 3D printing technology has been used for designing light-weight wing structures for macro- and micro-size Unmanned Aerial Vehicle (UAV) (Moon et al. 2014; Tan and Moon 2014).

3. Challenges and promises

Despite the benefits of AM and rapid advancements in the past decades, AM technology still faces many

challenges for MFD fabrication in terms of material, resolution, marketing, etc., as discussed in the followings.

3.1. Material

3.1.1. Availability

Many AM technologies suffer from the limited choice of materials available for the production of functional devices. One approach to use diversity of materials is the generation of master microstructures using 3D printing technology with the 3D printable resins. Ren and co-workers followed such process to finally fabricate microfluidic devices with PDMS or other materials such as hydrogel (Ren et al. 2013). Chan and co-workers took the same measure and a post-treatment technique to prepare 3D-printed masters suitable for PDMS molding (Chan et al. 2015). Fig. 3 briefly describes their strategy for single-step molding of 3D networks.

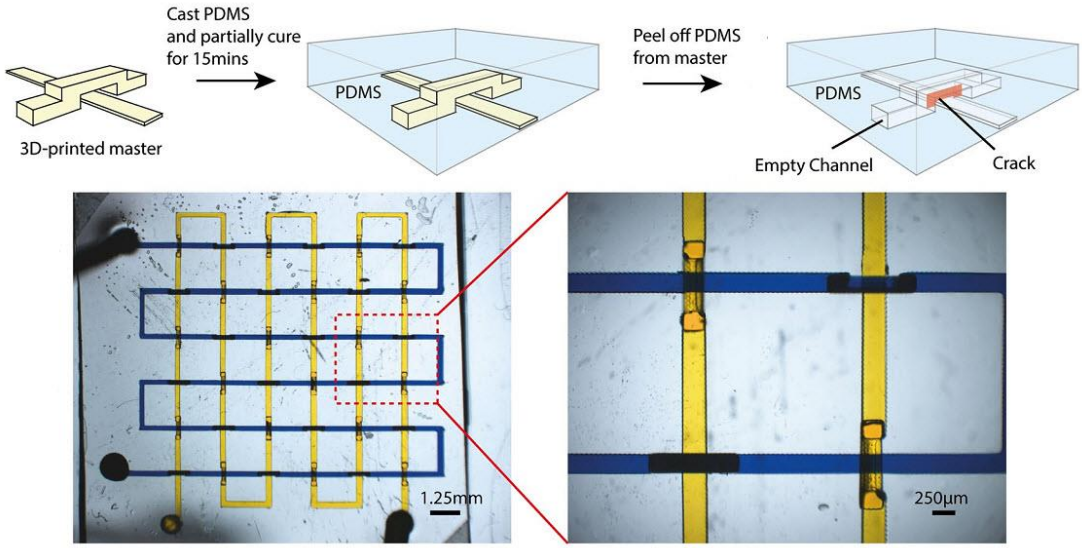

Fig. 3. Using single-step soft lithographic approach to fabricate cross-over geometries. The crack formed during peeling process can be healed after heating. (Top row) A schematic of the fabrication technique of cross-over links. (Bottom row) Permanently healed 3D chip composed of basket waving network with yellow and blue dye solution for optical detection. As shown by the bottom right image, no mixing occurs due to the cross-over feature of the network. Reproduced with permission from Springer (Chan et al. 2015).

More recently, Saggiomo and co-workers proposed a low-cost easy scaffold-removal fabrication method to manufacture truly 3D microfluidic networks using ABS and PDMS (Saggiomo et al. 2015). In this fabrication method the ABS plastic scaffold are first 3D-printed and then suspended into liquid PDMS. Finally the scaffold is dissolved in a solvent, leaving the final structure. To overcome the limitations of material properties and surface finish, other approaches like multidirectional deposition and non-planar layer building (Bourell et al. 2011; Choi and Chang 2006; Dutta et al. 2011; Mason 2006; Milewski et al. 1998; Pan et al. 2012a; Pan et al. 2014; Ruan et al. 2010; Song et al. 2015; Zhao et al. 2013) have been proposed and adopted in many variations of the conventional AM processes.

3.1.2. Biocompatibility

Recently, Ho and co-authors reviewed various 3D printing technologies in bio-microfluidic devices (Ho et al. 2015). According to their study the advent of 3D printing is a huge benefit for medical and biological applications and will change the perceived limitations in the design of experiment for biological assays.

Current materials for AM processes have shown less strength and durability, and their mechanical properties are known to degrade over time. However, most of them are polymer-based which makes them suited for disposable medical and biological applications (King 2009). Biocompatible polymers based on polycaprolactone (PCL), are widely used the field of tissue engineering. For instance, custom-built 3D printing is used to fabricate bioartificial patient-specific bone grafts by employing PCL (Temple et al. 2014). In another study, PCL based composites have been introduced, and were shown to be promising materials in the field of bone regenerative medicine (Gonçalves et al. 2015). Hydrogels, another biocompatible material, were also applied for 3D printing of components that have applications in tissue engineering, drug screening, and organ on a chip models (Stanton et al. 2015). However, despite those advances, there still remains a lot to be discovered in the field of biocompatible material for AM processes.

