CHAPTER 3

Microfluidic "Lab-on-a-Chip" Sensing in Food Safety and Quality Analysis

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3.1 Introduction

Food essentially comprises various natural or synthetic chemicals that supply energy, flavours or extended preservation time. Among these food compositions, excessive food additives, toxins, bacteria, pathogens, heavy metals, pesticide residues, antibiotics and biogenic amines may lead to negative effects on the human body, leading to serious issues in almost all physiological systems,[1–3] such as digestive systems, neurosystems and reproductive systems. In addition, overconsumption of fats, proteins, peptides and carbohydrates in foods may lead to health issues, such as obesity, diabetes and cardiovascular diseases. In terms of national security, contaminated food products can easily cause large spread of disease, causing tremendous threat to the consumers. In China, there have been numerous outbreaks related to foods due to contaminated food sources or misconduct of

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the food producers.[4–6] In the United States, many incidents caused by bacterial infections originating from contaminated poultry, meat, produce and water have also been reported.[7–9] Thus, analysis of the food contents to ensure their safety for consumption is critical. Food contamination can be monitored using analytical equipment. However, using bench-top instruments makes it either inconvenient to conduct field tests or costly to equip food production facilities. An approach to overcome the problems associated with conventional equipment is to use microfluidic ''lab-on-a-chip'' systems, which offer several advantages over conventional methods.

Lab-on-a-chip (LOC) is an emerging technology that aims at miniaturizing chemical and biological processes and conducting various operation procedures, including sample sorting, bioanalysis and flow manipulation.[10–12] LOC technology integrates multiple microfluidic components, such as sensors, microvalves, micromixers, microchannels and micropumps, into a single chip with miniaturized footprint, allowing high detection sensitivity and speed with a reduced cost. As a result, microfluidic LOC technology has been used in many applications, such as clinical analysis,[13–16] drug discovery,[17–19] environmental monitoring[1,20,21] and forensics,[22–24] showing great promise in replacing the bulky equipment in the laboratory.

LOC devices possess many advantages over conventional analytical instruments and are very suitable to be used to analyze different food compositions. First, their high surface area-to-volume ratio allows surface modification to increase detection sensitivity and rapid antibody–antigen reaction. Second, sample consumption can be largely reduced due to the miniaturized dimension of microfluidic devices. Furthermore, detection throughput can be increased by integrating additional parallel sensing channels and introducing automated fluid handling systems. Lastly, the cost of food analysis can be minimized by reducing reagent consumption and chemical waste. In this chapter, food analysis approaches based on LOC technology are introduced. The major materials, structures and main operation principles of LOC devices will be introduced, followed by some representative applications of LOC devices in determining various food compositions to ensure food safety and quality (Figure 3.1).

3.2 Materials, Structures and Fabrication Methods of LOC Devices

3.2.1 Major Materials Used in Microfluidic LOC Devices

Microfluidic LOC devices can be made of a wide range of materials, such as glass, silicon, polymer and paper, all of which can be used to construct structural components (*e.g.*, device substrates, microchannels, microvalves and micromixers). In addition, metals such as gold (Au), platinum (Pt), copper (Cu), aluminium (Al) and silver (Ag), inorganic compounds (*e.g.*, silver chloride (AgCl) and zinc oxide (ZnO)), composite materials (*e.g.*, carbon paste) and other nanoparticle pastes can be used to build electrodes and

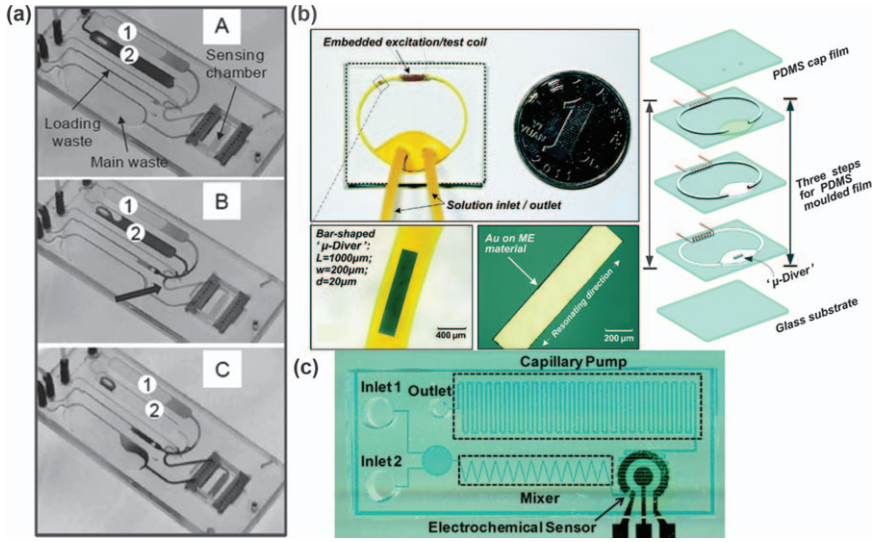

Figure 3.1 Examples of integrated microfluidic LOC devices used for food safety and quality analysis. (a) A multiplexed biosensor for the detection of antibiotics in milk. (Reproduced from ref. 25 with permission from the Royal Society of Chemistry). (b) A fully integrated system to determine foodborne pathogens. (Reproduced from ref. 26 with permission from the Royal Society of Chemistry). (c) An integrated device for rapid detection of toxins. (Reproduced from ref. 27 with permission from the Royal Society of Chemistry).

sensors. Other active components based upon organic and inorganic semiconductor materials can be used to construct active components, such as photo emitters, detectors and transistors in LOC devices.

3.2.1.1 Glass and Silicon

Glass and silicon can be patterned to form microstructures or used directly as the substrates to construct microfluidic chips.[11,28] The advantages of glass and silicon include resistance to organic solvents, ease in metal deposition, high thermal conductivity and stable electro-osmotic mobility. Their applications include on-chip reaction, droplet formation, solvent extraction and *in situ* fabrication. However, high fabrication cost and complex fabrication processes (etching and bonding) limit the use of glass and silicon as structural materials in LOC devices. In addition, as silicon is not transparent, it is not suitable to act as the substrate material when optical measurement is involved. Furthermore, as glass and silicon are not gas permeable, chips constructed on the basis of glass and/or silicon channels and chambers are not suitable for cell cultivation in the long term. The aforementioned

limitations of glass and silicon urge the development of other chip materials based upon polymers that are more compatible for broader biological applications.

3.2.1.2 *Elastomers and Plastics*

Compared with inorganic materials, polymers, which are inexpensive and easy to process, have become the most commonly used material for the production of microchips. According to their physical properties, polymers can be classified as elastomers,[29,30] thermosets[31,32] and thermoplastics.[33,34]

Elastomers consist of cross-linked, entangled polymer chains, which offer reversible deformation in response to external forces. The most commonly used elastomer in microfluidic systems is polydimethylsiloxane (PDMS),[35–38] which features simple fabrication processes and low cost. Liquid PDMS precursor can be thermally cured at temperatures below 120 °C, and can be moulded into micro-/nanostructures using templates obtained by patterning photosensitive polymers or other simplified fabrication methods, such as laser milling, micromachining and 3D printing. PDMS can be bonded with glass or other PDMS layers through plasma or UV-assisted surface treatment, followed by direct contact bonding. This eventually can form permanent chemical bonds to allow device sealing. However, absorption of small molecules onto the walls of PDMS channels and water evaporation due to gas permeability of PDMS yield negative influence on the applications of PDMS-based devices. Various modification strategies, including chemical vapour deposition, silanization and surfactants may be used to minimize molecule absorption and water evaporation.

The thermosetting materials represented by SU-8 photoresist[39,40] and polyimide[41,42] can crosslink to form an irreversible rigid polymer network when they are heated or radiated. These thermosetting materials are stable at high temperature and possess high resistance to solvents and high optical transparency. The high strength and capability to conduct photopolymerization enable the materials to fabricate free-standing or three-dimensional structures with high aspect ratios. Thermosetting materials can be used to fabricate the entire microfluidic chips or used as the templates for other elastomers and thermoplastics.

