Review

Journal of Biosystems Engineering

J. of Biosystems Eng. 41(2):116-125. (2016. 6)
http://dx.doi.org/10.5307/JBE.2016.41.2.116

eISSN : 2234-1862
pISSN : 1738-1266

Applications of Microfluidics in the Agro-Food Sector: A Review

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Received: April 16th, 2016; Revised: May 17th, 2016; Accepted: May 19th, 2016

Abstract

Background: Microfluidics is of considerable importance in food and agricultural industries. Microfluidics processes low volumes of fluids in channels with extremely small dimensions of tens of micrometers. It enables the miniaturization of analytical devices and reductions in cost and turnaround times. This allows automation, high-throughput analysis, and processing in food and agricultural applications. **Purpose:** This review aims to provide information on the applications of microfluidics in the agro-food sector to overcome limitations posed by conventional technologies. **Results:** Microfluidics contributes to medical diagnosis, biological analysis, drug discovery, chemical synthesis, biotechnology, gene sequencing, and ecology. Recently, the applications of microfluidics in food and agricultural industries have increased. A few examples of these applications include food safety analysis, food processing, and animal production. This study examines the fundamentals of microfluidics including fabrication, control, applications, and future trends of microfluidics in the agro-food sector. **Conclusions:** Future research efforts should focus on developing a small portable platform with modules for fluid handling, sample preparation, and signal detection electronics.

Keywords: Animal monitoring, Environmental monitoring, Food safety, Food processing, Microfluidics

Introduction

Increasing studies have focused on using microfluidic devices to solve scientific problems that could not be solved with conventional technologies. Whitesides (2006) defined microfluidics as the science and technology of systems that process or manipulate very small volumes of liquids, typically ranging from nanoliters to attoliters. Microfluidics uses channels with dimensions of tens of micrometers to manipulate tiny amounts of fluids. The advantages of microfluidics include low cost, short reaction and analysis time, high sensitivity, small footprints, and high-throughput (Whitesides, 2006; Chen et al., 2013). The miniaturization of devices provides additional advantages, such as using small amounts of reagents and integrating several analytical steps (Mairhofer et al., 2009).

Microfluidics was initially applied in analysis. Currently, the application areas have expanded to diagnostics, drug discovery, cell biology, chemical synthesis, medicine, biotechnology, and ecology. Microfluidics is one of the most promising technologies for diagnostics because it offers affordability, accessibility, sensitivity, specificity, ease-of-use, speed, and robustness (Mao and Huang, 2012). In food and agricultural industries, microfluidics is used for food safety, food processing, and animal sciences (Neethirajan et al., 2011).

Microfluidics is a multidisciplinary technology encompassingphysics, chemistry, biochemistry, micro or nanotechnology, engineering, and biotechnology. In order to successfully develop new applications, it is necessary to understand the distinct characteristics of microfluidics including laminar flow, large surface-to-volume ratios, and dominant surface phenomena like capillary forces or surface tension.

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This review discusses fabrication, control, and application examples of microfluidics. Additionally, the future trends in microfluidics were discussed.

Fabrication of microfluidics

Generally, inert substrates are considered for fabricating microfluidics to prevent unfavorable interactions. In addition to selecting a proper substrate, handling agricultural or food samples requires considering a variety of liquid properties such as surface tension, viscosities, contact angle, tendency to adhere to charged surfaces, and adsorption onto the surface (Mark et al., 2009).

The earliest microfluidic devices were fabricated with silicon or glass by using modified semiconductor manufacturing process and microelectromechanical systems (MEMS) (Lliescu et al., 2012). Presently, a silicone elastomer, namely polydimethylsiloxane (PDMS) is one of the most frequently used substrates for microfluidics as it is inexpensive and it has good optical transparency and biocompatibility. The usage of this material allows for soft lithography for fabricating and replicating microfluidic structures using elastomeric stamps, molds, and conformable photomasks (Xia and Whitesides, 1998). Since the soft lithography technique does not require complex laboratory facilities, it is simple, inexpensive, and readily accessible to several researchers. Figure 1 illustrates the typical fabrication process of PDMS microfluidics. Usually, a thick photoresist is used as the PDMS replication mold. A photolithographic process is used to make the microfluidic mold for the PDMS channel. A liquid PDMS elastomer, such as Sylgard 184 (Dow Corning Co., MI, USA), is poured to create a PDMS lid containing microfluidic channels. The cured and replicated PDMS lid is carefully detached from the replication mold and then bonded to a glass slide to create a microfluidic channel device.