With more and more novel materials being invented every day, we should expect to see an explosive growth in utilizing 3D printing to realize devices for biomedical studies. For example, in a recent study he and co-workers modified a desktop 3D sugar printer which enables fast print of microfluidic chips that is suitable for cell culture studies, opening up potential use of their technique for biological assays (He et al. 2015). Another study by Rogers et al. has reported a novel polyethylene glycol diacrylate (PEGDA) stereolithographic resin (Rogers et al. 2011), and pneumatic microvalves (Rogers et al. 2015; Rogers et al. 2014) produced in that resin using SL. PEGDA, similar to PDMS, exhibits several properties desirable for microfluidic and bioMEMS applications, and is demonstrably less prone to nonspecific protein adsorption than PDMS.

3.1.3. Transparency

In many recent developments (Anderson et al. 2013; Au et al. 2015; Bonyár et al. 2010; Comina et al. 2013), the stereolithographic resins used to realize new advances in lab-on-a-chip fabrication (Comina et al. 2013) and microfluidic machinery (Au et al. 2015) are proprietary in nature, providing reliability and convenience at the expense of transparency and manufacturer lock-in. The opaque materials used in many AM processes hinder the liquid flow visualization, as shown in Fig. 4, necessary in most microfluidic devices.

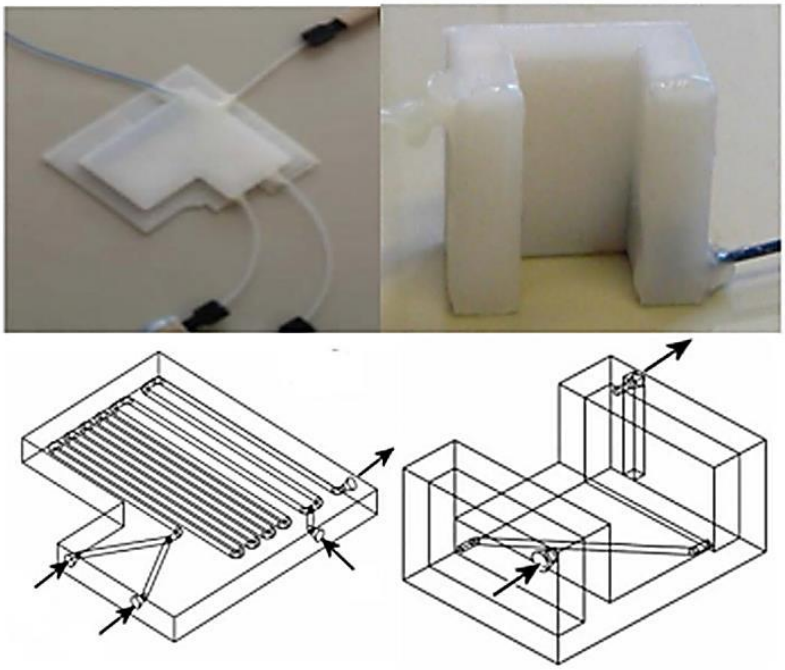

Fig. 4. 3D-printed reactionware for polyoxometalate syntheses with the CAD drawings given in the bottom row; (Left) three inlet device and (Right) one inlet device. Reproduced with permission from RSC (Kitson et al. 2012).

Researchers have taken measures to improve the optical transparency of printed devices. Employing dynamic mask SL, Shallan and co-workers fabricated transparent 3D microfluidic to millifluidic devices including micromixers by taking advantage of colorless resins (Shallan et al. 2014). This proprietary resin consists of a modified acrylate oligomer and monomer, an epoxy monomer, a photoinitiator, and additives. Likewise, a colorless resin used by Au and co-workers to stereolithographically print fluidic valves and pumps for cell culture applications (Fig. 5) (Au et al. 2015). For future directions, developing devices for on-chip observation may require chemical polish. This becomes highly important as conventional methods for polishing are difficult to perform for inaccessible areas (Gross et al. 2014).

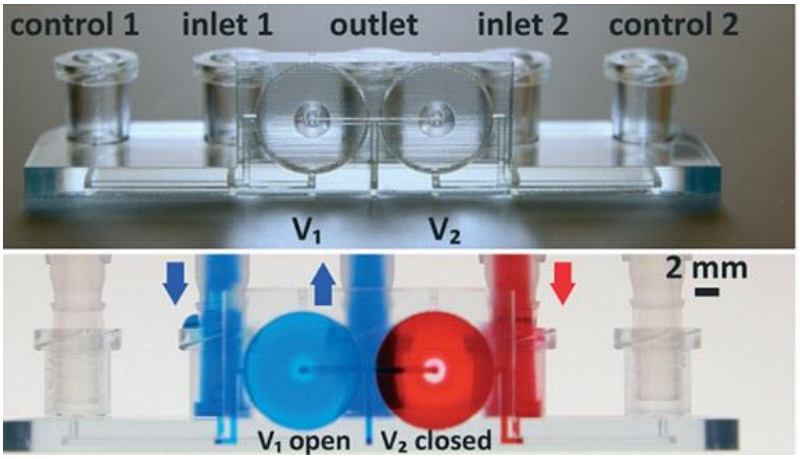

Fig. 5. (Top) 3D-printed two-valve switch and (Bottom) dye-filled switch in an actuation state. Reproduced with

permission from RSC (Au et al. 2015).

