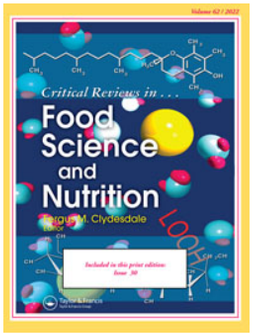

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REVIEW

Toward intelligent food packaging of biosensor and film substrate for monitoring foodborne microorganisms: A review of recent advancements

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ABSTRACT

Microorganisms in food do harms to human. They can cause serious adverse reactions and sometimes even death. So it is an urgent matter to find an effective method to control them. The research of intelligent- biosensor packaging is in the ascendant in recent years, which is mainly promoted by reflecting on food safety and reducing resource waste. Intelligent biosensor-packaging is an instant and efficient intelligent packaging technology, which can directly and scientifically manifest the quality of food without complex operation. In this review, the purposes of providing relevant information on intelligent biosensor-packaging are reviewed, such as types of biosensors for monitoring foodborne microorganism, the suitable material for intelligent biosensor-packaging and design and fabrication of intelligent biosensor-packaging. The potential of intelligent biosensor-packaging in the detection of foodborne microorganisms is emphasized. The challenges and directions of the intelligent biosensor-packaging in the detection of foodborne pathogens are discussed. With the development of science and technology in the future, the intelligent biosensor-packaging should be commercialized in a real sense. And it is expected that commercial products can be manufactured in the future, which will provide a far-reaching approach in food safety and food prevention.

HIGHLIGHTS

- Several biosensors are suitable for the detection of food microorganisms.
- Plastic polymer is an excellent choice for the construction of intelligent biosensor packaging.
- Design and fabrication can lay the foundation for intelligent-biosensor packaging.
- Intelligent biosensor-packaging can realize fast and real-time detection of microorganisms in food.

KEYWORDS

Biosensor; foodborne microorganisms; food safety; intelligent package

Introduction

"Bread is the staff of life"—and food safety is a global concerning issue. The safety of food plays an important role in ensuring the human health. As billions of people around the world need to be fed, it is still one of the major challenges to produce a sufficient number of safe food that are free from bacteria, viruses and protozoan pathogens, as well as harmful residues, pesticides and allergens in the present (X. Sun et al. 2022). Although many regulations and laws have been promulgated, food is still vulnerable to contaminate with the globalization of food production and circulation. Common contaminants include pathogens, pesticides, heavy metals and mycotoxins in food. Food spoiled by harmful microorganisms is one of the most common phenomenon, and it is stated by the World Health Organization that contamanitated food with harmful microorganisms can cause approximately 200 diseases (Soon, Brazier, and Wallace 2020). So it is vital to detect whether harmful bacteria exist in food. Generally, microorganisms are detected by traditional bacteria culture and colony counting based approach. Although the result can be correct and precise, it is a tedious and time-consuming work. With the development of technology, there are novel approaches for detecting microorganisms, such as nucleic acid-based polymerase chain reaction technology (PCR), enzyme -linked immunosorbent assay (ELISA) and so on, which require advanced equipments and highly skilled personnel. They seriously hinder practical application for in-situ detection of food spoilage in the circulation chain. The emergence of biosensors can solve this predicament. Biosensors have priority over those methods in the aspects of simplicity and quickness (Soni, Ahmad, and Dubey 2018). In addition to these advantages, it characters low cost, excellent selectivity, high sensitivity (Ali et al. 2020). But those sensors do not detect bacteria in real-time monitoring. Therefore, it is urgent to find a low-cost, real-time and more sensitive analysis method.

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Recently, the burgeoning intelligent biosensor-packaging provides an excellent platform for food safety. And it can not only monitor the situation of food in real time, but also easily reflect food quality with observation. Yousefi et al. constructed an intelligent biosensor package based on DNAzyme to detect pork contaminated with target bacteria, which can clearly and simply monitor *E. coli* in pork matrix in real time (Yousefi et al. 2018). However, there are some troubles with widespread application, such as choosing appropriate substrate and immobilizing bimolecular.

In this review, we summarize the biosensors for the detection of microorganisms in food, analyze the substrates suitable for the intelligent biosensor- packaging and discuss design and preparation of that package (Figure 1). This review has performed with the purpose of providing relevant information on intelligent biosensor-packaging, which will boost the further development of intelligent biosensor-packaging and provide a reference for intelligent packaging.

Biosensors for monitoring foodborne microorganisms

Biosensor is a system based on the combination of recognition molecule and target, which can observe its change by some means. Its fundamental principles can be described as biological recognition and converting signal. It has the characteristics of rapidity, simplicity and good sensitivity (Lowe 1989). So it is widely used as a way of rapid detection, especially in the detection of food pollutants (Umapathi et al. 2021). And one of the most common detection of them is foodborne microorganisms (2022; Eyvazi et al. 2021; Fu et al. 2021). The process of monitoring foodborn microorganisms in biosensors can be seen as Figure 2. The food samples are pretreated and added to various types of biosensors. Different recognition molecules are used to identify the target bacteria, and then analyze with the help of different detection equipments.

Optical biosensor

Optical biosensor can be described as a detected device that performs by exploiting the reaction between light field and biorecognition components. The identified elements of optical biosensors mainly contain biological material, such as enzymes, antibodies, antigens, receptors, nucleic acids, whole cells and tissues.

