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Microfluidic technology and its application in the point-of-care testing field

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ABSTRACT

Since the outbreak of the coronavirus disease 2019 (COVID-19), countries around the world have suffered heavy losses of life and property. The global pandemic poses a challenge to the global public health system, and public health organizations around the world are actively looking for ways to quickly and efficiently screen for viruses. Point-of-care testing (POCT), as a fast, portable, and instant detection method, is of great significance in infectious disease detection, disease screening, pre-disease prevention, postoperative treatment, and other fields. Microfluidic technology is a comprehensive technology that involves various interdisciplinary disciplines. It is also known as a lab-on-a-chip (LOC), and can concentrate biological and chemical experiments in traditional laboratories on a chip of several square centimeters with high integration. Therefore, microfluidic devices have become the primary implementation platform of POCT technology. POCT devices based on microfluidic technology combine the advantages of both POCT and microfluids, and are expected to shine in the biomedical field. This review introduces microfluidic technology and its applications in combination with other technologies.

1. Introduction

In early 2020, the outbreak of the coronavirus disease 2019 (COVID-19) caused incalculable losses of people’s lives and health, and damaged the economy around the world. As of August 2021, there were 220 million confirmed cases worldwide, and the pandemic had caused a cumulative 4.56 million deaths around the globe (WHO, 2021b). Three novel coronavirus variants that are more transmissible and more virulent have since been found. Currently, the virus is still raging in some countries and regions, seriously threatening the lives and health of people around the world and posing a huge challenge to the global public health system (WHO, 2021a). The World Health Organization (WHO) Independent Panel on COVID-19 Pandemic Preparedness and Response has predicted that this pandemic will cause $10 trillion in damages globally by the end of 2021 (WHO, 2021c). This sudden disaster exposed the shortcomings of traditional diagnostic methods. Most traditional disease detection methods, although accurate, require expensive and sophisticated instruments, are operated by professional technicians in the laboratory, and have complex and time-consuming processes. In this global pandemic situation, traditional detection methods appear to be unsuitable for rapid diagnosis, and it is extremely urgent to develop timely diagnoses that can be used to quickly screen for viruses, especially in underdeveloped and remote countries and regions where it is difficult to meet the above strict experimental conditions of traditional detection methods (Yager et al., 2006). Therefore, the development and promotion of rapid and simple infectious disease detection methods are of great significance for maintaining public health worldwide.

Point-of-care testing (POCT) is a rapid detection method that is utilized at the sampling site or next to the patient (Pandey et al., 2017). With the advantages of being instant, rapid, simple, portable, and automatic, POCT can eliminate the complex process of sample processing in a laboratory and is not restricted by the environment. Hence, it has become a research hotspot in the in vitro molecular diagnosis field in recent years (Zarei, 2018). The diagnostic techniques commonly used in POCT detection primarily include immunochromatography, colloidal gold, dry chemistry, and loop-mediated isothermal amplification (LAMP) (Pandey et al., 2017). In recent years, with progress in science and technology, the development of sensing technology, advanced manufacturing technology, and the Internet of things, miniaturized, convenient, and integrated microfluidic chip technologies (Su et al., 2015) and biosensor technologies (Nasser et al., 2018) are increasingly applied to POCT detection, which have obvious advantages for the field.

This review primarily focuses on the key technologies of microfluidics, biosensing technology, and LAMP technology in the field of POCT diagnosis (Fig. 1).

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Microfluidic technology is a science and technology used to accurately control and manipulate micro-nano fluids in a micro-nano scale space (Whitesides, 2006). The microfluidic chip, also known as a lab-on-a-chip (LOC), is one of the technology platforms and devices used for the realization of microfluidic technology (Figeys and Pinto, 2000). Microfluidic chips can integrate the basic functional units involved in biological and chemical experiments—such as sample preparation, reaction, separation, and detection—on a chip of several square centimeters (Thorsen et al., 2002). Fluid flows to each reaction unit through microchannels. The basic characteristics and advantages of microfluidic chips are the flexible combination and large-scale integration of multiple technical units on tiny platforms that is an inter-discipline involving physics, chemistry, materials science, medicine, mechanics, optics, mechanical engineering, micromachining, and biological engineering (Juncker et al., 2002; Thorsen et al., 2002).

The concept of microfluidic technology was originally derived from Micro Total Analysis Systems (μ-TAS) and proposed by Swiss scientists Manz and Widmer in 1990 (Manz et al., 1990). Initially, it was primarily used in chemical and biological analysis (Manz et al., 1990). The most obvious characteristics of liquid at the micro-nano scale are laminar flow and electroosmosis (Beebe et al., 2002), which are required to manipulate and drive fluid. Only a small amount of samples in μ-TAS can complete the separation and detection process with high sensitivity and resolution, low cost, and short time-consumption (Beebe et al., 2002).

To be specific, the processing, reaction, detection, and other processes of the samples to be tested are all integrated into the microchannel, but to implement the microfluidic technology, the approach for designing the microfluidic chip structure was not clear at that time (Lin et al., 2006). As Manz’s team realized capillary electrophoresis and fluid analysis on flat microchips, the main configuration of the μ-TAS was defined as the centimeter scale flat microchip (Harrison et al., 1992; Manz et al., 1991). In 1994, Ramsey et al. improved the injection method of capillary electrophoresis and improved the chip performance (Jacobson et al., 1994a, b). In that year, the first μ-TAS conference was held in the Netherlands. In 1995, Caliper, the world’s first microfluidic company, was founded in the United States. In the same year, the Mathies team from the University of California, Berkeley, realized DNA sequencing on a microfluidic chip, and then a polymerase chain reaction (PCR) was integrated on the microfluidic chip (Woolley et al., 1996; Woolley and Mathies, 1995). The Mathies team’s research showed the commercial value of microfluidic technology (Woolley et al., 1998). In 1998, Whitesides and Xia introduced polydimethylsiloxane (PDMS) to make microfluidic chips using rapid prototyping and soft lithography, thereby reducing the manufacturing cost of microfluidic chips (Xia and Whitesides, 1998). In 2000, the 4th International μ-TAS conference was held, and this indicated that the spring of microfluidic technology was near. In 2001, Lab on a Chip, a professional journal in the field of microfluidics, was founded. In 2002, Quake et al. realized the large-scale integration of microfluidic chips (Manz et al., 1995). In 2003, microfluidic technology was named as “one of the 15 most important inventions that will affect the future of humans” by Forbes magazine. In 2004, Business 2.0 magazine called microfluidic technology “one of the seven technologies that will change the world”. Since then, many microfluidic companies have been established successively, and research on microfluidic technology has become a hot topic. Microfluidic chips are beginning to shine in biochemistry, immunity, molecular diagnosis, gene sequencing, and other fields.