3.2. Resolution

Powder-based techniques offer reasonably high resolution. However, as to the fabrication of microfluidic devices, removal of the excess materials used in the process makes these techniques unsuitable for internal microfluidic channels (King 2009). For instance, parts printed with SLM require extensive cleaning, due to powders being stuck on solid details, making the production of micro-cavities highly challenging. Resolutions of metallic AM processes like SLS and EBM are also limited by the focus of power source and powder sizes (Clare et al. 2008; Gong et al. 2012), and the spatial resolution is typically in the range of $10 \sim 500\ \mu m$.

Liquid-based techniques, such as the FDM process, are popular for small parts production. However, the achievable resolution is not suited for microengineering (King 2009). FDM printed parts have a rough surface due to the printing method which involves tracing the outer shape of the reference CAD model with circular cross-section plastic pieces. In fact, the achievable resolution for FDM process is limited by the xy-plotter, the z-stepper motor, the thickness of the filament, and the extrusion nozzle diameter (Fig. 6) (O'Neill et al. 2014).

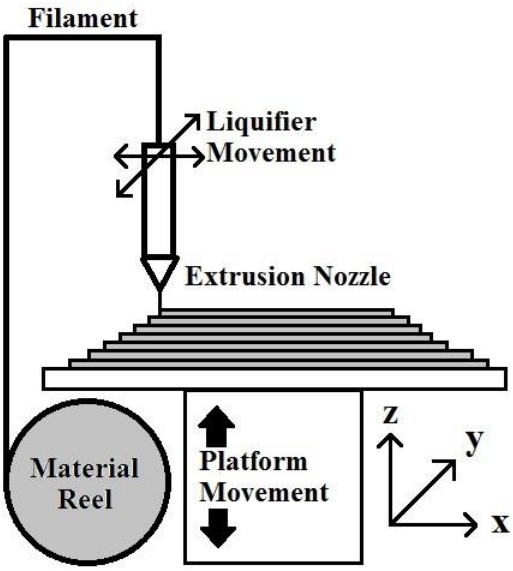

Fig. 6. Principle of FDM operation; the heating chamber liquefies polymer, and then it is fed to the system as filament.

Moreover, resolution is often inversely proportional to the price of job throughput, build area, and speed. Achieving higher printing speed requires faster print head movement, using large bead size and/or utilizing parallel processes (Keating 2014). Recently, O'Connor and co-workers addressed the effect of orientation on the hydrodynamic characteristic of 3D-printed polymer microchannels (O'Connor et al. 2015). This research group used a Project HD 3500 3D-printer with a layer resolution of $\pm 16\ \mu m$. They came to the conclusion that, unlike conventional etched silicon approaches, contemporary polymer 3D printing techniques may produce cross-sections with less resemblance to the nominal shapes and this might get even worse when changing the print orientation (vertical to horizontal). However, due to the speed of the prototyping, they are useful at the early stages of design and experiment.

With the rapid development of AM technology, the most advanced approaches like micro-stereolithography (Cheng and Lee 2009; Choi et al. 2006; Choi et al. 2009; Ikuta et al. 1996; Park et al. 2011; Sun et al. 2005; Xu et al. 2006), and two-photon technology (Galajda and Ormos 2001; Kawata et al. 2001; Maruo and Kawata 1998; Park et al. 2006) could achieve sub-micron and even nano-scale resolution.

3.3. Commercialization

Table 1 represents a list of commercialized 3D printers that have been applied for the fabrication of components in microfluidics networks. Three main factors including resolution, build area, and build speed determine the price for AM machines (O'Neill et al. 2014). Most commercially available printers utilize FDM technology that is, due to the cost, more attractive to the consumers compared to laser-based machines that possess high resolution and large build volumes. While a typical FDM has shown relatively low accuracy and speed in comparison to SL and MIP-SL, it only costs about $500 (Pryor 2014).

Table 1 also shows that machines employing SL (or Digital Light Processing (DLP), a slight technology variation) tend to have a finer xyz resolution than their FDM and SLM (Selective Laser Melting) counterparts, which make them suitable for micro-scale cavity printing. As to the printing material, the machines listed in Table 1 can take a range of materials, from regular PLA to more proprietary materials, like the E-Shell series from EnvisionTEC. The quality of 3D-printed parts increases as the materials become less common; proprietary materials are designed to print with greater accuracy than their more common counterparts, ABS, PLA and others. Recently, machines like the Objet Connex 3D printer from Stratasys and ProJet 5500X from 3D Systems have entered into the market with the capability of printing multiple photopolymers in one build (Stratasys ; 3DSystems). This progress will contribute significantly to expand the material choice for 3D-printed components.

