Chapter 8

Nanoliter/Picoliter Scale Fluidic Systems for Food Safety

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Microfluidic technology is one of the latest platforms for detection of chemical and biological hazards. This technology may provide a higher sensitivity couple with the best specificity provided by targeting DNA molecules. This chapter introduces existing microfluidic systems and reviews some ongoing researches relevant to food safety. Microfluidic devices provide high throughput and large-scale analysis by multiplexing and parallelization of analyses on a single device.

Introduction

Food production is highly efficient and provides the convenience and variety of foods demanded by current lifestyles. To prevent food shortage and deliver low-cost and safe food and drinking water with low environmental impact, new sets of engineering and scientific processes, products and tools are being utilized. Microfluidic systems (also known as micro total analysis systems, µTAS, or lab-on-a-chip, LOC) are considered emerging technologies that provide high throughput and large-scale analysis via integration of multiple steps, multiplexing and parallelization on a single device (1, 2). Microfluidic systems is one of the latest platforms applied to the detection of chemical or biological contaminants in foods (Figure 1) (3–10).

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Figure 1. Timeline and cornerstones in the evolution of detection systems used for food safety.

Microfluidic System for Food Safety

Conventional Detection Method

Different methodologies have been developed to detect and identify biological and chemical agents in food and water. The main biological agents are bacterial pathogens, parasites and viruses. Chemicals agents include primarily natural toxins or intentional adulterations (11–16).

Pathogenic organisms have historically been detected by culture and colony counting method. The target bacterial pathogen present in food or water samples is cultured and isolated in a nutritive medium. Current molecular methods allow for the detection of bacterial pathogens in food before the actual isolation. Although the isolation of the pathogen is labor intensive and time consuming, the regulatory agencies in the USA require the actual isolation to determine if a sample is indeed positive (17–20). To decrease time and effort of detection of pathogens without compromising the reliability of the results, enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR) have been widely used to detect pathogen specific proteins and nucleic acid sequences, respectively. Although these techniques dramatically reduce the time for detection (21–23), key issues, such as cost, automation and simplicity, still remain a limitation.

To detect chemicals hazards in food samples, analytical chemistry methods such as gas chromatography (GC), high performance liquid chromatography (HPLC) (24) and mass spectrometry have been applied to get the best sensitivity
(11) and rapid detection (25). Although with advantages, these techniques remain slow, expensive, and require extensive sample preparation and trained personnel. In addition, the physical dimensions of the required equipment does not allow on-the-spot detection of chemical hazards (21–23). The need for cheap, high throughput and portable analytical systems has encouraged the development of new technologies and more suitable analytical methodologies (26, 27).

**Microfluidic System-Based Method**

*Whole Cell Detection*

Foodborne pathogenic bacteria in animals, plants and water have been found to be an important source of contaminations for humans worldwide (12–16, 28). Strains of pathogenic Escherichia coli, *Campylobacter, Salmonella, Listeria, Staphylococcus, Clostridium* and *Bacillus*, the major foodborne pathogenic bacteria, are responsible for millions of foodborne illnesses, thousands of deaths, and several billion dollars in productivity losses, medical costs, and premature death (29). It is therefore of great importance to develop methods to efficiently detect pathogenic bacteria (30).

**Microfluidic System for Cell Sorting and Capture**

Microfluidics presents several characteristics that make it a suitable technique for isolating pathogens from suspended particle mixtures. Microfluidics is also an important platform to develop portable devices for accurate and rapid detection systems with limited amount of reagents (31–36). One main limitation to the use of whole cell detection in microfluidic system is the difficulty to detect low amounts of cells from nanoliter volumes. Among the methods used for whole cell detection, dielectrophoresis techniques have the capability to concentrate the target cells on a specific area and thus amplify the signal for efficient detection (37, 38). For example, Gagnon & Chang (2005) have developed a microfluidic device (Figure 2A) composed of microchannels align atop a thin continuous serpentine microwire of platinum. When alternating current passes through the wire, it generates an electro-osmotic flow that traps and concentrates the cells on the microwire area, and directs the cells towards a designated points of the device, while the suspended particles are swept towards the outlet along the fluidic flow (37). This system requires less than 100 μl of solution and can concentrate $10^3$ particles/ml in few seconds.

