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 with describing microfluidic devices with biomedical applications. Even though microfluidics have been developing rapidly over the past decade, the uptake rate in biological research has been slow. This could be due to the tedious process of fabricating a chip and the absence 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, the fast building time and ease of learning has simplified the fabrication process of microfluidic devices to a single step. This could possibly aid the field of microfluidics in finding its "killer application" that will lead to its acceptance by researchers, especially in the biomedical field. In this paper, a review is carried out of how 3D printing helps to improve the fabrication of microfluidic devices, the 3D printing technologies currently used for fabrication and the future of 3D printing in the field of microfluidics.

Introduction

For the past decade, some researchers have believed that microfluidics has the potential to influence[1] or even change[2] the way biological research is being conducted. Microfluidics is defined as the handling and analysing of fluids on the micrometer scale.[2] The ability to combine several laboratory functions onto a single chip gives microfluidic devices a significant advantage over traditional assays used in cell biology. These devices, commonly referred 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 the substantial cost and sample volume, (iii) accurately manipulating the cell microenvironment to obtain the maximum information, and (iv) providing scalability and batch screening of multiple samples. Different microfluidic systems are making inroads into biomedical research, from those with a relatively simple function to multiple function analytical systems used in a wide range of applications, 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 use in biological research.[9] One possible explanation is that this technology is still searching for its "killer application" that can outperform currently available traditional methods.[10–12]

There are currently different techniques for fabricating microfluidic 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 pieces of equipment, are labour intensive (multiple step processes to make final product), cause time wastage when making a change in design, and suffer from limited availability of biological materials. For small-scale production such as analysis in a laboratory environment, soft

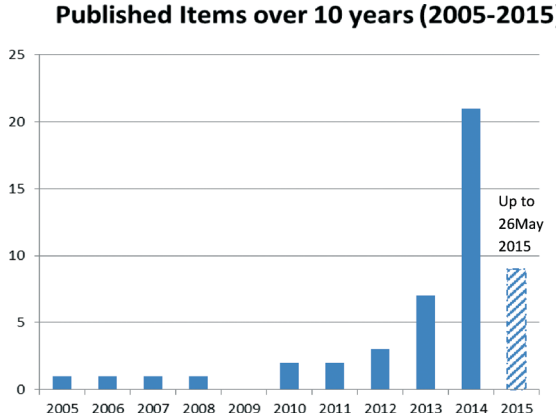

Fig. 1 Microfluidics publications involving 3D printing from 2005 to 2015. The data shown reflect the most recent data searched (26 May 2015) from the Web of Science category. The following general search string was used: topic = (microfluidics) and (3D printing) and published = (2005 to 2015).

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lithography, a multiple step process, is the current gold standard. A quick and easy fabrication of microfluidic devices will be preferred[13] as cell biologists may not necessarily 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 microfluidic devices into a single step. Publications on microfluidics with 3D printing have been on the rise in the last five years (Fig. 1). There are several other notable advantages associated with 3D printing for the fabrication of microfluidic devices over conventional methods, including the embedding of a tissue scaffold with high porosity, high resolution and defined pore structure into the device, using a range of different materials. A wide range of biomaterials, such as living cells and growth factors, could also be direct printed with a 3D printer.[14–18] The recent development of 3D printing capability has paved the way for the printing of intricate and minute 3D layered structures. It offers the 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 100–300 μm 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 its acceptance by researchers, especially in the biomedical field.

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 carried out of 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 techniques such as bioprinting, and its biological applications. Finally, an analysis of the latest research into the improvement of 3D printed microfluidics is provided.

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

Some of the key functions of a microfluidic device are sample preparation, separations of liquids, detection and fluid manipulation. More information on each specific function can be found in a paper by Woolley.[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 helps to reduce analysis time but also improves throughput.[5] Pumps, valves and mixers are added onto the device to help in manipulating the fluids. Samples are then sensed and detected using optical (laser induced fluorescence), electrochemical (conductivity, amperometry and potentiometry), mass spectrometry or biosensor methods involving a transducer.

