Lab on a Chip

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3D Printed Microfluidics for Biological Applications

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The term ''Lab-on-a-Chip,'' is synonymous to describing microfluidic devices with biomedical applications. Even though Microfluidics have been developing rapidly for the past decade, the uptake rate in biological research has been a slow one. This could be due to its tedious process to fabricate chip and the absent of a "killer application" that would outperform existing traditional methods. In recent years, three dimensional (3D) printing has been drawing much interest from the research community. It has the ability to make complex structures with high resolution. Moreover, fast building time and ease of learning has simplified the fabrication process of microfluidics devices to a single step. This could possibly aid the field of microfluidics in finding its "killer application" that will lead to the acceptance by researchers especially in the biomedical field. In this paper, a review is done on how 3D printing helps to improve the fabrication of microfluidics devices, 3D printing technologies current used for fabrication and the future of 3D printing in the field of microfluidics.

Introduction

For the past decade, some researchers believed that microfluidics has the potential to influence[1] or even change[2] the way biology research is being conducted. Microfluidics is defined as the handling and analysing of fluids at the micrometer scale level[2]. The ability to combine several laboratory functions onto a single chip gives microfluidic device a significant advantage over traditional assays used in cell biology. These devices commonly refer to as miniaturized total analysis systems (μTASs)[3, 4] or lab-on-chip (LoC) technologies are capable of (i) streamlining complex assay protocols, (ii) reducing substantial cost and sample volume, (iii) accurately manipulating the cell microenvironment to obtain maximum information, and (iv) providing scalability and batch screening of multiple samples. Different microfluidics systems are making inroads into biomedical research, some with relatively simple function to multiple function analytical systems used in a wide range of application including cellular analysis, genomics, proteomics and metabolomics, immunoassays, point of care (POC) diagnostics[5, 6] and organs on chips[7, 8]. However, even with the many advances in this technology, it has not been highly adopted for the use in biological research[9]. One possible explanation is that this technology is still in search for its "killer application" that can outperform current traditional methods available[10-12].

There are currently different techniques for fabricating microfluidics devices such as micro-machining, soft lithography, embossing, in situ construction injection moulding and laser ablation[2] which are used for large scale replication and production. Some of these techniques require much space to hold multiple equipment, labour intensive (multiple step processes to make final product), time wastage to facilitate the change in design, and limited biological materials available. For small scale production such as analysis in a laboratory environment, soft lithography, a multiple step process is the current gold standard. A quick and easy fabrication of microfluidic devices will be preferred[13] as cell biologist may not necessary have the time to learn the fabrication process[9]. Recently, advancements in 3D printing in terms of resolution and speed have helped to simplify the fabrication process of microfluidics devices into a single step. Publications on microfluidics with 3D printing have been on the rise in the last five years (Figure 1). There are several other notable advantages associated with 3D printing for the fabrication of microfluidic device over conventional methods including embedding of tissue scaffold with high porosity, high resolution and defined pore structure into the device, a range of different material. A wide range of Biomaterial such as living cells and growth factors could be direct printed with the 3D printer[14-18] as well. The recent development of 3D printing capability has paved way for intricate and minute 3D layered structures that can be printed. It offers great topography flexibility of having multiple 2D layers stacked onto each other. This allows high precision construction of a multi-layered transport/micro-vascular channel network in a range of 100-300μm with an accuracy of tens of microns[14, 15, 19]. This could possibly aid the field of microfluidics in finding the "killer application" that will lead to the acceptance by researchers especially in the biomedical field.

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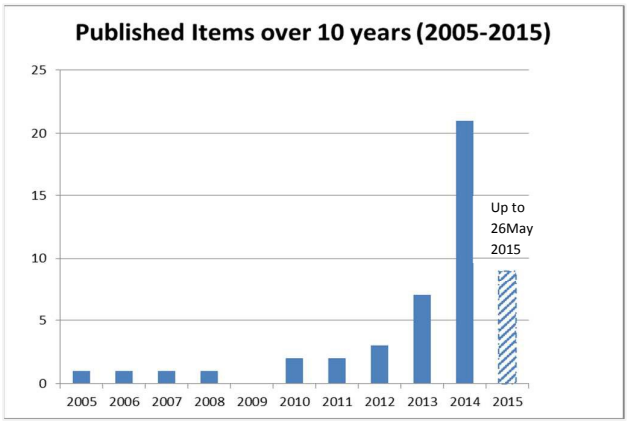

Figure 1 Microfluidics publication involving 3D printing from 2005 to 2015. The data shown reflect the most recent data searched (26 May 2015) from the web of science category. The following search general string was used: Topic= (microfluidics) AND (3D printing) AND Published = (2005 to 2015)

The development of technologies to enhance the capabilities of investigators in biology and medical research has always been an important goal for the microfluidics community [20]. In this review, an examination is done on how 3D printing improves the process in making fully functional chips, the current 3D printing technologies for fabricating microfluidics chips with a focus on newer technique such as bioprinting and its biological applications. Finally, an analysis of the latest research on the improvement of 3D printed microfluidics.

How 3D printing can play an important role in the fabrication of microfluidics devices

Some of the key functions of a microfluidic device are sample preparation, separations of liquids, detection and fluid manipulation. For more information on each specific functions, it can be found in wolley paper[5]. Different functions help to determine the desired analysis capability and dictate the design of the microfluidic device. The ability to extract or purify, label or separate the sample within the device help to reduce analysis time but also improve throughput[5]. Pumps, valves and mixer are added onto the device to help in manipulating the fluids. Samples are then sensed and detected by optical (laser induced fluorescence), electrochemical (conductivity, amperometry and potentiometry), mass spectrometry or biosensor involving a transducer.