2.2. Materials of microfluidic chips

Microfluidic technology initially relied on silicon materials used in microelectronics and micro-electro-mechanical systems (MEMS) (Manz et al., 1995). Lithography is the most commonly used technology to fabricate silicon and glass chips in MEMS, and this technology has since matured. Therefore, silicon and glass were the first substrate materials used to manufacture microfluidic chips (Hoffmann et al., 2009; Verpoorte et al., 1992). However, silicon is expensive, opaque, as fragile as glass, and has poor air permeability, making it difficult to meet many applications in biochemical analyses. It is gradually being replaced by softer polymer materials (Becker and Gärtner, 2008; Mou and Jiang, 2017; Ren et al., 2013) with a wider variety of manufacturing methods. PDMS, also known as silicon rubber, is the most widely used microfluidic chip material in the research stage. It has good air permeability, good optical transmittance, good biocompatibility, low price, simple molding, and is the ideal microfluidic chip material. Other polymer materials, such as thermoplastic polyethylene methyl methacrylate (PMMA), also known as acrylic or organic glass, have excellent optical transmittance and easy surface modification. PMMA and polycarbonate (PC) are common materials for the mass production of microfluidic chips. Cycloolefin copolymers (COC) are a type of amorphous transparent materials.
polymers with a ring olefin structure. It has a much lower water absorption than PMMA, similar transparency to PMMA, and better heat resistance than PC. It is easy to form and seal, resistant to acid and alkali chemical corrosion, and has good dielectric properties. Hence, COC is often used in the semiconductor and medical device industries. Polytetrafluoroethylene (PTFE), also known as Teflon, is a good hydrophobic material and is used as a hydrophobic coating. Polyethylene terephthalate (PET) has often been used as a hot-pressing bonding film for sealing chips. Polypropylene (PP) is a translucent material, commonly used to make pipe fittings, centrifugal tubes, saliva collectors, and other consumables. Thermoplastic polyurethane (TPU) is a non-toxic, good biocompatible, high modulus, high strength, hydrophobic, and opaque material that can prevent water and protein absorption. It is often used in microfluidic microchannels, artificial hearts, and other devices. Polyimide (PI) foam is a high-temperature insulating material that can be used as the thermal insulating layer of microfluidic chips. In addition, low-cost microfluidic polymer materials, such as polystyrene (PS) and polyvinyl chloride (PVC), are also available. In recent years, with the rise in 3D printing technology, photosensitive resin has become an optional material for microfluidic chips. Hydrogel material (Nie et al., 2020) is a new type of biological material that can be used for repairing biological tissue and bone, cell transplantation, and skin wound healing materials. Owing to its unique physical and chemical properties, hydrogel materials have become a hot topic in biology, chemistry, materials, and other fields. Paper microfluidic chips (Dou et al., 2017a) based on filter paper are cheaper, more environmentally friendly, simpler to manufacture, and more flexible in the structure design. In recent years, paper microfluidic chips have gradually stepped onto the stage of microfluidics. As the functions of microfluidic chips have become more diversified and integrated, composite microfluidic chips made of two or more materials and microfluidic chips with special functions made of nanomaterials have also become prominent (Domachuk et al., 2010; Ren et al., 2014).

2.3. Fabrication techniques of microfluidic chips

Photolithography and etching are traditional manufacturing techniques of microfluidic chips and are primarily used to make silicon and glass chips. With the rise in polymer-based microfluidic chips, injection molding, molding, hot pressing, soft lithography, laser ablation, photolithography galvanofomung afbormung (LIGA), 3D printing, and other manufacturing technologies are increasingly used in microfluidic chip processing (Becker and Gärtner, 2008; Scott and Ali, 2021).

2.3.1. Photolithography and etching

Photolithography and etching techniques used in semiconductor and integrated circuit chips are the basic processing techniques of microfluidic chips. These two technologies are mature and have been widely used in silicon and glass chip processing, and they can produce high precision micro-nano-structures at micro-nano scales. However, these two technologies have high requirements for the processing environment, requiring professional technicians to operate in an ultra-clean room. In addition, the operation process is complex, and the equipment is expensive. However, photolithography and etching are still currently some of the most commonly used high-precision processing technologies.

The steps of photolithography generally include coating, pre-baking, exposure, development, post-baking hard film, and other processes (Becker and Gärtner, 2008; Dendukuri et al., 2007). First, the photore sist is uniformly covered on the surface of a substrate material (typically silicon) using spin coating and then dried on a hotplate. The next step is to expose the substrate coated with photore sist under ultraviolet (UV) light through a mask. The pattern on the mask is then transferred to the substrate material by developing and drying.

Photolithography simply transfers the pattern on the mask to the substrate in two dimensions, and subsequent etching processes are required to obtain the final three-dimensional structure. Etching is a process in which a photosist is used as a masking layer, and the etched material is stripped from the substrate by physical or chemical methods to carve the desired pattern microstructure with a certain depth (Hnatovsky et al., 2005). According to the different etching processes, etching is divided into wet etching and dry etching. Wet etching is the etching method in which the etched materials will be stripped off by a chemical reaction between the etching solution and the etched substance. Its advantages are its high selectivity, good uniformity, and wide applicability, and its main disadvantage is a lack of high fidelity. Wet etching is suitable for most glass, metal, plastic, and other materials (Hnatovsky et al., 2005). Dry etching is an etching method that uses plasma and other high-energy beams to directly attack the surface film and react with it. It is an anisotropic etching method characterized by high fidelity, and is only targeted at characteristic materials. However, the equipment for dry etching is expensive. Hence, dry etching is rarely used in manufacturing microfluidic chips. After etching, the photosist on the surface should be removed. The methods for photosist removal on the surface of a pattern include the solvent method, oxidation method, plasma method, and the ultraviolet decomposition method (Dendukuri et al., 2007). After the above steps, a microfluidic chip with a microchannel structure is obtained.

2.3.2. Injection molding

Injection molding is the most commonly used method for mass processing devices. The process of injection molding is to place the raw material in an injection molding machine and then heat the raw material into a liquid state and press the liquid materials into the mold. Finally, it is cooled, solidified, and demolded to obtain the chip structures (Attia et al., 2009; Mair et al., 2006; Sen et al., 2019). The key to the injection molding method is the preparation of the mold. The mold preparation process is typically complex, and it requires many steps, high technical requirements, a long production cycle, and a high cost. However, a good mold can produce 300,000 to 500,000 polymer chips (Mair et al., 2006). Injection molding has good repeatability, a short production cycle, a low cost, and is suitable for the mass production of microfluidic chips. The mold production process consists of preparing the silicon-based negative mold using the photolithography and etching methods, and then preparing the nickel alloy positive mold using the electroforming method. After a series of fine processing, the mold can be installed on the injection molding machine to produce the microfluidic chips-based polymer substrate.

2.3.3. Molding

Molding is a common method for preparing the microfluidic chips-based thermosetting polymer (Becker and Gärtner, 2008; Novak et al., 2018). The process of the molding method is to first use photolithography and etching to produce a positive mold and then pour liquid polymer materials on the positive mold and heat it to cure. The polymer is then peeled off the mold to obtain a microchip with microstructure patterns. The materials of the positive mold are silicon and glass. PDMS is the commonly used polymer material in molding in addition to thermosetting plastics, such as phenolic resin, epoxy resin, organic silicon resin, polyurethane, polyacrylic acid, and fluorine plastics. The choice of materials is the key to the molding method (Sen et al., 2019). The adhesion between the mold and the polymer material should be weak and easy to de-mold. Although molding materials are limited to thermosetting polymers and heat-resistant volatile polymers, its process is simple and rapid and is suitable for low-cost chip fabrication, especially for the rapid iterative state of laboratory research.

2.3.4. Hot embossing

Hot embossing is a microfluidic chip fabrication method that uses temperature and pressure to rapidly replicate microchannels (Abgrall et al., 2007; Locascio et al., 2006). The key of the hot pressing process is to align the polymer substrate with the mold and then put them into the
Although the materials of hot embossing are limited to thermoplastic polymers, such as PMMA, PC, COC, PP, and PTFE. Hot embossing molds can be silicon-based, glass-based, or micromachined metal-based. Although the materials of hot embossing are limited to thermoplastic material, the equipment required is simple and easy to operate (Yin et al., 2018). Therefore, hot embossing can be used to replicate chips in large quantities and is widely used.