Thermoplastics can reversibly change their geometry by heating the materials to glass transition temperature, and can be moulded using metal or silicon templates, allowing mass production for commercial applications. Typical thermoplastics used in LOC include polymethyl methacrylate (PMMA),[43,44] polycarbonate (PC),[45,46] polystyrene (PS)[47,48] and polyethylene terephthalate (PET).[49,50] These materials show minimum gas permeability, making them unsuitable for cell cultivation and characterization in the long term. However, their improved solvent resistance as compared with PDMS allows them to be used in the situation where the analytes are dissolved in solvents.

3.2.1.3 Hydrogels

Hydrogels contain 3D networks of hydrophilic polymer chains that span the aqueous media. They are highly porous with controllable pore sizes, allowing small molecules and particles to diffuse through. The combination of aqueous nature and high permeability makes hydrogels perfect for encapsulating cells for 3D culture. Hydrogels can be categorized as synthetic hydrogels (*e.g.*, polyethylene glycol (PEG)[51] and polyacrylamide (PAM)[52]) and natural hydrogels. Natural hydrogels include gelatine[53] and collagen[54] derived from animals as well as agarose[55] and cellulose[56] derived from plants. Animal-derived hydrogels contain factors that promote cell adhesion and proliferation. In contrast, plant-derived hydrogels and synthesized hydrogels lack cell adhesion sites and have to introduce extra grafting structures in order to improve cell adhesion. Fabrication of hydrogels can be achieved by a direct writing approach followed by moulding in the concave templates. Hydrogels commonly act as cell culture media,[57] delivery agents[58] and reaction chambers[59] in LOC devices.

3.2.1.4 Paper

Paper contains highly porous structures made of cellulose, and achieves liquid wicking through capillary forces without using any external force. Selective areas of the paper can be modified hydrophobically, allowing liquid to flow through the hydrophilic regions in a controlled manner. Fabrication of paper-based microfluidic devices can be achieved either by lithographic approaches to yield high resolution patterns or by inkjet printing methods in which functional regions can be defined by ink printing. Typical detection methods for paper-based LOC include colorimetry,[60,61] luminescence[62,63] and electrochemical detection.[64,65] Using paper to construct LOC devices offers several advantages. First, paper can act as a passive pump to transport liquid solution and as a filter to remove large particles without the need for external power and components. Second, the large surface-to-volume ratio of paper allows sufficient capability of reagent storage by simply soaking the paper, followed by drying. Moreover, the low cost and ease of fabrication makes paper very attractive as a material for massive application of LOC devices.

3.2.2 Major Structures and Components

A comprehensive LOC platform can perform standard laboratory functions, which include sample transportation, mixing, reaction, separation and recycling. These functions can be realized by a combination of various components, such as microvalves (Figure 3.2a), microseparators (Figure 3.2b), micropumps (Figure 3.2c) and micromixers (Figure 3.2d), all of which are connected by microchannels to construct functional devices. The following

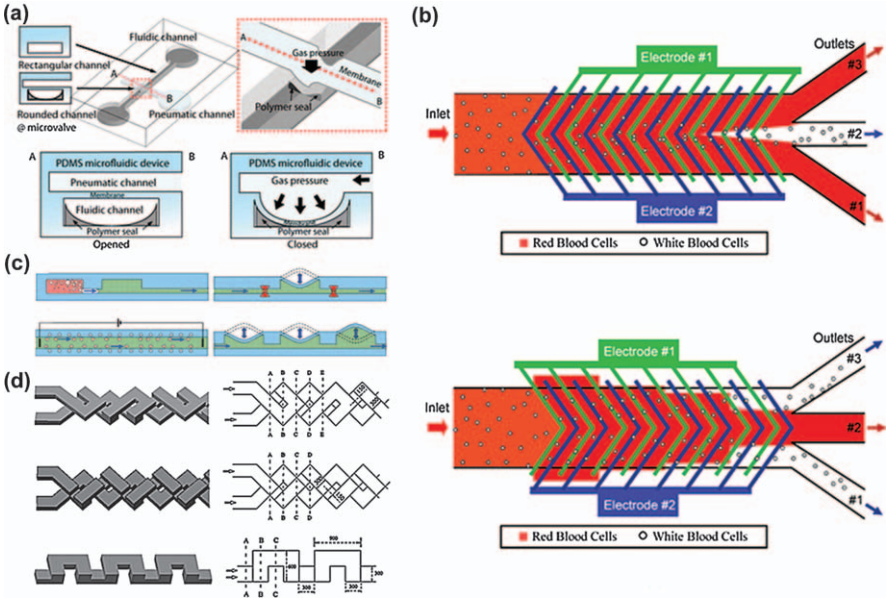

Figure 3.2 Representative images of the major components in a microfluidic chip, including (a) microvalves (reproduced from ref. 70 with permission from the Royal Society of Chemistry), (b) microseparators (reproduced from ref. 71 with permission from the Royal Society of Chemistry), (c) micropumps (reproduced from ref. 72 with permission from the Royal Society of Chemistry), and (d) micromixers (reproduced from ref. 73 with permission from the Royal Society of Chemistry).

sections provide a brief overview of each of the aforementioned microfluidic components. As there are numerous formats and operation principles of individual components, detailed information about these components can be referred to more comprehensive and specific reviews by Zhang *et al.*[66] and Wu *et al.*,[67] as well as books by Nguyen *et al.*[68] and Geschke *et al.*[69]

3.2.2.1 *Microvalves*

Microvalves can control and limit flow in LOC devices. Ideal microvalves feature low leakage, low power consumption, fast response, linear operating capacity and wide adaptation. According to the functions of microvalves, they can be divided into one-way valves[74–76] and switching valves.[77,78] Check valves are typically only represented by one-way valves, which offer two working states, namely channel closed and open. However, there are many types of switching valves, such as three-way valves and multi-position selector valves. According to the actuation mode of valves, they can be categorized as active valves and passive valves. Active valves use external driving forces to achieve open and closed operation, and are typically realized by actuation methods, such as pneumatic actuation,[79] thermal

expansion,[80] piezoelectric effect,[81] shape memory alloy,[82] magnetic and electrostatic[83] and electromagnetic[84] actuation. Passive valves do not require external power or control,[85] but they rely upon the change of flow and pressure of the fluid itself. Detailed introduction about the use of microvalves in LOC devices can be found in reviews by Kwang *et al.*[86] and Au *et al.*[87]

3.2.2.2 *Microseparators*

Microseparators are components that separate different biomolecules according to their physical properties such as mass, size, diffusion coefficient, magnetic susceptibility and polarity. Different molecules in mixtures are commonly separated by electrophoresis,[88,89] in which molecules with different mobility under an electric field can be separated according to their spatial location or dynamic speed. The use of electrophoresis in continuous flow devices can select charged molecules and particles in the samples and move them into a specific stream of flow that can be separated downstream.[90] Besides the electrophoresis approach, some separation methods apply force fields generated through electric, magnetic and acoustic approaches or with a rotational CD to create centrifugal force.[91,92] Other methods rely upon passive hydrodynamic phenomena in microchannels, such as sedimentation, cross-flow filtration, hydrodynamic filtration or centrifugation in curved channels.[93] In addition, microfilters can be considered as the passive type of microseparators that discriminate particles based upon their geometrical sizes. Reviews about different separation technology used in LOC devices have been written by Gossett *et al.*[94] and Bhagat *et al.*[95]

3.2.2.3 *Micropumps*

Micropumps are used in LOC to create a pressure difference in order to drive the motion of fluid within the microchannels. Common operation principles of micropumps include piezoelectric, thermopneumatic, electrostatic and electromagnetic actuation. Detailed reviews about the principles and operation of micropumps can be found in multiple literatures.[87,96,97] The micropumps can be categorized into two types, namely mechanical displacement micropumps and electro- or magnetokinetic micropumps. The former applies oscillatory or rotational forces on the fluid through moving solid–fluid or fluid–fluid boundaries using diaphragm displacement, fluid displacement and rotation, while the latter generates steady flows due to the continuous application of energy from electro-osmotic, electrohydrodynamic, magnetohydrodynamic and electrowetting effects.