The PDMS has certain drawbacks such as the difficulty of fabricating high aspect-ratio channels and automating mass production process, the hydrophobic nature, and the high oxygen and water permeability. Hence, researchers have turned their attention to polymethylmethacrylate (PMMA), polycarbonate, and cylic olefin polymers. These substrates are harder, less permeable, and amorphous thermoplastic polymers that can be injection molded or hot embossed for mass production (Lliescu et al., 2012).

Two or more sheets of substrates should be assembled by adhesion for targeted functionality. The most widely used PDMS can easily be bonded to a glass plate even without treatment. In order to increase the bonding strength, either of the PDMS (that is the cover or both the sheets) are treated to obtain a hydrophilic surface. This is commonly achieved by a plasma treatment. Thermoplastic polymers (e.g. PMMA) can be bonded together by thermal pressure bonding. In this bonding method, the substrates are heated above the glass transition temperature and pressed together. In addition to the above bonding methods, other techniques such as using adhesives, solvent bonding, or ultrasonic welding methods can also be used. In the case of using adhesives, it is necessary for the adhesive to have a high viscosity to prevent the blocking of the channel by overflow (Becker and Gartner, 2008).

Prior to assembling the microfluidic device, the surface of the microfluidic channel is treated with special reagents (e.g., capture antibodies) to give some functionality such as the detection of pathogenic bacteria (Figure 2).

Driving and control of fluids

Liquids in the microfluidic channel can be driven by capillary force, pressure gradient, centrifugal force, and electrokinetic phenomena. The liquid movement driven by the capillary force is controlled by the wettability and feature size of the porous or microstructured devices. The capillary platform is simple, low cost, and robust, but it is important for assay protocols to follow a fixed and limited process scheme. Liquid transport mechanisms

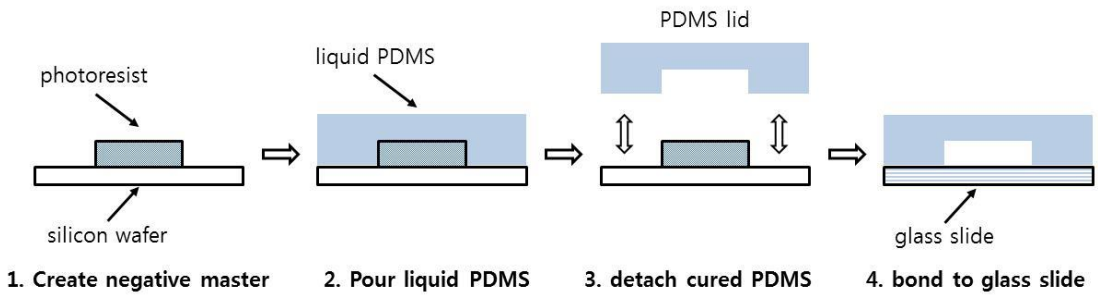

Figure 1. Fabrication process of a PDMS microfluidic device.

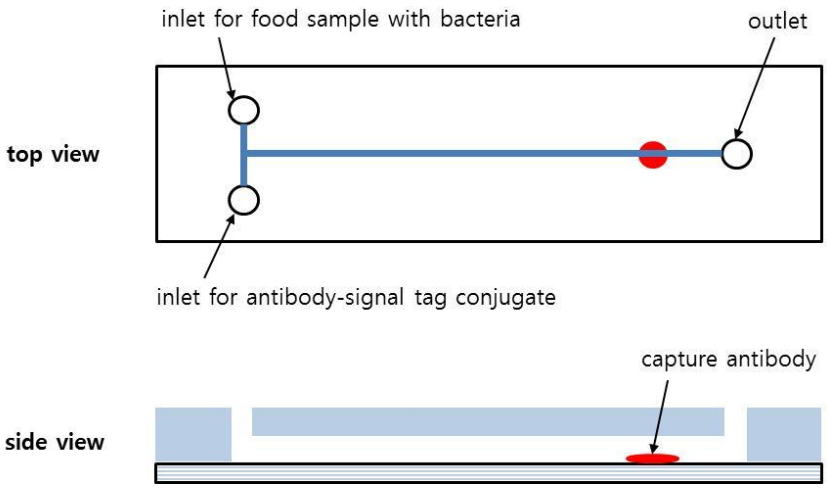

Figure 2. Schematics of a microfluidic device for food safety.