Once the design and functions are determined, the conventional and easiest way to rapid prototype the microfluidic device is the PDMS casting based 3D moulding (soft lithography). To fabricate the microfluidic device, firstly, computer aided design (CAD) or other engineering drawing software have to be utilized to design the required channel patterns, later the channels would be moulded on a SU-8 master or a piece of metal using laser cutting method. After the fabrication of the mould, polydimethylsiloxane (PDMS) polymer will be filled into master mould and cure for over 2 hours. After the curing procedure, the PDMS will be peeled from the master and cut into the shape of the required device. Lastly, oxygen plasma is introduced to enhance the bonding strength between the PDMS and the glass[17]. This whole process is extremely time consuming and much of the fabrication process involves manual operations which further comprise the accuracy of the microfluidic device[21, 22]. With 3D printing, time needed is greatly reduced as the process can be done with just one machine and being fully automated, it can be easily replicated. In order for making 3D printing of microfluidics more applicable for biomedical field, certain factors like cost, resolution/speed and materials have to be taken into account to ensure optimum results.

As mentioned, the fabrication of micrometer-scale features on master mould is a tedious process for rapid device prototyping. It is relatively time-consuming and expensive to produce multiple high resolution (<10μm feature size) photomasks and it is rather challenging to align and expose sequential layers of photoresist for the soft lithography fabrication process[23]. However with 3D printing, it does not depend on masks for creating the micropattern, instead it takes the input from CAD software. Hence, it is able to produce arbitrarily defined structures in a fully 3D space, with no significant increase in fabrication complexity and time[23, 24]. Spivey and team made a single cell capturing device for observing the cellular aging process of *Schizosaccharomyces pombe*. Using the modified digital micromirror device-based projection printing (DMD-PP) technology to create the master mould, he was able to develop micrometer-scale devices that required intricate or unconventional geometries, such as curves or sloping/irregular top surfaces and 3D structures with micrometer-scale features such 4μm catch channels (Figure 2). This enabled him to fabricate high-throughput microfluidic platform for aging studies and long time-scale single-cell analysis in fission yeast[23]. Other teams have looked into other machines such as the inkjet printing for making of 'millifluidic' chip for microliter droplet generation[25] and stereolithography for PDMS chip as flow cell[24].

Once the masks have been fabricated, it will take a few hours to possibly one or two days of production, depending on the amount of chips required. However, the process for getting the final microfluidics design is a tedious one. Initial microfluidic testing may reveal design flaws and performance deficiencies require the user to modify the design, therefore incurring significant delay and stretch the development time with an increase in cost. Besides making moulds, the ability of 3D printing offers the opportunity to fabricate the whole microfluidics device in a single step with no need for and assembly as with PDMS. Recently, 3D printed microfluidic devices with integrated membrane-based valves was fabricated[26]. The author used his own material formulation with 3D printing machine and was able to fabricate the first active microfluidic device within an hour, with valve made together with the device[26]. This helped to reduce time needed for the designing process. The author believed with 3D printing, development landscape of microfluidics will change permitting a "fail fast and often" strategy in which early and rapid empirical feedback was used to guide and accelerate device development[26]. More details of the different 3D

printing technologies and biological applications uses with 3D printed microfluidics will be covered in the following section.

The use of CAD models to create the microfluidic device gives the user the ability to integrate other commercial parts whose dimension are known or can be measured to be attached to the chip. Jayda and team were trying to show the ease of integrating different electrochemical detection schemes for 3D printed devices[27]. In the paper, they were able to show two designs of microfluidics chips adapting to a variety of electrode materials (platinum, gold and silver) added to a threaded receiving port for a wide range of applications (neurotransmitter detection, NO detection, measuring oxygen tension in red blood cells). Other functionalities include fluidic interconnects and membrane inserts to enable signalling molecule detection. Unlike the physical format of soft lithographic masters, the part files for 3D printing are standardized, i.e., the part can be exchanged with and transferred to any lab that has access to a CAD program and 3D printer[27] making of different components for medical uses. This module like approach to the experimental design is removable and can be easily reused after exposure to biological sample.

Another way 3D printing can help is the fabrication of Microfluidics Interface (MFI) technology to help improve existing chips. MFI was developed for the proper integration of on-chip devices with multiple functions and materials. Song-I Han and Ki-Ho Han used the SLA to make the MFI, providing a simple method for realizing complex arrangements of plug-in microfluidic interconnects, integrated microvalves for micro fluidic control and optical windows for on-chip optical processes[28]. Using the SLA as well, Hwanyong Lee and team were able to make a polymer MFI for the high-performance on-chip integrated reverse transcription (RT)-microchip which was able to perform two genetic functionalities of RNA extraction and cDNA synthesis[29].

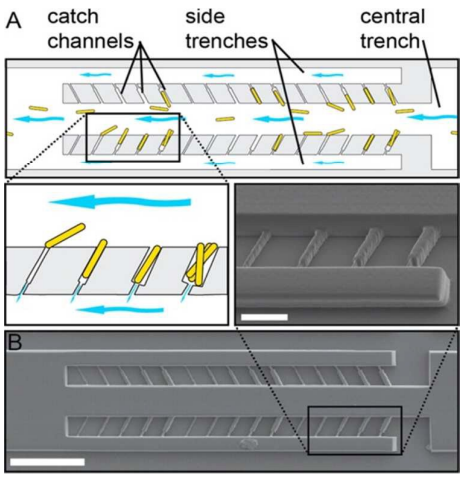

Figure 2. (A) Schematic diagram the fission yeast lifetime microdissector. Blue arrow indicate the flow from right to left while Yellow rods are the yeast drawn into the channels and retained them via suction. (B) The PEGDA master structure with variable catch channel dimension. Scale bar is 100µm. Higher magnification image of the master structure showing (from left to right) 3, 4, 5, and 6µm catch channels. Scale bar is 20µm. Picture taken from ref 23 with permission from American Chemical Society.