Once the design and functions are determined, the conventional and easiest way to rapidly prototype a microfluidic device is PDMS casting-based 3D moulding (soft lithography). To fabricate the microfluidic device, firstly, computer aided design (CAD) or other engineering drawing software has to be utilized to design the required channel patterns; later, the channels are moulded on a SU-8 master or a piece of metal using the laser cutting method. After the fabrication of the mould, polydimethylsiloxane (PDMS) polymer is used to fill the master mould and is cured for over 2 hours. After the curing procedure, the PDMS is 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 compromise the accuracy of the microfluidic device.[21,22] With 3D printing, the 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 to make the 3D printing of microfluidics more applicable for the 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 a master mould is a tedious process for rapid device prototyping. It is relatively time-consuming and expensive to produce multiple high resolution (<10 μm feature size) photomasks and it is rather challenging to align and expose sequential layers of photoresist for the soft lithography fabrication process.[23] However, 3D printing 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 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 as 4 μm catch channels (Fig. 2). This enabled him to fabricate a high-throughput microfluidic platform for aging studies and long time-scale single-cell analysis in fission yeast.[23] Other teams have looked into other technologies, such as the inkjet printing for making 'millifluidic' chips for microliter droplet generation[25] and stereolithography for PDMS chips as flow cells.[24]

Once the masks have been fabricated, it takes a few hours to possibly one or two days for production, depending on the number 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 that require the user to modify the design, therefore incurring significant delays and stretching the development time, with an increase in cost. Besides making moulds, 3D

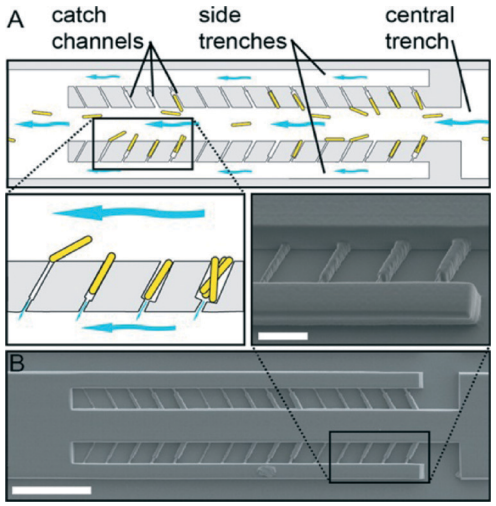

Fig. 2 (A) Schematic diagram of a fission yeast lifetime microdissector. Blue arrows indicate the flow from right to left while yellow rods are the yeast drawn into the channels and retained *via* suction. (B) The PEGDA master structure with variable catch channel dimensions. Scale bar is 100 μm. Higher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

printing offers the opportunity to fabricate the whole microfluidic device in a single step without the need for assembly process. Recently, a 3D printed microfluidic device with integrated membrane-based valves was fabricated.[26] The author used his own material formulation with a 3D printing machine and was able to fabricate the first active microfluidic device within an hour, with valves made together with the device.[26] This helped to reduce the time needed for the design process. The author believed that with 3D printing, the development landscape of microfluidics will change, permitting a "fail fast and often" strategy in which early and rapid empirical feedback is used to guide and accelerate device development.[26] More details of the different 3D printing technologies and biological applications with 3D printed microfluidics will be covered in the following section.

The use of CAD models to create a microfluidic device gives the user the ability to integrate other commercial parts, whose dimensions are known or can be measured, into the chip. Erkal 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 microfluidic chips adapted 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, or 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] for making different components for medical uses. This module-like approach to the experimental design means that parts are removable and can be easily reused after exposure to a biological sample.

Another way 3D printing can help is in 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] Also using the SLA, Hwanyong Lee and team were able to make a polymer MFI for a high-performance on-chip integrated reverse transcription (RT)-microchip which was able to perform two genetic functionalities, RNA extraction and cDNA synthesis.[29]

3D printing technologies and applications

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

3D printing is also known as additive manufacturing, 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 involves 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 is then saved in .STL format and transferred to the 3D printer. In the 3D printer, the .STL file is sliced into a certain number of 2D sequential cross-sectional slices depending on the resolution of the printer, and finally the printer additively rebuilds the 3D objects layer-by-layer based on the 2D cross-sectional slices.[34]

There are many ways of making microfluidic devices, but the focus here will be on the manufacture of microfluidics devices in a one-step process. The one-step process/manufacturing refers to directly making devices, 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 on both industrial and commercial markets; some, like stereolithography (SLA) and the 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, the 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 microfluidic devices mainly use photopolymer resins 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 use non-photocurable techniques, like the FDM, and while soft hydrogels use bioprinters.