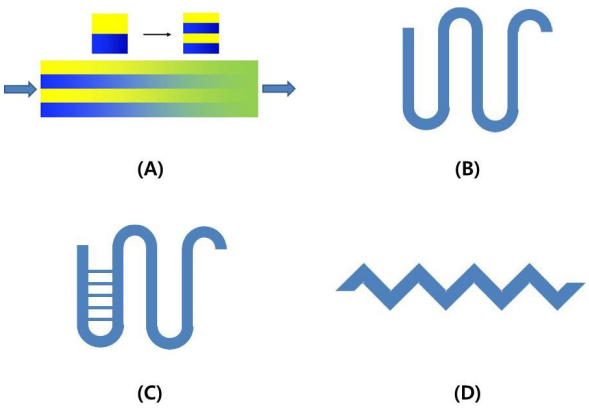

Figure 3. Various types of passive mixers (A) Lamination (B) Serpentine (C) Intersecting channels (D) Zigzag channels.

based on the pressure gradient can be implemented by using external or internal pressure sources such as syringes, pumps, micropumps, gas expansion, and pneumatic displacement of membranes. Pressure driven liquids in the microchannel are generally characterized as laminar flow. This property enables the pressure driven microfluidic devices to have parabolic velocity profiles, diffusion mixing, and multiphase flows. This platform has advantages for continuous sample processing and versatile liquid controls. However, it has shortcomings such as requiring a relatively large external pressure source and connections to the pressure source. Centrifugal forces are controlled by the angular speed of a rotating microstructured device. In electrokinetics, platform liquids are controlled by electric fields acting on electric charges, or electric field gradients acting on electric dipoles. Among the electrokinetics effects, electroosmosis can be used to move the complete liquid bulk with a planar velocity profile (Mark et al., 2009). An electrokinetics platform effectively separates and concentrates particles in the sample liquids. However, it is necessary for the platform to reduce the transport time for practical purposes (Chang, 2006).

Diffusion is the main mixing method since fluid flow in a microfluidic channel is dominantly laminar. In diffusion mixing, the diffusion zone between two different liquid streams expands as it flows down the channel and takes a very long time. The flow velocity, diffusivity of the mixing components, width and depth of the channel, and structures of the microfluidic systems (Atalay et al., 2011) influence channel length and reaction time to complete mixing.

Various mixing methods were developed to achieve thorough and rapid mixing. Some methods applied external force to perturb the sample species, while other methods utilized special configurations to increase the contact area and contact time of the sample species (Lee et al., 2011). A high mixing performance was observed in embedded ultrasonic transducers used to generate acoustic or ultrasonic waves to stir the samples (Liu et al., 2003; Yaralioglu et al., 2004). Inducing local instabilities in the flow stream by using dielectrophoretic activity (Choi et al., 2009), electrokinetic time pulse (Zhao and Yang, 2011), and pressure perturbation with velocity pulsing (Niu and Lee, 2003) was also effective in enhancing the mixing performance. Passive mixing methods use special configurations to speed up the mixing rate (Figure 3). Wedge shaped inlet structures were used to assist flow lamination through inertia effects (Buchegger et al., 2011). Serpentine or zigzag shapes are frequently used as microchannels to increase the ratio of the channel surface area to its volume. Based on these basic designs, intersecting

channels could be added to split, rearrange, and combine flow streams (He et al., 2001; Melin et al., 2004). Additionally, three-dimensional serpentine structures were developed to promote a chaotic mixing effect (Liu et al., 2000; Vijayendran et al., 2003).

Computer simulation could be used to optimize the design of microfluidic devices. This helps in understanding the transport phenomena and in optimizing geometries, structures, and dimensions. Hence, the cost and time to develop a microfluidic device could be reduced (Atalay et al., 2011).