Only a handful of the machines in Table 1 offers layer thicknesses in the range which allows the fabrication of MFDs. Comina and co-workers employed a micro-stereolithography MiiCraft printer with $\sim 56\ \mu m$ and $\sim 50\ \mu m$ for lateral and vertical resolutions, respectively, to produce templates for PDMS fabrication (Comina et al. 2013). Moreover, the smallest cross-section for printed monolithic channels in optically transparent 3D microfluidic to milifluidic devices has been achieved in another study, using MiiCraft a DMD-based 3D printer (Shallan et al. 2014). The MiiCraft was used to fabricate 3D microfluidic devices including a 3D micromixer and gradient generator (Fig. 7) with lower cost compared to the previously published designs using conventional fabrication methods.

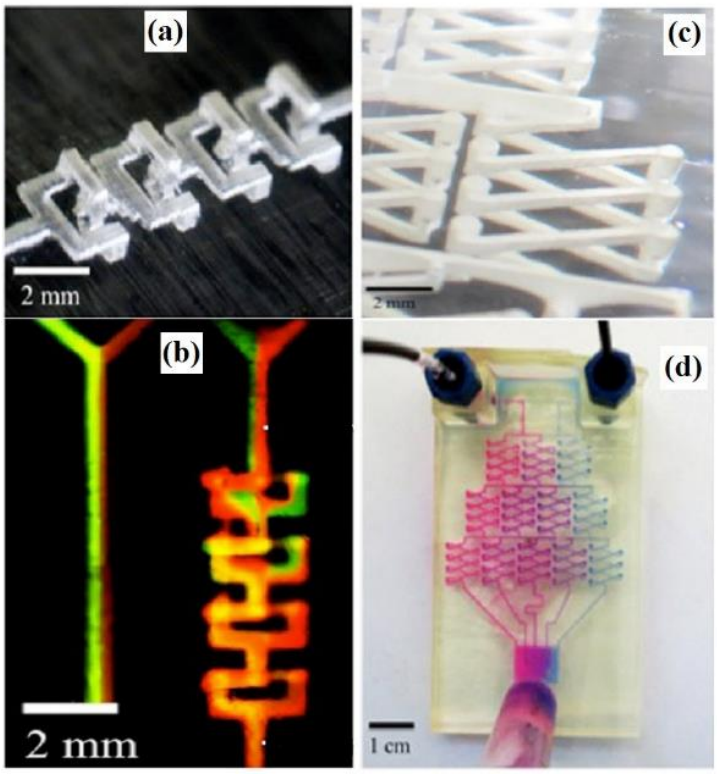

Fig. 7. A 3D micromixer in operation. (a) Four 3D-printed mixing units. (b) Fluorescent image of the mixing progress within the micromixer. (c) One 3D-printed unit of the gradient generator. (d) Performance visualization of the gradient generator using rhodamine B and bromothymol blue as dyes. Reprinted with permission from (Shallan et al. 2014), Copyright 2014, American Chemical Society.

Chan and co-workers have also used a MiiCraft 3D printer for the fabrication of 3D master microstructures (Chan et al. 2015). The masters are finally utilized for the generation of 3D microfluidic devices through a convenient soft lithographic technique. Rogers and co-workers reported the fabrication of 3D-printed microfluidic devices with integrated membrane-based valves (Rogers et al. 2015). They used a stereolithographic B9Creator v1.1 3D printer, and fabricated for the first time active elements in microfluidic systems. More interestingly, the custom resin formulation developed in this study opens up potential for polymer modification to be used in immunoassays or nucleic assays.

Very interestingly, Tumbleston and co-workers have recently introduced a method called “Continuous Liquid Interface Production” or CLIP for the fabrication of 3D objects continuously, instead of “layer-by-layer” fashion, with high speed prototyping in the order of minutes(Tumbleston et al. 2015). They concluded that CLIP may decrease the manufacturing cost of the polymer-based objects, and can be used to fabricate parts from soft elastic materials, ceramics, and biological materials. This method allows for faster SL process and opens up potential for mass production via additive manufacturing and extends the applicability of AM processes to many areas of science and technology (Tumbleston et al. 2015).

Table 2. Examples of commercialized 3D printers applicable for microfluidic devices fabrication.

Type	Brand	Model	Price ($)	Material	Max Size (mm)	Layer Thickness (μm)	Speed (mm/s)
FDM	Ultimaker	Ultimaker 2	2,499	PLA, ABS, U PET	210×210×205	20	$30-300$
FDM	Cubify	CubePro	2,799	PLA, ABS, NYLON	285.4×270.4×230	$70-300$	15
FDM	Lulzbot	TAZ	2,200	PLA, ABS, NYLON	298×275×250	$75-350$	200
FDM	Airwolf	AW3D HD	2,995	PLA, ABS, NYLON	304.8×203.2×304.8	60	150
FDM	Cubify	Cube	999	PLA, ABS	152.5×152.5×152.5	70	15
FDM	Type A	Series 1	2,749	PLA, ABS	305×305×305	$50-250$	$50-250$
FDM	Afinia	H800	1,899	PLA, ABS	254×203.2×203.2	100	30
DLP	EnvisionTEC	Perfactory Micro	~12,000	E-Material (Proprietary)	60×45×100	50	0.007
DLP + SL	MiiCraft	MiiCraft+	1,200	ProBV-003 (Proprietary)	43×27×180	$5-200$	2.7×10^{-5}
SL	FormLabs	Form 1+	3,299	UV Resin	125×125×165	$25-200$	~0.0085
SL	B9Creator	V1.2 HD	4,595	Castable Resin	57×32×203	30	$\sim 0.003-0.006$
SL	Kudo3D	Titan 1	2,999	UV Resin	190.5×109.22×254	~100	0.019
SL	Cubify	Projet 1200	4,900	UV Resin, FTX Visijet	42.9×149.9×26.9	30	~0.004

