Review

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Review on Biosensors for Food Safety

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Abstract

Background: Frequent outbreaks of foodborne illness have been increased awareness of food safety. CDC estimates that each year roughly 48 million people gets sick, 128,000 are hospitalized and 3,000 die of foodborne diseases in US. In Korea, 6,058 were hospitalized and 266 incidents were reported in 2012. It is required to develop rapid methods to identify hazard substances in food products for protecting and maintaining safety of the public health. However, conventional methods for pathogens detection and identification involve prolonged multiple enrichment steps. **Purpose:** This review aims to provide information on biosensors to detect pathogens in food products to enhance food safety. **Results:** Foodborne outbreaks continue to occur and outbreaks from various food sources have increased the need for simple, rapid, and sensitive methods to detect foodborne pathogens. Conventional methods for foodborne pathogens detection require tremendous amount of labor and time. Biosensors have drawn attentions in recent years because of their ability to detect analytes sensitively and rapidly. Principles along with their advantages and disadvantages of a variety of food safety biosensors including fiber optic biosensor, impedimetric biosensor, surface Plasmon resonance biosensor, and nano biosensor were explained. Also, future trends for the food safety biosensors were discussed.

Keywords: Biosensor, Fiber-optic, Food safety, Impedimetric, Nano-biosensor

Introduction

Foodborne illnesses are a major health problem around the world. Frequent outbreaks of *Salmonella* with chicken, pork, tomatoes, and *Staphylococcus aureus* with rice rolled in laver, sandwiches, and *Vibrio* with clam and other raw fish have been increasing the awareness of agro-food safety. It is estimated that each year foodborne illness causes approximately 48 million illnesses, 127,000 hospitalizations, 3,000 deaths in the USA alone (CDC, 2011; Gould et al., 2013). A majority of the illnesses are caused by viruses (58%) and bacteria (33%) (CDC, 2011). Increasing public awareness for food safety makes rapid and accurate evaluation of meat, poultry, fish, vegetables and their products more important. To reduce foodborne outbreaks and economic losses rapid and sensitive detection methods are necessary.

Conventional pathogenic microorganism detection relies on culture-based methods those are considered "gold-standard" but labor and time consuming. Sometimes, it takes as many as 3-10 days for detection results. The increasing use of rapid food safety testing is receiving more and more attention. The major reason for this trend is that the food industry requires quick and accurate results. The rapid detection of contaminants in food is critical for ensuring the safety of consumers. Recent advances in technology make detection and identification faster, more sensitive and more specific than traditional method. Rapid assays are possible using immunoreactions such as Enzyme-linked immunoassay (ELISA) and polymerase chain reaction (PCR). However, they still require enrichment, several lengthy steps, expensive laboratory instruments, and experienced operators (Hart et al., 2011; Hossain et al., 2012). Among the newer technologies biosensors have shown great potential for rapid detection of foodborne

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pathogens.

Biosensors have ability to detect analytes sensitively and rapidly. A biosensor is an analytical device incorporating a biological material with a physiochemical transducer to generate a measurable signal from the analytes (Lazcka et al, 2007; Su et al, 2011). The biological that responds to the target compound can be antibodies, nucleic acids, enzymes, aptamers, and imprinted polymers. The transducer can be optical, electrochemical, piezoelectric, and magnetic.

In this review, technology trends and recent developments in biosensors for agro-food safety are discussed. Principles along with their advantages and disadvantages of a variety of food safety biosensors including fiber optic biosensor, impedimetric biosensor, surface Plasmon resonance biosensor, and nano biosensor were explained. Also, future trends for the food safety biosensors were discussed.

Fiber-optic biosensor

Fiber-optic biosensors provide specific, reproducible, and reliable detection of various biomolecules. Fiber-optic biosensors use light transmittable tapered fibers to send excitation laser light and receive emitted fluorescence, usually from a labeled antibody. The fluorescent light excited by an evanescent wave generated by the laser is quantitatively related to the number of labeled biomolecules in close proximity to the fiber surface (Marazuela and Moreno-Bondi, 2002). Figure 1 shows the schematic description of the fiber-optic biosensor.

These biosensors have been used to rapidly detect various microorganisms including: *Vaccinia* virus (Donaldson et al., 2004), *Escherichia* coli O157:H7 (DeMarco et al., 1999), Bacillus globigii (Anderson et al., 1999), *Salmonella* enteritidis (Bhunia et al., 2003), and *L.* monocytogenes (Tims et al., 2001; Geng et al., 2004).

