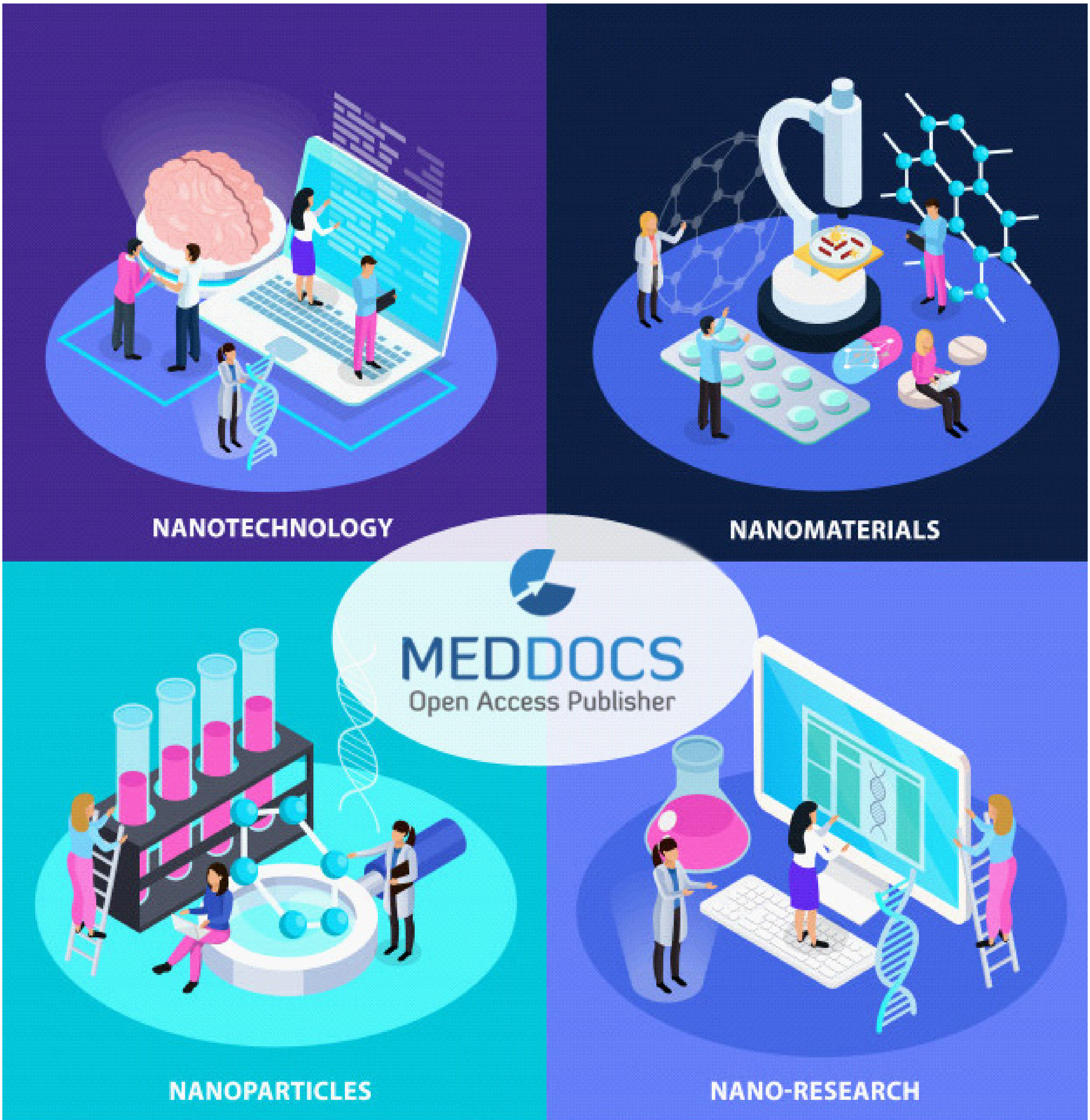

IMPORTANCE & APPLICATIONS OF NANOTECHNOLOGY

Nonobiosensor for Food Safety: Microbial Detection

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Abstract

Food safety and health has been a priority in human life from the past to the present. Because if it is disrupted, a lot of financial and human costs will be imposed on the society. Based on this, the methods of identifying food pathogens gradually expanded And more efficient biosensors were created by researchers. Over time, the use of nanotechnology and its integration with biosensors has multiplied the capabilities of biosensors. In other words, nanobiosensors are a combination of biotechnology and nanotechnology that based on the changes in the system, they are able to identify a specific biomaterial in small quantities. Today due to this feature, nanosensors are used to identify a variety of bacteria and pathogens in food.

Keywords: Biosensors; Food safty; Biosensors; Pathogens.

Introduction

Food safety is an important issue in the food industry. This is essential to protect consumers from food contamination and spoilage, as food is exposed to a variety of chemicals that may be harmful to humans health [1]. With the advent of the industrial revolution and the excessive use of chemicals, antibiotics and toxins caused severe damage to humans and helped to emerging antibiotic-resistant bacteria [2]. The incidence of various diseases due to foodborne pathogens has caused many problems around the world and more so in developing countries. Bacterial pathogens (such as Escherichia coli O157:H7, Salmonella typhimurium, Campylobacter jejunum, Bacillus*cereus*, *Legionella pneumophila*, *Staphylococcus aureus*, Streptococcus, and Norwalk Like viruses (NLVs 3) can lead to disease). And more interestingly, the incidence of these diseases has not decreased since 1994 [3,4]. Global statistics show 600 million foodborne illnesses with 420,000 deaths in 2010, with the highest rates of diarrhea occurring in low-income areas such as Africa and Southeast Asia (WHO 2015).

As mentioned, food contamination poses a potential risk to human health. Therefore, strict monitoring of food resources should be applied. The desire for fresh and natural foods free of pathogens, preservatives and less additives has increased the demand for fast measurement methods [5]. On the other hand, in many foodborne pathogens, even very low concentrations of contamination are involved, so we need rapid and sensitive diagnostic methods to ensure food health [6]. Some rapid detection methods have been developed to detect the presence of microorganisms