3D printing technologies and applications

3D printing has found its applications in the fields of engineering, art, and manufacturing sectors[14, 15, 30]. With its tremendous advantages, usage in the field of biology science has also been recognized rapidly. 3D printing of the scaffold for moulding human organs is a new strategy for the in vitro tissue engineering study[31]. 3D printers are able to print complex structure with high definition, which makes the moulding of the real organ realistic[8, 32]. In addition, 3D printing of the microfluidics devices enables the capability of studying the complex biological phenomenon in a precise controllable manner, as the micrometer size of the channel only allows small volumes of fluids pass over a very short distance[6, 15, 31]. Therefore, it opens a new avenue for diverse biology applications. Cytotoxicity test of the chemicals, cellular stress assays, DNA sorting, single cell behaviour study and cell manipulation studies, these are part of the new applications for the microfluidic devices in cell biology [5, 20, 33].

3D printing also known as addictive manufacturing is defined by the ASTM as the process of joining materials to make objects from 3D model data, usually layer upon layer, as opposed to subtractive manufacturing methodologies (ASTM F2792). The 3D printing process involved two main processes: design modelling and design production. The first step is the use of CAD or other commercial engineering drawing software for 3D object model construction[15, 34]. The model file will then be saved in .STL format and transferred to the 3D printer. In the 3D printer, the .STL file will be sliced into a certain amount of 2D sequential cross-section slices depending on the resolution of the printer and finally the printer rebuilds the 3D objects layer-by layer additively adding based on the 2D cross-sectional slices[34].

There are many ways of making microfluidics devices, but the focus here will be on manufacturing of microfluidics device in a one step process. One step process/manufacturing is referred to direct making of the device from the digital data to the final structure with a single machine[13]. However, the devices may undergo further physical or chemical treatment for surface modifications or cleaning up.

There are many different techniques for 3D printing available both industrial and commercial market, some like Stereolithography (SLA) and Fused Deposition Method (FDM) are well-established while others like Electron Beam Melting (EBM) or Bioprinters are up and coming. The factors in determining the machine of choice are resolution (accuracy), speed, material and build size. In this paper, focus will be on two key 3D printing technologies, one that makes the channels directly and another that removes material to make the channel. Currently, 3D printing technologies that make microfluidics devices mainly use photopolymer resin as their materials. These include SLA, DMD-PP, Inkjet printing, two photon polymerization (2PP) or two photon ablation. In addition to photocurable materials, other materials such as thermoplastics and elastomers uses non photocurable techniques like the FDM, and for soft hydrogel, the bioprinters.

3.1 Stereolithography (SLA)

The first commercialized 3D printing machine was established by Chuck Hull in 1988[35]. A UV laser was utilized to scan and trace over the certain area to cure the fluid resin material. The material is then hardened by the high power lasers or UV light and the build platform shifts down in the z-direction by one layer. A sweeping blade will recoat a fresh layer of resin over the cross section of the part and the next layer is traced with the laser. This process is repeated till the structure is completed. A major advantage of SLA fabrication is the high precision on the surface resolution. Normally, layers of photo-curable resins are exposed to UV laser beam. By controlling the positions of the laser focus, polymerization of the resin can be controlled to achieve desired structure and design. Several non-linear effects such as the polymerization of resin near the focal length of the beam as well as temperature sensitivity must be controlled[14, 15, 30]. With further improvements such as galvanometer-based vector scanning[14, 19], SLA systems has become highly commercialized and most widely used 3D printing machine[15, 19, 22]. The SLA systems can produce high resolution products while keeping the cost low due the relatively low usage of the liquid medium. Moreover, the SLA printer is being designed to be smaller, faster and cheaper, aiming towards future personal use[15, 19, 30]. An example will be the Form1+type high-resolution 3D printer developed by the Form Labs Company. This personal desktop SLA model is able to achieve industrial precision standards, at a more affordable price. It is able to print layers of up to 25µm with a minimum feature size as low as 300µm. The minimum 10µm movement of the laser beam during scanning of the methacrylate photopolymer resin allows final products with smooth surface finish[15]. Thus, with the size of the machine greatly reduced and functions combined into one, microfluidic devices can be made much easier and efficiently.

A novel immunomagnetic flow assay on-a-chip was designed by Lee and team[36]. This study stands as an outstanding example of how works that were previously confined to the laboratory due to their size, can now be brought out with aid of 3D printing. Cylindrical 3D micro channel named as High-capacity Efficient Magnetic O-shaped Separator (HEMOS) was printed using a commercial 3D Viper SLA system based on stereolithography. The geometry of the cylinder reduces linear flow velocity enabling handling of high volumes. Antibody-Immobilized Magnetic Nanoparticle Clusters (AbMNCs) were mixed with the sample which then forms AbMNCs-Bacteria complex, in this case the bacteria is Salmonella. A high magnetic force was applied on the walls of the HEMOS to separate out and attracts the AbMNCs--Salmonella complexes, thus capturing the bacteria to the sides of the HEMOS (Figure 3). A simulation was also carried out to determine influence of magnetic arrangement on the HEMOS in the study. The microfluidic chip was capable of handling 10 mL in 24s which implies that it can handle 1.5 litre in just about 1 hour (enough to handle samples from 150 patients). Recently, using the same device with slight modification Lee and team were able to detect *E.coli* in milk as well[37].