2.3.5. Soft lithography

Soft lithography is a new micropattern copying technology that emerged in the 1990s (Faustino et al., 2016; Novak et al., 2018). Compared with traditional lithography, it uses soft molds, such as elastomeric stamps, to replace the hard film in lithography to realize the copy and transfer micropatterns. Its core is the elastomeric stamp (Faustino et al., 2016; Xia and Whitesides, 1998). PDMS is the most commonly used elastomeric stamp material. The elastomeric stamp can be made by photolithography or molding. Soft lithography can be used to manufacture complex three-dimensional structures and irregular curved surface graphics, and its manufacturing accuracy can be up to the 30 nm–1 μm level (Sen et al., 2019; Xia and Whitesides, 1998). Soft lithography is a type of technology suitable for laboratory processing that can be applied to biopolymer, colloid, glass, ceramics, and other materials. Owing to the limitation of PDMS materials, soft lithography technology also has some disadvantages, such as the limitation of multi-layer micro-machining, and a too large or too small aspect ratio will lead to a distortion of the microchannels (Novak et al., 2018).

2.3.6. Laser ablation

Laser ablation is a non-contact micro-machining technology that uses a mask, polymer substrate, and laser light source (Becker and Gärnter, 2008). The processing mechanism of laser ablation is that the laser acts on the substrate material exposed at the bottom through the mask, using the laser energy to ablate the exposed part of the substrate material to finally obtain the same pattern on the substrate as the mask (Becker and Gärnter, 2008; Faustino et al., 2016; Hsieh et al., 2017). Laser ablation also can directly draw the designed pattern without a mask. The computer-aided design (CAD) pattern data are imported into the corresponding software, the appropriate parameters are established according to the pattern and material, and protective glasses are worn. The laser light source is then opened to directly act on the substrate to finally obtain the required pattern (Huff et al., 2010; Scott and Ali, 2021; Waddell, 2006). This method is simple and does not require an ultra-clean environment and complex process work. In addition, it can obtain a high precision pattern. However, because laser ablation has high requirements for equipment operation, the laser light source is dangerous to a certain extent, and experimental personnel need to take protective measures, such as wearing protective clothing and goggles. In addition, the operational process should be performed strictly in accordance with the specifications (Scott and Ali, 2021; Waddell, 2006). All of these processes result in the low efficiency of laser ablation, and this is not suitable for mass production and limits its application potential.

2.3.7. LIGA

LIGA is a German abbreviation for Lithographie, Galvanoformung, and Abformung, meaning photolithography, electroforming, and molding. LIGA refers to the process of using X-ray lithography and micro-electroforming to manufacture precision molds and replicate microstructures in large quantities (Khan Malek, 2006; Wang et al., 2021). LIGA is primarily used to manufacture microfluidic chips with a high aspect ratio. LIGA technology is composed of photolithography, electroforming, and microreplication. Quasi-LIGA technology uses an UV light source to replace synchrotron radiation X-ray in LIGA technology, and then it conducts the subsequent micro-electroforming and microreplication process. It does not need synchrotron radiation X-ray lithography and a special X-ray mask, and this aspect is conducive to the mass production of micromechanical devices. According to the different process routes of UV deep lithography, quasi-LIGA technology can be divided into three types: multilayer lithography-LIGA, silicon deep etching-LIGA, and SU-8 deep lithography-LIGA (Khan Malek, 2006; Wang et al., 2021).

2.3.8. 3D printing

Additive manufacturing (AM), also known as three-dimensional printing (3D printing) or rapid prototyping (RP), is a new manufacturing technology developed in recent years, which enables rapid prototyping by directly printing the 3D objects layer by layer (Amin et al., 2016; Au et al., 2016; Nielsen et al., 2020; Thayer et al., 2020). Compared with other manufacturing technologies, 3D printing has many advantages. First, in terms of material selection, traditional processing technologies primarily use silicon, quartz, glass, polymer, and other materials. Currently, biomaterials, such as ceramics, metals, and carbon matrix composites, are rarely used in traditional processing methods (Amin et al., 2016; Au et al., 2016). This aspect limits the application of micromachining technologies in the biomedical field. With the rise of 3D printing, many biological materials are used in 3D printing, and this expands the scope of materials in the field of micromachining. In addition, resin, metal, and hydrogels are also widely used (Tetsuka and Shin, 2020). Second, these existing machining technologies have difficulty machining complex three-dimensional structures, resulting in many designs that cannot be realized. This limits the arbitrary structure development of microfluidic chip technology. However, 3D printing can print 3D structures that are difficult to create using traditional processing modes. The designed drawings are imported from CAD to the connected software, and then the printed materials are placed on the printing platform (Nielsen et al., 2020; Thayer et al., 2020). The machine can then print out the patterns layer by layer. The printing process is simple and fast, and the cost is low (Zhu et al., 2020). Third, the processing of microfluidic chips had difficulty simultaneously meeting the requirements of a high processing accuracy, fast processing speed, and low processing cost. Owing to the high degree of automation and customization, 3D printing can realize the rapid fabrication of microfluidic chips in small batches without mold. The increasingly mature 3D printing technology is a new development opportunity for the field of micro-processing, such as bio-printing, organ chips, human organs and tissues, artificial cells, and mechanical arms, and will accelerate the pace of biological manufacturing (Bishoph et al., 2016; Ho et al., 2015; Hong et al., 2017; Mandrycky et al., 2016; Murphy and Atala, 2014).

According to the different formulation principles and materials, 3D printing technology suitable for microfluidic technology can be divided into photocuring, material jetting, and fused deposition modeling (FDM) (Hu et al., 2016). The classifications and characteristics of various 3D printing technologies are shown in Table 1 (Borrello et al., 2018; Hu et al., 2016; Ho et al., 2015; Karakurt et al., 2020; Layani et al., 2018; Ligon et al., 2017; Mandrycky et al., 2016; Murphy and Atala, 2014; Placone and Engler, 2018; Quan et al., 2020; van der Linden et al., 2020; Wang et al., 2016; Wu et al., 2019; Yuan et al., 2020).

The principle of photocuring molding is photopolymerization, and its printing material is photosensitive resin. Photocuring is primarily divided into two types: the stereo lithography apparatus (SLA) and digital light procession (DLP) (Quan et al., 2020). SLA is currently one of the most precise 3D printing technologies. Its formation process is as follows: During the printing process, the SLA requires a filled liquid tank filled with liquid photosensitive resin. A liquid photosensitive resin is first cured using a polymerization reaction under UV light. The laser
Beam then scans and solidifies layer by layer. The printing components of the structure are formed in the liquid tank, and their size is limited by the size of the liquid tank. The printing speed is also limited by the scanning speed (Karakurt et al., 2020; Wang et al., 2016). DLP is different from SLA. DLP uses UV light and a digital micromirror as the light source, while SLA uses a scanning micromirror as the point light source. As a result, DLP scans faster, but has a lower resolution than SLA (Borrello et al., 2018; van der Linden et al., 2020; Wu et al., 2019).