4. Emerging applications of 3D-printed MFDs

4.1. 3D templates, components and devices

As discussed above, additive manufacturing (AM) technologies have seen use in the construction of master molds for soft lithography (Chan et al. 2015; Comina et al. 2013). The use of AM techniques to create the masters used to mold a finished device in PDMS, instead of typical photolithographic techniques (Whitesides et al. 2001; Xia and Whitesides 1998), avoids the issues of high cost and limited feature topology associated with photolithography. Hot embossing and wet/dry etching, two other methods of producing masters, offer excellent reproducibility and feature resolution, but still struggle to produce complex 3D topologies. Template manufacture via AM techniques can not only solve this problem, but also reduces cost and in many cases that does not require a cleanroom.

Meanwhile, AM has also seen use in generating fluidic components and devices directly. Au and co-workers have developed a pneumatic microvalve similar to the Quake microvalve (Au et al. 2015). Multiplexing these valves allowed the production of useful automated microdevices without recourse to PDMS or silicon, including peristaltic pumps, multiplexers, and cell-culture chambers.

4.2. A discretized approach to microfluidic design

The well-known hydraulic-electric circuit analogy has been an established method for a long time with the purpose of deeply understanding problematic phenomena in fluid mechanics (Esposito 1969). Provided that flow is laminar, viscous and incompressible conditions which can reasonably be assumed to hold within a microfluidic device, Oh and co-authors critically reviewed the analogy between microfluidic networks and electrical circuits (Oh et al. 2012). This very useful analogy allows for the estimation of pressure drop, flow rate, and hydraulic resistance prior to fabrication, and accurately predicts channel dimensions, driving pressure, velocity, etc., required to obtain a desired flow rate, shear stress, or mixing ratio in the fabricated device.

By taking advantage of the electrical-circuit analogy microfluidic networks can be assembled from a standard selection of individual valves, pumps, reservoirs, and so on. Bhargava and co-workers have exploited this analogy to construct an interchangeable library of generic microfluidic parts, including mixers, chip-to-world interfaces, 2D/3D junctions, and integrated IR sensors (Bhargava et al. 2014). Fig. 8 represents some standard components which can serve as microfluidic elements such as junctions, mixers, splitters, etc. Moreover, as visible in Fig. 8, a gradient generator is produced by assembling smaller components which is equivalent to a circuit consisting of two parallel resistors. While the size of the individual components is in the domain of minifluidics, the channel sizes and liquid volumes involved are firmly in the realm of microfluidics. The large size of these components is beneficial where prototypes for proof-of-concept, visualization, verification of numerical simulation, or pedagogy are desired. Components can be attached and detached by hand, and flow paths through such devices are easily visible with the naked eye or through low-power magnification. The simple, modular design allows interested researchers to produce their own components, as complex or simple as is necessary for the task at hand. More importantly, this modular design enables researchers to easily swap out alternative designs or flow patterns as the need arises – something which would, in any other case, require the device to be completely rebuilt.