such as ATP biomolecules and catalase-based methods for detecting pathogens in food products [7-12]. In addition, colorimetric measurements and ATP detection have replaced traditional methods such as pH measurement and live microorganisms. A colorimetric sensor is also provided to monitor corruption. In this method, the health of the food is measured in response to the change in pH under the influence of temperature and the resulting color change [13]. Traditional food poisoning methods require sample preparation, which can be very tedious and time consuming. However, with the advancement of technology and the emergence of biosensors in

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the detection of food microbes, both the sensitivity of the test has increased and the detection time has decreased [14].

A sensor is a device with a proportional output that is displayed with a measurable physical, chemical, or biological quantity. A sensor system is a collection of processes and information processed from sensed data. Due to the need for sensors and sensory systems, these tools are used in many industrial, commercial and service sectors, etc. In addition, today with the expansion of small nanoscale structures and the use of such new material technology, new technologies in sensor construction have expanded [15]. Important sensors with advanced manufacturing technology include intelligent olfactory sensors (electronic nose), taste (electronic language) and biological sensors (biosensors) that are able to accurately detect specific compounds. Such capability has made these sensors a very useful tool in accurately and quickly identifying various compounds in even the smallest of combinations [16.] The biosensor is a group of sensors that are based on biomaterials and are based on the selective identification of specific biomaterials with the help of physicochemical detectors. In fact, the basis of a biosensor's work is to turn a biological response into a message. By combining biotechnology with electronic extrusion, these sensors react only with a specific material, and with the help of photoelectrons and microelectrodes, and by reacting with a specific material at concentrations lower than myelin, provide a suitable tracking and analyzing tool. Finally, the response of this specific reaction is expressed in the form of messages and analyzed by a microprocessor [17]. In other words, biosensors are analytical tools that use the intelligence of biological materials to identify and react to certain compounds. The product of this reaction can appear as a chemical, optical or electrical message [18].