Material jetting consists of spraying the printing material onto the printing platform and then allowing it to solidify. In this method, the printing is done layer by layer. Material jetting can be divided into inkjet printing and photopolymer jetting, according to the different materials used. Inkjet printing uses melted wax material as the printing material to cool and cure. The photosensitive resin is used as the cured material of the photopolymer jetting that is deposited to the printing platform using a layer spray and cured by UV light irradiation. The entire printing process needs to repeat the above steps many times (Layani et al., 2018; Placone and Engler, 2018). DLP, digital light projection; DMD, digital mirror device; FDM, fused deposition modeling; LENS, laser engineered net shaping; LMD, laser metal deposition; LOM, laminated object manufacturing; MJM, multi-jetting mold; SLA, stereo lithography apparatus; TPP: two photon polymerization; UV, ultraviolet.

| Additive manufacturing (3D printing) | Principles | Classifications | Properties | Characteristics | References |
|-------------------------------------|------------|----------------|------------|----------------|------------|
| Photocuring                         | The liquid photosensitive resin is used as the printing material, and the liquid resin is selectively cured by photopolymerization to achieve molding | SLA, DLP, TPP | SLA: UV light + scanning galvanometer; DLP: UV light + digital micromirror | The printing size is related to the size of the liquid tank, high accuracy, slow speed, and photosensitive resin | (Ligon et al., 2017; Mandryczyk et al., 2016; Murphy and Atala, 2014; Placone and Engler, 2018; Quan et al., 2020; van der Linden et al., 2020) |
| Material jetting                    | The printing material is sprayed to the printing platform continuously and then solidified. The model is built layer by layer | Inkjet printing, polymer jetting, MJM | Inkjet printing: waxy materials; MJM: photosensitive resin | Polymers, waxy materials | (Borrello et al., 2018; Hu et al., 2016; Ho et al., 2015; Karakurt et al., 2020; Layani et al., 2018; Ligon et al., 2017) |
| Binder jetting                      | Powder materials and adhesive are used as the forming materials. The powder materials are evenly distributed on the forming platform, adhesive droplets are sprayed by the inkjet printing nozzle, and the powders are bonded together by adhesion | / | / | / | (Borrello et al., 2018; Hu et al., 2016; Ho et al., 2015; Karakurt et al., 2020; Layani et al., 2018; Ligon et al., 2017) |
| Material extrusion                  | The nozzle extrusion wire is used to build a two-dimensional plane. The two-dimensional plane is then used to form a three-dimensional entity | FDM, 3D microextrusion, 3D plotting | The step grain of forming surfaces is obvious and difficult to improve | Silica gel, organic, or inorganic slurry, hydrogel, polymers, or even cells | (Hu et al., 2016) |
| Powder bed fusion                   | The powder material is melted using an energy beam such as a laser, or an electron beam is scanned and forms an entity after the material is interred and bonded | SLS, SLM, EBM | SLM: laser melts the polymer; SLM: laser melts metal powder EBM: electron beam melts metal powder | Good mechanical properties, expensive equipment, high cost, slow speed | (Borrello et al., 2018; Hu et al., 2016; Ho et al., 2015; Karakurt et al., 2020; Layani et al., 2018; Ligon et al., 2017) |
| Sheet lamination                    | The sheet coated with the adhesive is fed to the printing platform and it is bonded with the previous layer by the hot pressing roller | LOM | It is difficult to obtain a precise surface, and the application field is limited | Fast speed, low cost, paper, and composite materials | (Borrello et al., 2018; Hu et al., 2016; Ho et al., 2015; Karakurt et al., 2020; Layani et al., 2018; Ligon et al., 2017) |
| Directed energy deposition          | Uses a laser, plasma, electron beam, and other different heat sources to selectively melt powder or filamentous materials. Layer by layer deposition is used to form the solid | LMD, LENS, DMD | Suitable for large size machining and industrial automation, parts repair, and parts additions | Parts repair, parts additions | (Hu et al., 2016; Ho et al., 2015; Karakurt et al., 2020; Layani et al., 2018; van der Linden et al., 2020; Wang et al., 2016; Wu et al., 2019; Yuan et al., 2020) |

Like microfluidic technology, additive manufacturing technology also became an emerging technology at the end of the last century. It has developed rapidly in the past decade. Currently, many 3D printing technologies can be used to print microfluidic chips. SLA technology has a high printing accuracy, but the equipment is expensive and the printing speed is slow. DLP improves the printing rate, and the printing accuracy is also high, and it is one of the promising 3D printing technologies in the future. The FDM technology has wide applicability, low cost, and many commercial applications, but the printing accuracy is not high, which suitable for disposable microfluidic chips. 3D printing technology has significant application potential in medical devices, bioanalytical analysis, organ chips, and the life sciences. These microfluidic devices processed by combining microfluidic technology with 3D printing have a more flexible structural design and access to a more diverse material selection. At the beginning of the study, 3D printing can significantly improve the processing speed and accuracy and reduce the manufacturing cost, which in turn speeds up the iteration of the research. In the future, microfluidic chip manufacturing based on 3D printing technology will help POCT products develop rapidly.
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There are an increasing amount of research institutes and enterprises that are studying additive manufacturing in China and abroad. In China, the team of Yang Huayong, academician of the Chinese Academy of Sciences at Zhejiang University, has developed 3D-printed hydrogel materials and has made many innovations in organ printing, biocells, and microfluidic device printing. In addition, the 3D printer independently developed by the team can also achieve high precision and rapid printing, and it has been sold in China and abroad. Nanjia Zhou’s team at Westlake University has achieved the highest precision electronic 3D printing in the world, and Nanjia Zhou is awarded the MIT Technology Review’s “35 Innovators Under 35” in 2019. It is worth mentioning that in 2020, Zhou successfully commercialized his scientific research achievements and founded a 3D printing company named West Lake Future Manufacturing Enovate 3D. In addition, BMF Precision Tech Inc., and other companies that focus on high precision and rapid 3D printing, have developed the world’s leading 3D printing technology with ultra-high printing accuracy. Their self-developed 3D printing equipment has been used in many top universities and scientific research institutions around the world. As an emerging manufacturing method, 3D printing has great prospects in microfluidic field.

2.4. Bonding techniques of microfluidic chips

The final step in the microfluidic chip manufacturing process is bonding, and this is used to encapsulate the chip. Direct and indirect bonding techniques can be used according to different bonding techniques. Common direct bonding methods include thermal bonding (Abgrall et al., 2007; Wang et al., 2011; Yin et al., 2018), surface modification bonding (Bhattacharyya and Klapperich, 2007; Zhou et al., 2010, 2012), and ultrasonic bonding (Zhang et al., 2010b), and indirect bonding techniques include solvent bonding (Brown et al., 2006; Gan et al., 2011; Hsu and Chen, 2007; Koedsjojo et al., 2008; Sun et al., 2007) and adhesive bonding (Thompson and Abate, 2013; Wu et al., 2005).

According to the different chip materials, the bonding methods of silicon and glass chips include thermal bonding (Abgrall et al., 2007; Xu et al., 2012), anode bonding (Xu et al., 2012), and low temperature bonding (Bart et al., 2009; Shoda et al., 2020), while the bonding methods of polymer microfluidic chips include thermal bonding (Hu et al., 2016; Wang et al., 2011; Yin et al., 2018), solvent bonding (Brown et al., 2006; Gan et al., 2011; Hsu and Chen, 2007; Koedsjojo et al., 2008; Song and Park, 2017; Sun et al., 2007), adhesive bonding (Thompson and Abate, 2013; Wu et al., 2005), plasma bonding, and UV irradiation bonding (Bhattacharyya and Klapperich, 2007; Hui et al., 2005; Tsoa et al., 2007; Wu et al., 2005; Yin et al., 2015). Thermal bonding is the most common method of bonding. Its specific operation process is to place the chips and cover plate in parallel alignment in the thermal bonding machine, and then vacuum the chamber and set the bonding pressure, temperature, and time. The chip and cover plate are bonded by applying pressure. However, owing to the temperature close to the material of the glass transition temperature, thermal bonding may cause material degeneration and the channel to collapse. Anodic bonding (Xu et al., 2012) is a permanent bonding method of silicon and glass chips. The specific process is to connect the negative electrode on the side of the glass, and then the silicon chip is connected to the positive electrode. The positive and negative ions move to the anode and cathode respectively at a high temperature, and the positive and negative charges are combined under electrostatic attraction, resulting in the bonding of glass and silicon chips. Low temperature bonding (Bart et al., 2009; Mair et al., 2007; Song and Park, 2017; Tsoa et al., 2007) is the use of hydrofluoric acid (HF), epoxy adhesive, and PDMS as a binder applied a certain pressure and at low temperature to achieve bonding between the glass chips. Owing to the viscosity of the material itself, the PDMS microfluidic chip can be directly aligned with the same or different materials of the cover sheet, but this bond is reversible and unreliable. PDMS microfluidic chips and cover plates treated by plasma or UV can be irreversibly bonded (Shiroma et al., 2017; Zhang et al., 2010a). For the PMMA microfluidic chips, organic solvents are used to seal them at low temperature and atmospheric pressure, which is suitable for rapid mass production (Brown et al., 2006; Gan et al., 2011). However, attention should be paid to the solvent entering and blocking micro-channels. The characteristics and comparison of the various bonding methods are shown in Table 2 (Bhattacharyya and Klapperich, 2007; Brown et al., 2006; Gan et al., 2011; Hsu and Chen, 2007; Koedsjojo et al., 2008; Sun et al., 2007; Thompson and Abate, 2013; Wang et al., 2011; Wu et al., 2005; Zhang et al., 2010b; Zhou et al., 2010, 2012).