Optical biosensors mainly include fluorescent sensors, chemiluminescence sensors and surface Plasmon resonance sensors. Fluorescence sensing can be more specific according to different molecules or substances. For example, there are biosensors based on aptamer and fluorescent dye (G. X. Zhang, Liu, et al. 2020), nanometer material (Dou et al. 2021) and so on. The way aptamer-based sensors detect pathogen is that the fluorophore and aptamer bind in a labeled or non-labeled manner. When the target microorganism appears in the sample, the aptamer binds to it and then the fluorophore changes, which can reflect the concentration of organism (Hong et al. 2021). Duan et al. developed an aptamer sensor for detection of *Vibrio parahaemolyticus* and *Salmonella typhimurium* in shrimp and chicken (Duan et al. 2014). It was a fluorescent aptamer sensor based on AccuBlue dye. The design principle was based on the fact that the fluorescence intensity of the dye increases after it is inserted into dsDNA. The result was close to the gold standard, indicating that this method can be applied to the analysis of pathogens in real food samples.

As to nanomaterial-based sensors, gold nanoparticles are widely applied in detecting microorganisms. Gold nanoparticles and antibodies can be biologically coupled by physical adsorption, covalent bonding or some linker molecules. Once the analyst is captured by the antibody on the surface of the gold sensor, the antibody conjugate can be used to enhance the signal, thus improving the detection limit. In order to rapidly detect *Salmonella typhimurium*, Wu et al. prepared an enzyme-linked antibody-aptamer sandwich method based on gold nanoparticles. The detection limit was 1×10^3 CFU/mL. Compared with other enzyme-linked methods, this method improved the detection range and

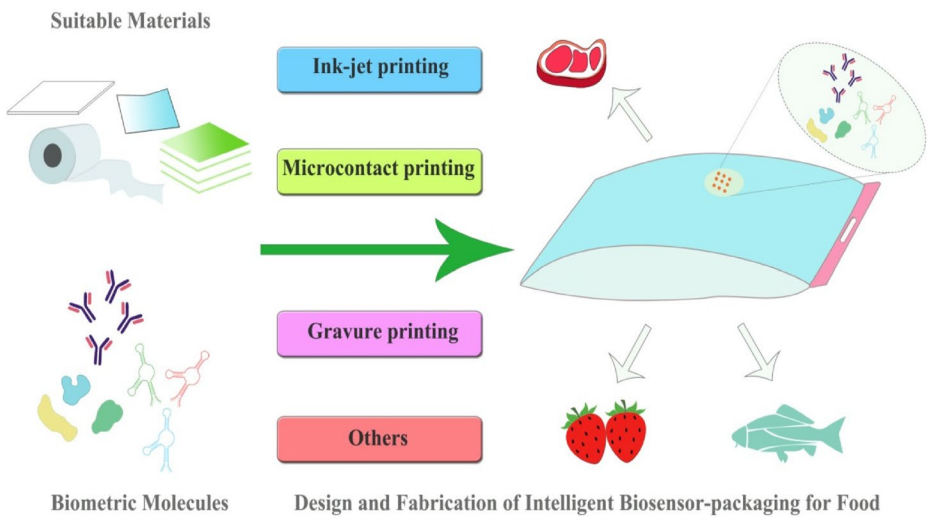

Figure 1. The schematic of intelligent biosensor-packaging.

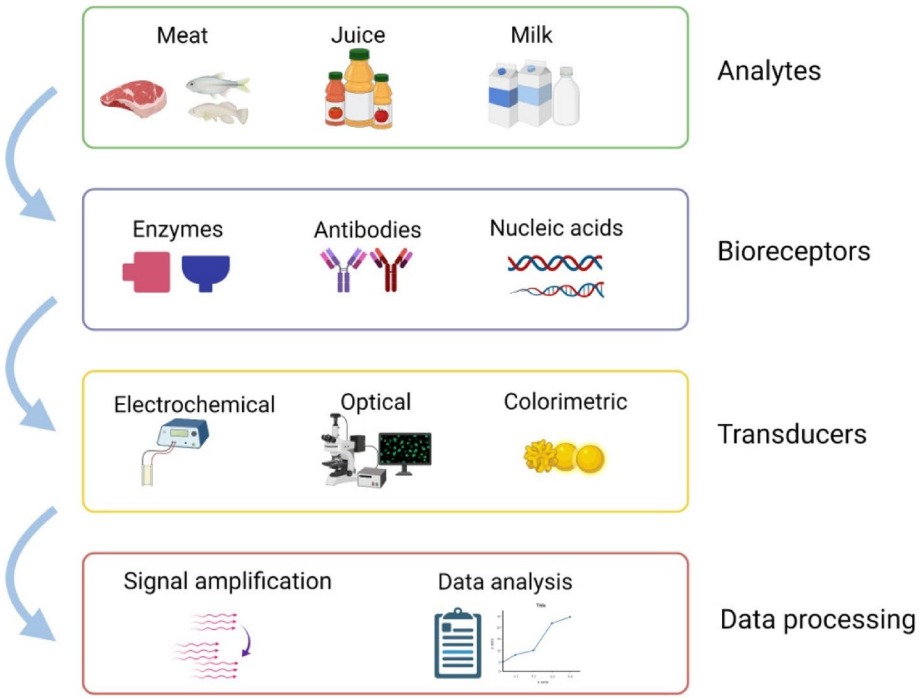

Figure 2. The process of biosensing.

sensitivity. The method performed well in milk samples (W. Wu et al. 2014).

Chemiluminescence refers to the phenomenon of light radiation produced by chemical substances in chemical reactions. The foodborne microorganisms in the target sample are detected by the change of chemical reaction between substances. Hao et al. designed a chemiluminescent aptamer sensor with aptamer as recognition molecule and functionalized gold nanoparticles as signal probe to detect *Salmonella typhimurium* (Hao et al. 2017). Under the best conditions, the detection limit for the target bacteria was 10 CFU/mL.