Another microfluidic system for sorting and capturing pathogenic bacteria has been developed by Cheng et al. (2007) (38). This microfluidic system is composed of four different parts where different micro electrodes are used to filter, focus, sort and trap bacterial cells by creating a dielectrophoretic (DEP) force field cage, as shown in Figure 2B. In the first part (filtering section), parallel electrodes trap debris and particles with positive DEP (pDEP) while negative DEP (nDEP) particles, such as bacteria, pass to the focusing section where nDEP forces focus them toward the center of the channel. Particles are then sorted by three oblique
DEP gates in function of their size and their specific charges and are trapped and concentrated in three different channels where they are detected. Although this device is slower (~50 minutes) than Gagnon and Chang’s device (37), it has a similar sensitivity as it can detect 500 cells within 100 µl, but has also the possibility to sort microorganisms in function of their size and electrical charges (38). All these microfluidic devices are more sensitive and faster (seconds vs. hours) than other optical biosensors, and therefore are suitable for the development of portable systems for rapid and accurate detection of microorganisms, especially when limited volume of sample (<100 µL) are available.

Figure 2. Microfluidic chips for whole cell capture (A) and sorting (B) for harmful bacteria detection. Reproduced with permission from Reference (37) (Copyright 2007 Wiley-VCH Verlag GmbH & Co. KGaA) and from Reference (38) (Copyright 2007 American Institute of Physics), respectively.

Impedimetric Biosensor

Impedimetric biosensors are other systems for concentration and detection of microorganisms present in food and water. They rely on the insulating properties of bacterial cell membranes. Impedimetric biosensors are composed of a solid...
electrode to which cells attach through specific or non-specific adsorption (Figure 3) (39). After attachment to the electrode, the natural capacitance (0.5-1.3 μF/cm²) and resistance (10^2-10^5 Ω/cm²) of the cell membrane (40) effectively reduce the current that reaches the electrode, and hence increase the impedance of the interface. The adsorption of the bacteria on the electrode is then revealed through the detection of either a shift in impedance or a change in capacitance or admittance at the bulk of the electrode interface (41).

Traditionally, macro-sized electrodes are used to measure impedance (42–44). To improve the sensitivity, microelectrodes, such as interdigitated array microelectrodes (IDAM), have been developed (45–47). IDAM consist of a series of parallel microband electrodes that are connected together, forming a set of interdigitating electrodes with low ohmic drop, fast establishment of steady-state, rapid reaction kinetics, and increased signal-to-noise ratio (45–47). In addition, their small dimensions (0.1–0.2 μm in height, 1–20 μm in width; 2–10 mm in length; 1–20 μm gap between the microbands) make them easy to combine with microfluidic systems to improve their performance (33, 48–55).

![Figure 3. Working principle of impedance detection technique with (A) or without (B) the use of bio-recognition elements for bacterial cell adherence on the electrodes. Adapted with permission from Reference (39). Copyright 2009 Elsevier.](image)
The detection techniques based on the specific adsorption of bacteria on the surface of the electrodes requires a functionalization of the electrode surface with bio-recognition elements (or bioreceptor), such as antibodies specific to pathogenic bacteria. When bacteria attach to the antibodies, the insulating properties of the cell membrane induce measurable changes in the electrical properties (23). The main advantage of the use of antibodies as bio-recognition elements is their selectivity. The critical part in the construction of antibody-based impedimetric biosensors is the immobilization method, because it can profoundly affect the analytical performance of impedance biosensors. Physical adsorption without the use of chemical linker is the simplest immobilization method. It depends on the non-specific interactions (such as non-covalent bridges, hydrophobic interactions, and van der Waals forces) of the antibody with the surface. However, the low stability and the random orientation of the antibodies immobilized on electrodes result in the decrease of the binding bacteria and a high detection limit \(10^5\) cfu/ml (56), which restrict the use of this method to a limited number of applications. To improve the sensitivity of the biosensor, self-assembled monolayer (SAM) of chemical linkers have been use to immobilize antibodies on the electrodes (57–59). Recently, Tan et al. (2011) have integrated an electrochemical impedance spectroscopy (EIS)-based immunosensor, composed of an alumina nanoporous membrane with immobilized antibodies specific to foodborne pathogen, with a PDMS microfluidic system for the rapid detection of \(E.\ coli\) and \(Staphylococcus aureus\), as shown in Figure 4A (60). This microfluidic immunosensor based on the nanoporous membrane impedance spectrum could achieve rapid bacteria detection within 2 hours with a sensitivity of \(10^2\) cell/ml in pure culture.