Within the category of mechanical displacement pumps, diaphragm displacement pumps contain deformable diaphragms actuated by piezoelectric, electrostatic, electromagnetic, pneumatic and thermopneumatic approaches. The deflection of the diaphragm during the expansion and compression strokes causes volume and pressure changes within the microchambers, and

achieves dynamic adjustment of the liquid amount within the chambers. Another type of mechanical displacement pump is the fluid displacement pump, which uses secondary fluid in direct contact with the working fluid and achieves working fluid manipulation without using a diaphragm. The secondary fluid is usually made of ferro-magnetic liquid or gas. Rotary micropumps consist of a toothed gear rotating in a fluid chamber with an inlet and an outlet port.

Electro- and magnetokinetic micropumps directly convert electrical and magnetic energy into the directional fluid motion. Electrokinetic pumps pull ions within the microchannels and, in turn, drag along the bulk fluid by momentum transfer due to viscosity. Magnetokinetic pumps typically apply the Lorentz force on the bulk fluid to drive the microchannel flow.

3.2.2.4 Micromixers

Fluid mixing in microchannels is important for many biological and chemical applications. Mixing purely by spontaneous particle diffusion within microchannels is slow, but the introduction of passive and active mixers can greatly reduce the distance over which particles travel, and increase the efficiency of mixing.[98,99] Active mixers demand the involvement of external energy, while passive mixers rely purely upon fluid dynamics in the specific geometry of microstructures in microfluidic channels. A type of passive mixer contains grooves on the floor of the microfluidic channels. These grooves align asymmetrically, and generate a transverse component to the flow within the microfluidic channels. This transverse component when combined with the axial pressure gradient along the direction of flow motion yields vigorous relative movement of fluid elements, causing increased contact area between the flowing streams and enhanced diffusion. Another passive mixing design uses serpentine channels, leading to both inertial forces and centrifugal forces when the fluid flows through curved channels. These two combined forces can also generate transverse flow to enable effective mixing. Active mixers use approaches such as electrowetting, nonlinear electrokinetic effects, acoustic streaming and bubble movement to facilitate mixing. However, active mixers often involve complex structures that demand complicated fabrication processes and external power sources. Thus, applications of active mixers in LOC devices are limited due to the technical challenges and cost efficiency. In contrast, passive mixers are easier to fabricate, and are more suitable for species that are sensitive to electrical, mechanical or thermal agitation. In addition, passive mixers also feature robustness and stability, and can be easily integrated with microfluidic systems. Thus, they are more favourable in LOC devices. Detailed reviews about the principles and operation of micromixers can be found in several recent literatures.[100–103]

3.2.3 Fabrication Approaches

Fabrication of LOC adopts techniques, including CMOS, MEMS and other micromachining processes (*e.g.*, moulding, milling and cutting). Recent

development in additive manufacturing also yields direct pattern generation through 3D printing that includes stereolithography, fused deposition and inkjet printing. This section offers brief reviews of some typical fabrication approaches in microfluidic LOC devices. More state-of-the-art fabrication techniques can be found in reviews by Anderson *et al.*[104] and Abgrall *et al.*[105]

3.2.3.1 *Surface Micromachining*

Micromachining includes techniques such as film deposition, photolithography, etching and bonding of microchips.[106–108] These techniques can be used to process various materials, such as Si, glass, metals, polymers and semiconductors used in LOC devices to form structural components (*e.g.*, microchannels, valves, diaphragms, cantilevers and pumps) as well as electrical functional components (*e.g.*, electrodes, heaters and detectors). The deposition of materials is achieved through either chemical or physical processes. In chemical processes, reactions between gas phase and liquid phase chemicals under a certain temperature and pressure yield thin layers of polymers, inorganic materials or semiconductors in a controlled manner. While in physical processes, raw materials can be evaporated or sputtered onto the target surface without the involvement of chemical reactions. Photolithography defines selective regions of materials with a photoresist that can be spin-coated and exposed to form photoresist patterns. The underneath materials of the photoresist are further processed through either dry or wet etching using the photoresist as a mask layer, forming patterns on the materials in correspondence to the photoresist. The bonding of the processed materials onto different substrates (*e.g.*, glass and silicon) can be achieved by methods such as surface plasma treatment, UV ozone activation and anodic bonding.

3.2.3.2 *Soft Lithography*

Soft lithography (Figure 3.3) uses elastomeric stamps to replace hard stamps in photolithography for the fabrication of micro-patterns and structures.[109,110] It can be used to create complex 3D structures and can be applied to many materials including polymers, colloid materials, glass and ceramics. In general laboratory environment, its feature size can reach 30 nm to 1 μm with relatively simple setup as compared with the lithography method as aforementioned. The essential components of soft lithography are elastomeric stamps, which are usually made of PDMS through moulding. Typical techniques in soft lithography include microcontact printing, capillary moulding, replica moulding, micro transfer moulding and solvent-assisted moulding. Two representative techniques are contact printing and capillary moulding, which generate non-structured, chemically modified surfaces and a topographically modified physical micro-/nanostructure, respectively. Contact printing uses an elastomeric PDMS stamp, which is soaked in a molecular ink, to contact with a substrate, resulting in transfer printing of the ink onto the substrate surface. In contrast, capillary

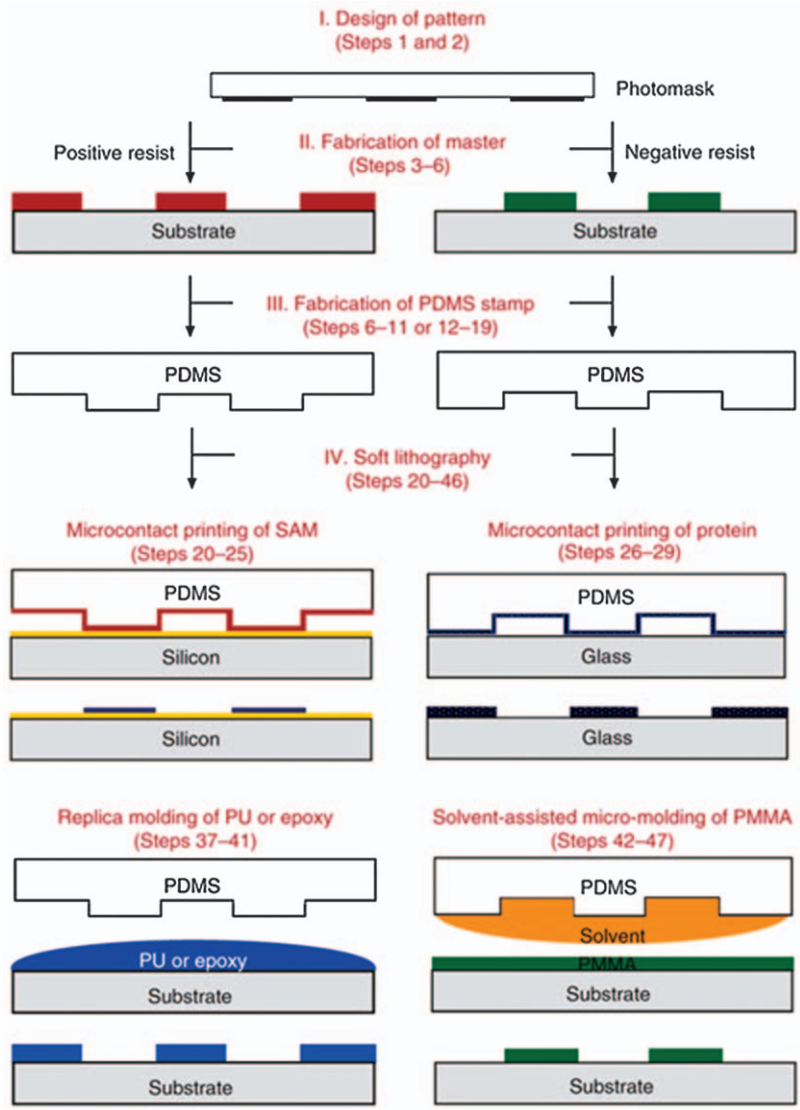

Figure 3.3 Schematic illustration of the four major steps involved in soft lithography and two major soft lithographic techniques – microcontact printing and replica moulding.
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moulding uses a patterned PDMS mould to contact with the polymer surface. Heating the polymer above its glass transition temperature allows the polymer to melt and fill the void space of the PDMS mould, generating a negative replica of the mould. Besides thermal approaches, pattern formation in capillary moulding can also be achieved by solvent-laden polymers

of UV-curable polymers through solvent evaporation or UV exposure to achieve sub-100-nm soft lithography.[111]