Improvements in the portability and automation of a fiber-optic biosensor (RAPTOR™, Research International, Monroe, WA, USA) increased the usefulness of this detection device. The RAPTOR can perform four assays on the same sample allowing replicate measurements of the same analyte or simultaneous detection of four different targets. The RAPTOR uses four 635 nm diodes to excite each of four, 4.5 cm long fiber-optic probes. The fibers are assembled in a coupon which has fluidic channels for automated operation. Fluorescent molecules bound on the surface of the sensing region are excited by an evenescent wave generated by the laser. Photodiodes collect emission light at wavelengths over 670 nm. The emission signal is recorded in pico amperes (pA) and related to concentration of analyte. The automated RAPTOR system has been used to detect *Salmonella* typhimurium (Kramer and Lim, 2004), *Giardia* lamblia (Anderson and Rowe-Taitt, 2000), and *L.* monocytogenes (Kim et al., 2007).

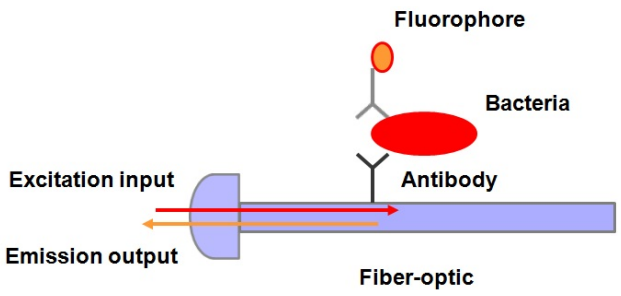

Figure 1. Schematic description of the fiber-optic biosensor.

Impedimetric biosensor

One common rapid detection method is based on impedance characteristics of electrodes in a medium where bacteria reside. Impedance measurement methods have analyzed both the resistive and capacitive properties of the medium or electrodes. Most research conducted with conventional impedimetric methods has focused on the changes in electrical impedance of a medium resulting from the bacterial growth (Easter and Gibson, 1985; Gibson, 1987). Impedance changes of the medium result from the release of ionic metabolites from live bacteria. Some researchers measured impedance changes of both the medium and electrodes (Yang et al, 2003; Yang et al., 2004). Impedimetric biosensors have been widely adapted as an analysis tool for the study of various biological binding reactions because of their high sensitivity and reagentless operation. The impedimetric biosensor, which was devised to increase the selectivity the sensitivity by incorporating a biologically functionalized detection layer on the surface of the electrode, usually measures electrode or interface impedance. The impedimetric biosensor enables qualitative and quantitative monitoring of bacteria by measuring the changes in the electrical impedance due to the presence of bound molecules.

Usually impedimetric biosenors are constructed as an interdigitated micro-electrode (IME) shape by using semi conductor fabrication process (Figure 2). The IME has hundreds of interdigitated electrode fingers which have very small electrode width and gap sizes to improve sensitivity.

To measure responses from analytes with an impedimetric biosensor, a cyclic function of small amplitude and variable frequency is applied to the biosensor. At each of the frequencies applied, the resulting current is used to calculate the impedance by a transducer. Since, the impedance has a real and an imaginary component, analytical analysis of the impedance response is complicated. Often researchers build an equivalent circuit of the biosensor to interpret the impedance data by electrical modeling (Yang et al., 2004; Lindholm-Sethson et al., 2010). Based on the modeling results, typically a most significant frequency value is selected and used to extract information related to the analyte presence from the impedance responses. In fact, this ease to use approach is widely accepted, but using one frequency value to analyze the response would lead to loss of valuable information because the signal from the analyte may involve a range of frequencies. Multivariate analysis of impedance data has the possibility to detect unknown weak interactions in the low frequency regime and these specific interactions might contribute to lower detection limits in analytical applications with multivariate regression (Lindholm-Sethson et al., 2010; Kim et al. 2013).

A variety of impedimetric biosensors have been constructed to monitor various biological reactions at the surface of electrodes by immobilizing biomolecules such as enzymes, antibodies, nucleic acids, cells, and microorganisms (Guan et al, 2004). Recently, an aptamer based impedance biosensor was used to detect lipopolysaccharide (LPS) often referred to as endotoxin (Su et al., 2012). In fact, some impedimetric biosensors have been used to detect various microorganisms including *E*. coli O157:H7 (Radke and Alocilja, 2005; Varsheny and Li, 2007) and *Salmonella* typhimurium (Kim et al., 2003; Kim et al., 2013).