Surface Plasmon resonance (SPR) is an analytical technique to detect the change of refractive index of metal surface materials (Nguyen et al. 2015). The detection process of surface plasmon resonance biosensor refers to fixing the identification element on the surface of metal chip, and then monitoring the binding or dissociation of target and ligand in the liquid solution flowing on its surface to change the refractive index of its surface, so as to achieve the detection of target. A SPR-based sensor was established to detect *E. coli* and *Salmonella* in cucumber and hamburger samples. The detection limits of *Salmonella* and *E. coli* in cucumber extract were 17 CFU/mL and 57 CFU/mL, respectively. The detection limits of the two bacterias in Hamburg were 11.7 CFU/mL and 7.4×10^3 CFU/mL, respectively (Vaisocherová-Lísalová et al. 2016).

Colorimetric sensor

The design of colorimetric sensor is based on the reaction of target substances and their metabolites with specific components, the characteristics of some chemical substances and material such as gold nanoparticles (Bustamante et al. 2019; G. Liu et al. 2018).

Here, several widely studied colorimetric sensors will be introduced in details. First of all, gold nanoparticle-based colorimetric has been studied in many researches. The reason why it attracts many scholars is that gold nanoparticles can be easily functionalized and displayed different colors when they are diverse size, shape and aggregation status, which match with the colorimetric sensor exploiting color to detect the target (Aldewachi et al. 2017). In one research, they used the different charging conditions of bacteria under different pH to interact with positively charged gold nanoparticles, which showed different aggregation states and optical properties and detected different foodborne microorganisms through color changes. This method was used for colorimetric detection of a variety of foodborne bacteria. At low pH, the detection limits of *E. coli*, *Salmonella*, *Staphylococcus aureus* and *Vibrio parahaemolyticus* were 4.4×10^7 CFU/mL, 6.6×10^6 CFU/mL, 1.6×10^7 CFU/mL and 5.8×10^6 CFU/mL, respectively (Du et al. 2021). Besides, this biosensor also worked by chelation, chemical bonding, base pairing, electrostatic interaction, and hydrogen bonding between ligand and target (Xiaoyi et al. 2018).

Another colorimetric biosensor is based on the enzymes. The sensor focuses on the catalysis of enzymes and detects the presence of target bacteria through the discoloration reaction of some substrates catalyzed by enzymes. Catalase exists in almost all organisms and catalyzes the colorless peroxides' substrate to produce colored TMB in the presence of hydrogen peroxide (X. Wang et al. 2014). So this sensor can be detected by the above-mentioned reaction if there are harmful bacteria in food. Yu et al. constructed a biosensor for specific detection of *Staphylococcus aureus* (Yu et al. 2020). They used dsDNA-SGI complex to catalyze the oxidation of tetramethylbenzidine under light irradiation in the

presence of SYBR Green I. As a result, a sensitive photocatalytic colorimetric response to SA was produced. The test they established can detect 100 CFU/mL of *Staphylococcus aureus* in milk samples. Besides, this method can complete the detection within 5.5 hours, which was faster than traditional methods.

However, the developed biosensor used a complex optical system. Based on this, a biosensor for detecting Salmonella without complicated optical system had been developed. Kim et al. designed a biosensor that can detect *Salmonella* without a complex optical system. Stem-oriented DNA bound to target DNA and was stretched to expose biotin. After binding to biotin, streptavidin bound to a reflex Janus particle. The system can be used to analyze *Salmonella* in the range of 0-100 nM with high sensitivity and selectivity, and the detection limit was 2.48 pm (Kim et al. 2019).

Electrochemical biosensor

After capturing the target to be measured, the biometric element in the electrochemical biosensor will cause the change of current, potential or conductivity, so as to realize the qualitative or quantitative analysis of the analyzed objection (Stasyuk et al. 2019). There are several widely studied electrochemical sensors (Silva et al. 2020). For instance, aptamer-based electrochemical biosensors are one of them. They prepared an electrochemical biosensors composed by gold nanoparticles and aptamers for detecting Salmonella with the detection limit of 3 CFU/mL, indicating the sensor had the advantages of good detection accuracy and specificity (Xiaoyuan Ma et al., 2014).

DNA-based electrochemical biosensors have also been studied. Bai et al. constructed a cocoon-like electrochemical biosensor with DNA nanostructure as a signal tag (Bai et al. 2020). When *E. coli* existed in surroundings, the aptamer bound to it and separated from the gold surface. The cocoon-like DNA bound to the capture probe. Therefore, the concentration of *E. coli* can be detected according to the change of electrochemical signal. The detection range of the electrochemical biosensor is 10-10^6 CFU/mL. The detection limit was estimated to be 10 CFU/mL.

Although these biosensors have good sensitivity, researchers have been committed to developing more sensitive biosensors and trying to transform them into practical applications. An electrochemical immunosensor based on graphene nanomaterials and gold nanoparticles was constructed. It is suitable for detecting veterinary antibiotics in animal-derived food (A. Wang, Cui, et al. 2022). The more applications can be seen in the Table 1.

Constructing intelligent biosensor-packaging

Selecting substrate and technologies are key steps in device manufacturing because they are the basis for the construction of biosensors. Then the material properties of the substrate determine what biometric molecules are to be supported (Khan, Lorenzelli, and Dahiya 2015). More importantly, the key to construction of intelligent biosensor-packaging is to effectively deposit biomolecules on the substrate. So it is necessary to present some details about them.

The suitable materials for intelligent biosensor-packaging

The substrate materials suitable for the preparation of intelligent biosensor-packaging should not only support the requirements of biomolecule deposition, but also meet the functions of ordinary packaging, such as oxygen insulation, low air permeability and so on. Common substrates used for intelligent biosensor-packaging include synthetic plastic polymers, natural polymers and other materials (Figure 3)

Table 1. The application of biosensors in food detection.