In 2007, Varshney et al. (61) developed an impedimetric biosensors based on the non-specific absorption of bio-recognition element, based on gold IDAM for the detection of \(E.\ coli\) O157:H7 in food samples. In this method, cells are first separated, re-suspended and concentrated with the help of magnetic nanoparticle-antibody conjugates (MNAC), functionalized here with anti-\(E.\ coli\) antibodies (Figure 4B), and uniformly spread on the surface of IDAM. The impedance sensor detected a minimum of \(7.4 \times 10^4\) and \(8.0 \times 10^5\) cells/ml of \(E.\ coli\) O157:H7 in pure culture and ground beef samples, respectively (39). The integration of this technology within a microfluidic system increased the sensitivity of detection to \(1.6 \times 10^2\) and \(1.2 \times 10^3\) cells/ml of \(E.\ coli\) O157:H7 present in pure culture and ground beef samples, respectively with very low volume of sample (60 nl) (61).

When compared with impedance macrosystem (62), microfluidic slightly increase the sensitivity of the detection with (60) or without specific immobilization (63) \(4.2 \times 10^2\) cells/ml, \(10^2\) cells/ml and \(10^2\) cells/ml in pure culture respectively) but is faster (about 2 hours, less than 2 hours and 35 minutes respectively) and requires lesser amounts of reagents (ml scale, \(\mu l\) scale and nl scale, respectively).
On-Chip Fluorescent Staining and Cell Counting

Fluorescence-based cell detection presents several advantages when compared with other techniques, including sensitivity, multicolor for simultaneous multi cell type detection, stability low hazard, availability and low cost (64, 65). It has been previously used for the detection of pathogenic bacteria. Recently, Yamaguchi et al. (2011) developed a simplified and rapid on-chip method using a microfluidic cytometry device for rapid quantification of bacteria cells, such as E. coli, in water (66). The microfluidic chip is composed of a T-shape introduction section, followed by a serpentine mixing channel, an alignment part composed of crossing channels, and a detecting part. Samples are injected and fluorescently labeled, and anti-E. coli O157:H7 antibodies are injected through the inlet of the T-shape introduction part. Bacterial cells in the sample are fluorescently stained during flow through the mixing part. After mixing, the cells are aligned by injection of a sheath fluid (PBS) through two microchannels, and fluorescently stained cells are detected in the detecting part. The authors demonstrated the capability of this simple fluorescent-based technique to detect a low number of bacterial cells (10^4 cells/ml). Although the sensitivity of the system is not significantly different from conventional epifluorescence microscopy (EFM), the use of microfluidic presents several advantages to EFM: it is simpler as it does not requires pretreatment apparatus, it is rapid and results can be obtain a limited amount of time (one hour) and it has the possibility to be integrated with small light source such as light-emitting diode (LED) for on-site analysis.

![Image](https://via.placeholder.com/150)

**Figure 4.** Impedimetric biosensors for bacteria cell detection. **A.** Sensor based on the use of bio-recognition element. Adapted with permission from Reference (60). Copyright 2011 Elsevier. **B.** Sensor based on non-specific binding of bacteria. Adapted with permission from Reference (61). Copyright 2007 Elsevier.
Metabolites Release Detection

Detecting any pathogenic bacteria is a reliable method to prevent food poisoning. However, in some cases, although bacteria are killed during thermal process, bacterial toxins (such as staphylococcal enterotoxin B (SEB) or botulinum neurotoxin (BoNT) can remain active and be associated with food poisoning. To detect toxin, chemiluminescence methodologies such as ELISA-based and enzyme-substrate-based methods have been used for their sensitivity and reliability (67–72).