3.1 Stereolithography (SLA)

The first commercialized 3D printing machine was established by Chuck Hull in 1988.[35] A UV laser is utilized to scan and trace over a certain area to cure the fluid resin material. The material is then hardened using 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 of the surface resolution. Normally, layers of photo-curable resins are exposed to a UV laser beam. By controlling the positions of the laser focus, polymerization of the resin can be controlled to achieve the desired structure and design. Several non-linear effects such as the polymerization of the 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 have become highly commercialized and are the most widely used type of 3D printing machine.[15,19,22] SLA systems can produce high resolution products while keeping the cost low due to the relatively low usage of the liquid medium. Moreover, SLA printers are being designed to be smaller, faster and cheaper, aiming towards future personal use.[15,19,30] An example is 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 25 μm with a minimum feature size as small as 300 μm. The minimum 10 μm movement of the laser beam during scanning of the methacrylate photopolymer resin allows final products with a smooth surface finish.[15] Thus, with the size of the machine greatly reduced and its functions combined into one, microfluidic devices can be made much more easily 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. A cylindrical 3D micro-channel named as a 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 the linear flow velocity, enabling the handling of high volumes. Antibody-Immobilized Magnetic Nanoparticle Clusters (AbMNCs) were mixed with the sample, which then forms a AbMNCs–Bacteria complex, where in this case the bacteria is *Salmonella*. A high magnetic force was applied to the walls of the HEMOS to separate out and attract the AbMNCs–*Salmonella* complexes, thus capturing the bacteria on the sides of the HEMOS (Fig. 3). A simulation was also carried out to determine the influence of the magnetic arrangement of the HEMOS in the study. The microfluidic chip was capable of handling 10 mL in 24 s, which implies that it can handle 1.5 litres in just about 1 hour (enough to

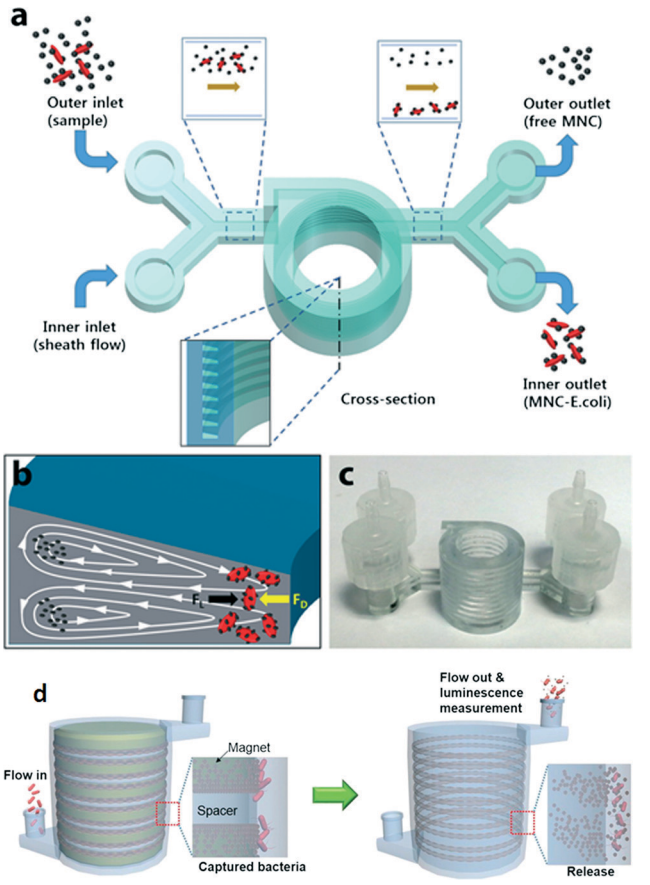

Fig. 3 (a) Schematic illustration of separation of captured bacteria by inertial focusing. (b) Illustration of Dean vortices in a channel with trapezoid cross-section. (c) Photograph of the 3D printed device. Picture taken from ref. 37 with permission from Nature Publishing Group. (d) Schematic illustrations 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 the American Chemical Society.