Biosensor	Identification element	Target bacteria	Detection range	Detection limit	References
Chemiluminescence biosensor	aptamer	*Salmonella typhimurium*	3.2-3.2 × 10^6 CFU/mL	10 CFU/mL	(Hao et al. 2017)
	Aptamer	*E. coli* O157:H7		130 CFU/mL	(D. Sun et al. 2022)
Plasma resonance sensor	Antibody	*Salmonella typhimurium*	10^2-10^6 CFU/mL	10^2 CFU/mL	(Farka et al. 2016)
	Peptides	*E. coli* O157:H7	1.0 × 10^3-5.0 × 10^7 CFU/mL	5.0 × 10^2 CFU/mL	(Zhou et al. 2018)
	Antibody	*Salmonella typhimurium*	0.9-5.9 log CFU/g	0.9 CFU/g	(Bhandari, Chen, and Bridgman 2019)
	Aptamer	*Vibrio parahemolyticus*	10^2-10^7 CFU/mL	10 CFU/mL	(Y. Sun et al. 2019)
	Antibody	*Salmonella typhimurium*	10-10^5 CFU/mL	35 CFU/mL	(Guo et al. 2020)
	Protein	Positive and negative bacteria		$10^{1.5}$ CFU/mL	(Naseri et al. 2022)
Colorimetric biosensor	DNA	*Staphylococcus aureus*		0.17nM	(Xing et al. 2022)
		E. coli O157:H7		10^3 CFU/mL	(Dester, Kao, and Alocilja 2022)
	Aptamer	*Staphylococcus aureus*	7.6 × 10^1-10^7 CFU/mL	1.09 CFU/mL	(Qi et al. 2022)
	Aptamer	*E. coli* O157:H7	0.02-50.0 nM	0.01 nM	(Li, Deng, et al. 2015)
	Antibody	*Salmonella*	1.0 × 10^1 -5.0 × 10^4 CFU/mL	5 CFU/mL	(Lu, Pang, and Xie 2017)
	Peptide	*E. coli* O157:H7		5 nM	(Ropero-Vega et al. 2022)
Electrochemical biosensor	DNA	*E. coli* O157:H7	2.67 × 10^2 -2.67 × 10^6 CFU/mL	2.67 × 10^2 CFU/mL	(Mao et al. 2006)
		E. coli O157:H7	1.0 × 10^{-19}-1.0 × 10^{-6}mol/L	3.33 × 10^{-20} mol/L	(Shi et al. 2022)
		Salmonella	1.8-1.8 × 10^5 CFU/mL	1 CFU/ml	(Bacchu et al. 2022)
	Bacteriophage	*E. coli* O157:H7	1-10^4 CFU/mL		(El-Moghazy et al. 2022)
Piezoelectric biosensor	Antibody	*E. coli* O157:H7	10^3-10^8 CFU/mL	10^3 CFU/mL	(Su and Li 2004)
	Aptamer	*Staphylococcus aureus*	4.1 × 10^1-4.1 × 10^5 CFU/mL	41 CFU/mL	(Lian et al. 2015)

(D. Wang, Ba, et al. 2018; Suaifan, Alhogail, and Zourob 2017). Below, we will briefly discuss the characteristics and applications of these substrate materials respectively.

Substrate material from synthesis

Polymer materials can be used as the supporting platform of biosensors depending on their unique advantages, such as light weight, flexibility, portability, biocompatibility and low price (Lanzalaco and Molina 2020). At the same time, they are often used in food packaging because of their excellent mechanical properties. The polymers commonly used in this biosensor include poly (ethylene terephthalate) (PET), polylactic acid (PLA), polydimethylsiloxane (PDMS) and Cyclic olefin polymers (COP).

Polyethylene terephthalate (PET) is not only an amorphous thermoplastic, but also a semi-crystalline thermoplastic, which is common in the field of food packaging. At the same time, this polymer has high hardness, stiffness, and strength, which are sufficient to support the construction of biosensors. Besides, it also has high dimensional stability and it is used in the temperature range from −40 °C to 100 °C (Li-Na 2013). The mechanical properties of polyester film make it the preferred substrate for various processing technologies. In addition, the processing tolerance of PET film makes it a good substrate for all kinds of applications. In the biosensor, the material is suitable to be used as the substrate of electrochemical biosensor and optical sensor. From the point of view of unlabeled and non-high temperature processing, Salinas et al. developed a zinc oxide thin-film biosensor with recyclable plastic PET as a substrate and antibody as recognition molecule (Salinas et al. 2022). They showed that the recyclable plastic substrate PET was compatible with flexible electronics, which paved the way for applications in low-cost biosensors. In addition, they used a polymerization chain reaction approach to verify the reliability of the biosensor. Ren et al. constructed a PET-based biosensor for sensitive detection of *E. coli* in a large number of food substrates (Ren, Cabush, and Irudayaraj 2020). In paper, they first treated the PET pad with sodium hydroxide and hydrogen peroxide in turn, and then coupled the antibody to the PET pad. After that, the treated PET mat was rolled up and put into a test tube. Finally, the bacteria-containing solution of the treated lettuce sample was added into the test tube with an injection needle. The PET pad was taken out and placed in a solution containing gold nanoprobes, a certain concentration of horseradish peroxidase solution and a solution of Tetramethyl benzidine in turn. Finally, the concentration of *E. coli O157:H7* in the sample was determined by absorbance at 650 nm. The lowest concentration of *E. coli O157:H7* can be lower than that of 100 CFU/mL.