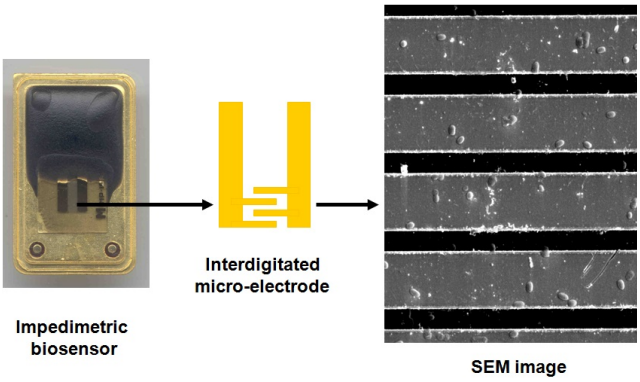

Figure 2. Schematic description and magnified image of the impedimetric biosensor.

Surface plasmon resonance biosensor

Surface plasmon resonance (SPR) biosensors are capable of direct monitoring the antigen-antibody reactions in real time without requiring additional labeling reagents. The SPR biosensors have been widely adapted as an analysis tool for the study of various biological binding reactions because of their high sensitivity and reagentless operation.

SPR sensors utilizes a thin, gold-coated transparent materials exposed to a laser or a polarized beam of light that produces surface plasmon resonance phenomenon. The SPR phenomenon is highly sensitive to changes in the thickness of attached analytes on the surface of the thin metal. A SPR biosensor that has a layer of biological active binding site on the surface of the metal could detect antibody-antigen bindings on the sensor surface by measuring either a resonance angle or a refractive index value (Hoa et al., 2007).

SPR biosensors have been used to detect various biological agents including *Salmonella* group B, D, and E (Bokken et al., 2003), *Listeria* monocytogenes (Koubova et al., 2001), *Salmonella* enteritidis (Koubova et al., 2001; Betty et al., 2002), *Salmonella* typhimurium (Betty et al., 2002; Medina, 2004; Oh et al., 2004), *Escherichia* coli O157:H7 (Fratamico et al., 1998), and *Salmonella* paratyphi (Oh et al., 2004).

Although many SPR applications were studied on biological binding reactions, expansion of SPR application has been hindered by high cost SPR systems designed for central laboratories. Arrival of low-cost portable SPR sensor (Spreeta, TI, USA) enabled field use and increased accessibility of the SPR system. The Spreeta sensor contains all the optical components necessary to measure refractive index changes related to SPR phenomena in the compact enclosure. Figure 3 shows the schematic description of the portable SPR biosensor.

The feasibility of applying the Spreeta sensor to detect

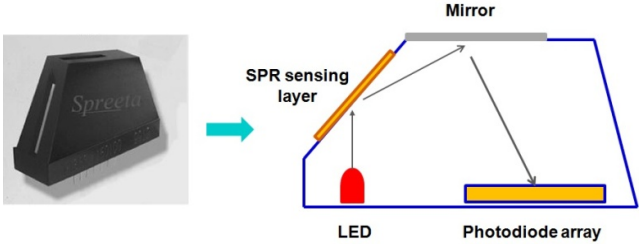

Figure 3. Schematic description of the portable SPR biosensor (Spreeta).

various biological analytes including mouse IgG (Chinowsky et al., 2003), *Staphylococcus* aureus enterotoxin B (Naimushin et al., 2002), peanut allergens (Lai and Yeung, 2001), and *Escherichia* coli enterotoxin (Spangler et al., 2001) has been demonstrated.

Nano-biosensor

Recently, nanotechnologies have been drawing attention and many groups have reported the application of nanotechnologies for food safety area. Especially, fluorescent nanoparticles or quantum dots were successfully applied to improve the performance of existing biosensors. Organic fluorescent dye-based optical immunoassays have been widely used for the rapid detection of foodborne pathogens because of their sensitivity and ease of operation (Geng et al., 2004; Kim et al., 2007). However, methods that rely on organic fluorescent dyes are limited by their sensitivity to photobleaching, limiting long-term analysis. They also have a narrow excitation bandwidth and overlapping emission profiles from different fluorophores in multiplexed applications (Kampani et al., 2007).

Nanotechnology has the potential to solve the limitations of organic fluorophores. Fluorescent nanoparticles or quantum dots (QDs) have several advantages over conventional organic dyes including high quantum yield and brightness, photostability, and resistance to chemical degradation. Semiconductor QDs exhibit size- and composition-dependent fluorescence properties that are suitable for multi-target and highly sensitive imaging with a single excitation wavelength (Tallury et al., 2010). The water-solubility of QDs makes them suitable for biological applications including imaging, detection, and biomolecular conjugation. Figure 4 shows the schematic description of the QD based nano-biosensor.