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]

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 a controllable digital mirror which can reflect the laser light in an entire plane, enabling the curing of an entire layer at a time. The key device is the digital mirror device (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 raised vertically upwards. This allows the machine to print parts without height restriction; however, material resins with high viscosity may affect the lifting process. The process is completed when the entire product has been printed. Compared to SLA systems, DMP-PP uses a mask projection for photopolymerization and the building of the structure is bottom-up. This is unlike SLA, which requires the movement of the stage as well as the laser; by integrating a convex lens, this could greatly reduce the fabrication time,[15] as shown in Table 1. The resolution of the features is lower as compared to SLA.

A 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 *in vitro* microchip, made of a hydrogel with a honeycomb structure, was capable of mimicking the 3D vascular morphology of an *in vivo* micro-environment. The 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. An increase in channel width led to a decrease in 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 a complex bio-environment, which is otherwise impossible to realize using ordinary conventional methods.

Table 1 The different types of 3D printing technologies currently used for making chips, their energy source (referring to the energy that is required to join the material), materials that are compatible with the machines, advantages and disadvantages of the machines, and applications in the fields of microfluidics and biology

3D printing	Energy source	Materials	Adv	Dis	Application
Stereolithography (SLA)	Laser/UV	Photocurable resin/polymer – Acrylonitrile Butadiene Styrene (ABS) like, *etc.*	High resolution, good surface finish	Requires post curing and removal of support structures	Making of master mould[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 mould[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 materials available	Pathogen detection of bacteria[45] Pathogen detection of viruses[46]
Inkjet	UV	Photocurable resin/polymer	Fast build speed, multi material printing	Removal of support materials from the channels is tedious	Making of master mould[25] Versatile chips for different types 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, extrudes as filament only, viscous solution may clog system	Making of vascular channels[52–58]

3.3 Two-photon polymerization (2PP)

Two-photon polymerization (2PP) uses femtosecond (Fs) laser pulses that directly write the pattern into a volume of photosensitive resin. The 2PP process is similar to SLA in which light triggers a chemical reaction leading to the polymerization of the photosensitive resin.[40–42] The majority of the materials are transparent in the near-infrared and highly absorptive in the UV spectral range. For SLA, the polymerization process takes place near the surface of a photosensitive resin due to single-photon 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 directly record or write any desired polymeric 3D pattern into a volume of photosensitive material. A simplified diagram is shown to illustrate the difference between one-photon and 2-photon activated processes (Fig. 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 for an inert gas atmosphere. However, due to the tracing method, the speed is slower compared to SLA. Despite 2PP being a relatively new technology, its application areas are expanding rapidly. 2PP is used for micromechanical systems, microfluidic devices, biomedical devices and scaffolds for tissue engineering.[40–42]

Besides the isolation of microbial particles, microfluidic devices can aid in the dynamic observation of microorganisms. Some bacteria contain a flagellum or pilus which aids the bacteria in moving from one point to another. Midorikawa and his team have developed a device also known as a nanoaquarium with femtosecond laser direct writing for the inspection of mobility of 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 the observation time and prevent the evaporation of water as compared to traditional methods, where small microorganisms are grown on a petri dish.