ELISA-Based Microfluidic System

ELISA is a reliable technique for the detection of low quantity of toxin (67–70). However, the large amount of reagent, space and time required to perform one ELISA are cost problems that limit the quantity of tests performed simultaneously outside a well-equipped laboratory. Microfluidics offers a cheap alternative to conventional ELISA methodology (73–75). The possibility to realize on a same chip the different step of the ELISA makes it a very attractive method to bring toxin detection systems on-site. For example, Yang et al. (2010) have developed a lab-on-a-chip system (Figure 5A) for a Carbon Nanotube (CNT) based immunoassay with optical detection of staphylococcal Enterotoxin B (SEB) for food safety applications (73). In this chip, four biosensing elements were combined: (1) CNT technology for primary antibody immobilization, (2) Enhanced Chemiluminescence (ECL) for light signal generation, (3) a cooled charge-coupled device (CCD) for detection and (4) polymer lamination technology for developing a point of care immunological assay for SEB detection. To combine those four elements, the authors have fabricated a six layer, 3D fluidic structure constructed with a rigid 3.2 mm PMMA (acrylic) core laminated with additional layers of thin polycarbonate (PC) film bonded with adhesive. The LOC contains two main analytical elements: an eight channel microfluidic design for the loading of the different samples and buffers and an interchangeable immunosensor surface where the ELISA reaction is done. Eight samples are introduced into the wells and go through the microchannels to the interchangeable immunosensor surface, which is composed of a polycarbonate strip with anti-SEB antibody immobilized on CNT. SEB contained in the sample first binds to the anti-SEB primary antibody of the interchangeable polycarbonate strip. After incubation and washing, horse radish peroxidase (HRP) labeled secondary antibodies are added. The HRP is then analyzed by ECL using a CCD detector. The combination of the four biosensing elements at a microscale level presents various advantages when compared with conventional ELISA technology as the assay can be performed without a laboratory, with a minimal amount of reagents (and consequently minimal exposure to users of toxins and other biohazards) and with a high sensitivity (approximately 0.1 ng/ml of SEB).
Enzyme-Substrate Reaction-Based Microfluidic System

When the metabolite released by bacteria is an enzyme, an alternative detection method in the food or drink is to detect its enzymatic activity using, for example, fluorescent probes. Frisk et al. have developed a microfluidic device for the detection of BoNT/A with high sensitivity, on-site portability and multiplexing capabilities (Figure 5B) (74, 75). This device is composed of three parts: an input port, a reaction microchannel, and a detection port. In the reaction microchannel, the surface is coated with a fluorescently labeled peptide enzymatic substrate of BoNT/A. The sample is introduced into the input port and flows through the microchannel. There, BoNT/A catalyzes the cleavage reaction of the fluorescent labeled peptide that diffuses into the detection port where the evaporation of the solution preconcentrates the fluorescent-labeled fragments for detection. The evaporation-based concentration system leads to 3-fold signal amplification over 35 min and allows the detection of as little as 3 pg/ml of the toxin in buffer and 3nM in food (75). Because of the small size of the system, the platform could be easily modified and adjusted for detecting other serotypes of BoNT, for example by adding microchannels coated with fluorescent-labeled substrate specific to each serotypes. In addition, the integration of this device with a portable light source would enable real-time and on-site monitoring.

Nucleic Acid Sequence Detection

Molecular methods for the detection of nucleic acids (DNA and RNA) have been regularly used for detection of pathogenic bacteria in food because of their high sensitivity, selectivity and short time (30). With the use of smaller volumes and sample amounts, microfluidic systems increase the detection sensitivity and improve the experiment throughput. Several microfluidic platforms have been develop to perform molecular methods including nucleic acid hybridation (76–79), isolation (80–82), and amplification (83, 84).