3.2.3.3 3D Printing

3D printing is represented by a series of printing techniques that are capable of fabricating polymeric structures using additive manufacturing approaches (Figure 3.4). Popular 3D printing techniques include stereolithography,[113] two-photon polymerization,[114] fused deposition modelling[115] and inkjet printing,[116] among which the former two techniques are based upon photocurable resins. Photocurable resins solidify under light exposure supplied through a UV laser or near-infrared femtosecond laser in a certain spectral range. Fused deposition modelling uses a stacked layer of

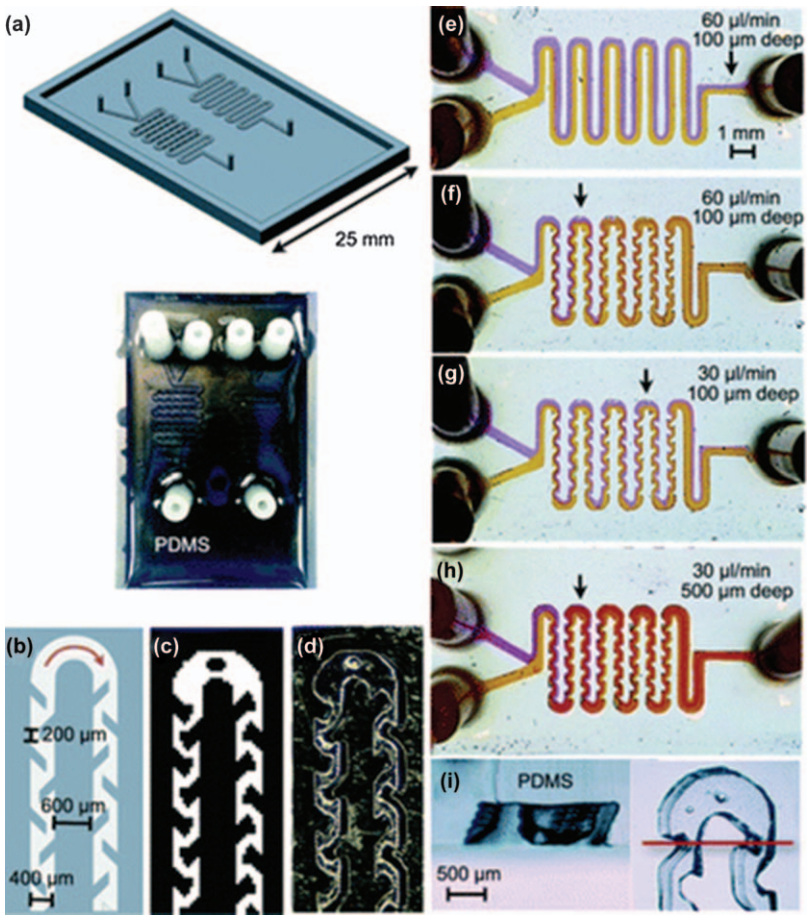

Figure 3.4 A 3D printed template that is used for cast moulding a PDMS microfluidic micromixer (reproduced from ref. 118 with permission from the Royal Society of Chemistry).

thermoplastic materials, which are melted by heaters and extruded through nozzles onto the stages. The extruded materials cool down and solidify on the stages to form models. Materials that can be inkjet printed require appropriate viscosity and surface tension, allowing the formation of droplets and the ability to flow through nozzles with diameters ranging from 5 to 50 μm. The inks of inkjet printing typically contain fully dissolved chemicals that can solidify under the exposure of UV light or heat, or contain nanoparticles that form a connective matrix when sintered by heat, microwave, laser or light.[117]

3.3 Methods Used in LOC Detection of Food Safety and Quality Analysis

3.3.1 PCR and Isothermal Amplification

The supreme specificity of nucleic acids allows sensitive detection of pathogens and recognition of some biomolecules in foods using DNA or RNA. However, biologically relevant samples typically contain very small amounts of nucleic acids, making direct detection almost impossible under many circumstances. Therefore, various nucleic acid amplification methods that aim at dramatically increasing the numbers of nucleic acids in the test samples have been developed. Currently, there are numerous nucleic acid amplification methods available, but the most widely used methods are the polymerase chain reaction[119] (PCR) and isothermal methods.[120]

The cycling temperatures in PCR-based microfluidics must be precisely controlled to obtain desirable temperature kinetics for DNA amplification. During the PCR process, a template of double-stranded DNA is first broken apart at the denaturation temperature (90 to 95 °C), resulting in single-stranded DNA for subsequent amplification. The temperature is further reduced to an annealing temperature (~55 °C), allowing annealing of DNA primers to serve as the starting molecules for DNA synthesis and flanking the DNA sequence to be amplified from both sides. Primer extension is performed by increasing the lower annealing temperature to a medium temperature, which is usually at 72 °C. Using microfluidic LOC technology can create miniaturized PCR (μPCR) devices to amplify DNA. μPCR devices can be categorized into static chamber (SC) and continuous flow (CF) devices. In an SC device, the sample is static in a chamber, which undergoes thermal cycling through precisely controlled heaters. In a CF device, the sample moves through fixed temperature zones to achieve the required thermal cycling, leading to faster DNA amplification and lower power consumption by eliminating the temperature ramping processes. Moreover, the comparable dimensions of μPCR devices and heating elements allow more effective temperature control, thus rapid heating and cooling rates.

The isothermal amplification approaches can achieve DNA amplification at a fixed temperature,[121–124] and involve multiple featured techniques, such as loop-mediated isothermal amplification (LAMP),[125] rolling circle amplification (RCA),[126] strand displacement amplification (SDA),[127] recombinase

polymerase amplification (RPA)[128] and helicase dependent amplification (HDA).[129] A shift from PCR towards isothermal methods in microfluidic LOC devices is observed due to the simplicity and reduced thermal budget of the isothermal methods. The main advantage of isothermal methods over PCR is the elimination of the need for thermocycling, as isothermal methods are realized under a stable and constant temperature over time.[130]

3.3.2 Immunoassay

Immunoassay is a technique that exploits the sensitivity and specificity of antibody–antigen interactions for the detection of relevant analytes, and is the fundamental approach used in food analysis to detect specific particles and molecules. In immunoassay, the recognition site of a target antigen by a specific antibody-binding site is called the "epitope". Antigens contain numerous epitopes. However, antibodies can only bind to unique epitopes, resulting in highly specific interaction to achieve antigen recognition from other interfering biomolecules in test samples. Immunoassays can be classified into heterogeneous and homogeneous approaches. In heterogeneous immunoassays, antibodies immobilized on the solid surface interact with nearby antigens. Thus, influence from the unbound antibodies and other reagents can be eliminated. In homogenous immunoassays, both antibodies and antigens are freely suspended in solution. The physical or chemical changes due to bonding can be used to distinguish the bound and unbound antibodies.

Immunoassays have been utilized in a variety of sensor formats, resulting in the construction of immunosensors with different detecting techniques. An immunosensor generally detects immunoassay events through either optical or electrochemical signals. One of the widely used configurations for the capture of target antigens is known as the sandwich structure. The generation of an immunosensor signal from antigen capture is predominantly realized using some types of labels conjugated on a secondary antibody. The selection of labels depends upon the specific detection methodology, and generally includes fluorescent labels, enzymes, paramagnetic particles and metallic colloids. Immunoassay based upon label-free detection approaches have also been developed and offer many advantages, including reduced complexity of chemical reaction and capability to achieve more miniaturized systems. Some of the detection methods on the basis of the labelled or label-free immunoassays are introduced in the following section.

3.3.3 Detection Methods

The most common detection technique used in microfluidic immunoassays is optical detection, which includes approaches of laser-induced fluorescence (LIF) and chemiluminescence (CL) detection based upon labelled biomolecules. Another popular approach is electrochemical detection, which is capable of miniaturization with simplified external read-out.

In addition, some label-free detection methods, such as surface plasmon resonance (SPR), quartz crystal microbalance (QCM) and magnetoelectronic detection, have also been used in LOC devices.