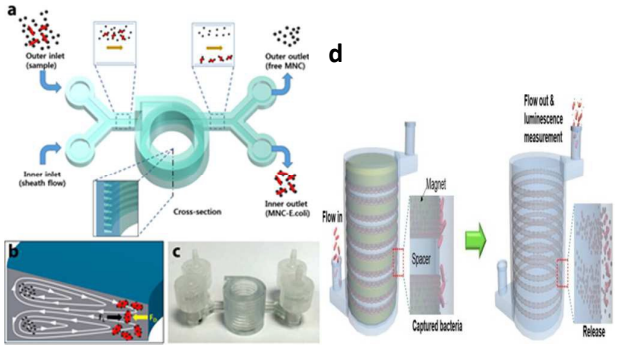

Figure 3. (a) Schematic illustration of separation of captured bacteria by inertial focusing. (b) Illustration of Dean vortices in a channel with trapezoid cross-section. (c) Photograph of the 3D printed device. Picture taken from ref 37 with permission from Nature Publishing Group. (d) Schematic illustrations of 3D immunomagnetic flow assay. The magnet-spacer assembly was placed in the opening of the HEMOS. Picture taken from ref 36 with permission from American Chemical Society.

3.2 Digital Micromirror Device-based Projection Printing (DMD-PP)

Digital Micromirror Device-based Projection Printing (DMD-PP) technology is a projection system that has controllable digital mirror which can reflect the laser light in an entire plane, which enables the curing of the entire layer at one time. The key device is the digital mirror devices (DMD) that produces the image. To build a part, the .STL file is sliced and the sliced layer is converted into a bitmap file. The bitmap image is black and white, black representing areas that are void and white representing the material. When the image is projected onto the resin, only the illuminated white portion will cure the resin. Once the layer is cured, the build platform is raise vertically upwards. This allows the machine to print parts without height restriction, however, material resin with high viscosity may affect the lifting process. The process is completed when the entire product have been printed. Comparing to the SLA systems, DMP-PP uses a mask projection for photo-polymerization and the building of structure is bottom up. Unlike the SLA which requires the movement of the stage as well as the laser, by integrating the convex lens, this could largely reduce the fabrication time [15] shown in Table 1. The resolution of the feature is lower as compared to the SLA.

3D micro structure was designed using a 3D bio printer which employs DMD-PP technology[38]. The DMD-PP technology is capable of printing materials with theoretical negative Poisson ratio[39]. The 3D i*n vitro* microchip made of hydrogel with a honeycomb structure was capable of mimicking 3D vascular morphology of in-vivo micro environment. HeLa cancer cell line was cultured inside the micro channel and its metastatic properties were analysed. It was found that the cells migrated at different speeds inside channels of different width. Increase in channel width led to decrease of migration speed of the cells. It can be inferred from the above observation that cancer cells are capable of moving faster in smaller veins than in large arteries. Thus, 3D printing has enabled us to create complex bio environment which are otherwise impossible to realize using ordinary conventional methods.

3.3 Two-photon polymerization (2PP)

Two-photon polymerization (2PP) uses femtosecond (Fs) laser pulses that direct write the pattern into the volume of photosensitive resin. The 2PP process is similar to the SLA in which light triggers a chemical reaction leading the polymerization of the photosensitive resin[40-42]. Majority of the material are transparent in the near-infrared and highly absorptive in the UV spectral range. For the SLA, the polymerization process takes place near the surface of a photosensitive resin due to single photo polymerization (1PP). As a result, it is only possible to build 3D structures layer by layer. However for 2PP, two photons are being absorbed simultaneously by the photoinitiator enabling them to act as one photon to start polymerization. This allows the laser to direct record or writes any desired polymeric 3D pattern into the volume of photosensitive materials. A simplified diagram is shown to illustrate the difference between one photon and 2 photon activated process (Figure 4). Due to the threshold behaviour and nonlinear nature of the 2PP process, resolution (structure size) beyond the diffraction limit of the optics used to focus the laser beam can be realized by controlling the laser pulse energy and the number of applied pulses. 2PP allows better resolution spot size and reduces the need of an inert gas atmosphere. However, due to the tracing method, the speed is slower as compared to SLA. Despite 2PP being a relatively new technology, its applications areas are expanding rapidly. 2PP are used for micromechanical systems, microfluidics devices, biomedical device and scaffold for tissue engineering[40-42].

Besides the isolation of microbial particles, microfluidic devices can aid in the dynamic observation of microorganisms. Some bacteria contain flagellum or pilis which aid the bacteria from moving from one point to another. Midorikawa and his team have developed a device also known as nanoaquarium with the femtosecond laser direct writing for the inspection of mobility microorganisms such as Euglena[43] and Phormidium[44]. Using the direct laser writing, followed by annealing and successive wet etching, they were able to produce different structures such as microchannels and micromirrors in the glass chips. This allowed them to reduce observation time and prevent the evaporation of water as compared to traditional methods where small microorganisms grown on petri dish.

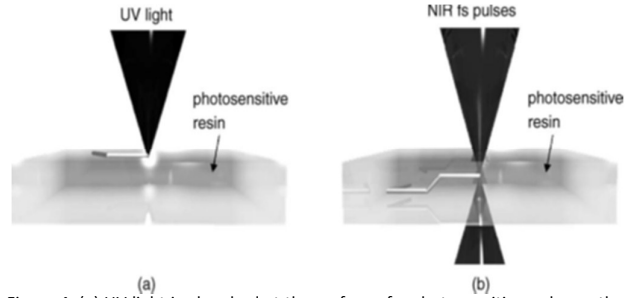

Figure 4. (a) UV light is absorbed at the surface of a photosensitive polymer thus leading structures only on the surface. (b) NIR light can be focused into the volume of the UV-sensitive resin and structures are only formed at the focal point. Picture taken from ref 42 with permission from Elsevier

Moreover, the small amount of water enables them to analyse infinitesimal quantities of chemical substances dissolved in water. The used of Fs direct writing allowed quick making of prototypes of various nanoaquariums with different structures allowing highly functional observations and analysis of the dynamics of mobility microorganism[44].