Biosensors are made up of three main components. The first part is a sensitive biological receptor or biological element that can be a biological substance such as enzymes, cells, and nucleic acids and selectively react only with a specific substance. The second part is called the converter, which determines the type and amount of reaction of a particular substance with biological receptors by various physico-chemical methods and sends it to the processor with the help of appropriate signals. And the third part is the processor, which is also responsible for displaying the result of the process. (Figure 1) The function of biosensors is a combination of various sciences, including biochemistry, molecular biology, chemistry, physics, electronics, and computer science, which have made them a powerful tool for identifying biological molecules [19].

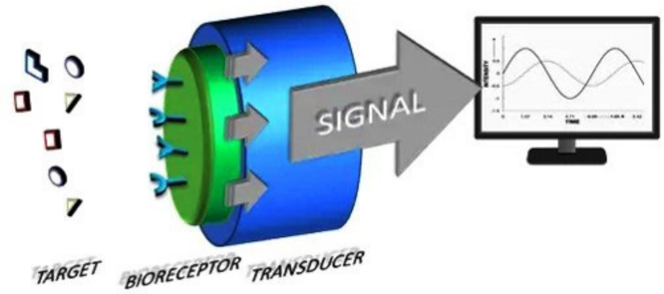

Figure 1: The general structure of biosensors [16].

Type of biosensors

Biosensors have different classifications, for example, they are divided into enzymatic, microbial, and antibody-based biosensors based on the biochemical elements tested. The next section describes the classification of biosensors based on converters.

Electrochemical biosensors

Electrochemical sensors are one of the most common biosensors, and countless advances have led to a good diagnostic process [20]. Features such as high sensitivity, rapid analysis with precision of analytical measurements have brought electrochemical biosensors as a new diagnostic tool in technology [21]. The performance of electrochemical sensors is such that the electrochemical analyzer at the working electrode surface is oxidized or reduced. This electron flux produces an electrochemical signal and is measured by a tracker [22]. Electrochemical biosensors have a variety of uses including for food and beverage analysis. Electrochemical biosensors have also been introduced based on the Clark-type oxygen electrode to determine the pathogens *E. coli*, *S. aureus*, and *Enterococcus serolicida* [23]. Electrochemical sensors themselves are divided into three groups: Amperometric, Potetiometry, and Impedimetric.

Amperometric

This is the most common electrochemical method used in biosensors, based on the relationship between the concentration of the analyte and the intensity of the current, and works when the analyte produces a direct or indirect current in the electrode. Figure 2 showes tracking the reduction or oxidation of a particular reaction. Parameters such as oxygen consumption can also be measured using Clark's electrochemical converters [24]. Immunosensors can quickly detect Salmonella and Staphylococcus bacteria based on the lack of antibody activity on the surface of an electrode [25].

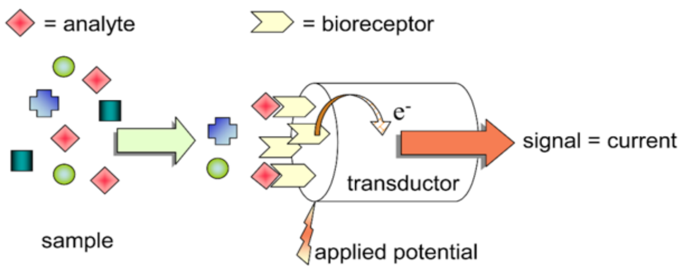

Figure 2: Schematic design of amperometric biosensors [26].

Potentiometry

In potentiometric biosensors, the potential difference between the working electrode and the reference electrode is measured and the charge accumulation at zero current created by the selected connection at the electrode surface is investigated [27].