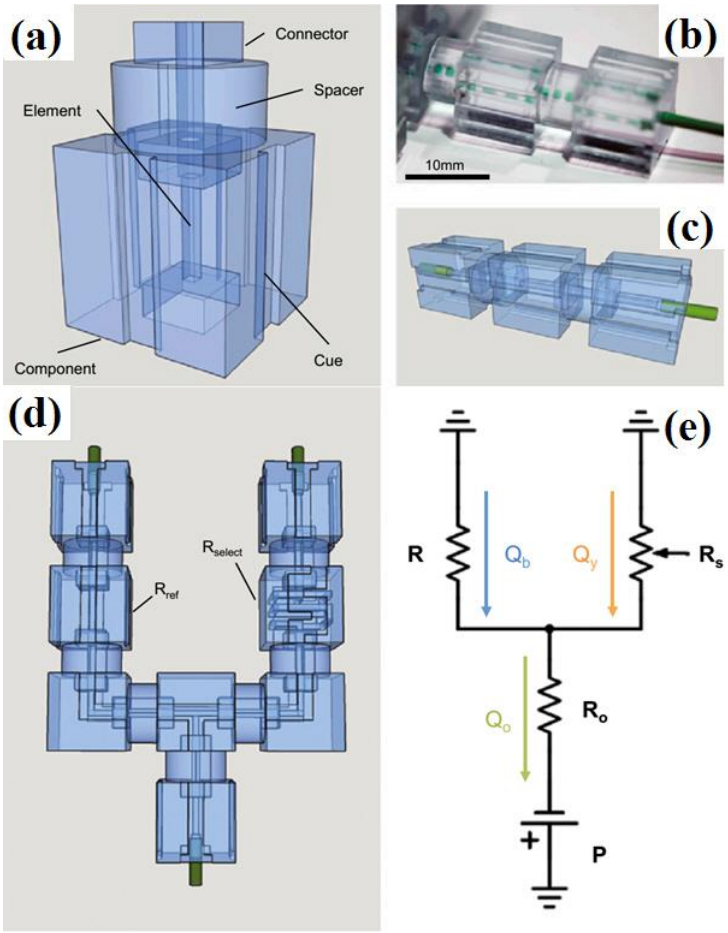

Fig. 8. (a) CAD assembly of a straight pass microfluidic element. (b) The flat connection of the element and the port allows for easy visualization of the connecting junction. (c) Detail of the chip-to-world interfacing. (d) CAD assembly of a concentration gradient generator. (e) Equivalent circuit diagram for the concentration generator depicted in (d). Adapted with permission from (Bhargava et al. 2014).

This "Lego brick" approach to microfluidic design greatly simplifies manufacturing and prototyping. Components are cheaply mass-manufactured through stereolithography (Bhargava et al. 2014) or other additive manufacturing techniques, and defective parts are easily replaced. Taking into account the durability of the resulting modular microfluidic network, the fabricated components can be either permanently or reversibly sealed with commercially available pipe sealant and adhesives, or simply immured in PDMS.

However, the electrical flow analogy does not hold in all scenarios and is not always a reliable tool for MFD design: time-variant, 2D/3D, and multi-phase flows still require Computational Fluid Dynamics (CFD) tools (Asproulis et al. 2012; Kalweit and Drikakis 2008) for a more accurate prediction of their behavior. This analogy also does not describe spatial variations in velocity, or other properties which are necessary in studying the functionality of many microfluidic systems such as micromixers (Yazdi et al. 2014, 2015b, a; Hashmi and Xu 2014), separation devices (Bhagat et al. 2010; Bhagat et al. 2011) and biomicrofluidics (Kwon et al. 2014; Zhang et al. 2015). While these modular devices currently suffer from increased size, weight and complexity compared to a monolithic device, the many advantages of a discretized approach to MFD manufacturing and design are not to be overlooked.

4.3. Combined additive/subtractive manufacturing

Subtractive manufacturing, despite its shortcomings, remains a powerful and extremely precise method of manufacturing devices with features at the micro- to nano-scale. Many of its disadvantages, especially those relating to topological restriction, can be mitigated or avoided by using additive techniques where possible.

However, the use of additive manufacturing is not without its own unique challenges. Feature resolution and aspect ratios in particular have not yet matched the precision of photolithographic etching, and even two-photon SL cannot yet produce topologies such as a hollow shell (Xiong et al. 2012). The use of both additive and subtractive methods together could lead to new devices not otherwise feasible to produce with a one-mode approach, although material incompatibilities between modes may made this difficult in practice.

Xiong and co-workers have found a high-resolution photoresist manufactured by Nanoscribe GmbH (IP-L 780) to be uniquely suitable for this 'combined arms' approach (Xiong et al. 2012). They first manufactured a desired part through two-photon photopolymerization at 780 nm. Then, after curing and drying, the same laser was used at higher power for femtosecond laser ablation of the finished part. The authors demonstrated several novel geometries using this two-photon polymerization/femtosecond laser ablation (TPP+FLA) process, including a Bragg diffraction grating and a monolithic array of 3D spiraling microchannels. Fig. 9a and 9b, gives the authors' detailed characterization of these geometries.

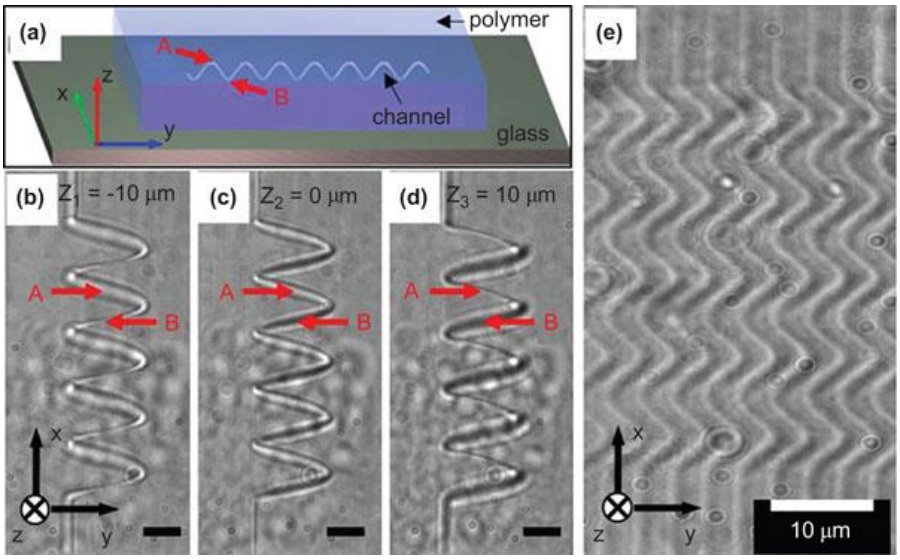

Fig. 9a. Details of geometries produced with the TPP-FLA fabrication method. (a) Schematic of a 3D spiral microchannel. (b-d) X-Y cross-sectional view of a fabricated spiral microchannel, scale bar 10 μm. (e) An array of such microchannels. Reproduced with permission from Macmillan Publishers Ltd (Xiong et al. 2012).