3.4 Fused Deposition Modelling (FDM)

The principles of FDM are based on surface chemistry, thermal energy and layer manufacturing technology[30]. Thermoplastic materials are melted by a heating element into a semisolid form. It is then extruded out through the nozzle on to a stage layer by layer. As the material is extruded, it cools and solidifies to form the model. Once the first layer is completed, the stage lower by one layer and the process is repeated. The FDM method is the cheapest method available currently and is the future for home printer applications. The setback of FDM would be between each laid down layers, air space and fusion lines are always present and it can affect the final resolution of the product[14, 30].

Pathogens are usually referred to microorganisms that cause diseases in humans. Many researchers have been looking at different methods to study how microorganisms cause infection. One important goal is to isolate microbial particles such as whole bacteria, cells, ATP, oxygen and other essential biomolecules in the hopes of developing an early detection diagnostic device that is compact and low cost to prevent infection from taking place. A Fused Filament Fabrication (FFF) based 3D printed chip which was suitable for bacterial cultivation, DNA isolation, PCR, and detection of amplified gene using gold nanoparticle (AuNP) probes was employed to detect Methicillin resistant Staphylococcus aureus (MRSA) bacteria by Chodabova and team[45]. A commercial 3D printer "Profi3D Maker" was used in the study to print the acrylonitrile butadiene styrene microfluidic chip consisting of a reaction chamber, two channels and a dosing capillary. The heating element, temperature sensor and fan were kept in a thermostatic box enclosing the chip. Detection of bacterial DNA was carried out by using gold nanoparticles (AuNP) capable of binding to the target DNA site the mecA gene. mecA gene is the specific gene of the MRSA bacteria which has to be identified and amplified. The detection was based on colorimetric analysis of the outcome of mecA gene and AuNP. The chip provides a one-step approach for detection of the harmful pathogenic bacteria MRSA and due to its ultra-cheap and portable property, it can be readily implemented as an on-site diagnostic tool. The same chip can be further modified by varying the functional sites of the AuNPs to detect other bacteria based on their gene expression. Another group have also looked into microfluidics devices as mini bioreactor similar to Chodabova to aid in the analysis of virus with the use of quantum dots[46].

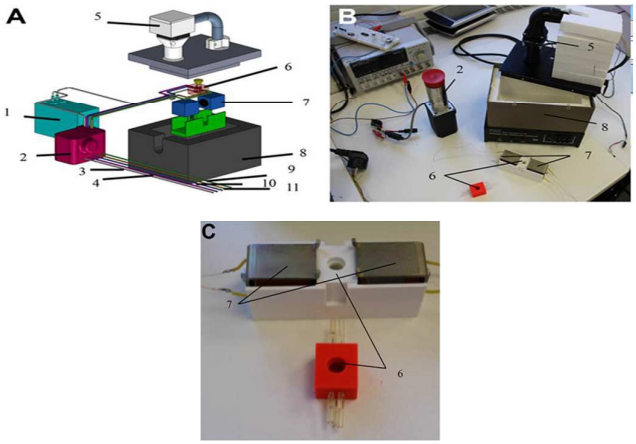

Figure 5 (A) Scheme of 3D-printed chip for detection and confirmation of MRSA presence using binding of MRSA to the gold nanoparticles with specific primers in the chip, (B) system for the identification of MRSA in the sample, and (C) reaction chamber of 3D-printed chip: 1—spectrophotometric detector, 2—pump with the valves, 3—outlet, 4—the first inlet hose, 5— thermoregulatory system, 6—cultivation chip, 7—electromagnet, 8—thermoisolating box, 9—the second inlet hose, 10—the third inlet hose, and 11—the fourth inlet hose. Picture taken from ref 44 with permission from John Wiley and Sons.

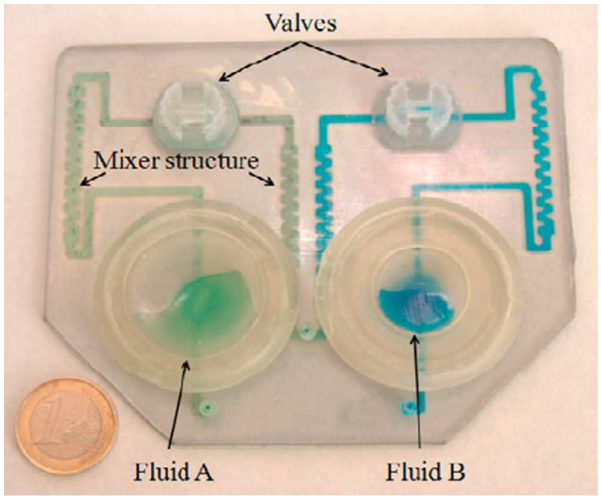

Figure 6. Cervical microfludics prototype with fluid mixer and homogenizer. The reagent and the sample will be stored in two reservoirs and expelled by fingertips for mixing. Picture from ref 47 with permission from Elsevier .