3.3.3.1 *Optical Approaches*

The optical detection method is one of the most widely used methods in microfluidic detection. Some typical approaches include LIF, CL, spectroscopy and SPR. Microfluidic systems are mostly utilized for sample handling, such as loading, pre-treatment and separation. Such systems can be easily interfaced with external detection techniques or conventional optical detection instruments (*e.g.*, inverted fluorescence microscope, digital CCD cameras spectroscopes, light emitter and detectors and smartphones). However, the requirement of large detection equipment hinders the miniaturization and integration of entire detection systems, making on-line and in-field detection very challenging. Efforts in directly combining optical detection methods with microfluidics have been successfully demonstrated in many research works.

Fluorescence-based biosensors are by far the most prevalent type of biosensors encountered in microfluidic applications due to their ease of implementation. In the florescence process, a substance emits light as an effect of the absorption of light of a shorter wavelength. Specifically, a substance absorbs photons, which can lead to the shift of electrons to unstable higher energy levels. Subsequently, this can result in the return of the energy levels to the ground state and release energy in the form of photons. The benefit of fluorescence detection includes low detection limit, high selectivity and a wide array of fluorescence labels for tagging biomolecules. LOC devices based upon fluorescence detection have been developed with integrated microlenses, waveguides, light sources and detectors, resulting in highly compact systems that are suitable for portable detection. Additionally, miniaturized LOC devices with planar waveguides offer increased optical paths, leading to improved detection sensitivity. Fluorescence detection has been widely used in the determination of bacteria,[131] pathogens,[132–134] toxins[135] and biogenic amines[136] in different agri-food products.

Chemiluminescence is the emission of light as a result of a chemical reaction. In general, a chemiluminescent reaction can be generated by two basic mechanisms. In a direct reaction, two reagents, usually a substrate and an oxidant in the presence of some cofactors, react to form a product or intermediate with or without the presence of a catalyst. Then, some fractions of the product or intermediate will be formed in an electronically excited state, which can subsequently return to the ground state with emission of a photon. On the contrary, indirect or sensitized CL is based upon a process of energy transfer of the excited species to a fluorophore. This process makes it possible for those molecules that are unable to be directly involved in a CL reaction to transfer their energy to a fluorophore that is, in turn, excited followed by returning to its ground state with photon emission.

Surface plasmon resonance is a label-free optical detection method, which involves a resonant oscillation phenomenon that occurs at the interface between two media with distinct signs of dielectric constants. Such an interface can be formed by metals (Au or Ag) and liquids. SPR carries a mathematic form similar to that of the evanescent wave, and generates a decayed electrical field that is reduced exponentially as the field is moved away from the interface. SPR is a surface effect, which is sensitive to physical and chemical changes localized at the interface, while excluding other changes in the bulk regions. Beside the planar SPR sensors, metallic nanoparticles exhibit SPR effects and can serve as immunosensors. The surface plasmon present at a metal–ambient interface absorbs light at a specific wavelength, resulting in a resonance frequency that depends upon the shape of the nanoparticles and the ambient refractive index. In a general design, the surface of gold nanoparticles is immobilized with antibodies, which can bind with antigens and cause mass accumulation on the nanoparticles and shift of resonance frequency.

3.3.3.2 *Electrochemical Approaches*

Electrochemical detection (ED) is the most attractive alternative to optical detection because of its inherent sensitivity, capability to be miniaturized without loss of performance and high compatibility with the microfabrication technique. Electrochemical detection measures current, voltage, conductance or impedance changes in the process of affinity bonding between receptor/ligand or antigen/antibody systems or enzyme catalyzed chemical reactions. An electrochemical sensor typically contains a simple electrode configuration, including a working electrode, a counter electrode and a reference electrode. Functionalization of electrodes with enzymes can make use of their ability to selectively catalyze chemical reactions. Electrochemical detection offers less expensive read-out than optical systems and can be easily miniaturized and incorporated into microfluidic systems.

Among all electrochemical approaches in microfluidic systems, amperometric detection is one of the most common methods. It detects current changes due to generation and consumption of electroactive species during chemical reactions and affinity binding processes. Device miniaturization and high integration lead to fast response time and efficient collection of electroactive species due to shortened transportation distances. Conductometry is another widely used approach in ED systems. Such detection involves measurement of the conductance between two or four electrodes, through which an alternating current is passed. Conductometry allows convenient detection of ionic species, which are often not readily detected by other techniques, down to nM levels. Conductivity detection can be accomplished either by direct contact between test samples and sensing electrodes or by a contactless mode in which the electrodes do not come into contact with the solutions. Contactless detection is more favourable as it can avoid multiple issues (*e.g.*, current

leakage, bubble generation and surface contamination) associated with the contact mode.[137]

In food microfluidics, ED has been successfully implemented in both amperometry[138,139] and conductometry,[140,141] as well as other ED approaches, such as impedance measurement[142,143] and voltammetry.[144,145] The large number of electroactive analytes in foods, the suitability of conductometry for detecting ionic food analytes and the capability to detect both transparent and opaque samples lead to the broad use of ED in food microfluidics.

3.3.3.3 Other Approaches

Two other non-optical and label-free detection approaches with high sensitivity and capability to conduct wireless detection are quartz crystal microbalance (QCM)[146,147] and magnetoelastic sensors[26,148] (ME). QCM measure mass deposition due to the absorption or release of small molecules onto the surface of the microbalance through changes in frequencies of a quartz crystal resonator. ME sensors are made of amorphous ferromagnetic alloys, which exhibit an ME resonance when excited by an external time-varying magnetic field. When target molecules make contact with the sensor surface, mass changes cause a shift of the resonance frequency, which can be measured remotely through a pick-up coil.

3.4 Applications in Food Safety and Quality Analysis

Microfluidic devices possess many unique features, such as low sample consumption, fast detection speed and miniaturized footprint, and these make microfluidic devices very attractive for food safety and quality analysis. There are many natural or synthesized compositions in foods that can generate positive or negative effects on the human body. In this section, LOC devices that measure or act on selective compositions that are important for the safety and quality of foods are presented. Additional information has also been provided in some systematic reviews.[149–151]

3.4.1 Food Additives

Food additives (*e.g.*, preservatives) can improve the preservation time of foods. In addition, food additives (*e.g.*, dyes, thickeners and whiteners) can improve food appeal. Although many food additives are applied under regulation and are typically safe in small quantity, extended use of food additives or intentional use of unregulated food additives may pose potential health risks, such as cancer, heart disease and bowel symptoms. Some typical food additives that have been measured by microfluidic LOC devices include nitrite[152,153] and food dyes.[154,155]

Nitrite exists in beverages and food products as a preservative because it provides highly effective protection against food poisoning microorganisms.

However, high concentrations of nitrite in the human body cause diabetes and nervous system disorders.[156] He and coauthors presented a microfluidic system that contained laser-etched microchannels and a microreactor of 1.8 μL in volume to determine nitrite in foods through the CL phenomenon.[157] Nitrite reacted with ferrocyanide to yield ferricyanide, which later reacted with luminol to generate CL light. CL light generated due to the chain reaction of ferrocyanide, nitrite and luminol in an acidic medium could be recorded by a luminescence analysis within 2 minutes, resulting in a linear detection range of the nitrite concentration from 8 to 100 $\mu g\,L^{-1}$ and a detection limit of 4 $\mu g\,L^{-1}$. Shiddiky and others developed two simple and fast methods for the extraction of nitrite in food samples.[153] The methods were characterized by UV-visible spectroscopy and electrochemical measurement. Microchip electrophoresis with electrochemical detection coupled with a copper/silane complex-modified carbon paste electrode has been employed to detect nitrite. In real sample detection, the sensor was demonstrated to successfully measure nitrite in ham and sausage samples with a wide concentration range from 1 to 150 ppm.

To detect food dyes in various products, Lee and colleagues developed a LOC device integrated with on-chip pre-concentration, separation and electrochemical detection.[154] The device contained three parallel channels: the first two were used for amplification and the last one was for electrochemical detection. Amperometric detection was performed using a glassy carbon electrode coupled horizontally with the microchip at the outlet of the separation channel. The sensitivity of this method was improved by approximately 10 800-fold compared to a conventional micellar electrokinetic chromatographic analysis. Reproducible response was observed during multiple injections of samples with detection limits between 1.0 and 5.0 nM for all food dyes.