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 manufacture of prototypes of various nanoaquariums with different structures, allowing highly functional observations and analysis of the dynamics of mobility of microorganisms.[44]

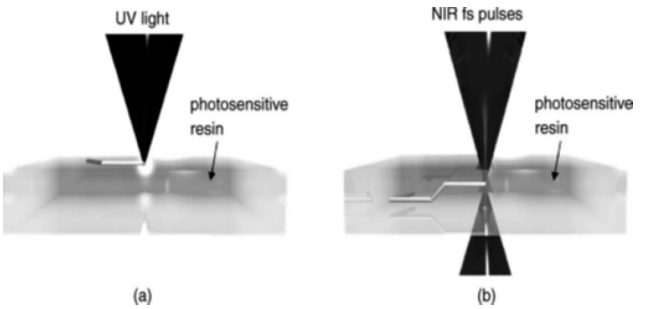

Fig. 4 (a) UV light is absorbed at the surface of a photosensitive polymer, thus leading to structures only on the surface. (b) NIR light can be focused into the volume of the UV-sensitive resin and structures are only formed at the focal point. Picture taken from ref. 42 with permission from Elsevier.

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 semi-solid form. They are then extruded out through the nozzle onto 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 moves 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 is that between each of the laid down layers, air spaces and fusion lines are always present and this can affect the final resolution of the product.[14,30]

Pathogens are usually referred to as 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 an amplified gene using gold nanoparticle (AuNP) probes was employed to detect Methicillin resistant *Staphylococcus aureus* (MRSA) bacteria by Chudobova and team (Fig. 5).[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 (AuNPs) capable of binding to the target DNA site, the mecA gene. The 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 the mecA gene and AuNPs. The chip provides a one-step approach for detection of the harmful pathogenic bacteria MRSA and due to its ultra-cheap and portable properties, 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 microfluidic device as mini bioreactors, similar to Chudobova, to aid in the analysis of viruses with the use of quantum dots.[46]

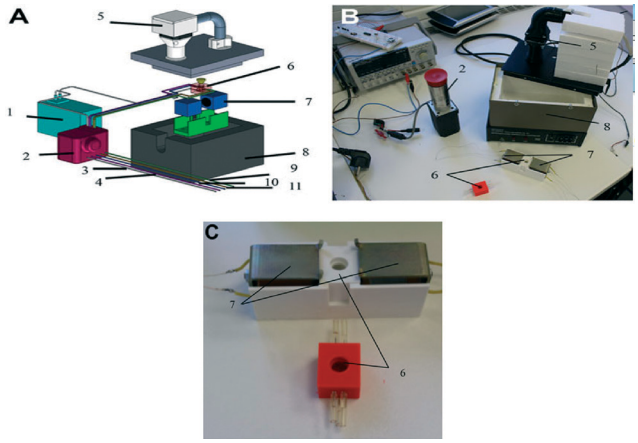

Fig. 5 (A) Scheme of 3D-printed chip for detection and confirmation of MRSA presence using binding of MRSA to gold nanoparticles with specific primers on the chip, (B) system for the identification of MRSA in the sample, and (C) reaction chamber of 3D-printed chip: 1—spectrophotometric detector, 2—pump with valves, 3—outlet, 4—first inlet hose, 5—thermoregulatory system, 6—cultivation chip, 7—electromagnet, 8—thermoisolating box, 9—second inlet hose, 10—third inlet hose, and 11—fourth inlet hose. Picture taken from ref. 45 with permission from John Wiley and Sons.

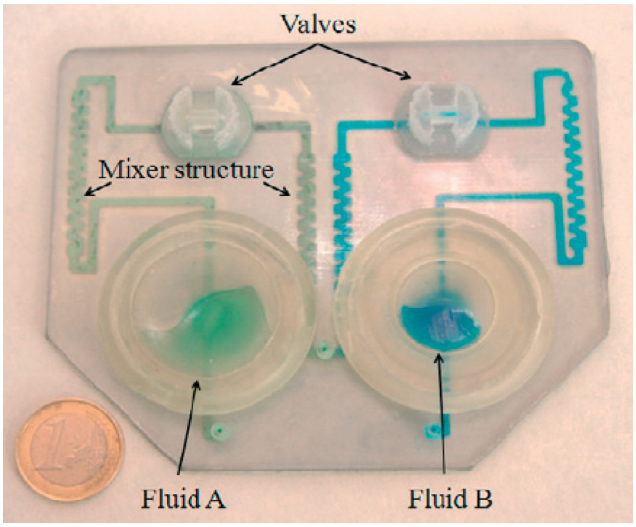

Fig. 6 Cervical microfludics prototype with fluid mixer and homogenizer. The reagent and the sample will be stored in two reservoirs and expelled by fingertips for mixing. Picture from ref. 47 with permission from Elsevier.