3. Applications of microfluidic technology in POCT

3.1. Detection technologies of microfluidic chips

The signal collection and detection devices of microfluidic chips are important parts of microfluidic functional components. To complete the detection process on a microfluidic chip with several square centimeters, there is no doubt that the requirements for detection methods and devices are more stringent, at least to meet the characteristics of small size, high sensitivity, and rapid response speed (Lin et al., 2006; Manz et al., 1995; Thorsen et al., 2002; Whitesides, 2006). The detection technologies of microfluidic chips include laser-induced fluorescence detection (Ryu et al., 2011), UV absorption spectroscopy detection (Petersen et al., 2002; Verpoorte, 2002), chemiluminescence detection (Wu et al., 2016), electrochemical detection (Kim et al., 2005; Vandaveer et al., 2004; Verpoorte, 2002), mass spectrometry detection (Thorslund et al., 2005), plasma emission spectroscopy detection (Jenkins et al., 2005), and ultrasonic detection (Franke et al., 2016), and biosensor detection (Azimi et al., 2021). Optical detection and electrochemical detection are currently the most widely used detection methods. Laser-induced fluorescence detection is the earliest, most widely used, and most sensitive optical detection method (Irawan et al., 2007; Whitesides, 2006). The detection principle is that atoms absorb light of a certain wavelength under an excitation light source of a specific frequency. The atoms are transformed from low energy level to high energy level, and they release a low-frequency fluorescence. Laser-induced fluorescence is a method used to induce electron transition to produce fluorescence by using a laser as the excitation light source (Pennathur and Fyenson, 2008). The Whitesides team reported a PDMS chip system using fluorescence detection (Whitesides, 2006).

Chemiluminescence detection is also a highly sensitive detection method (Wu et al., 2016). It determines the content of substances by detecting the luminescence intensity using the luminescence phenomenon where the ground state molecules absorb energy to transition to the excited state and back to the ground state in the form of light radiation during the process of a chemical reaction. Compared with optical detection, chemiluminescence detection does not require a light source, and the equipment is simple and easy to miniaturize and integrate, which is more suitable for microfluidic chips detection. Lin’s group designed a rotary scan chemiluminescence detection device for multi-channel detection (Mao et al., 2006).

Electrochemical detection is a detection method that converts the chemical signals of substances to electrical signals for analysis and testing (Vandaveer et al., 2004). It has the advantages of good sensitivity, simple equipment, and low cost. Mathies et al. designed it to detect analytes using sheath flow technology and capillary electrophoresis (Woolley et al., 1997).

3.2. Microfluidic chips based on LAMP

The combination of microfluidic technology and nucleic acid diagnosis is one of the most promising application directions of microfluidic technology. The combination greatly simplifies the complicated steps of nucleic acid amplification and detection and eliminates the heavy equipment, so it is very suitable for POCT molecular diagnosis (Easley...
The nucleic acid amplification process of isothermal amplification technology only needs to be completed at a constant temperature. It does not need a complex heating and cooling temperature system, and this greatly reduces the reaction time, which is more suitable for miniaturized instruments. Therefore, the combination of microfluidic technology and isothermal amplification technology can improve the detection sensitivity and detection flux at the same time by taking advantage of the advantages of both, and this gives it great development potential in the field of molecular POCT (Chang et al., 2013; Chen et al., 2010; Jung et al., 2015; Liang et al., 2019; Pumford et al., 2020; Xu et al., 2018).

The LAMP technology proposed by Notomi et al., in 2000 is based on the principle of using four specific primers and one chain to replace the active DNA polymerase to amplify nucleic acids at approximately 65 °C. The amplification efficiency reached 109–1010 copies in tens of minutes. Four specific primers were designed for six regions of target genes, and nucleic acid amplification could be completed using a chain replacement DNA polymerase (Bst DNA polymerase) at a constant temperature (approximately 65 °C) for several tens of minutes (Notomi et al., 2000). When a nucleic acid is synthesized in large quantities, pyrophosphate ions that precipitate from diethyl-nitrophenyl thio- phosphate (dNTP) combine with Mg²⁺ in the reaction solution to produce magnesium pyrophosphate precipitation with high specificity. Amplification can be determined by directly detecting the precipitation in the reaction solution to produce magnesium pyrophosphate precipitation with high specificity. Amplification can be detected by detecting the precipitation degree of the naked eye or a turbidimeter. The reaction is judged by the turbidity of the magnesium pyrophosphate precipitation (Mori et al., 2001, 2004). RNA amplification can be achieved by adding the reverse transcriptase to the DNA amplification reagent (Lin et al., 2019). LAMP has high specificity, sensitivity, stability, reliability, a quick response time, simple steps, and easy detection, which meet the requirements of microfluidic system integration (Fujino et al., 2005; Hataoka et al., 2004; Ihira et al., 2004). In addition, microfluidic technology can provide a flexible structure platform for LAMP, such as independent reaction microcavities and serpentine flow channels for sample transfer. This not only speeds up the reaction speed and saves reaction reagents, but also avoids cross-contamination and aerosol leakage. In recent years, researchers have paid an increasing amount of attention to the combination of LAMP and microfluidic chips.

A classic LAMP microfluidic chip in China is the centrifugal disc constant temperature microfluidic chip designed by CapitalBio technology. This chip has 24 detection holes, each hole volume is 1.4 μL, and the results can be obtained in 30 min. It can realize the parallel detection of multiple indicators, which greatly reduces the detection time and cost. In 2012, Kong et al. designed a portable POCT microfluidic chip based on LAMP technology for bacterial identification. The chip has a simple structure and constructs microchannels on the PDMS. By the bonding of air plasma and the glass plate, reagent distribution is conducted by using the capillary siphon effect, and fluid control is conducted by a torque valve. During the amplification process, the results can be obtained with the naked eye using a water bath at 65 °C for 45 min. POCT identification of bacteria in time with “sample in, result out” was also realized (Fang et al., 2012). Jiàng’s team proposed a microfluidic chip for the quantitative detection of pathogens based on LAMP technology in 2010, known as micro LAMP (μLAMP). The structure is a PDMS-glass composite microfluidic chip with eight conical inlet/outlets channels that can quickly and sensitively provide the specific and quantitative identification of the target nucleic acid, meeting the requirements of multi-channel parallel detection. The amplification reaction conditions are a 63 °C water bath for 1 h, and the results can be observed with the naked eye and confirmed by gel electrophoresis. A multi-integrated optical fiber, digital optical fiber sensor, and optical transducer through a turbidity absorbance measurement can realize the real-time quantitative analysis of the product (Fang et al., 2010). Bau’s group reported a LAMP reactor integrated isolation membrane for POCT detection of infectious diseases. This disposable cassette includes a single LAMP reaction chamber in which nucleic acids can be separated, concentrated, purified, and amplified. The isolation membrane can capture the nucleic acids that can eliminate the elution step. The LAMP reactor can be used to detect nucleic acids associated with other pathogens borne in saliva, urine, and other body fluids as well as in water and