3.5 Inkjet

Inkjet printing is a non-contact technique capable of reproducing digital image data on a substrate using picolitre droplets. The technique is similar to the mechanism of a commercial inkjet printer just that photoresin or wax is jetted out instead of ink. Jetting heads will release materials onto the tray and the material is cured by the UV attached to the jetting head. Once the material is cured, the build tray will be lowered and the next layer is built. The advantages of this system include high quality and accuracy, fast build speed and ability to print multi - materials.

One of the first microfluidics chip to be produce using the inkjet system is reported by Bonyár[47]. It was designed to be use as a transportation device for cervical sample from the clinic to the laboratory. The device contains a mixer and homogenizer for gynaecological cervical sample preparation Figure 6).

Anderson and team was able to fabricate a fluidic device with the inkjet printer (Object Connex 350) which enables flow and incorporates a membrane above the channels in order to study drug transport and cell viability. The design incorporates up to eight channels, each with their own membrane insertion port. This allows drugs in this case (linezolid and levofloxacin) to cross over the membrane to interact with the cells. This simple design is capable of allowing the study of drug transport and cell viability in a parallel manner. With up to 8 channels printed in a single chip, time taken for screening of drug concentrations will be reduced[48].

3.6 Bioprinting

Microarrays are used for cellular investigation with high-throughput screening like drug screening, in vitro toxicology tests and functional genomic studies. However, the inability to recapitulate a complex cellular structure still remains. A solution could be the use of bioprinting which allows for the cells and biomaterials to be placed in a specific spatial arrangement.

Customized 3D printed scaffold for tissue regeneration or even patterning biological materials such as DNA and cells can be done with the bioprinter. Currently, bioprinters on the market such as The regenhu BioFactory® or EnvisionTEC 3D-*Bioplotter*® or NovoGen MMX Bioprinter™ have different heads attached for the printing of different materials, giving them a huge advantage for making multi-materials cell environment. The printing heads can be classified into two groups, dispensing and jetting.

Dispensing is described by the release of material usually in filament form, this includes extrusion. For extrusion, pressure is used to force the material through nozzle in a controlled manner to construct a 3D structure. Once material is deposited, solidification of the material through physical or chemical means provides sufficient mechanical integrity to fabricate 3D structures. Depending on the printer, either the printer head or stage will move while dispensing the material to form the pattern. The materials used are usually highly viscous hydrogel.

Jetting is described by the release of material in droplet form for better precision. It uses either the inkjet head or microvalve technology. This technique can be divided into two main categories: Continuous inkjet (CIJ) where a steady stream of small droplets are produced when pressure oscillations are being applied to the stream and droplets is either defected by an electrostatic field onto a substrate or not deflected and collected for reuse. Drop on demand inkjet where ink droplets were produced when required. A volumetric change in the fluid initiates the droplet formation done either by thermal or piezoelectric. In thermal inkjet printing, rapid local heating generates bubble within the ink chamber that ejects a small droplet while piezoelectric inkjet printing is used to create a pulse resulting in droplet ejection. In the case of microvalve printing, simple droplet based deposition or extrusion style printing mechanism where fluids under constant pneumatic pressure are dispensed from tips by opening and closing a small valve, which can be controlled mechanically, electrically

or magnetically. Once material is deposited, solidification of the material through physical or chemical means provides sufficient mechanical integrity to fabricate 3D structures. Depending on the printer, either the printer head or stage will move while dispensing the material to form the pattern. The materials used are usually highly viscous hydrogel.

The primary goal of human-on-chip is to simulate the normal human physiology and micro environment needed for organ growth in vitro[49]. The integration of organ on chips enables the mimicking and stimuli generation of mechanical stresses, chemical gradients and other in situ conditions required. There are both single organ-on-chip for mimicking a particular organ function and multi organ-on-chip for studying the interaction between multiple organs. For generating such organ on chips conventional cell culture techniques were employed previously before the advent of 3D bio printers[31]. 3D bioprinters use agarose, cells and other biomaterials for making the scaffold. Additional benefit of the bioprinter is because of the multiple heads, multiple materials can be printed. Initially, PDMS chips used for creating organs-on-chips lacked the ability to form complex intricate geometries and used bio compatible materials during chip development[50, 51]. Lately, a fully functional hydrogel micro channel was bio printed as an in-vitro replacement for blood vessel [52]. This was achieved by using a NovoGen MMX bio printer and cell culture was carried out inside this micro channel to test its bio compatibility. The cell viability was analysed at the end of each day and found to be better inside the channel than in ordinary hydrogel blocks (figure 7) thus proving the ability of a printed microchip to recreate in-vivo environments for cell survival, division and differentiation. Currently, the main area of study is to create vessels by printing sacrificial materials and removing it once tissue is made. Artificial material is either removed physically by mechanical pulling or vacuum, or by chemical means of heating to melt. Other sacrificial materials include collagen precursor[53], direct method with fugitive organic ink[54-56], gelatin[57] and carbohydrate glass[58] with different bioprinters.

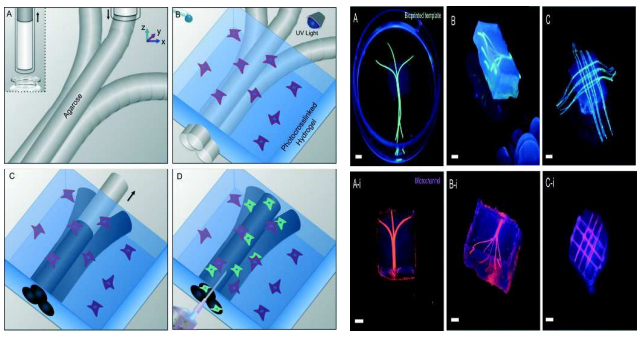

Figure 7. Schematic representation of bioprinting of agarose template fibers and subsequent formation of microchannels via template micromolding. A) A bioprinter equipped with a piston fitted inside a glass capillary aspirates the agarose. After gelation in 4 °C, agarose fibers are bioprinted at predefined locations. B) A hydrogel precursor is casted over the bioprinted mold and photocrosslinked. C) The template is removed from the surrounding photocrosslinked gel. D) Fully perfusable microchannels are formed. Picture taken from ref 52 with permission from Royal Society of Chemistry.