Food safety

Microfluidic systems can be used to rapidly and sensitively detect pathogens, toxins, chemical residues, and heavy metals in food because they are able to conduct measurements from small volumes of complex fluids with efficiency and speed.

Wang et al. (2012) detected genetically modified *E. coli* strain expressing green fluorescent protein with a simple PMMA microfluidic device. The limits of detection (LOD) of the device for PBS, milk, and spinach samples were 50, 50, and 500 CFUs/mL, respectively. Tan et al. (2011) developed a flow-through type PDMS microfluidic immunosensor with antibody immobilized nanoporous alumina membrane. The microfluidic immunosensor could detect food pathogens *S. aureus* and *E. coli* O157:H7 in a PBS solution 10^2 CFU/mL within 1-2 h. Yang et al. (2010) developed an eight channel microfluidic device to detect *Staphylococcal Enterotoxin* B (SEB) in food. Six layers of black acrylic were used to fabricate the device by using lamination technology. An anti-SEB antibody-carbon nanotube (CNT) mixture was immobilized onto a polycarbonate strip and bonded to the device. A syringe was used to instill the flow of sample fluids to analyze eight samples simultaneously. The device could detect 0.1 ng/mL of SEB in soy milk.

Many reports have indicated that microfluidic devices are effective in enhancing the sensitivity of bacteria detection by concentrating the pathogens in a small volume and removing interfering foreign materials from the sample (Ramadan and Gijs, 2012). Varshney et al. (2007) successfully detected levels of *E. coli* O157:H7 cells as low as 1.2×10^3 cells in a ground beef sample in a time period of 35 min by using a poly(dimethylsiloxane) (PDMS) microfluidic flow cell and an impedance biosensor. The sensitive impedance change could be measured by concentrating the *E. coli* cells in a detection microchamber with the immunoseparation of magnetic nanoparticles. Beyor et al. (2008) also focused on *E. coli* isolation from a dilute sample with a grass microfluidic device by using the immunomagnetic separation method.

Mycotoxins are toxic secondary metabolites of fungi that could contaminate food and cause severe health problems. Conventional detection methods for mycotoxins are time consuming and necessitate expensive laboratory instruments. Microfluidic device based mycotoxin detection methods were studied to overcome these drawbacks. Hu et al. (2013) detected low levels of aflatoxin B1 spiked in corn extraction solutions with a PDMS microfluidic device. Serpentine shaped microfluidic channels were used to reduce the diffusion length and time of aflatoxin adsorption to smectite-polyacrylamide nanocomposite on glass slides. Hervás et al. (2009) presented electrochemical microfluidic chips to determine the mycotoxin zearalenone (ZEA) in baby foods. The microchip consisted of a glass plate with a four-way injection cross, a long longitudinal channel, and relatively short side arms fabricated by using wet chemical etching and thermal bonding techniques. The study detected very low concentration levels of ZEA (less than 1 ppb) in approximately 200 s. The authors of the study also proposed a microfluidic chip with an electrokinetic magnetic bead-based electrochemical immunoassay to detect zearalenone (ZEA) in foods. The microfluidic chip had a double-T mixing junction to sequentially perform the immunointeraction and enzymatic reaction. Detection of the target ZEA analytes in a maize sample was performed in less than 15 min with an LOD of 0.4 μg/L (Hervas et al., 2011). Arévalo et al. (2011) developed a flow-through type microfluidic immunosensor for quantifying citrinin (CIT) mycotoxin in rice samples. The device incorporated an electrochemical immunosensor based on a glassy carbon (GC) electrode. The LOD of the device was 0.1 ng/mL, and the analysis time and the total assay time were 2 min and 45 min, respectively. Galarreta et al. (2013) demonstrated the detection of ochratoxin-A (OTA) with a metallic nanostructure embedded within a PDMS microfluidic channel. The nanostructured metallic platform was inscribed by electron beam lithography on a glass coverslip surface for surface-enhanced Raman spectroscopy (SERS) measurements. Another group (Novo et al., 2013) demonstrated PDMS microfluidics with integrated silicon photodiodes for the chemiluminescence detection of OTA in wine and beer. The microfluidic

device had two U-shaped channels to simultaneously analyze a reference solution and an OTA contaminated solution. The LOD for the beer and red wine extracts were 0.1 and 2 ng/mL, respectively.