Nucleic Acid Hybridation

Recently, various microfluidic DNA-based probes were coupled to different measurement techniques, including SPRi (85), conductance impedance (86), and fluorescence (87). Ben-Yoav et al. (2012) have developed a microfluidic-based electrochemical biochip shown in Figure 6A, which contains an array of individually addressable 25 nl reaction chambers, fabricated with micro-electromechanical systems (MEMS) technology (88). Each chamber contains a grid of 3×3 sensors and each row of 3 sensors also contains a counter and a reference electrode to complete the three-electrode system. Three unique single stranded DNA (ssDNA, 30-mers) probes were functionalized onto
patterned electrodes of the chip to detect complementary DNA hybridization events using EIS analysis. This impedimetric biosensor-based technique is similar to those for whole cell detection but uses ssDNA as bio-recognition elements. This biosensor is able to detect ssDNA targets on the nM scale and with low cross-reactivity (13%).

![Diagram of microfluidic chip](image)

**Figure 5.** Microfluidic chip for metabolite detection. **A.** ELISA-based microdevice. (a) schematic figure of the device with indication of the sample wells (1), rectangular loading frame to simplify reagent application (1a), channels (2), detection wells (3), interchangeable surface with the antibody ligand-CNT (3a), joining element for all eight channels (4), waste chamber (5) and the chip outlet (6). (b) top view of the chip. (c) image of the immunosensor on polycarbonate strip and (d) bottom view. Adapted with permission from Reference (73). Copyright 2010 The Royal Society of Chemistry. **B.** Enzyme-substrate reaction-based system. (a) schematic representation of the microfluidic chip with the input port, the reaction microchannel and the detection port. Close up shows the formation of the self-assembled monolayer of the BoNT/A enzymatic substrate on Au surface. (b) 40 arrayed detection system for throughput screening. Adapted with permission from Reference (75). Copyright 2009 American Chemical Society.

**Nucleic Acid Amplification**

Polymerase Chain Reaction (PCR) is a molecular technique for nucleic acid amplification that can replicate a specific fragment of a target nucleic acid and create several million DNA copies within a few hours. It is thus a key element in food safety to identify pathogens, allergens, and GMOs present in small quantity (89, 90). However, it remains difficult if not impossible to detect the presence of a single pathogen using this technique. Microfluidics has been integrated with
PCR to dramatically increase sensitivity. Ottesen et al. (2006) have developed a microfluidic digital PCR platform allowing single cell detection (83). The platform is composed of 1,176 individual independent 6.25 nl reaction chambers, allowing the realization of 1,176 independent PCR reactions. PCR was carried out on a conventional flat-block thermocycler. Amplification was monitored using 5’ nuclease probes to generate a fluorescent signal detected with a modified microarray scanner (Figure 6B).

In some devices, entire bacteria are introduced into the detection PCR platforms, lysed by thermal (91), chemical (92), physical (93, 94), and electrical methods (95), and their nucleic acid is then amplified on site. In 2008, Cheong et al. developed a one-step real-time PCR method for pathogen detection (84). In this design, Au nanorods were used to transform near-infrared energy into thermal energy and subsequently lyse the pathogens. Next, DNA was extracted and amplified in the PCR chamber. The entire process does not need to change or remove reagents, which improved the overall efficiency of the device.

Among all nucleic acid amplification techniques, isothermal techniques for amplification of DNA/RNA have recently drawn interest (96, 97) since they do not require high temperature and large variation of the temperature. This approach to a simpler and more energy efficient model holds with the goal of microfluidic systems to reduce cost and labor of routine experiments, making isothermal amplification techniques an excellent choice for nucleic acid detection LOC. Recently, we have developed, in our laboratory, a microfluidic chip for detection of pathogenic bacterial using the isothermal real-time helicase-dependent amplification (HDA) technique (unpublished data). The chip is a microfluidic platform composed of a 16×24 array of nanoliter-scale microchambers where real-time HDA for single cell analysis can be performed.