The future of 3D printing of microfluidic devices

The integration of 3D printing with microfluidics has been gaining popularity recently (Figure 1). Based on the publications, a research trend is shown in Figure 8. Initially 3D printing is used for prototyping of moulds. This fast output gives it a huge advantage over traditional methods. As the technology improves in 2010, direct making of chips with simple channels were being produced. Applications of these chips for biological study are mostly seen in 2013 onwards. With improvement of materials, chips with functional mixer and valves can be printed directly. 3D printing provides a promising prospect for fabricating microfluidics devices, but its current limitations such as hardware, materials and cost are areas that still need improvement for the increase of uptake by biologists. With further research, we feel that 3D printing will be able to produce higher fidelity chips, more components such as mixer and in the future a fully functional part. The following section will be explaining some of the issues mentioned and how researchers are looking to improve on.

When choosing a material for making a microfluidic system, three factors are taken into consideration, function, degree of integration and application[5]. Other factors for biological application include cellular compatibility, supportability (oxygen, nutrient diffusion etc.), optical transparency and mechanical properties[5, 59]. Key materials for the making of microfluidics devices are silicon and glass initially[59]. These materials were chosen because of their excellent inertness, high strength and thermoconductivity. However, these materials are non-permeable to gases which are not suitable for long term cell culture. PDMS was first introduced in the late 1990s, for academic laboratories due to its reasonable cost, rapid fabrication and ease of implementation[59]. In addition, the high permeability to gas, elasticity and better optical properties allowed it to be the most common substrate for cell-related amidst glass and silicon. Although there are many materials available, not all are printable. Some possible materials include elastomers, plastics, hydrogels and paper. Despite PDMS being a widely used material for microfluidic, other materials have to be employed as it is unable to be 3D printed directly yet.

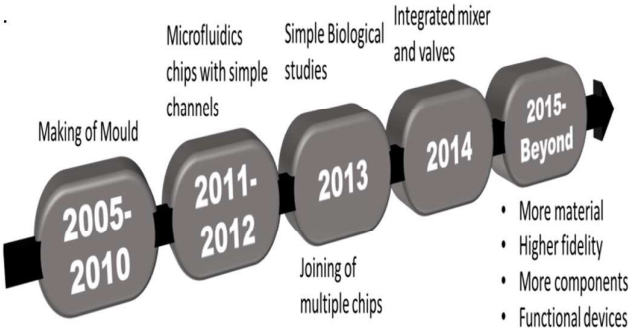

Figure 8 Research trend on 3D printing microfluidics chips since the first relative publication in 2005. The trends of using 3D printing as a rapid prototyping device to printing functional chips with the specific function of the user

As mentioned for SLA, DLP and 2PP photo curable resin/polymer is needed for making of microfluidics chips. Photopolymers are polymers that undergo an interaction with light to alter its physical or chemical properties[60]. Its low-cost, tunability and transparency made it a preferred choice for researchers in making microfluidics with 3D printing. However, most materials for 3D printing are commercialised and requires optimisation before using in biological applications. Feng zhu and team decided to investigate on commercial materials for the Multi-jet modelling system and the SLA systems. For the MJM, VisiJet Crystal (rated United States Pharmacopoeia (USP) Class VI) while SLA is using the clear photopolymer, Watershed 11122 XC (Watershed) and Dreve Fototec 7150 Clear (Dreve Otoplastik GmbH). The zebrafish embryo trapping microfluidic devices was printed with both printers and compared for the cell viability. It was found that that VisiJet Crystal (HD3500+) and DSM Watershed (Viper Pro) materials are toxic to zebrafish embryos for long term studies (more than 3 days) even after post treatment of the materials. Initially, Dreve Fototec material was toxic to zebrafish embryos, but after soaking in 99% ethanol for 24 hours, they were completely inert to zebrafish embryos[61]. This showed that more studies must be done on the materials and proper sterilisation techniques must be carried out when working with biological materials. Currently most 3D printing technologies print one material, thus by making new materials that can be printed together on the same machine, microfluidics chip with multiple functions can be printed. For example, Paydar and team used the commercial Objet printer to make the first microfluidic interconnect using multiple materials. Basically, they combined a flexible elastomer (TangoBlack®,Objet Geometries Ltd., Rehovat, Israel) O-ring with a rigid, plastic (VeroBlack®, Objet Geometries Ltd., Rehovat, Israel) body that has barbed clips for