Guo et al. (2015b) used a PDMS microfluidic device to detect pesticide residues in vegetable samples. The device consisted of a microchamber detection inlet and a microchannel outlet. It was installed on a gold interdigitated array microelectrode to detect chlorpyrifos in leek, lettuce, and cabbage samples. The study detected pesticide residues with lower LOD by using the microfluidic chamber. Babrak et al. (2015) developed a 96-well microfluidic immunoassay plate device for the detection of Botulinum neurotoxins (BoNTs). Each well of the plate consisted of a tapered spiral microchannel that provided increased surface area, faster reaction kinetics, and occupied only 5 μl of sample volume.

Microfluidics could be used to prepare samples for the analysis of food contaminants. Adami et al. (2016) suggested the utilization of microfluidics to separate components such as fats and proteins in milk that could interfere with the detection of aflatoxin M1. For example, a combination of inertial and hydrodynamic forces could be used for the separation of fat particles at high flow rates in the microfluidic channel. Table 1 lists the characteristics of microfluidic devices that were developed for food safety.

Food processing

Processing with a device that was closer to the size of the structural elements of foods (i.e., 1-100 μm) was beneficial in designing novel food microstructures to meet market demands. Microfluidics is suitable for these operations and microfluidic systems were developed to generate emulsions and foams, and for fluid mixing and dispersion (Skurtys and Aguilera, 2008). Tetala et al. (2009) developed a three-phase microfluidic device for the small-scale purification of alkaloids from plant extracts. The device was employed in the purification of alkaloids (strychnine and brucine) from Strychnos seeds. Cuadros et al. (2012) used a microfluidic device to produce calcium alginate fibers that were used in food engineering as texture and microencapsulation agents with uniform diameters (approximately 300 μm and 550 μm). The microfluidic device was fabricated by using microfabrication techniques and syringe pumps for injecting the solutions. An inner metal needle and an outer polycarbonate capillary tube were co-axially assembled inside the device to create the coaxial flow. The $CaCl_2$ solution from the outer capillary joined the alginate solution in the inner capillary at the interface and formed the calcium alginate gel on the fiber surface.

A PMMA microfluidic device with two intersecting channels was used to produce food foams to obtain proper void fractions and flow rates (Laporte et al., 2016)

Table 1. Microfluidic devices for food safety

Target	Device type	LOD	Notes	Reference
E. coli	Simple linear channel, PMMA	50 and 500 CFUs/mL for milk and spinach buffer	Genetically modified strain	Wang et al. (2012)
S. aureus and *E. coli* O157:H7	Flow through, PDMS	10^2 CFU/mL in PBS		Tan et al. (2011)
Staphylococcal Enterotoxin B	Simple linear channel, PMMA	0.1 ng/mL in soy milk	Multiple channels	Yang et al. (2010)
E. coli O157:H7	Simple linear channel, PDMS	1.2×10^3 ground beef	Interdigitated array microelectrode	Varshney et al. (2007)
Zearalenone	Single linear channel, glass	Less than 1 ppb in baby food	Embedded glassy carbon electrode	Hervás et al. (2009)
Zearalenone	Double-T mixing junction	0.4 μg/L in infant food		Hervas et al. (2011)
Citrinin	Flow-through, stainless steel	0.1 ng/mL rice	Embedded electrode	Arévalo et al. (2011)
Ochratoxin-A	Simple linear channel, PDMS	2.5 μM	Nanostructure embedded	Galarreta et al. (2013)
Ochratoxin-A	U-shaped two channels, PDMS	0.1 and 2 ng/mL for beer and red wine extracts	Integrated silicon photodiodes	Novo et al., (2013)
Chlorpyrifos	Simple linear channel with microchamber, PDMS	1 ng/mL in PBS	Interdigitated array microelectrode	Guo et al. (2015b)
Botulinum neurotoxins	Tapered spiral channel	30 pg/mL in human serum	Commercial 96-well plate	Babrak et al. (2015)