3.4.2 Toxins

Toxins produced by microorganisms are chemical substances existing in foods contaminated by certain bacteria and fungi. Toxin contamination can occur throughout the entire food production chain, from processing to transportation and storage. Toxins are extremely hazardous to human health. Representative toxins include botulinum neurotoxins (BoNTs)[158] and mycotoxins.[159] BoNTs are considered as the most poisonous substances known to humans, and are the cause of the life-threatening neuroparalytic disease botulism. On the other hand, major fungal genera produce may contain mycotoxins, which are the secondary metabolites of fungi. There are various types of mycotoxins, such as aflatoxins (AFs), deoxynivalenol (DON), zearalenone (ZEA), fumonisin B1 (FB1), ochratoxin A (OTA) and citrinin (CIT).

There are many microfluidic LOC devices that have been developed to measure BoNTs.[27,160,161] A representative sensor measured botulinum neurotoxin type A (BoNT/A) using self-assembled monolayers (SAMs), which

consisted of an immobilized synthetic peptide.[162] Microchannels in the sensor provided multiple functions, including facile fluid manipulation, sample incubation, analyte concentration and fluorescence detection, avoiding sample transfer and loss between different cells that achieve individual functions. Peptide SAMs were exposed to varying concentrations of BoNT/A or its catalytic light chain (ALC), resulting in enzymatic cleavage of the peptide substrate from the surface. Fluorescence detection was achieved down to 20 pg mL^{-1} for ALC and 3 pg mL^{-1} for BoNT/A in 3 h.

Several LOC devices have been developed to achieve rapid and accurate determination of low levels of mycotoxins in foods.[163–165] A research team developed a fully automated device with CL read-out system to detect OTA in green coffee extract.[166] Peptide-linked OTA and biotin conjugates were synthesized and immobilized in an array of 4×6 microspots, and used for an indirect competitive immunoassay. The device only required a CCD camera to detect CL signal. Thus, the complexity of the external detection system can be significantly reduced. The miniaturization and automation largely increased mass transport and reduced detection time (down to 12 mins), resulting in a detection limit of 0.3 g L^{-1} for OTA. Parker and others developed an electrochemical-based microarray for the detection of aflatoxin M1 (AFM1) in milk by using an antibody-modified screen-printed carbon working electrode with a carbon counter electrode and an Ag/AgCl reference electrode.[167] A simple pre-treatment technique of incorporating 18 mM calcium chloride in PBS to the milk samples eliminated the interfering signal in milk. The resulting immunosensor achieved a detection limit of 39 ng L^{-1} with a linear dynamic detection range up to 1 μg L^{-1}. Arévalo and colleagues developed a microfluidic electrochemical immunosensor coupled with flow injection system that can be used for rapid, sensitive and selective quantification of CIT at trace levels in rice samples using ED.[168] Due to the high selectivity of the antibody used in the detection, no sample pre-treatment was required to purify the samples. The detection principle was based upon a competitive immunoassay. Specifically, CIT in rice and CIT deposited on a sensor electrode competed for the monoclonal mouse anti-CIT IgG antibody (mAb-CIT) present in solution, followed by a series of reactions that generate current inversely proportional to the amount of CIT present in the rice samples. The electrochemical detection was carried out within 2 min and the total analysis time did not exceed 45 min, leading to a detection limit of 0.1 ng mL^{-1}.

3.4.3 Bacterial and Foodborne Pathogens

Numerous cases of foodborne illness can be attributed to pathogens, such as Shiga-toxin producing *Escherichia coli*, *Salmonella enterica*, *Staphylococcus aureus*, *Campylobacter jejuni* and *Listeria monocytogenes*. LOC biosensors offer ideal tools to realize portable and real-time biosensing of pathogens in farms, packaging/processing facilities, delivery/distribution systems and at consumer levels.[169–171]

Several types of LOC biosensors, including immunoassay and PCR-based, have been developed and tested for the detection of foodborne pathogens. Strachan and coauthors developed a PMMA microfluidic chip integrated with on-board infrared-mediated PCR amplification. The device can be seamlessly integrated with a particle-based, visual DNA detection system for specific detection of *Salmonella enterica* in less than 35 minutes.[172] Microfluidic control was achieved using a capillary burst valve and a manual torque-actuated pressure system. The capillary burst valve that was fabricated using laser ablation can confine the PCR reagents to a chamber during thermal cycling. The manual pressure system mobilized the fluid from the PCR chamber to the detection reservoir containing oligonucleotide-adducted magnetic particles. Interaction of amplified products specific to the target organism with the beads in a rotating magnetic field allowed immediate detection (<30 s) based upon hybridization-induced aggregation of the particles and simple optical analysis.

A more integrated device for pathogen detection has been presented by Kim *et al.*[173] to determine *Salmonella* in milk. In this centrifugal microfluidic device, three main steps have been implemented in a single microfluidic disc, including DNA extraction, isothermal RPA and pathogen detection. The devices used a single laser diode to achieve wireless control of multiple functions (*e.g.*, valve actuation, cell lysis and non-contact heating) in the isothermal amplification step, resulting in a compact and miniaturized system. To achieve high detection sensitivity, *Salmonella* cells were first pre-enriched in phosphate-buffered saline (PBS) and milk samples before loading onto the disc through antibody-coated magnetic beads. The entire procedure, from DNA extraction through to detection, was completed within 30 min in a fully automated fashion with detection limits of 10 CFU mL^{-1} and 10^2 CFU mL^{-1} in PBS and in milk, respectively.

Impedance-based electrochemical detection has also been presented. Boehm and others have developed a simple and rapid method for the detection and identification of *E. coli.*[174] The developed device could detect impedance changes when *E. coli* in suspension passed through a microfluidic chamber immobilized with monoclonal antibodies. Continuous perfusion of bacteria suspension through the chamber not only identified specific bacteria but also enhanced the detection sensitivity of the chamber by accumulating bacteria on the chamber wall over time. The sensor could detect 9×10^5 CFU mL^{-1} *E. coli* in the solution by consecutive perfusions, and could increase its detection limit to 10^4 CFU mL^{-1} of *E. coli* when a shallower chamber (2 μm high) was used.

In addition to detecting pathogen, microfluidic devices can also be used for pathogen sorting by isolating pathogens from suspended particle mixture using dielectrophoresis.[175] By converging fluid flow through alternating current electro-osmotic flow in a microfluidic device,[176] the target pathogens can be directed towards the stagnation points, while the suspended particles can be swept towards the outlet along the fluidic flow. Using 3D electrodes to create a dielectrophoretic force field cage, bacterial cells inside a

microfluidic channel can be captured more efficiently through tailoring the orientation of the 3D electrodes and by creating a dielectrophoretic force field cage.[177] In a device with 3D electrodes, whose orientation was tailored to achieve a spatial force field, the rate for sorting and collecting three different types of pathogens reached ~300 particles per second.

3.4.4 Antibiotics

Antibiotics are valuable tools for reducing animal disease and suffering from bacterial infections and pathogens as aforementioned. However, the extensive use of antibiotics has led to drug resistance of pathogens, which can be difficult to eliminate and affect human health in a prolonged time period. Applications of antibiotics must be used responsibly in both humans and animals to prevent the spread of drug-resistant bacteria.[178]

Serval microfluidic LOC devices have been developed with the capability to simultaneously detect multiple antibiotics.[179,180] For example, a multiplexed immunoassay-based antibiotic sensing device integrated in a LOC format has been developed to simultaneously determine three antibiotic families (*i.e.*, sulfonamides, fluoroquinolones and tetracyclines) in raw milk.[25] The device contained a polymer-based self-contained microfluidic cartridge and measured antibiotic levels based upon a multi-antibiotic competitive immunoassay. Immunoassay solution was pressure driven externally through a syringe pump and a multi-position valve. Pre-filled microfluidic cartridges were used for a positive/negative binary test for the simultaneous detection of three antibiotics. For result interpretation, any signal lower than the threshold value (100 $mg\,L^{-1}$) was considered to be negative for a given antibiotic. The reliability of the multiplexed detection system was assessed by a validation test conducted on a series of six blind milk samples. The test result had ~95% accuracy. The whole immunoassay procedure was fast (<10 minutes) and easy to handle (automated actuation). In another example, a microarray biosensor integrated with a commercialized Xantec HCX sensor and a flow cell has been demonstrated to quantitatively and simultaneously determine multiple antibiotic families.[181] The biosensor was based upon an imaging surface plasmon resonance (iSPR) platform, and can measure aminoglycosides, sulfonamides, fenicols and fluoroquinolones. By multiplexing seven immunoassays in a competitive format, all the target compounds can be detected in ppb levels in buffer and in 10-fold diluted milk, offering a promising alternative for multi-analyte food profiling.