Sample Preparation

Sample preparation steps for PCR are of first importance to achieve high sensitivity and specificity. Hong et al. (2004) have developed nanoliter-scale nucleic acid processors for DNA and mRNA purification (80). Two different microfluidic chips were fabricated to isolate variable numbers of cells, lyse the cells, and purify their DNA or mRNA. All the steps were realized in one single microfluidic chip without pre- or post-treatment of the sample. For mRNA purification, oligo-dT polymer magnetic beads were introduced into the separation chamber to form an affinity column. After cell lysis in the cell chamber, lysate is flushed over the affinity column and mRNA was recovered from the column. For DNA purification, the chip was composed of three independent processors, as shown in Figure 6C. In each processor, lysis buffer, dilution buffer, and E. coli in culture medium are introduced into a metering section. The three solutions are then pushed into a microfluidic mixer where cells are lysed. Lysate is then flushed over the DNA affinity column, and the genomic DNA is then recovered from the chip for PCR amplification. This methodology allows DNA recovery with a reduced number of bacteria (< 28 bacteria), and thereby increases the sensitivity of this process three to four orders of magnitude over that of conventional methods.
Microfluidic-Based Assay for Nutritional Hazard Detection from Chemical Origin

With the increased role of chemistry and chemical engineering in agriculture and food sciences, the presence of hazardous chemicals in the food, drink, and soil became a threat for human health and increased the importance of constant monitoring of ecosystems. A similar method to the one previously described for the detection of toxins with enzymatic activity can be used to detect harmful chemicals. With such a method, chemical reactions that provide detectable signal in function of the concentration of the target substrate can be used to monitor the presence of harmful chemicals. Several applications, based on a chemical detection concept, have been integrated in microfluidic
systems (98–100). Recently, He et al. developed a microflow injection analysis (µFIA) system for the determination of nitrite in food, based on the reaction of ferrocyanide with nitrite in acidic medium (99). The microfluidic chip has three main parts: an introduction part composed of a Y-inlet for the introduction of the reagent (luminol) and the working solution (the sample in ferrocyanide and HCl solution), a mixing part, and a detection component. In the mixing part, nitrite from the sample metabolizes ferrocyanide to ferricyanide in acidic medium and chemoluminescence reaction of luminol with ferricyanide is sensed in the detection part.

Use of Microfluidic System To Improve Food Production

Microfluidic for Bacterial Plant Pathogen’s Infection Studies

Microfluidic systems have the ability to recreate fluidic microenvironments that can be found in nature (101–107). This unique feature can be used to study cell behavior in a controlled environment, when it is difficult or impossible to do it in vivo or with conventional methods (108, 109). For example, microfluidic systems have been developed to recreate xylem vessels of plants and study the infection process and strategy of the plant pathogen bacteria Xylella fastidiosa (108, 109), which lives inside the xylem vessels of different plants, such as grapes, citrus, coffee, and almond (110). Using polydimethylsiloxane (PDMS) microfluidic chambers, researchers have mimicked the plant xylem vessel and demonstrated that the pili of X. fastidiosa are implicated in cells adherence (108) and migration against flowing currents of growth medium (109). With conventional methods, such as atomic force microscopy or laser tweezers, the molecular and biochemical aspects of the plant pathogen is difficult to study because of the large size of the cell chambers or the time required for the experiments. Scaling-down the experiment to the microscale reduces the size of the observation chamber and the time of the observation, overcoming the limitations of conventional apparatus.

Microfluidic System for Animal Food Production

Embryogenesis-based biotechnologies have various promising applications in the field of food production, from in vitro fecundation (IVF) for livestock breeding to embryos development studies (111, 112). Conventionally, experiments are performed manually, in 96 to 384 well plates (113–116), and monitored with imaging methodology (117), which keeps high-throughput analysis and imaging of embryos and juveniles challenging (113, 115, 117, 118). However, miniaturized LOC technologies overcome these limitations by providing an automated platform for the handling and immobilization of micron-size organisms (113, 119–122), and for high-throughput screening (123) that simplify the different steps of fecundation and embryogenesis (124, 125). For example, Ma et al. (2011) have reported a novel microdevice that integrates each step of IVF, including oocyte positioning, sperm screening, fertilization, medium replacement, and embryo culture (111). The chip (Figure 7A) is composed of an oocyte positioning
region and four symmetrical straight channels, crossing at the oocyte positioning region for sperm introduction, monitoring and selection. Oocytes are singly introduced in the 4 × 4 units. The fertilization process and early embryonic development of the individual zygote is traced with microscopic recording and analyzed by in situ fluorescent staining. In this system, the embryo growth rate and blastocyst formation are similar to the conventional Petri dish method, and healthy blastocysts developed in the microdevice can be easily retrieved through a routine pipetting operation and used for further embryo transfer. The present system presents the advantage to avoid the use of centrifugation that can increase the production of oxygen species and decrease the sperm motility, both negatively influencing the fecundation of the oocytes. In addition, healthy blastocysts can easily be collected and used for further embryo implantations or studies.