Polylactic acid (PLA) is a widely used biodegradable polymer, which is formed by the polymerization of lactic acid or the polymerization of cyclic dimer of lactic acid (Murariu and Dubois 2016). Like PET, PLA is a common food packaging material. And it can also be oriented by processing, and chain orientation can increase the mechanical strength of the polymer. When the crystallinity, polymer structure and molecular weight change, the mechanical properties of PLA vary greatly. It can not only be prepared into soft elastic materials, but also can be processed into hard high-strength materials, which can meet a variety of applications (Avérous 2008). In several biosensors, PLA can be used to prepare the substrate of colorimetric sensors. A PLA nanofiber film embedded with biotin was prepared by electrospinning technology. Li et al. made use of the specific

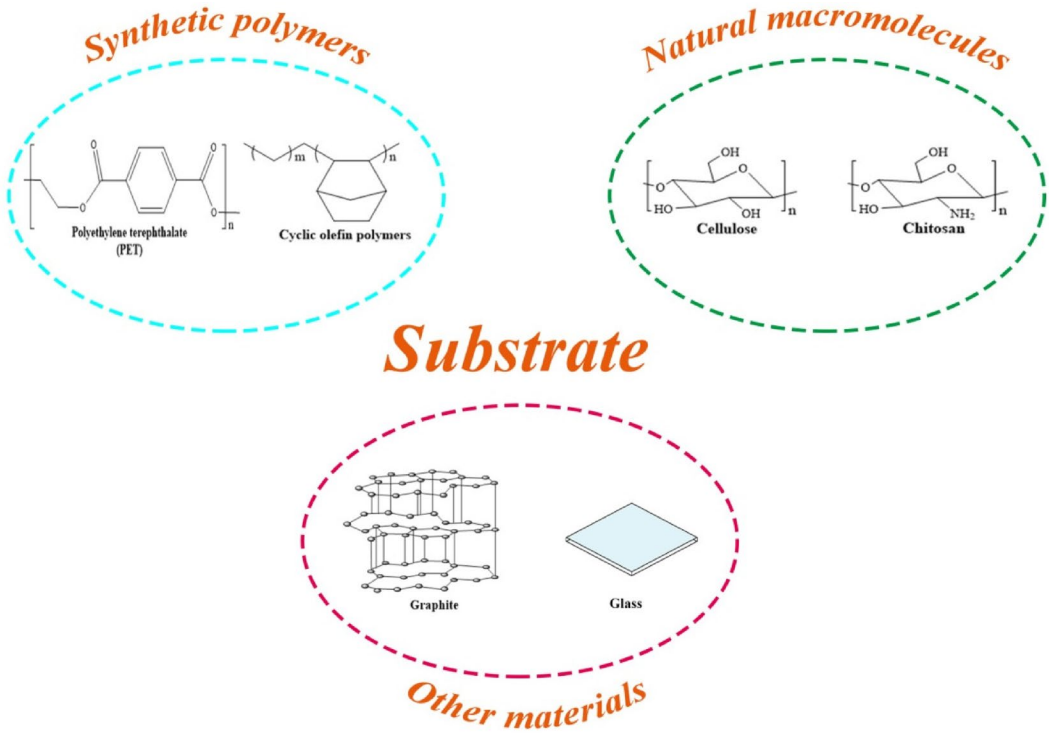

Figure 3. The suitable materials for intelligent biosensor-packaging.

characteristics of biotin and streptavidin to prepare PLA nanomembrane which can specifically bind to streptavidin (D. Li, Frey, and Baeumner 2006). Finally, they can combine with biotinylated *E. coli* DNA probe. It was a good way to incorporate biotin into PLA nanofiber to prepare the substrate of biosensors. In addition to blending biotin into nanofibers, they added biochar to optimize the electrical conductivity and thermal stability of PLA nanofibers. After biochar nanoparticles were prepared at high temperature, they used casting method to prepare composites with electrical conductivity. It was found that the composites containing 85% biochar had higher electrical conductivity, which lays a good foundation for the development of biosensors for intelligent food packaging applications (Sobhan, Muthukumarappan, and Wei 2020).

Polydimethylsil oxane (PDMS) has low cost, chemical inertia, thermal stability and oxygen permeability. In addition, PDMS has excellent elasticity and optical transmittance. Because of these characteristics, PDMS has an advantage over most other polymer substrates. PDMS thin films are widely used in flexible biosensors. It is suitable for supporting electrochemical sensors. However, there are still some obstacles to the simple preparation of PDMS, such as curing. For solving this problem, researchers proposed a liftoff method to prepare PDMS membrane (D. Wang, Ba, et al. 2018). In this work, they used a certain range of spin velocity to describe the spin curing process of PDMS film. The PDMS film had good biocompatibility, which had a good application prospect in the field of biosensors. In a relevant study, they proposed a PDMS microfilm biosensor based on surface stress. In addition, different functional materials were used to functionalize the biosensor (Sang and Witte 2010). Finally, *E. coli O157:H7* was used to test the biosensor. The results showed that the biosensor treated with 11-mercapto-1-alkanoic acid had best effect. And the biosensor can distinguish the survival state of *E. coli*.

Cyclic olefin polymers (COP) are synthesized by addition polymerization of many cycloalkyl dienes and olefin precursors. COP not only have good optical properties, but also good biocompatibility (Niles and Coassin 2008). In thermoplastics, the high transparency and low self-fluorescence of COP make it one of the most popular substrate materials for the production of fluorescence-based biosensors. Yousefi et al. constructed a biosensor based on COP. They printed DNAzyme on the food packaging material COP for real-time monitoring of *E. coli* in pork. They used real-time fluorescence intensity to determine the concentration of *E. coli O157:H7* in pork. The results showed that the real-time monitoring biosensor based on food packaging was effective and specific for the detection of microorganisms in food (Yousefi et al. 2018).