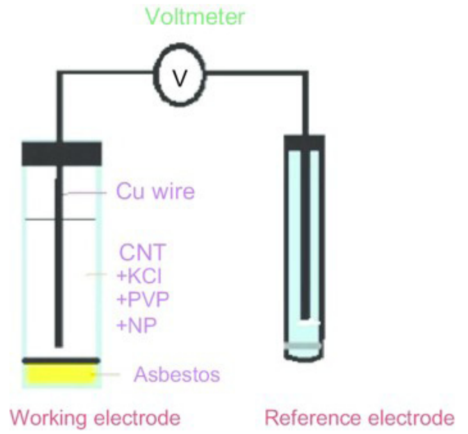

Figure 3: Schematic design of Potentiometric biosensors [27].

Impedimetric

Impedance is one of the first physicochemical methods to detect bacteria in food that was previously used as an unconventional method and for less than 24 hours to detect bacteria. This was done by measuring changes in resistance to electric current from an environment or a solution caused by bacterial growth. Microbial metabolism increases conduction current and reduces impedance [28]. This method has been welcomed by the Association of Formal Analytical Chemists (AOAC) for the diagnosis of Salmonella in food. Paul and his colleagues used this method to diagnose the pathogenic *Bacillus cereus* caused in food [29].

Optical biosensors

Optical biosensor performance involves measuring changes in amplitude, phase, frequency, or light. One of the most important advantages of using optical biosensors is that it makes it possible to analyze the sample over long distances. This type of biosensor is more common in analyzeres due to its high sensitivity and can detect toxins and pathogenic bacteria quickly. Fluorescent and SPR is one of the optical biosensors that have developed significantly due to their high sensitivity, which are described below [30].

fiber optic

In this method, light is emitted from an electron excited by the absorption of light, and in fact its function is based on the emitted light. In this method, antibodies combine with fluorescence. The most common combination of fluorescein is isothiocyanate [31]. Figure 4 shows a schematic of the fiber optic biosensor. Identification of the demand analyte is possible by changing the intensity of input and output light

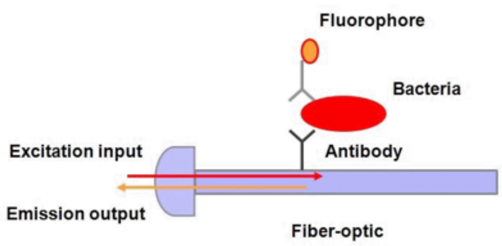

Figure 4: Schematic design of fiber optic biosensors [32].

SPR

The function of this type of optical biosensors is to measure changes in refractive index. This refractive index is due to the structural change of the thin layer of the metal surface. These biosensors have been used to diagnose pathogenic bacteria [33]. Rapid detection of drugs and antibiotics, beta-agonists, and antipyretic drugs in meat can also be performed by SPR biosensors [34,35]. Figure 5 shows a schematic diagram of an SPR biosensor. In this biosensor, changing the angle of reflection helps to identify the target analyte.

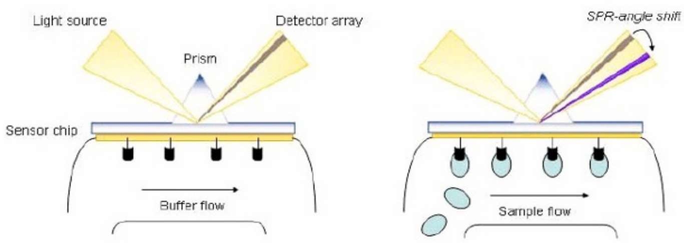

Figure 5: Schematic design of SPR biosensors [36].