3.4.5 Heavy Metals

Due to the pollution in water and soil, heavy metals can enter into the food chain through plants and animals, and accumulate within the food, leading to various health issues (*e.g.*, kidney damage, nervous system damage and cancers).[182] Heavy metals, such as cadmium (Cd), chromium (Cr), lead (Pb), arsenic (As), copper (Cu), nickel (Ni) and mercury (Hg), are among the

elements that are toxic and can be found within contaminated foods. Therefore, the detection of heavy metal contents in food is necessary and has been achieved by multiple LOC devices.[183–186]

One of the simplest approaches to detect heavy metals in food is by conducting colorimetry detection. Wang and coauthors developed a 3D paper-based microfluidic device to measure Cu, Ni, Cd and Cr in water.[187] The device contained stacked layers of wax patterned paper and double-side adhesive tapes, and allowed fluid transportation through capillary force without the need of external pumps and power. The ions of the heavy metals can react with metal-selective chromogenic reagents and induce colour changes that can be captured by any portable cameras. The resolution of such a colorimetry LOC device was 0.29, 0.33, 0.19 and 0.35 ppm for Cu(II), Ni(II), Cd(II) and Cr(VI), respectively. Due to the miniaturized size and no demand for large external supporting equipment, this paper-based LOC device can be very useful for determination of heavy metals on a daily basis.

More quantitative results can be achieved using electrochemical detection. For example, an electrochemical sensor with a three-electrode configuration was developed to determine Mn, Zn, Cd and Pb by anodic stripping voltammetry. The sensor consisted of an Ag/AgCl reference electrode, an Au auxiliary electrode and a special bismuth working electrode, which could be used to minimize the hydrolysis effect of the device and extend the potential window to the −0.3 to −1.9 V range with respect to the Ag/AgCl reference electrode.[185] The sensor can detect highly electronegative metals (*e.g.*, Mn) and offers superior measurement capabilities for even mildly electronegative metals (*e.g.*, Pb and Cd). In addition, the sensor can detect multiple analytes simultaneously in the low μM concentration range that is relevant to the biophysiological levels of these heavy metals.

Optical detection approaches including fluorescence and CL have also been used in quantitative determination of heavy metals in water and foods. A miniaturized lead sensor has been developed by combining a lead-specific DNAzyme with a microfabricated device containing a network of microfluidic channels coupled *via* a nanocapillary array interconnect.[188] The interconnect can manipulate fluid flows and deliver small volumes of samples to DNAzyme in a spatially confined detection window. The DNAzyme was interrogated using LIF detection in this window, showing linear response over a Pb^{2+} concentration range of 0.1–100 μM, and a detection limit of 11 nM. Nogami and others developed a capillary electrophoresis (CE) micro-device with CL detection using the reaction of 1,10-phenanthroline and hydrogen peroxide for separation and determination of two metal ion groups, the platinum metal group (Ru(III), Rh(III), Pd(II), Os(VIII), Ir(III) and Pt(IV)) and the fourth periodic transition metal group (Cu(II), Fe(II), Co(II) and Ni(II)) in water.[189] The microchip consisted of two microchannels that crossed at the intersection and four reservoirs that were located at the ends of the channels. The metal ions in the sample solution migrated in the channel along with 1,10-phenanthroline in a solution. The solution mixed

with hydrogen peroxide in a different reservoir to emit CL, which can be detected by a photomultiplier tube located just above the reservoir.

3.4.6 Pesticide Residues

Pesticide residue refers to pesticides, such as insecticide, fungicide, herbicide and nematocide, all of which remain on or in food after they are applied to food crops. Exposure of the general population to these residues may commonly occur through consumption of treated food sources (*e.g.*, meat, poultry, fish, nuts and vegetables). These pesticide residues exhibit bioaccumulation, leading to build-up of harmful materials to dangerous levels, causing either acute symptoms (*e.g.*, nausea, abdominal cramps, diarrhoea, dizziness, anxiety and confusion) or chronic issues (*e.g.*, respiratory problems, neurological disorders, skin conditions, depression, miscarriage, birth defects and cancer).

The herbicide glyphosate is widely used in a great variety of crops. In direct contact with humans, this species can generate dermal irritation and damage to the respiratory, ocular, endocrine and cardiovascular systems. Additionally, recent studies demonstrated that there is a direct relationship of long exposure to glyphosate with the formation of cancerous cells in human. Silva and colleagues reported a method for rapid, simple, direct and reproducible determination of glyphosate and its major metabolite aminomethylphosphonic acid (AMPA).[190] The microchip was made of polyester with the capability to conduct contactless capacitive detection and measure conductivity and electrophoresis separation of the analytes. The polyester microchip contained microchannels (150 μm in width and 12 μm in depth) that were used for sample injection and separation. Under an excitation voltage of 4.5 Vpp at 320 kHz and pH 8.8, the detection limits for glyphosate and AMPA were 45.1 and 70.5 $\mu mol\,L^{-1}$, respectively. A paper-based sensor for detecting the presence of organophosphate pesticides (*e.g.*, Malathion and Paraoxon) in water has been developed by Sicard and others.[191] The sensor applied the inhibition effect of the organophosphates to the hydrolysis reaction of indoxyl acetate with acetylcholinesterase (AChE). The blue-coloured reaction product indigo was used to determine the concentration of organophosphates in the tested samples. Using a cell phone camera and imaging analysis software, the blue pixels of the sensor images can be counted to quantify the concentration of the pesticides. The colour change in response to a concentration of 10 nM could be identified using this colorimetry approach. Electrochemical detection of pesticides using a LOC device has been demonstrated by Islam and others.[192] The device conducted electrophoresis separation, followed by in-channel pulsed amperometric detection of three common triazine herbicides (*i.e.*, simazine, atrazine and ametryn). Redox reaction of these herbicides generated current peaks in the amperometric curve at a distinct time. Both the amplitude of the peaks and the time were recorded to indicate different species and concentrations. The response time for the detection of these herbicides was less than 90 s, and the limit of detection for the sensor

was determined to be 0.35, 0.45 and 0.55 nM for simazine, atrazine and ametryn, respectively.

3.4.7 Migrants from Packaging Materials

Synthetic chemicals used in food packaging may pose long-term health issues to the human body. Many of these packaging substances can leak into the food, causing chronic and continuous exposure to substances, whose long-term impact is either known to be harmful or still under investigation. Some known toxicants, such as formaldehyde, a cancer-inducing substance, are legally used in food packaging materials (*e.g.*, plastic bottles and tableware). Other chemicals such as bisphenol A (BPA) and tributyltin are known to disrupt hormone production, but they are still used in food packaging materials. Thus, to identify the trace of these packaging materials in foods is very critical.

Formaldehyde can be found in preservatives and food packaging materials. It can cause minor to serious problems, such as pain, vomiting, coma and possible death, when a large amount of formaldehyde is taken. Weng and colleagues developed a rapid and simple microfluidic analytical device to determine formaldehyde in eight different samples with only 2 μl sample consumption in one minute.[193] The device was based upon a heated PDMS microfluidic chip, which contained four reaction reservoirs and one substrate reservoir. Samples in the reservoirs were illuminated by 410 nm violet light, whose absorption through the samples can be measured to determine formaldehyde content. A novel integrated microfluidic chip with stacked layers of PMMA structures was developed for the detection of formaldehyde in Chinese herb samples.[194] The test sample and a fluorescence derivatization reagent were mixed in a circular mixing chamber and then flowed through a serpentine reaction channel heated to a temperature of 30 °C by an on-chip heater. The reaction could be completed in 4 min, and the device can be observed in a LIF detection system. The formaldehyde concentration (from 1 to 50 ppm) of the sample was then inversely derived from the measured value of the fluorescence intensity.