Substrate material from nature

The paper-based biosensor has become a hot topic because of its convenience and rapidity. Cellulose is the main body of paper preparation. Cellulose refers to a linear homopolymer consisting of repeated β-D-glucopyranose units. During mechanical or chemical mechanical treatment under high pressure, cellulose fibrillates, resulting in the formation of cellulose microfibers (CMF) or cellulose nanofibers. Nanocellulose refers to fibers with nanometer size in at least one dimension. It retains the structural properties of the original cellulose and contains rich active groups, so that it can be modified by covalent bond, physical adsorption and surface graft polymerization to further improve its properties (Q. Zhang, Liu, et al. 2020). Nanocellulose has excellent characteristics, such as high optical properties, good mechanical properties and easy modification properties (Moon et al. 2011). Based on these properties, nanocellulose can be applied to biosensing (Golmohammadi et al. 2017). Ranjbar et al. designed and constructed a nanofiber-based electrochemical biosensor for the detection of *Staphylococcus aureus* (Ranjbar and Shahrokhian 2018). They prepared a composite nanomaterial in which carbon nanoparticles and gold nanoparticles were incorporated. Finally, the aptamerized composite material was coated on the electrode. The prepared biosensor had a wide linear range with a sensitivity of 1 CFU/mL.

Bacterial cellulose (BC) is a microfiber secreted by various bacteria and is a biopolymer. It also has some advantages that can be used as a substrate material. The characteristics of BC include remarkable mechanical properties, high plasticity and three-dimensional nano-network structure (Moon et al. 2011; Lin et al. 2013). The material is suitable for electrochemical biosensor and optical biosensor. Farooq et al. constructed an electrochemical biosensor for the detection of *Staphylococcus aureus* (Farooq et al. 2020). The method was to immobilize the phages on bacterial cellulose. Bacterial cellulose was first impregnated with carboxylated multi-walled carbon nanotubes and then modified with polyethyleneimine to provide a positive charge for phage immobilization. The biosensor detected *Staphylococcus aureus* as 5 CFU/mL in milk within 30 minutes. In addition, the high specificity of the biosensor for *Staphylococcus aureus* was proved. All the results validated that the constructed biosensor had the advantages of simplicity, sensitivity and specificity. This tool can apply to the early detection of *S. aureus* in food.

Chitosan is a random copolymer prepared by alkaline deacetylation of chitin, which is made up of D-glucosamine and N-acetyl glucosamine units connected by β-1 amino 4 glycosidic bonds (Muxika et al. 2017). It often appears as a food packaging film in food. In addition, the polycationic properties of chitosan allow the formation of different complexes. And chemical derivatization is allowed to modify or enhance its lumber ability (Lizardi-mendoza, Monal, and Valencia 2016). The structure and biocompatibility of chitosan make it possible to be applied in many fields. Among them, it is suitable to be used as a substrate for biosensors, such as electrochemical biosensors or colorimetric sensors. In order to detect *Clostridium perfringens*, Qian et al. prepared DNA biosensor (Qian et al. 2018). The basis of their construction was the modification of chitosan with CeO_2. In this way, the prepared composites not only have a certain specific surface area and electrical conductivity, but also high DNA adsorption capacity. It manifests that the biosensor has a great prospect in food quality monitoring.

Others

When biosensors are used to detect the binding of analyst, biological macromolecules, such as antibodies and DNA, are usually required to closely contact with the underlying substrate. Selecting substrate has an effect on the fixation of biomolecules. In electrochemical biosensors, biometric molecules are usually fixed on the electrode, so it is considered to be the base material of biosensors (Suni 2021). Conductive metals can be used as electrode materials. But these metals such as gold will affect the binding stability of biomolecules. Therefore, we will not discuss it here. Instead, carbon is a commonly used electrode material for electrochemical biosensors because of its good electrical conductivity and biocompatibility. Carbon electrodes also have different forms in electrochemical biosensors, such as glassy carbon, graphite and so on. In a relevant work, the aptamers with recognition function can be combined to the glassy carbon electrode modified by graphene oxide and gold nanoparticles. Finally, the assembled electrochemical biosensor can specifically identify common foodborne pathogens, and its detection speed and result had great potential (Xiaoyuan Ma et al., 2014). Besides, silicon is also a nonmetallic material. In microelectronic devices, it always has been the first choice for fabrication of them because of its low cost and easy to obtain. However, because of its poor biocompatibility, its application range is limited. Silicon carbide has no such shortcoming. Silicon carbide is chemically inert and biocompatible, which helps it play a good role in the preparation of substrate in biosensors (Saddow et al. 2011). In addition to these materials, glass is also a common packaging in food and a common substrate in optical biosensors. Tan et al. studied the immobilization of protein on glass. It was difficult to fix it on glass, so they improved this situation by coating some materials on the glass (Tan et al. 1999). The results showed that the glass coated with phenylsilane or polystyrene film had the best fixing effect for protein. In addition, compared with traditional methods, this method was simple and low in cost. Wang et al. deposited gold nanoparticles directly on indium tin oxide film coated glass, and then immobilized superoxide dismutase on gold nanoparticles for preparing electrochemical biosensors (L. Wang et al. 2008).

Design and fabrication of intelligent biosensor-packaging

The intelligent biosensor-packaging is mainly consisting of biosensor and base material, which is an instant and efficient intelligent packaging technologyand directly and scientifically, manifests the quality of food without complex operation. There are some methods for building this package. But these means have different advantages and disadvantages. Next, we will discuss and analyze the techniques used to build this smart packaging, including ink-jet printing, microcontact printing, gravure printing and others (Figure 4).