piezoelectric biosensors

The basis of these sensors is based on the measurement of frequency change. The deposition of mass on the surface changes the frequency of the crystal resonance. (Figure 6) These biosensors are a good tool for diagnosing animal diseases. Piezoelectric biosensors are a combination of antibodies with the sensitivity of quartz crystalline dust (QCM), so they quickly measure and detect the concentration of the pathogen. These crystals are activated by the immobilization of suitable antibodies on the surface and thus have the ability to bind to the target analyte. By attaching the analyte to the antibody, the mass accumulates on the crystal surface and reduces the oscillation frequency of the crystal. This change in frequency makes it easy to detect the pathogen [37]. Researchers have used many piezoelectric biosensors to detect pathogens from food. For example, Ye et al. used a flow analysis (FIA) analysis system to quickly and continuously determine the pathogen [38-41].

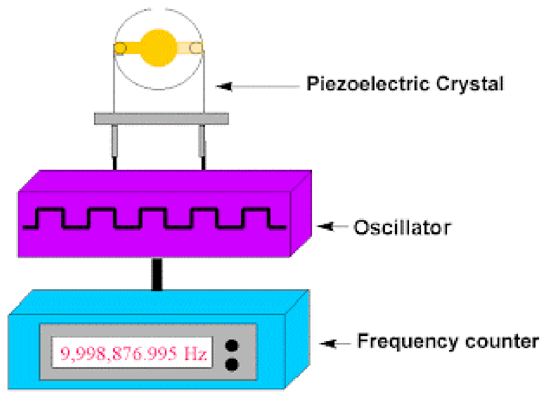

Figure 6: Schematic design of piezoelectric biosensors [42].

Thermal biosensor

Thermal biosensor performance is the measurement of heat with a chemical or biochemical. In this biosensor, changes in circulating fluid temperature are measured following the reaction of a suitable substrate with immobile enzyme molecules. (Figure 7). Calorie measurement has significant potential in biomaterial analysis. The two most common types of biosensors are adiabatic calorie and heat conduction, both of which are widely used. It's most important advantage is the possibility of continuous monitoring, and mini and micro designings [43]. Researchers have used various metabolites such as ethanol, glucose, lactate, triglycerides / peroxides, urea, ascorbate, cellulose and sucrose, and penicillin using immobility [44-47].

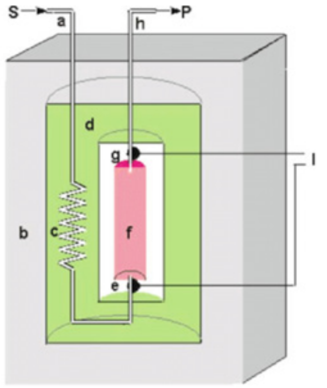

Figure 7: Schematic design of Thermal biosensors [48].

Nanobiosensor

Nanosensors are devices that are able to sense and analyze chemical or biological forces on a nanoscale. In other words, nanosensors are based on nanoparticles that are targeted at a ligand in which the ligand finds a specific substance. Due to the presence of nanoparticles in nanobiosensors and having a high contact surface, desirable and unique features such as high reactivity, increased electrical conductivity, magnetic properties are visible [49]. In other words, nanoparticles, due to their high surface-to-volume ratios, can detect high concentrations of markers in very small sample sizes. In addition, nanosensors use multi-parameter analysis to directly read diagnostic signals. Nano characteristics can be adjusted according to their shape. This means that nanotubes, nanowires, thin nanoparticles exhibit a variety of sensitivities [50].

Today, nanomaterials are used in the structure of biosensors, which has led to valuable advances in the performance of biosensors. One of the most important purposes of using nanomaterials in the structure of biosensors is to increase the level required to stabilize biomaterials, which increases the sensitivity of reactivity even at low potentials and helps the rapid transfer of reaction electrons to the electrode surface. On the other hand, despite the nanomaterials in the structure of biosensors, chemical mediators of electron transfer can be removed and tools simplified, which in turn is very important in the development of biosensors [51,52].