### Table 2

| Bonding methods | Classifications | Principles | Characteristics | References |
|-----------------|-----------------|------------|-----------------|------------|
| Direct bonding  | Thermal bonding | Heat to near the vitrification temperature of the material and apply pressure to achieve bonding | Without auxiliary binder, no contaminants produced; good mechanical and thermal properties after binding; high quality for surface, complex post-treatment | (Bhattacharyya and Klapperich, 2007; Brown et al., 2006; Gan et al., 2011; Hsu and Chen, 2007; Koersjdojo et al., 2008; Sun et al., 2007; Thompson and Abate, 2013; Wang et al., 2014; Wu et al., 2015; Zhang et al., 2010b; Zhou et al., 2010) |
|                 | Surface modification bonding | The surface of the material is modified by radiation treatment (X-ray, UV light) or plasma treatment (O₂, O₃, N₂) to achieve bonding | Low bonding temperature, high requirements for equipment | (Bhattacharyya and Klapperich, 2007; Brown et al., 2006; Gan et al., 2011; Hsu and Chen, 2007; Koersjdojo et al., 2008; Sun et al., 2007; Thompson and Abate, 2013; Wang et al., 2011; Wu et al., 2005; Zhang et al., 2010b; Zhou et al., 2010) |
|                 | Ultrasonic bonding | Bonding is achieved by melting the material using ultrasonic energy | Fast bonding speed, high efficiency, part-bonding; suitable for mass production of polymer chips; does not use adhesives, reduces pollution; simple process and low cost; increases the difficulty of chip manufacturing | (Bhattacharyya and Klapperich, 2007; Brown et al., 2006; Gan et al., 2011; Hsu and Chen, 2007; Koersjdojo et al., 2008; Sun et al., 2007; Thompson and Abate, 2013; Wang et al., 2011; Wu et al., 2005; Zhang et al., 2010b; Zhou et al., 2010) |
| Indirect bonding | Solvent bonding | A special solvent, such as acetone, is used to slightly dissolve the surface of the material and then pressure is applied to achieve permanent bonding | Room temperature bonding technique; small microchannel deformation, suitable for small batch production; high requirements for the chip surface cleanliness and flatness; suitable solvent selection | (Bhattacharyya and Klapperich, 2007; Brown et al., 2006; Gan et al., 2011; Hsu and Chen, 2007; Koersjdojo et al., 2008; Sun et al., 2007; Thompson and Abate, 2013; Wang et al., 2011; Wu et al., 2005; Zhang et al., 2010b; Zhou et al., 2010) |
|                 | Adhesive bonding | Bonding is achieved by adding an intermediate medium between the substrate and the cover (epoxy, UV glue, etc.) | Simple, low cost, suitable for chips of most materials; room temperature bonding technique; poor stability, adhesives may block microchannels | (Bhattacharyya and Klapperich, 2007; Brown et al., 2006; Gan et al., 2011; Hsu and Chen, 2007; Koersjdojo et al., 2008; Sun et al., 2007; Thompson and Abate, 2013; Wang et al., 2011; Wu et al., 2005; Zhang et al., 2010b; Zhou et al., 2010) |
food (Liu et al., 2011). Luo et al. designed a real-time microfluidic multiplex electrochemical LAMP chip for differentiating bacteria. This microfluidic system is called the multiplex electrochemical LAMP (μME-LAMP) system. The μME-LAMP system combines LAMP and an indium tin oxide (ITO) electrode-based microfluidic chip to analyze multiple genes qualitatively and quantitatively by measuring the electrochemical signal. This has the potential to be applied in clinical diagnosis (Luo et al., 2014). Lee et al. proposed a microfluidic chip coated hydrophilic film-based LAMP for the digital quantification DNA. This microfluidic chip requires no external pump and uses capillary force to drive the liquid flow that realizes the digital LAMP. The design has a promising future in POCT diagnosis (Ma et al., 2018a). LAMP technology can also be used to detect food-borne and water-borne pathogens to ensure the safety of food and drinking water (Tourtoulou et al., 2012; Zhou et al., 2014). In the multiplex detection of sexually transmitted diseases, microfluidic chips with disk or butterfly structures are used to improve the detection flux and efficiency, and these have been applied commercially (Ye et al., 2021; Zhou et al., 2014). LAMP-based microfluidic chips still have much for improvement and will definitely occupy a place in the POCT diagnostic field in the future (Ma et al., 2018b; Zhang et al., 2019).

4. Paper-based and hybrid microfluidic chips

The paper chip has many advantages such as low cost, simple processing, flexible structure, friendly environment, and good biocompatibility. The microfluidic paper chip combined with microfluidic technology, which integrates the advantages of both, is a development direction with good application prospects (Martinez et al., 2007; Pang et al., 2018). Paper-based microfluidic chips generally use filter paper as the substrate material, and the filter paper is treated with a hydrophilic or hydrophobic treatment as the reaction zone or circulation zone. The unique three-dimensional pore structure of paper material can provide capillary force to drive liquid flow, and the surface of material treated with the hydrophilic can adsorb reagents to stay and react there. In addition, the surface treated with the hydrophobic is conducive to liquid flow. The paper commonly used for the base materials includes Whatman series filter paper, nitrocellulose membrane filter paper, ink paper, chromatographic analysis paper, Kleenex paper, and various composite papers (Pang et al., 2018). Paper-based microfluidic chip processing methods include photolithography, wax printing, flexography, inkjet printing, screen printing, 3D printing, and laser cutting. Hydrophilic and hydrophobic treatment has a one-step method and a two-step method. The one-step method uses a one-time hydrophilic or hydrophobic treatment of all the material surface, and the two-step method refers to the use of physical deposition, plasma treatment, and other methods for the local treatment of materials. Unlike polymer-based microfluidic chips, some paper-based microfluidic chips do not need to be closed, and these are known as open-channel because the liquid in the paper-based microfluidic chips moves inside the paper fiber, which simplifies the processing process of microfluidic chips (Pang et al., 2018). In addition, the hybrid microfluidic chip combined with polymer, glass, hydrogel, and other materials integrates the advantages of a variety of materials, making the chip more comprehensive, and this is one of the most promising development directions of microfluidic chips in the future (Chinmadayyal et al., 2019; Dou et al., 2017a, 2017b).