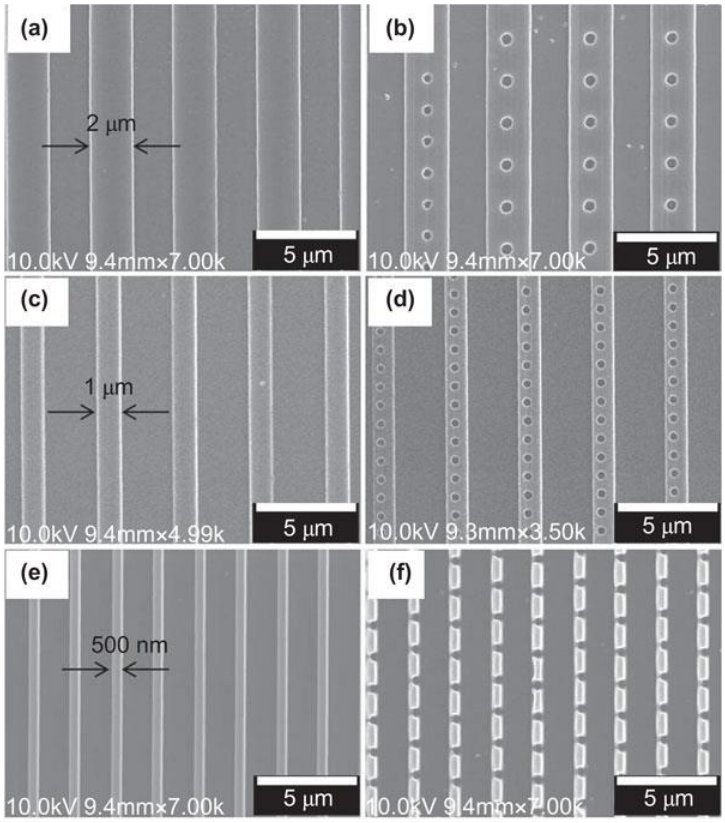

Fig. 9b. Details of geometries produced with the TPP-FLA fabrication method. (a, c, e) Arrays of polymer fibers of defined horizontal spacing, produced using TPP. (b, d, f) Arrays of polymer fibers with FLA produced holes of $\sim 500\ nm$ diameter, forming a Bragg grating structure. Reproduced with permission from Macmillan Publishers Ltd (Xiong et al. 2012).

4.4. Additive electronics components

Integrated electronic components, previously limited to MFDs manufactured in silicon or other conductive materials, are beginning to make their way into devices produced using AM methods. Sun and co-workers have produced a micro-scale battery using printable, highly-porous nanoparticle inks as electrodes (Sun et al. 2013). The extensive interdigitation of anode and cathode, combined with the space-filling nature of micro-scale manufacturing techniques, have resulted in one of the highest per-area power and energy densities reported from a battery to date. However, several hurdles must be overcome before this battery is ready to leave the laboratory: reported service life was on the order of 30 charge/discharge cycles, and the multi-step manufacturing process requires a 600 ℃ baking period in inert gas for nanoparticle sintering to occur, limiting further developments along these lines to laboratories possessing the appropriate facilities.

A technique for directly writing liquid metal microstructures at room temperature was recently proposed by Ladd and co-workers (Ladd et al. 2013). A binary eutectic GaIn alloy, with a melting point at or near room temperature, can be extruded under pressure from a syringe to form conductive microstructures that are load-bearing, flexible, and amenable to inclusion in PDMS devices and future soft robotics systems (Xu 2015). Wires of up to several cm in length were reported, and wire length was limited only by the travel range of the translation stage used as

an extrusion platform. Once exposed to atmosphere, the alloy rapidly formed a passivating oxide layer that served both as structural support for wires produced with this method, and yielded under tensile stress from the syringe to allow further wire elongation. Fig. 10 depicts free standing wires (top), as well as the general approach in fabricating structures by the extrusion of liquid metals. Various techniques such as rapid expelling of the liquid metal [(a) and (b)], stacking liquid metal droplets [(d), (e), and (f)], are then used to form structures as shown in the bottom section of Fig. 10.