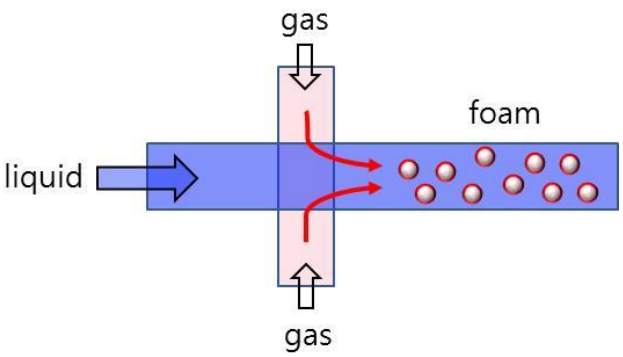

Figure 4. Schematics of a microfluidic mixing device for uniform foam generation.

(Figure 4). Food foams such as ice cream that are found in the food industry are popular because they affect tastes and textures (Campbell and Mougeot, 1999). The foaming operation involves the dispersion of gas bubbles in a liquid matrix. Traditional batch type mixers are inefficient and unable to control the rate of inclusion of air into the foam. The microchannel device could produce stable foams at the lowest void fractions because of a reduction in the bubble diameters (Laporte et al., 2016). Calcium alginate foams were also prepared by using a microfluidic T-junction device (Ahmad et al., 2012). The T-junction device could generate monodisperse microbubbles (mean diameter, ~154 μm) and produce stable highly porous foams. Researchers concluded that the porosity in the foam could be varied based on the initial bubble size.

Water environment monitoring

The proper monitoring of water quality is important to provide healthy drinking water. A threat to public health is continuously posed by waterborne microbial pathogens. A few researches were conducted on using microfluidics for environmental analysis. A concentration step is required for environment monitoring since there is an extremely small number of target pathogens in very large volume samples. Microfluidics provides the ability to prepare samples, and process and detect targets inside a single device in order to minimize the possibility of losing concentrated pathogens (Ramadan and Gijs, 2012).

A multichannel flow-through chemiluminescence device was developed to simultaneously detect multiple pathogens. The device was made of acrylonitrile-butadiene-typrene (ABS) and had six microchannels. The device was tested with *Escherichia coli* O157:H7, *Salmonella typhimurium*, and *Legionella pneumophila* in a water sample. The LODs were 1.8×10^4 CFU/mL for *E. coli* O157:H7, 2.0×10^7 CFU/mL for *S. typhimurium*, and 7.9×10^4 CFU/mL for *L. pneumophila* (Karsunke and Niessner, 2009). Yamaguchi et al. (2011) developed a PDMS microfluidic device for the semi-automated counting of *E. coli* O157:H7 in freshwater. The bacterial cells were fluorescently stained in the serpentine shape mixer and aligned by sheath fluid. The device could enumerate the bacterial cells in the range of 1×10^4 to 1×10^6 CFU/mL within 1 h.

Microfluidics enabled immobilization, maneuvering, and force measurement of a single-Cryptosporidium oocyst, which is a waterborne protozoa, as an isolated subject at a rate that was greater than 50 per hour with atomic force microscopy (AFM). Microchannelled force microscopy cantilevers could pick up and measure individual oocysts by suction by utilizing pressure control (McGrath et al. 2016).

Animal production and monitoring

Microfluidics can help to overcome conventional animal food production and monitoring methods. Conventional experiments in the area of embryogenesis-based bio-technologies are performed manually and monitored with an imaging system that makes high-throughput analysis difficult. Microfluidics devices are able to provide the automated handling of microorganisms, and high-throughput screening for fecundation and embryogenesis (Hamon et al., 2013).