Using microfluidics LOC devices can also realize fast and simple detection of BPA with low sample consumption. Kubo and coauthors developed a disc-shaped LOC device using a soft lithography approach.[195] The device contained 32 microchannels and chambers. Anti-PBA antibody was immobilized on microbeads, and introduced into the microchambers through the microchannels in the device. The anti-BPA antibody reacted with horseradish peroxidase (HRP)-conjugated BPA based upon competitive immunoassay. The unbound HRP in each microchamber could be detected using CL. As a result, BPA can be determined at a concentration range between 3.9 and 250 ng mL^{-1}.

3.4.8 Biogenic Amines

Produced by the decarboxylation of tyrosine and histidine, tyramine and histamine are among the most harmful biogenic amines found in fermented

beverages. These amines are produced as degradation products from microbial activities and are widely found in fermented foods and beverages, meat, fish and dairy products. Melamine is also a typical biogenic amine, and is used to produce a range of products such as plastics, laminates, coating agents, foams, pigments, glues and fire retardants.[196] However, multiple nationwide incidents associated with milk and beverage contamination in China have caused a national focus on melamine. In these incidents, melamine was illegally added to food products (*e.g.*, dairy products and animal feeds) to increase the apparent protein content, causing significant health effects on consumers (*e.g.*, blindness, kidney stones, reproductive damage and cancer).[197]

Detection of the aforementioned biogenic amines has been demonstrated using many devices. Jayarajah and others developed a portable microfabricated capillary electrophoresis (CE) instrument that was used for the determination of tyramine (3.3 to 30 mg L^{-1}) and histamine (1.0 to 3.0 mg L^{-1}) in fermented beverages.[198] The target molecules were labelled on their primary amino groups with fluorescamine in a 10 minute reaction, and the samples were analyzed directly, producing a detailed electropherogram in only 120 s on a glass CE device. A droplet-based microfluidic immunosensor has been reported to rapidly and accurately determine melamine in milk. The immunoassay was based upon the competitive reaction between native melamine and a melamine-fluorescein isothiocyanate conjugate against an anti-hapten antibody. The detection protocol provided a limit of detection of 300 ppb, which was below the maximum allowable melamine levels (2.5 ppm) defined by the US Food and Drug Administration and the European Commission.[199] Another detection method for melamine is based upon ultra-violet (UV) detection,[200] which also allows sensitive and fast screening of melamine in milk. This method uses the fracture sampling technique to directly detect sample milk through a narrow fracture and conduct separation through electrophoresis. The concentration of the melamine was later determined by UV absorption spectroscopy. This method can detect melamine in milk within 75 s. At the detection wavelength of 202 nm, the linear range for melamine was from 1.0 to 100 g mL^{-1} with a detection limit of 0.23 g mL^{-1}.

3.4.9 Food Allergens

With the rising incidence of people with food hypersensitivity such as food allergy or food intolerance, there is a need for highly sensitive, low-cost analysis methods based upon LOC devices.[201–204] Heyries and others developed a microfluidic device for CL detection of allergenic proteins in peanut and milk.[205] Three different proteins (*i.e.*, β-lactoglobulin, peanut lectin and human IgG) were immobilized in microchambers for detecting specific antibodies. The device can concomitantly detect three specific antibodies in pM levels with only 300 μL of sample consumption and

6 minute sample incubation time. An electrochemical LOC device was also presented to determine food allergens. In this device, detection of food allergen-induced changes in cell morphology and cell metabolism measurement can be performed simultaneously. Two types of cells have been used to observe their allergic response under antigen stimulus. Two microfluidic channels integrated with gold electrodes can be used to introduce cells and measure cell-secreted inflammatory cytokines through ELISA and cell impedance changes through cell-based electrochemical assay.[206]

3.4.10 Antioxidants

Antioxidants are present naturally in foods or manually added in dietary supplements to provide nutrient and health benefits to humans. For example, phenolic acids can be readily absorbed through the walls of the intestinal tract, and can be beneficial to human health conditions due to their antioxidant effect that prevents cellular damage due to free-radical oxidation reactions. They may also promote anti-inflammatory conditions in the human body when they are consumed regularly.

Many plant food-based antioxidants can be efficiently and rapidly determined using a microfluidic system based upon a peroxyoxalate (PO)-CL assay.[207] This assay uses a 9,10-bis-(phenylethynyl)anthracene (BPEA) as the fluorescent probe and hydrogen peroxide as the oxidant. Antioxidant plugs injected into the hydrogen peroxide stream result in inhibition of the CL emission, which can be quantified and correlated with antioxidant capacity. In the tested plant-food-based antioxidants, β-carotene was found to be the most efficient hydrogen peroxide scavenger, followed by α-tocopherol and quercetin. Although the method is inherently simple and rapid, excellent analytical performance is achieved with high sensitivity, large dynamic range and high precision. A microfluidic device has been investigated to estimate the total phenolic contents or antioxidant levels in honey based upon CL detection.[208] Several phenolic antioxidants such as quercetin, catechin, gallic acid, caffeic acid and ferulic acid can emit analytically useful CL signals in an enhanced potassium permanganate CL system. The detection limits can be achieved in a range between 2.4 nmol L^{-1} for gallic acid and 34 nmol L^{-1} for *o*-coumaric acid.

3.4.11 Food Authentication

LOC devices can also be used to analyze certain food compositions associated with religious beliefs, dietary habits and authentication to prevent fraud and misconduct to pursue maximized profits.[209] For example, detection of canine species in foods is important from the perspectives of health, religion and the fair-trade food business. Rahman and others used PCR and restriction fragment length polymorphism (RFLP) assay with LOC detection

platform for the authentication of canine DNA in processed foods to ensure Halal food regulation and animal rights protection.[210] In their work, a 100-bp fragment of canine mitochondrial Cytochrome b (*cytb*) gene was selected and amplified. The assay successfully detected 0.0001-ng canine DNA under pure state and 0.01% (w/w) canine meat spiked in chicken and beef burger formulations. The use of a LOC technique for the separation and quantification of milk proteins to prevent any impurity doping using low quality or diluted milk has also been presented.[211] A microfluidic chip with the capability to separate all major milk proteins (*e.g.*, a-lactalbumin, b-lactoglobulin, as-casein, b-casein and k-casein) was demonstrated. The separation and quantification of different proteins was realized by electrophoresis that was able to achieve a detection range from 0 to 0.3 mg mL^{-1} within 30 minutes.

3.5 Conclusions and Perspective

Current microfluidic LOC devices have demonstrated their use in determining various components in foods, allowing quantitative analysis of food safety and quality. The LOC devices feature miniaturized sizes and low sample consumption, and use optical, electrochemical and other electrical detection methods to specifically determine the concentrations of target molecules. The choice of materials and fabrication methods of LOC devices are flexible, including different combinations of various techniques according to the application needs.

However, it is also notable that the majority of the aforementioned LOC devices can only conduct single functions, while relying upon other external components to achieve comprehensive functions. More integrated microfluidic systems are still in high demand to achieve fully portable systems for daily use. Constructing miniaturized LOC systems with different components fabricated using various approaches is still challenging, and operation of these components with minimum external support still requires further research effort. It is worth mentioning that the use of cell phone cameras to assist optical detection and miniaturized batteries to achieve electrochemical detection represent two promising trends for LOC devices. In addition, use of porous structures, such as paper and sponge, can eliminate the conventional micropumps and microchannels in LOC devices for fluid handling, showing promising potential to achieve high integrated and miniaturized devices with simplified design. By resolving the aforementioned issues in terms of system miniaturization and simplification of device design and external setup, it can be expected that more portable LOC systems can be achieved to improve the efficiency and convenience of food safety and quality analysis. These portable LOC systems can facilitate improvement of personal health conditions by reducing intake of problematic foods and quantifying health compositions in foods, allowing more scientific dietary monitoring and fewer undesirable incidents with health risks.

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