In 2006, Wang et al. (2006) designed a disposable microfluidic cassette that can be used for DNA amplification and detection. This is an early nucleic acid detection system that combines microfluidic technology with transverse chromatography technology, greatly improving the detection efficiency. The disposable microfluidic cassette introduces a temperature-sensitive hydrogel valve as a control switch that can not only close the PCR chamber, but also inhibit the formation of bubbles. The detection method of the microfluidic cassette is infrared laser excitation of fluorescent particles to generate fluorescence. The entire process of cell lysis, DNA separation, purification, amplification, and detection can be completed on a single chip, realizing a comprehensive one-time independent and rapid detection of pathogens and body fluids. In 2007, Whiteside’s team at Harvard University first proposed a paper-based microfluidic chip and used it to analyze metabolites such as blood glucose, uric acid, and lactic acid (Martinez et al., 2007). Dou et al. (2017a) developed a PDMS-paper-glaze hybrid microfluidic chip for the detection of multiple pathogens in 2016. The chip has three layers. The upper layer of PDMS is used to transfer reagents, and it includes four microchannels and one injection port. The middle layer is the reaction zone, and it includes eight holes connected by four microchannels. The detection chamber, negative control cavity, and chromatography paper are also placed in the middle layer. The 3D porous structure of the cellulose paper is conducive to protect DNA primers from adverse environmental effects that can effectively prevent the formation of the aerosol leading to the loss of primers in the air. Hence, the polymer/paper-based composite chip has a longer service life. The bottom layer is supported by glass chips. The processing method of the hybrid chip is laser cutting, which is fast and easy to operate. The bonding method is oxygen ionic bonding to bond the glass and the PDMS. The inlet/outlet is encapsulated by epoxy resin. The by-product pyrophosphate is combined with manganese ions as a complex, and calcein is combined with manganese ions to produce fluorescence that can be directly observed with the naked eye in 1 h. The hybrid microfluidic chip can be used to detect multiple pathogens with high sensitivity and specificity at a low cost and high speed without instruments. Yin’s (Li et al., 2021) group introduced the kirigami technology to the 3D structure and extended the idea of two-dimensional paper cutting to three-dimensional space paper cutting and designed a universal three-dimensional paper cutting module with a variety of deformation modes. Li and Liu (2016) designed a three-dimensional multifunctional integrated paper-based microfluidic analysis system using origami technology that can realize the parallel detection of four tumor markers. The researchers believe that the modular design and assembly will not be limited by size and material, and their research is expected to find potential applications in the fields of reconfigurable metamaterials, robotics, and architectural design (Li et al., 2021). The introduction of origami technology provides new ideas for the development of paper-based microfluidic chips. The Li team combined LAMP technology, microfluidic technology, and biosensing technology to design a paper-polymer-based hybrid microfluidic nano-sensor based on the compact disc (CD) structure for the detection of meningitis and other infectious diseases. Costing just a few dozen cents and being just the size of a coin, the paper’s three-dimensional structure effectively stores primers for 73 days, facilitating subsequent amplification and increasing detection sensitivity and stability. This chip is small, portable, low-cost, fast, and can quantitatively detect a variety of pathogenic bacteria. It is very suitable for remote, underdeveloped, and poverty-stricken areas, and it is of great significance for the promotion of medical diagnosis in these areas (Dou et al., 2017b). In 2019, an autonomous capillary microfluidic chip (ACMC) designed by the Ning research group realized POCT instant myocardial infarction detection by using the capillary drive, self-focusing lens optical detection, and a customized mobile application. The test results are transmitted to the hospital and the user simultaneously (Liang et al., 2019). In addition, in the field of analytical chemistry, there are also some paper-based POCT combined with electrochemical biosensors for the detection of secretions and biomarkers (Gao et al., 2020; Li et al., 2020; Liu et al., 2019, 2021). Paper-based microfluidic chips have great application potential in POCT field.

5. Microfluidic biosensor

In recent decades, people are becoming increasingly health conscious, and the demand for prevention and diagnosis of some diseases, especially infectious diseases, is increasing. A biosensor integrated with a microfluidic chip for POCT diagnosis meets the requirement for rapid detection and has become a hot topic of research.
The combination of microfluidic technology and advanced biosensing technology has led to the invention of many outstanding miniaturized analytical platforms that can achieve precise control of micro/nano liquids and integrate various types of biological arrays on a miniaturized platform. This microfluidic integrated biosensor device has many advantages, such as low reagent consumption, shorter reaction time, automated sample preparation, high-throughput analysis, minimal hazardous material handling, parallel detection, high detection accuracy, and flexible design and miniaturization, portability, low cost, and one-time use (Ansari et al., 2016; Kumar et al., 2013; Liu et al., 2019).

Biosensor technology refers to a technology that can sense or respond to chemical and biological information and convert chemical or biological signals into electrical or optical signals that can be identified according to certain rules. POCT is an advanced technology that integrates biology, chemistry, materials, optics, microelectronics, and other interdisciplinary disciplines. It is fast, sensitive, low-cost, and the detection equipment is easy to automate and miniaturize. POCT has broad application prospects in medical diagnosis. The biosensor is a sensor that uses biological entities as recognition elements and converts biological signals into easily measured signals, such as light, electricity, heat, pressure, and mass, through specific targets to detect various biological entities. Biosensors primarily include three parts. One consists of the biological entities used for detection, including DNA, animal and plant tissues, bacteria, microorganisms, cells, enzymes, antibodies, proteins, nucleic acids, glucose, amino acids, and lactic acid. The second is a detection converter used to detect biological signals and convert them into other measurable signals. The third is the display device and signal processing device used for displaying the analysis results. Biosensors can be divided into two categories: a microarray biosensor and a microchannel biosensor, the latter being a biosensor that integrates a microfluidic chip. In addition, according to the different biomolecules, the microchannel biosensors can be divided into nucleic acid biosensors, enzyme biosensors, immune biosensors, bacterial biosensors, microbial sensors, cell sensors, and bionic sensors. According to the different signal detection principles, the microchannel biosensors can be divided into electrochemical biosensors, optical biosensors, thermistor sensors, field-effect transistor sensors, piezoelectric quartz crystal sensors, mass sensors, and surface plasmon resonance sensors. Biosensors can be used for the qualitative and quantitative analysis of biomolecules. Pregnancy tests and blood glucose meters are currently commercially successful biosensors, but most of the biosensors currently applied rely on laboratory instruments to realize sensing and are not suitable for POCT rapid detection systems. In recent years, scientists have become committed to developing biosensors that are portable, miniaturized, low-cost, and easy to operate. Therefore, microfluidic biosensors that combine microfluidic technology and biosensors have emerged at the right moment. Integrated microfluidic biosensor POCT equipment has great application potential in clinical testing, biochemical analysis, disease diagnosis, food safety, environmental monitoring, national defense, anti-terrorism initiatives, biological warfare agents, and in other fields. They are more efficient, specific, sensitive, portable, and easy to automate and obtain a result reading from (Ansari et al., 2016; Kumar et al., 2013; Li et al., 2017; Liu et al., 2019; Srinivasan and Tung, 2015). The schematic and applications of biosensors are shown in Fig. 2.

Sia et al. designed a microfluidic biochip based on optical detection for the diagnosis of HIV that brings hope for low-cost disease prevention and diagnosis in remote areas (Chin et al., 2011). Crook’s team combined a paper-based microfluidic chip with a biosensor, using printing and origami techniques to produce a three-dimensional paper-based microfluidic chip biosensor for adenosine detection (Liu et al., 2012). Rushling et al. used printed carbon electrodes as the substrate on a CD to make PDMS microchannels, and inserted silver and platinum electrodes to form electrochemical sensing for the detection of serum markers (Tang et al., 2012). There are also some studies on the detection of bioluminescent bacteria. These microfluidic biosensors are generally composed of microfluidic chips containing microchannels, active cells
for sensing the target, and transducers. Such studies can be divided into those with bacterial suspension, freeze-drying, and fixed types according to the state of bacteria. Bacterial suspension refers to bacteria in a suspended state in the liquid, bacterial lyophilization refers to bacteria that are made into lyophilized powder, and bacterial fixation is that bacteria are fixed to disposable sample pools, fiber optics, or chips. For example, the optical fiber monitoring system developed by Eltzov’s group to detect toxic pollutants in water is to fix bacteria on the optical fiber (Axelrod et al., 2016; Ma et al., 2020). Jouanneau et al. designed a microfluidic biosensor for detecting heavy metals. The microfluidic biosensor is suitable for bioluminescent bacteria in both states of preservation (Jouanneau et al., 2011). Nowadays, with the popularity of smartphones, the microfluidic biosensor integrated with mobile phones can provide real-time and synchronous feedback of detection results to users and the cloud by using the functions of mobile phones such as photography, video, sensing, and Bluetooth. Microfluidic biosensors integrated with smartphones are the new generation of smart biosensors, with huge market prospects (Axelrod et al., 2016; Xu et al., 2018). Ozcan’s team developed a POCT quick detection diagnostic device installed on a smartphone that obtains images through the camera and connects the phone customized application for synchronous processing. It then automatically outputs diagnostic results and sends them to users and medical institutions. Researchers can successfully use this equipment to detect HIV/AIDS, tuberculosis, malaria, and a variety of infectious diseases. It greatly facilitates the prevention, monitoring, and diagnosis of epidemic infectious diseases and also provides conditions for the popularization of the rapid diagnosis of infectious diseases in remote areas (Moon et al., 2009; Mudanyali et al., 2012).