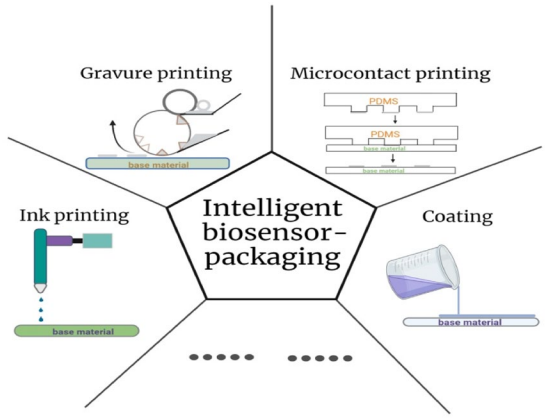

Figure 4. Design and fabrication of intelligent biosensor-packaging.

Ink-jet printing

Ink-jet printing is a non-contact and non-impact printing technology, which can spray ink onto the substrate through various types of nozzle printing heads and deposit ink on the substrate. According to different technologies, it can be divided into four inkjet printings, namely thermal, piezoelectric, electrostatic and acoustic (J. Li, Deng, et al. 2015).

There is a resistive element in the nozzle of the thermal print head. The heating of the element causes the ink to heat up and turn into bubbles. The bubbles continue to grow, so that the ink in the nozzle is ejected from the orifice. At the same time, the bubbles burst and the pressure becomes smaller. The ink fills the nozzles again, and this technique produces high nozzle densities and small ink droplets. The piezoelectric print head uses a corresponding electric field on the piezoelectric crystal material, causing the piezoelectric crystal to expand and squeeze the ink, and the ink droplets are ejected from the nozzle.

Electrostatic inkjet printing is different from piezoelectric inkjet printing. It first imparts a charge to the ink stream and then makes the ejected ink droplets in an external electric field, and controls the position of the ink deposited on the substrate by changing its potential. The disadvantage of this printing technique is that the inkjet printing ink has a much narrower range of applications, as the inks used in this printing method need to be conductive. Acoustic inkjet printing uses a transducer to emit sound waves and then uses a lens to collect the energy of the sound waves to form a pressure wave, which overcomes the surface liquid tension and discharges a drop of ink (Gonzalez-Macia et al. 2010; Parashkov et al. 2005).

In the past, inkjet printing was commonly used to print pictures and photos, but now it is used to print other functional materials, such as manganese dioxide, cell printing and thin film transistors.(Fan et al. 2021; Chung, Cho, and Lee 2019). Besides, a very interesting and beneficial application lies in the fabrication of biosensors. The fabrication of biosensors by inkjet printing and the deposition of biorecognition molecules on target substrates have become a widely used means and methods. Susana Díaz-Amaya et al. used inkjet printing technology to prepare disposable and fast paper-based biosensors (Díaz-Amaya et al. 2019).

They jetted the carboxylated aptamer ink onto a nitrocellulose substrate to prepare a convenient strip. At the same time, the results confirmed that even at extremely low concentrations of *E. coli* in ground beef it can have a good detection effect. In the aspect of detecting others pollutes, this technology was also a good tool. In Zhang's study, he operated a thermal inkjet printer to print pH dyes on cellulose paper and transparent film substrates to construct a colorimetric sensor (Y. Zhang and Lim 2016). Tests have shown that the indicator ink on paper was more sensitive than on plastic film. Moreover, Wu et al. constructed a paper-based biosensor capable of rapidly and sensitively detecting acetylcholinesterase inhibitors to detect organophosphorus pesticides in food (Y. Wu et al. 2017). The detection limit for oxygen and phosphorus was 0.01 ng/mL. And the corresponding oxygen and phosphorus in apple juice can be detected at the same time. There is no significant difference between the results of the biosensor and that of high performance liquid chromatography (HPLC), indicating that the paper-based test strip had great potential for application. It can be seen that inkjet printing has advantages in the preparation of biosensors for simple detection of target substances. These studies are all about the use of inkjet printing to prepare paper-based or flexible biosensors. In other words, inkjet printing closely links biosensors to flexible substrates. In the past, they used this technology to covalently couple DNAzyme molecules to cycloolefin polymer membranes containing epoxy functional groups and determine the content of microorganisms in their packaging by fluorescence intensity (Yousefi et al. 2018).

Microcontact printing

Microcontact printing technology is a contact printing, in which the process of printing is to use a template or stamp to contact the molecules to be deposited, and then transfer them to the substrate, thereby producing specified patterns and structures (Dias, Kingsley, and Corr 2014; Xie and Jiang 2011).

It is a common technology for preparing biosensors and a good helper for transferring biomolecules to substrates. Wang et al. transferred the two enzymes to the microelectrode by this micro-contact printing technique and prepared a double-enzyme combined sensing microprobe with high sensitivity and lower detection limit, which has been applied in neuroscience (B. Wang, Ba, et al. 2018). However, this technology also has some shortcomings. The surface of the PDMS stamp used for dipping biomolecule ink is hydrophobic. So the amount of dipping for hydrophilic molecules is less, which results in a shortage of biomolecules on the substrate surface. Taking this into account, Jang et al. developed a hydrophilic agar hydrogel coated on the surface of PDMS (Jang and Nam 2015). Compared with the traditional surfactant coating and plasma treatment, the transfer and uniformity of the agar hydrogel were improved. Moreover, some researchers also studied different ways to deposit bimolecular on the silanized surfaceand compared the two different methods. The results shows that the performance of the biosensor assembled by microprinting has been improved (González-Guerrero et al. 2013).