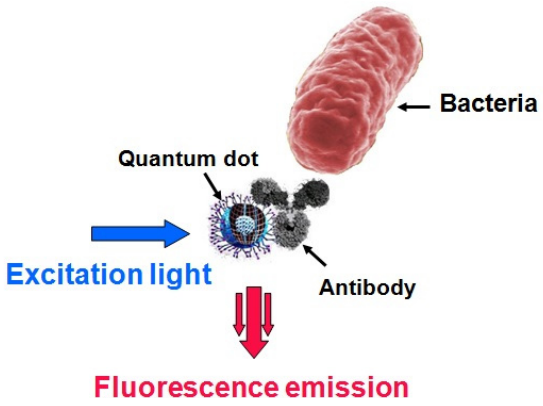

Figure 4. Schematic description of the QD based nano-biosensor.

Several groups have reported the application of QDs for pathogen detection including *Escherichia coli* O157:H7 (Su and Li, 2004; Wang et al., 2012; Zhu et al., 2012), *Salmonella* (Kuang et al., 2013), *Campylobacter jejuni* (Bruno et al, 2009), *Listeria monocytogenes* (Wang et al., 2007), and avian influenza viruses (Chou et al., 2012). The application of QDs has also been extended to multiplexed detection based on immunoassay formats. For example, Zhu et al. (2004) used different QDs for dual-color imaging *Cryptosporidium parvum* and *Giardia lamblia*. Zhao et al. (2009) improved on this concept and simultaneously detected 3 foodborne pathogenic bacteria, *S. typhimurium*, *Shigella flexneri*, and *E. coli* O157:H7. Use of QDs also improved the sensitivity of microorganism detection. Chalmers et al. (2007) achieved single-cell resolution of human oral bacteria in biofilms of *Streptococcus gordonii*. Hahn et al. (2005) claimed single-cell detection of *E. coli* O157:H7 using antibody-conjugated QDs.

Conclusions

This review aims to provide to provide information on biosensors to detect pathogens in food products to enhance food safety. Even though biosensors were feasible to detect various pathogens faster than conventional methods, they need skilled persons to prepare and use. In this context, lateral flow strip sensors showed great potential for the detection of food borne pathogens and have drawn much attention recently. The lateral flow strip sensors have the many advantages, such as the low-cost, rapid and sensitive detection, user-friendly operation, easy storage, and on-site detection. The strip sensor is based on the specific interaction between antigen and antibody and the colored particles such as gold nanoparticle, carbon, silica, polymer, liposome, quantum dot, magnetic bead, and etc (Posthuma-Trumpie et al., 2009). The strip sensor is constructed with four simple components; a sample application pad, a conjugate release pad, a nitrocellulose membrane, and an absorption pad. The detection is based on the retention and formation of visual spots of color-labeled antibodies in sensing zones on a membrane during sample flow through. The sample is driven up the strip sensor by capillary forces. Bacteria cells in the sample are combined to the color-labeled antibodies in the conjugate release pad, and continuously flow to the nitrocellulose membrane. And then, the cell-colored antibody

conjugates are reacted with immobilized antibody in the sensing spot to generate signals. Remaining sample is flow though up to the absorption pad. The strip sensors were used for the detection of *Salmonella* typhimurium (Lee et al., 2013), *Vibrio cholera* (Chua et al., 2011), *B. anthracis* spores (Wang et al., 2013).

To increase the sensitivity of detection, many researchers have used an immunomagnetic separation (IMS) method in combination with biosensing methods (Yang and Li, 2006; Dudak and Boyaci, 2008; Zhao et al., 2009; Liandris et al., 2011). In this technique, antibody-conjugated super-paramagnetic beads capture bacteria and are collected to the side of the sample container with an external magnet. The supernatant is removed to concentrate the target bacteria.

Most biosensor studies related to food safety detection have relied on laboratory instruments that are not suitable for field use or modification for optimization. In fact, the requirement of bulky and expensive laboratory instruments hinder commercialization of biosensors. To achieve successful commercialization of food safety biosensors, many efforts are needed to develop a portable biosensing system that is capable of point-of-care diagnosis at field with simple operation, low-cost, comparable sensitivity, and fast analysis time.

Conflict of Interest

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

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