3D printing	Energy Source	Materials	Adv	Dis	Application
Stereolithography(SLA)	Laser/UV	Photocurable resin/polymer- ABS like etc.	High resolution, good surface finish	Require post curing and removal of support structures	Making of Master mold [24] Microfluidic chips with active features [26, 62] Microfluidics Interface (MFI) [28, 29] Pathogen detection [36, 37] Biological assay (cell observations) [61]
Digital Micromirror Device-based Projection Printing (DMD-PP)	UV	Photocurable resin/polymer	Good resolution, fast build time compared to SLA	Limited build volume, peeling of parts from the tray may damage the chip	Making of Master mold [23] Cancer assay (studies on cell migration) [38]
Two-photon-polymerisation (2PP)	Femtosecond Laser	Photocurable resin/polymer	Very high resolution with small features	Slow build time	Biology observation on cell mobility [43, 44]
FDM	Thermal	Thermoplastics such as ABS, Polycarbonate, and Polyphenylsulfone; Elastomers	Cheap materials, ease of support removal	Slow build time, restricted accuracy, Not many transparent material available	Pathogen detection of bacteria [45] Pathogen detection of virus [46]
Inkjet	UV	Photocurable resin/polymer	Fast build speed, multi material printing	Removal of support materials from the channels is tedious	Making of Master mold [25] Versatile chips for different type of electrodes for gas detection [27] toxicity assay [48] Biological assay (cell

					observations) [61]
Bioprinting	Laser/UV	Hydrogels, viscous materials, photocurable resin	Multiple materials, cells can be printed as well	Low build rate extrude out as filament only, viscous solution may clog system	Making of vascular channels [52-58]

Table 1. The different types of 3D printing technologies currently used for making chips. The energy source refers to the energy that is required to join the material. Materials that are compatible with the machines. Advantages and disadvantages of the machines. Applications in the field of microfluidics and biology

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mechanical clamping onto fluidic chips. The key benefit of using 3D printing for interconnect fabrication is that the entire device, composed of clamp and gasket, is fabricated in a single step, obviating the need for manual assembly of O-rings[63]. With further improvement and better understanding of the materials chemistry, multiple functional chips might be able to be all printed within the same machine.

Another possible challenge is the hardware or 3D printing technologies available. With the ability of 3D printing, more complex structure can be printed or fabricated, however most of the technologies can only print one material at a given time. This may reduce the number of functions that can be done on the chip which then affects the take up rate. Depending on the 3D method used to fabricate the device, resolution of the small features on the device may be affected in the chip. Au and team did a comparative study between soft lithography and direct printing of microfluidics chip with SLA. A test device comprising of integrated female Luer connectors and different microchannel sizes was printed and compared with the device assembled by soft lithography[64]. The advantages of stereolithography when compared to soft lithography is that it is more convenient, faster, cheaper and allowed for producing complex 3D architectures overhanging structures which is not possible with PDMS moulding. However the resolution is decent and limitation by the laser beam (100 μm) prevented certain features to be produced as compared to soft lithography. The existing wide variety of PDMS microvalve and micropump designs might be difficult to replicate in plastic, so soft lithography may continue to be a dominant technique in microfluidic automation[64]. One way to solve the resolution issue can be the combination of two machines together to improve the resolution and speed as well. The use of 2PP can create very fine features but it uses a tracing method to make the parts which is very slow. Therefore, by combining with a conventional laser writer to manufacture the overall device structure and a direct-laser writer based on two-photon polymerization to yield finer details of different surface roughness, Stefan Hengsbach was able to fabricate biomedical microsystem to analyse the impact of micro textured surfaces on cell motility[65]. Hence, by combining with other technology, we can have better devices with improved features.

Currently, the cost of commercial machine is around $2000-$10000 depending on the systems requirements[22]. The high cost of the printers hinders the flexibility to experiment with different nonproprietary resins, as this may violate the warranty conditions. Shallen and team were able to show the use of a low-cost consumer-targeted 3D printer for the direct fabrication of enclosed microfluidic devices. The printer was used for the fabrication of a micromixer, a gradient generator, a droplet extractor, and a device for isotachophoresis[62]. This showed that with better improvement, low cost consumer printer would also be able to produce good quality chips. Another method that has been looked at was the assembly method. A sample library of standardized components and connectors can be manufactured using Stereolithography and assembled into a chip. This could present a solution based on discrete elements that liberates designers to build large-scale microfluidic systems in three dimensions that are modular, diverse, and predictable by simple network analysis techniques[66]. Paper recently has emerged as a promising microfluidic substrate due to its cheap cost and easy disposability and biocompatibility[59]. Xiao and team found an economical method which provides the potential to industrial production of 3D paper-based microfluidics in a printing house with mechanized procedures and standard industrialized stapling and printing equipment. Slightly similar to the LOM method for 3D printing, papers of the pattern of 2D paper based microfluidics was designed with computer-aided design software, (ii) the pattern was transferred to paper by wax-printing, (iii) the wax-printed paper was put into an oven to melt the wax. To fabrication of 3D paper-based microfluidics: (i) stacking the 2D paper-based microfluidics together according to the design of 3D paper-based microfluidics, (ii) binding the paper-based microfluidics to ensure a close contact of adjacent layers, (iii) cutting into individual devices[67]. Most software for 3D printing is engineering based therefore it might not be as user friendly for a biologist. With the continuous improvements made in terms of both software and hardware for 3D printed microfluidics, the search for the killer application might become a reality.

Conclusion

The field of microfluidics has progressed substantially since its introduction, with applications spreading across multiple fields and disciplines. The used of 3D printing for the fabrication of microfluidics will be a huge benefit for biological and medical applications. Au believe that 3D printing a “skill-less” fabrication technique has the potential to displace soft lithography as the technique of choice for the fabrication of microfluidic devices that do not require extensive, high-density automation and will allow biomedical scientists to have direct access to the “immediate manufacturing” of microfluidic devices[64]. This technology has the potential to not only change the way that researchers approach collaboration but also our perceived limitations of experimental designs, particularly in biological studies where spatial control of samples or cells is critical integrate into 3D printed microfluidic devices. With 3D

printing, the search for the killer application for microfluidics can be achieved sooner.

In other words, 3D printed microfluidics devices can dramatically lower the barrier for creating sophisticated microfluidic devices and offers a true rapid-prototyping ability with its attendant benefits to positively disrupt microfluidic development cycles.

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