Gravure printing

Gravure printing is a complete volume-to-volume, impact-based printing technology and the whole printing process is shown in the figure. In the printing process, the whole drum is ground by ink and the excess ink is hung up with a scraper. When the printing roller touches the substrate under high pressure, ink is deposited on the substrate (Maddipatla, Narakathu, and Atashbar 2020).

Based on this printing technology, fully printed biosensors for antioxidants was developed (Pavinatto, Paschoal, and Arias 2015). In this work, they deposited gold electrodes, and then printed custom inks containing enzymes on plasma-treated plastics by gravure. The activity of enzyme after printing was 15.5% and the detection limit was 200 mM. Instead, Jabrane et al. studied the possibility of preparing hydrogen peroxide biosensors by gravure printing enzymes and bacteriophages on paper, and also discussed the effect of shear force on enzymes (Jabrane et al. 2008). Experiments showed that these two biomolecules can be printed on paper. Although the printing technology had adverse effects on enzymes and bacteriophages, their results showed that large-scale production of the bioactive paper was possible. At the same time, they also prepared paper-based biosensors and immobilized bacteriophages on paper (Jabrane et al. 2011). The principle of this study was based on the characteristics of bacteriophage lysis of *E. coli*. In addition, in this work, they also found that if enough humidity was provided to the paper surface and the bacteriophages may be reactivated, so they can be used in high-humidity food to prevent them from being contaminated.

Others

Besides the printing technology mentioned above, there are some common methods for fixing recognition molecules. Drop casting is one of them. It refers to the contact of biometric molecules with the same modified substrate after modification. In essence, the fixation of biomolecules is based on the formation of chemical bonds between them. In a work, electrochemical DNA biosensor based on graphene oxide-chitosan nanocomposites was prepared and applied to the detection of *Salmonella typhimurium* (Singh et al. 2013). In this method, they used glutaraldehyde to covalently immobilize the aptamer. They first soaked the electrodes with glutaraldehyde for 60 minutes. After washing with water, aptamers were manually spotted onto the activated electrodes and incubated for 60 minutes at room temperature. In addition to aptamers, scholars tried to use polyethyleneimine to modify nanoporous electrodes and then immobilize the enzymes on the electrodes by drop casting (Besharati et al. 2022).

Encapsulation is also one of the methods to fix biological recognition molecules. It is often used to immobilize enzymes on electrodes. The advantage of it is that it can stabilize and adjust the characteristics of enzyme molecules And at the same time it can wrap the enzyme to avoid the interference of external environment (Brady and Jordaan 2009). Liu et al. encapsulated laccase in situ by electrospinning, and added polyoxyethylene-polyoxypropylene as

enzyme stabilizer and gold nanoparticles to enhance conductivity (Liu et al. 2011).

Recent implementations of portable integrated biosensors for food safety detection

Recently, with the development of technology and the increasing demand for real-time detection, portable detection devices have been widely studied because of their portability, convenience and low cost (Yousefi et al. 2019; S. Wang et al. 2016). In one study, a portable surface plasmon resonance biological analyzer for the detection of *E. coli O157:H7* was developed, which included an integrated biosensor and a microfluid cell with a three-way solenoid valve. In this analyzer, 3-mercaptopropionic acid was adsorbed on the gold surface in the form of covalent bond to react with the antibody of *E. coli O157:H7*. According to the test results, the sensitivity of the portable biological analyzer was much higher than standard kit, and the response value was linear with its concentration (S. Wang et al. 2016). Xu et al. constructed a portable fluorescent nano-biosensor system and used it to detect three kinds of bacteria from different regions at the same time (Xu et al. 2017). The results showed that the portable device can complete the detection within 1 hour and the result was reliable. Moreover, a miniaturized portable microfluidic device had been developed to detect *Listeria monocytogenes* in food. The equipment can be connected with impedance analyzer, thus realizing the field application of food detection. The detection range of *Listeria monocytogenes* was 1×10^2 CFU/mL-2.2×10^3 CFU/mL (Chiriacò et al. 2018). In addition, in order to detect *E. coli* in the field, Zheng et al. combined a simple photoelectrochemical aptamer biosensor with LED and electrochemical workstation to build a portable analyzer (Zheng et al. 2021). The detection limit of the analyzer was 200 CFU/mL. Although the portable detection equipment is easy to operate, convenient and fast, there are still some problems to be solved. For example, the detection object is not multi-dimensional and the integration is not high enough.

Conclusion and prospect

In review, intelligent biosensor-packaging can monitor the state of food in real time and convey the corresponding information to consumers. Meanwhile, it can intuitively allow people know whether food is edible, which can avoid food waste. More importantly, it is also beneficial to the economy. Compared with the traditional food inspection methods, intelligent packaging has the unique advantages of rapidness, simplicity and real-time monitoring.

But it also has some shortcomings, and its manufacturing process is still complicated and a little expensive. Meanwhile, it needs to rely on some auxiliary equipment. In summary, the potential of intelligent biosensor-packaging in the detection of foodborne microorganisms is emphasized. Design and fabrication of intelligent biosensor- packaging are also discussed in detail. The intelligent biosensor -packaging is stable during the shelf life of perishable packaged food and can monitor pathogens in real time. At the same time, it is hoped that the intelligent testing and packaging system will make some contribution to reduce the negative effects of foodborne diseases. Although this biosensor -packaging with multiple functions is needed, the concept is still in its infancy because of the high cost of preparation, the unpredictable stability of packaging and the lack of commercialization. With the development of science and technology in the future, the intelligent biosensor-packaging should be commercialized, which will provide a revolutionary approach in food safety and food prevention.

Disclosure statement

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