Figure 7. Microfluidic system for food production. A. microfluidic chip for IVF with 4×4 array of individual fecondation chamber. Reproduced with permission from Reference (111). Copyright 2011 ACS Publication. B and C. Microfluidic system for on chip embryogenesis studies in droplet-based microfluidic system (B) or continuous-flow system (C). Adapted with permission from Reference (126) (Copyright 2007 The Royal Society of Chemistry) and Reference (131) (Copyright 2011 PLOS), respectively.
To study embryogenesis, several microfluidic-based methodologies have been developed involving droplet-based microfluidic system (126, 127) or continuous-flow system (128–132) for screening and studies of fish embryos. Droplet-based microfluidic systems have been the first technology to be study fish embryogenesis in controlled environment. In 2006, Funfak et al. have developed a microfluidic system embryology based method (126). The system, shown in Figure 7B, is composed of a Teflon microtube in which fish egg-containing droplets are perfused with immiscible perfluoromethyldecasil (PP9). The development processes of fish embryos could be observed. After 5 days, larvae were collected from the droplets and transferred into breeding reservoirs for further experiments. More recently, Akagi et al. have introduced a miniaturized embryo array that automatically traps, immobilizes, and perfuses fish embryos (131). The devise, as it appears in the Figure 7C, is composed of a serpentine channels that contains embryo traps (each one designed for a single embryo), suction channels, and hydrodynamic deflectors. Embryos were loaded in the chip one-by-one, rolled on the bottom surface of the serpentine channels under the influence of drag force. Approaching an empty trap, the embryo was affected by the flow passing through the suction channel and directed toward the trap where it remained, without obstructing passage of other embryos in the main channel. The process was repeated with the second embryo and the following ones until all traps were occupied. After loading, the chip was perfused for up to 72 hours with normal and uniform development of all embryos observed. Although most of the microfluidic technology for fish embryological studies were performed on Danio rerio, one can easily transpose those technology to other fishes and other animals for food-purposed embryogenesis studies and engineering, such as fish spermatogonial transplantation for food production (112).

Summary

Microfluidic technologies are powerful tools that can revolutionize the entire food production system and introduce agriculture to a new era. In this chapter, we describe some of the systems and concepts that overcome a number of limitations imposed by conventional macroscale experimentations including the expensive and time-consuming methods, low throughput, and the need of large volume of reagents. With the miniaturization of the biomolecular, chemical, and biological processes on a single chip, the approach of the food production system is dramatically changed. This new set of tools for the supply of food aims to improve the production of low-cost and safe food and drink, and prevent a worldwide food shortage. However, a number of challenges still remain to achieve this new agricultural revolution. For example, although microfluidic systems dramatically downsize the space required in the lab to perform conventional experiments, the emptied space has been invaded by tubing, pumps, gas tanks, computers, and other components for chip handling making the “lab-on-a-chip” a “chip-in-the-lab”. In order to substitute conventional methods, progress to make microfluidic devices more accessible to conventional laboratories has to be made. In addition, these efforts will render microfluidic chips mobile and bring this technology into fields and farms to improve food production in multiple
areas. More importantly, mindsets need to change and participants of the food production system need to be convinced of the suitability of these systems by increasing the number of validation studies for the proposed processes. We are now at the door of a new era in agriculture, but a great deal of progress must be made to increase the utility of microfluidic systems.

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