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, except that photoresin or wax is jetted out instead of ink. Jetting heads release material onto the tray and the material is cured by the UV light attached to the jetting head. Once the material is cured, the build tray is lowered and the next layer is built. The advantages of this system include high quality and accuracy, fast build speed and ability to print multiple materials.

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

Anderson and team were able to fabricate a fluidic device with an 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, the 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 reproduce 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 scaffolds for tissue regeneration or even patterning of biological materials such as DNA and cells can be produced with a bioprinter. Currently, bioprinters on the market, such as The regenHU BioFactory®, 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-material cell environments. The printing heads can be classified into two groups, dispensing and jetting.

Dispensing is described as the release of material, usually in filament form; this includes extrusion. For extrusion, pressure is used to force the material through the nozzle in a controlled manner to construct a 3D structure. Once the 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 hydrogels.

Jetting is described as the release of material in droplet form for better precision. It uses either an inkjet head or microvalve technology. This technique can be divided into two main categories. In a continuous inkjet (CIJ), a steady stream of small droplets is produced when pressure oscillations are applied to the stream and the droplets are either deflected by an electrostatic field onto a substrate or not deflected and collected for reuse. In a drop-on-demand inkjet, ink droplets are produced when required. A volumetric change in the fluid initiates the droplet formation, either by thermal or piezoelectric

means. In thermal inkjet printing, rapid local heating generates a 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, a simple droplet-based deposition or extrusion style printing mechanism is used, 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.

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 organs-on-chips enables the mimicking and stimuli generation of mechanical stresses, chemical gradients and other required *in situ* conditions. There are both single organs-on-chips for mimicking a particular organ function and multi-organs-on-chips for studying the interaction between multiple organs. For generating such organs-on-chips, conventional cell culture techniques were employed previously before the advent of 3D bioprinters.[31] 3D bioprinters use agarose, cells and other biomaterials for making the scaffold. The additional benefit of the bioprinter is that 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 microchannel was bioprinted as an *in vitro* replacement for blood vessels.[52] This was achieved by using a NovoGen MMX bioprinter and cell culture was carried out inside this microchannel 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 (Fig. 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 them once the tissue is made. The artificial material is either removed physically by mechanical pulling or vacuum, or by chemical means of heating to melt. Other sacrificial materials include collagen precursors,[53] a direct method with fugitive organic ink,[54–56] gelatin[57] and carbohydrate glass,[58] with different bioprinters.

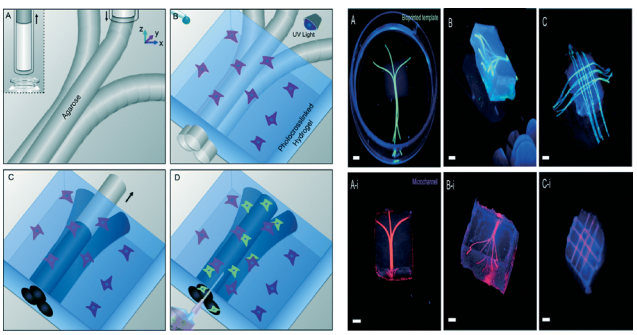

Fig. 7 (Left) Schematic representation of bioprinting of agarose template fibers and subsequent formation of microchannels via template micromolding. A) A bioprinter equipped with a piston fitted inside a glass capillary aspirates the agarose. After gelation at 4 °C, agarose fibers are bioprinted at 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. (Right) Photographs of the different bioprinted patterns. A-i) planar bifurcating pattern, B-i) 3D branching pattern and C-i) 3D lattice pattern. Green refers to the bioprinted template with agarose while Red refers to microchannels perfused with a fluorescent microbead. Picture taken from ref. 52 with permission from the Royal Society of Chemistry.