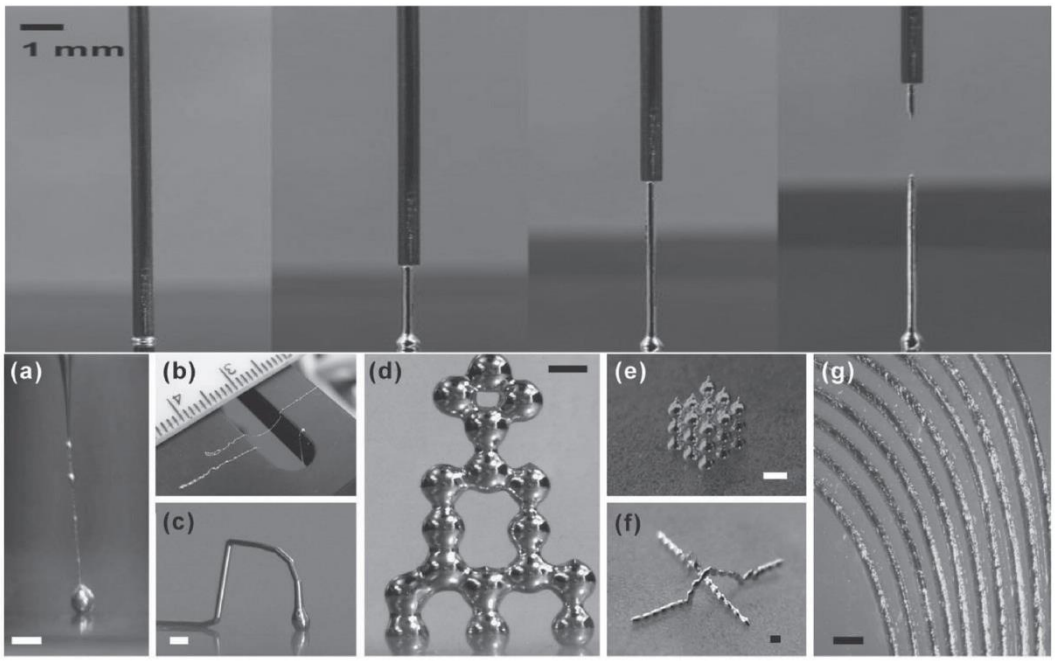

Fig. 10. (Top) Free standing liquid metal wires. (a) A thin liquid metal wire. (b) Liquid fiber suspended over a gap, showing strength. (c) Liquid metal arch. (d) A structure of liquid metal droplets. (e) Stacked droplets, forming a 3D cubic array. (f) Metal wires made of droplets of liquid metal. (g) Liquid metal composing in-plane array of wires. Adapted with permission from (Ladd et al. 2013).

5. Conclusion and future perspective

To summarize, we have presented to the best of our knowledge the state-of-the-art of 3D printing technologies as well as the application of these technologies in fabricating novel microfluidics and lab-on-a-chip devices. In fact, 3D printing has started to gain popularity in microfluidics community only a few years ago, and most of these studies have been focused only on creating the physical devices, such as channels with a defined shape.

In future, we expect that more and more research will come out in utilizing 3D printing for large-scale integration and multiplexing, thus facilitating the fabrication of devices that are more practical to control, repair, and interface with. Indeed, one of the problems in the microfluidics industry is the lack of standards for fluidic components and interfaces, like electronics industry does. 3D printing technology may bring changes to this situation by providing standard designs that can be easily adopted by others.

A new trend is emerging in the 3D printing industry to develop composite printing, and metal printing; these new types of materials should be able to enlighten brand new functions for microfluidics, such as hybrid

microfluidic/electronic systems, and novel microfluidic energy systems. Finally, with the rapid development in 3D printing technology, we should expect the printing resolution to be rapidly advanced in coming years: diffraction effects are already the biggest stumbling block in producing smaller feature sizes, and considerable progress has been made (Kawata et al. 2001) in producing features smaller than the diffraction limit.

Acknowledgements

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Appendix

Glossary of terms in manufacturing technologies

3DP	3 Dimensional Printing	A printing method in which a binder is printed onto a powder bed to fabricate a part.
CLIP	Continuous Liquid Interface Production	A variation of SL in which the 3D printing of parts is continuous and faster.
EBM	Electron Beam Melting	A 3D printing method similar to LENS, which uses electron laser beam to melt the powder beds.
EFAB	Electrochemical Fabrication	A layer-by-layer hybrid process comprising of electrochemical deposition and subtractive planarization to fabricate microstructures.
FDM	Fused Deposition Modeling	A 3D printing method based on extruding polymer that is fed as solid filament to the device.
FLA	Femtosecond Laser Ablation	A fabrication technique based on material removal from a target of interest using femtosecond laser beam.
LENS	Laser Engineered Net Shaping	A 3D printing method which employs powder delivery through a nozzle and subsequent laser melting the layers of the finished part.
LOM	Laminated Object Manufacturing	A 3D printing method based on laser cutting the layers of the finished part.
MIP-SL	Mask Image Projection SL	A variation of SL in which a Digital Micromirror Device (DMD) is used to project the 2D mask images on the resin surface.
Polyjet	-	A 3D printing method based on jetting photopolymers to finally being cured by UV light.
Prometal	-	A 3D printing method that uses an inkjet printing head to deposit binder onto a metal powder bed to form each layer of the finished part.
SL	Stereolithography	A 3D printing method based on curing layers of liquid photopolymer by exposing them to light (UV).

SLM	Selective Laser Melting	A 3D printing method based on selectively laser melting parts of a powder bed to fabricate layers of the finished component.
SLS	Selective Laser Sintering	A 3D printing method based on selectively laser sintering parts of a powder bed to fabricate layers of the finished component.
TPP	Two Photon Polymerization	A variation of SL in which the liquid photopolymer is cured using femtosecond laser energy.

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