Ma et al. (2011) adapted a microfluidic device with respect to in vitro fertilization (IVF) for animal study. The device consisted of four symmetrical straight microchannels crossing at the oocyte positioning region. Multiple steps including oocyte positioning, sperm screening, fertilization, medium replacement, and embryo culture with the device were integrated to increase the murine sperm motility from 60.8 ± 3.4% to 96.1 ± 1.9%. Wielhouwer et al. (2011) improved the embryonic development rate by using a microfluidic flow-through system instead of using a conventional static buffer replacement method. They achieved survival rates of 100% in zebra fish embryo with buffer flows of 2 μL per well per minute. Additionally, Choudhury et al. (2012) developed a multi-channel microfluidic perfusion platform for culturing zebra fish embryos. The silicon and glass microfluidic platform was composed of a microfluidic gradient generator, a row of eight fish tanks, and eight output channels. The fluidic gradient generator enabled a uniform and constant flow of culture media into the fish tanks that confined the individual fish embryos.

Microfluidics was used in livestock breeding to physically sort sperms and eggs. Microfluidic systems could sort sperms for sex and weight selection. This technology enabled genetic uniformity within livestock species, and provided an opportunity for breeders to select preferred traits (Sekhon 2012). Kempisty et al. (2014) developed a microfluidic device to analyze the impact of follicle size on oocyte quality. The microfluidic device consisted of a silicon-glass sandwich with embedded glass optical fibers for the spectral analysis of pig oocytes. They found that by using the device that the spectral characterization of oocytes was associated with follicular size in pigs. Huang et al. (2015) suggested utilizing a microfluidics device for animal breeding and selection through efficient single-nucleotide polymorphism (SNP) discovery. Given the utilization of genetic markers for high breeding values of economic traits, microfluidic systems can be used to predict potential genes associated with economic traits by using less reagents and reducing the time for genetic selection.

Microfluidics also can be used for early disease detection in animals. Dong et al. (2011) fabricated a glass microfluidic device with photolithographic wet etching technology to screen swing foot-and-mouth disease virus. The microfluidic device was composed of a channel and inner-microchannel dam slit structure to contain polystyrene microbeads that captured virus antibodies. The device exhibited nominal positiveness percentage values, analytical sensitivity, and assay reproducibility. Bhatta et al. (2012) demonstrated a microfluidic based optical sensing device that could detect the foot-and-mouth disease virus (FMDV). The microfluidic sensing device measured changes in the refractive index within specific sensing microchannels that were functionalized with anti-FMDV antibodies. Santis et al. (2011) used microfluidics technology for high-throughput accurate *Brucella* genotyping. The microfluidic platform improved the detection of amplified DNA fragments size in terms of handling and rapidity. Zhang et al. (2013) developed a microfluidic device to integrate immunomagnetic target capture, concentration, and fluorescence detection of the avian influenza virus (AIV). An optical fiber spectrometer was used to measure the fluorescence signal from the device. They obtained a low LOD up to 3.7×10^4 copy/μL with a sample consumption of 2 μL and a total assay time of less than 55 min.

Conclusions

This review aimed to provide information on the applications of microfluidics in the agro-food sector. Microfluidics uses micro-sized channels to manipulate tiny amounts of fluids. Microfluidic devices offer several advantages when compared to conventional methods. These advantages include low cost, short reaction and analysis time, high sensitivity, small footprints, and high-throughput, reduced use of reagents and samples (Arévalo et al., 2011). Microfluidics is potentially useful in performing and analyzing complex operations including diagnostics, detection of harmful materials, and biological screening. The integration of microfluidics and biosensors provides the significant ability to solve the shortcomings of conventional methods (Luka et al., 2015). Trends in microfluidic-based immunosensors include the simultaneous and parallel detection of multiple targets, and the improvement of sensitivity by using novel signal labels (Li et al., 2012).

Microfluidic systems could be easily blocked or the detection accuracy could be deteriorated since the matrices of agricultural products and foods are complex. However, most microfluidics devices for the detection of harmful agents in agricultural products and foods adapt off-device sample pretreatment. Future research efforts should focus on developing integrated sample pretreatment techniques (Guo et al., 2015a). The final goal of the microfluidics includes the implementation of a small portable platform with modules for fluid handling, sample preparation, and the electronics for measuring and treating the detector signals.

Conflict of Interest

No potential conflict of interest relevant to this article was reported.

Acknowledgement

This work was carried out with the support of "Cooperative Research Program for Agriculture Science & Technology Development (Project title: On site detection of Staphylococcal enterotoxin with a rapid kit based on bioprobes, Project No: PJ00998702)" Rural Development Administration, Republic of Korea.

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