The future of 3D printing of microfluidic devices

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

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 microfluidic devices were initially silicon and glass.[59] These materials were chosen because of their excellent inertness, high strength and thermoconductivity. However, these

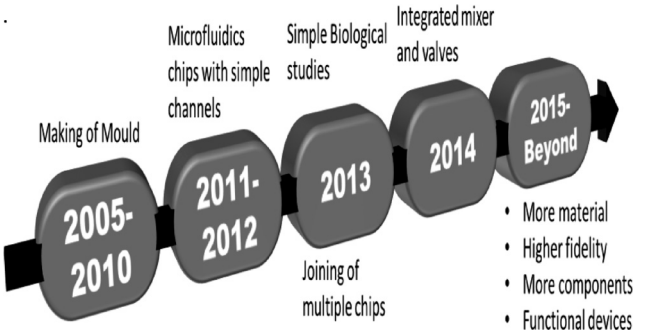

Fig. 8 Research trend in 3D printing microfluidics chips since the first relative publication in 2005; the trends of using 3D printing as a rapid prototyping device to printing functional chips with the specific function of the user.

materials are non-permeable to gases and 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 become the most common substrate for cell-related applications 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 microfluidics, other materials have to be employed as it is unable to be directly 3D printed yet.

As mentioned, for SLA, DLP and 2PP, a photo-curable resin/polymer is needed for making microfluidics chips. Photopolymers are polymers that undergo an interaction with light to alter their physical or chemical properties.[60] Their low-cost, tunability and transparency made them a preferred choice for researchers in making microfluidics with 3D printing. However, most materials for 3D printing are commercialised and require optimisation before use in biological applications. Feng Zhu and team decided to investigate commercial materials for the multi-jet modelling (MJM) system and the SLA systems. For MJM, VisiJet Crystal (rated United States Pharmacopoeia (USP) Class VI) was used, while SLA used the clear photopolymer, Watershed 11122 XC (Watershed) and Dreve Fototec 7150 Clear (Dreve Otoplastik GmbH). Zebrafish embryo-trapping microfluidic devices were printed with both printers and compared for cell viability. It was found that VisiJet Crystal (HD3500+) and DSM Watershed (Viper Pro) materials are toxic to zebrafish embryos in long-term studies (more than 3 days) even after post-treatment of the materials. Initially, the Dreve Fototec material was toxic to zebrafish embryos, but after soaking in 99% ethanol for 24 hours, it was completely inert towards 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 chips 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 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 materials chemistry, multi-functional chips might be able to be entirely printed within the same machine.

Another possible challenge is the hardware or 3D printing technologies available. With 3D printing, more complex structures 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 carried out on the chip, which then affects the uptake rate. Depending on the 3D method used to fabricate the device, resolution of 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 a microfluidics chip with SLA. A test device comprising 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 are that it is more convenient, faster, cheaper and allowed for production of complex 3D architectures overhanging structures, which is not possible with PDMS moulding. However, the resolution is moderate and the limitation of the laser beam (100 μm) prevented certain features from being 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 may be the combination of two machines together to improve the resolution and also the speed. 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 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 a biomedical microsystem to analyse the impact of micro-textured surfaces on cell motility.[65] Hence, by combining with other technologies, we can have better devices with improved features.

Currently, the cost of a commercial machine is around $2000–$10 000 depending on the system requirements.[22] The high cost of the printers hinders the flexibility to experiment with different nonproprietary resins, as this may violate the warranty conditions. Shallan 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 more improvement, a low-cost consumer printer would also be able to produce good quality chips. Another method that has been looked at is 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 has recently 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 for 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 Laminated Object Manufacturing (LOM) method for 3D printing, papers of the pattern of 2D paper-based microfluidics were designed with computer-aided design software, the pattern was transferred to paper by wax-printing, and the wax-printed paper was put into an oven to melt the wax. Fabrication of 3D paper-based microfluidics involved the following: (i) stacking the 2D paper-based microfluidics together according to the design of the 3D paper-based microfluidics, (ii) binding the paper-based microfluidics to ensure close contact of adjacent layers, and (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 believes that 3D printing as 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 manufacture 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 to integrate into 3D printed microfluidic devices. With 3D printing, the search for the "killer application" for microfluidics can be achieved sooner.

In other words, a 3D printed microfluidic device 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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