6. Some recent research on microfluidic chips

In addition, microfluidic technology has also been applied in disease diagnosis, bacterial screening, immune biochemical reaction, enzyme protein recognition, organoid technology, and other fields. In 2021, the ExoDFF (Tay et al., 2021) microfluidic chip developed by Hou et al. from Nanyang Technological University was used to evaluate the vascular health of diabetes patients. ExoDFF uses centrifugal force and hydrodynamics to separate vesicle cells from blood for analysis. Compared with a traditional blood separator, the cost of the ExoDFF chip is only a few yuan, and this greatly reduces the cost of detection. The chip is helps patients to monitor their vascular health instantly and quickly, and has a huge commercial potential (Tay et al., 2021). In July 2021, Science reported that a microfluidic glass chip called high throughput-microfluidic enzyme kinetics (HT-MEK) (Reardon, 2021) can test for mutations in more than 1000 enzymes at once in just a few hours. Its price is just $10, and it is approximately 7 square centimeters in size and contains 1568 micropores, each of which can test one enzyme. Droplet microfluidic technology is one of the commonly used microfluidic technologies for high-throughput detection. Wang Meng’s team from the Institute of Chinese Academy of Sciences (CAS) designed a droplet embedding microfluidic technology for high-throughput screening of Streptomyces with a capacity of 10,000 strains per hour. The work was published in Nature. Researchers from the Shenzhen Institute of Advanced Technology at CAS designed a microfluidic chip that can isolate circulating tumor cells (CTCs) and circulating fusion cells (CFCs) with high purity from whole blood samples in one step and perform high-throughput single-cell transcriptome sequencing. This work was published in Lab on a Chip. A digital microfluidic chip is a high flux microfluidic chip that uses the electrode array on the chip to form electrical signals to control the droplet. Its principle is to use the difference in the surface tension to control the droplet, and the core technology is how to efficiently and stably generate microdroplets. Recently, Ma’s group at the Zhejiang University School of Biomedical Engineering and Technology at the Chinese Academy of Sciences proposed a digital microfluidic chip that can divide a large droplet into three small droplets. The smaller droplet size can break the minimum volume limit. This technology successfully realizes the efficient separation of small droplets. Combined with other functional modules, a fully automatic digital microfluidic analysis platform was developed that can detect five samples in parallel in only 10 min. The platform can be used for immuno-diagnosis, disease monitoring, treatment guidance, and prognosis assessment. The relevant results were published in Lab on a Chip. Researchers designed a microfluidic platform that can accurately quantify and analyze the formation process of single-cell bulges and realize the rapid screening of mitochondria-specific drugs using microfluidic technology, which will have a positive effect on the development of new anticaner drugs. The work was published in Analytical Chemistry (Zhang et al., 2020). Fluid circuit boards were combined with microfluidic devices for automated cell culture and precise fluid control. Users have greater flexibility with this new modular microfluidic system, the results of which were published in Microsystems & Nanoeengineering (Vollertsen et al., 2020).

7. Summary and prospects

In recent years, POCT, as an important part of in vitro diagnosis (IVD), has attracted the focus of major universities, research institutions, and IVD enterprises. Microfluidic technology, as a multidisciplinary technology, has been used as the primary implementation platform of POCT technology, both from the support of the advantages of the technology itself and from the national level. With the outbreak of COVID-19, the country has been collecting nucleic acid rapid detection products, hoping to realize the purpose of adding the sample and directly observing the results, that is, the “sample in, result out”. POCT molecular diagnostic products that are fast, portable, highly sensitive, and specific have become new favorites in the market. Microfluidic technology has gradually become the primary implementation means of POCT diagnosis, owing to its rapid development, but also has challenges and opportunities. However, most of the current microfluidic products are confined to the laboratory stage of scientific research, and there are not many mature products on the market. The application of microfluidic technology is limited owing to ultra-high precision machining requirements, as well as the difficulty of precisely controlling liquid at the micro-nano size, the challenge of surface modification in micro-channels, and the problem of how to achieve rapid mass production at low cost. Cost and efficiency have always been problems to be solved. After years of research, a growing number of emerging technologies have begun to be used in microfluidic technology, which involves a new generation of manufacturing technology, chip material selection, amplification and detection methods, as well as sensing and other fields, such as 3D printing technology, LAMP technology, biosensing technology, paper-based microfluidic chips, nanomaterials-based microfluidic chips, and hybrid-based microfluidic chips. The introduction of a variety of functional materials makes it possible to develop multifunctional microfluidic chips that greatly expand the application field of microfluidic chips. Relevant researchers and practitioners should seize the opportunities and meet the challenges in time to make microfluidic technology shine in the biomedical industry (Augustine et al., 2020; Gai et al., 2011; Liu et al., 2019; Reinholt et al., 2014; Sackmann et al., 2014).

CRediT authorship contribution statement

Yaping Xie: Investigation, Writing – original draft. Lizhong Dai: Writing – review & editing, Writing – original draft. Yijia Yang: Conceptualization, Investigation, Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence
Ye, X., Li, Y., Wang, L., Fang, X., Kong, J., 2021. Talanta 221, 121462. https://doi.org/10.1016/j.talanta.2020.121462.

Yin, Z., Sun, L., Zou, H., Cheng, E., 2015. Nanotechnology 26, 215302. https://doi.org/10.1088/0957-4484/26/21/215302.

Yin, Z., Zou, H., Sun, L., 2018. J. Nanosci. Nanotechnol. 18, 2530–2535. https://doi.org/10.1166/jnn.2018.14341.

Yuan, J., Chen, C., Yao, D., Chen, G., 2020. Polymers 12. https://doi.org/10.3390/polym12112536.

Zarei, M., 2018. Biosens. Bioelectron. 106, 193–203. https://doi.org/10.1016/j.bios.2018.02.007.

Zhang, M., Wu, J., Wang, L., Xiao, K., Wen, W., 2010a. Lab Chip 10, 1199–1203. https://doi.org/10.1039/b923101c.

Zhang, Z., Wang, X., Luo, Y., He, S., Wang, L., 2010b. Talanta 81, 1331–1338. https://doi.org/10.1016/j.talanta.2010.02.031.

Zhang, H., Xu, Y., Poblerova, Z., Chang, H., Iliescu, C., Neuzil, P., 2019. Trends Anal. Chem. 113, 44–53. https://doi.org/10.1016/j.trac.2019.01.015.

Zhang, P., Yao, J., Wang, B., Qin, L., 2020. Anal. Chem. 92, 3095–3102. https://doi.org/10.1021/acs.analchem.9b04702.

Zhou, J., Ellis, A.V., Voelcker, N.H., 2010. Electroanalysis 31, 2–16. https://doi.org/10.1002/elps.200900475.

Zhou, J., Khodakov, D.A., Ellis, A.V., Voelcker, N.H., 2012. Electroanalysis 33, 89–104. https://doi.org/10.1002/elps.201100482.

Zhou, Q.-J., Wang, L., Chen, J., Wang, R.-N., Shi, Y.-H., Li, C.-H., et al., 2014. J. Microbiol. Methods 104, 26–35. https://doi.org/10.1016/j.mimet.2014.06.008.

Zhu, Z., Park, H.S., McAlpine, M.C., 2020. Sci. Adv. 6 https://doi.org/10.1126/sciadv.abg5575. eaba5575.