Various types of biosensors, such as metal nanoparticles, nanowires, and carbon nanotubes, are used to quickly diagnose pathogens. In any type of biosensing device, the diagnostic material is limited to the nanomaterial surface, and this hybrid interacts with a pathogen and improves the interaction between the pathogen and the diagnostic material by transmitting the signal, increasing sensitivity [53-56].

Type of the nanobiosensors

Optical Nanosensors

Metal nanoparticles such as gold, silver, copper, nickel, palladium, and iron increase electronic properties in the biosensor. Nano metal particles exhibit some size-dependent properties such as surface-to-volume ratio, local plasmonic resonance, superparamagnetic and quantum cells. Optical nanosensors of these properties depend on the size of the aggregate and the color change of the metal nanoparticles. Gold and silver nanomaterials are the most widely used, and Gold Nanoparticles (GNP) have been prominently used in research [57]. Studies have shown that the detection of some pathogenic bacteria such as *S. aureus*, *V. parhemolyticus* and *S. typhimurium*, *E. coli*, and organophosphates has been successful through fluorescence nanostructures [58,59]. In addition, metal nanoparticles have been designed to demonstrate the SPR biosensor system for the detection of antibiotics, mycotoxins, and pathogens such as *E. c*oli [60-62].

Electrochemical Nanosensors

In these biosensors, the surface of the electrode is modified with a nanomaterial, thus helping the electrons to move faster from the surface to the converter and receiver. In fact, the function of an electrochemical cell is performed by the movement of electrons from the electrolyte to the surface of the electrode [57]. Using titanium dioxide nanotubes (TiO2), the researchers designed electrochemical nanobiosensors, which were performed by detecting biological molecules [63].

Mechanical Nanosensors

The mechanical sensors based on mass changes of biological molecules. Mechanical nanosensors are more sensitive than optical and electrochemical nanosensors. Classical method is one of the mechanical biosensors, which the connection of the analyte increases the mass of the system, and in turn increases the mass of the system. In other types of mechanical biosensors, which are piezoelectric biosensors, the bending caused by the analytic connection changes the flow of the system. And this change in flow helps to identify the pathogen. The use of nanomaterials in these biosensors enhances mechanical change and provides a more appropriate response [64].

Application of nanobiosensor in bacterial detection

Gold nanoparticles, due to their high surface-to-volume ratio and high energy levels, stabilize and immobilize biological molecules, which preserves their biological activity. On the other hand, gold nanoparticles are able to rapidly transfer electrons between a wide range of electrophoresis materials. In addition, it enhances the Properties of light scattering and local magnetic field. Gold nanoparticle biosensors can be used in three categories: Optical biosensors, electrochemical biosensors, and piezoelectric biosensors [65]. In addition to gold, other metal nanoparticles such as platinum and silver have been used in biosensors due to their unique size and optoelectronic properties. The size of these nanoparticles affects light, magnetic, chemical and electrical properties. Therefore, they have different characteristics and are used in different technologies [63].

In recent years, nanoparticles have been widely used to detect microbes. In one study, fluorescent silica nanoparticles with antigenic labels were used in milk samples to detect Brucella IgG antibodies [66]. In addition, using a direct colloidal gold nanoparticles on a chip surface with 2 amino ethanol, a sensitive label less detection system was created. Thus, the new fusion protein was used as a binder for antibody immobilization using gold-binding polypeptides to protein A. To test the performance of gold nanoparticles, the GBP-ProA protein was attached to both bare and gold nanoparticles at the surface of the plasmon resonance biopsy chip via the GBP section. The results showed that the signal in the assembled chip of gold nanoparticles increased tenfold in the diagnosis of *S. typhimurium* compared to the state without gold nanoparticles [67].

As mentioned earlier, *Escherichia coli* O157: H7 is an important factor in foodborne illness. Therefore, rapid and accurate diagnosis of *E. coli* O157: H7 is very important to minimize potential problems. For this purpose, in a study, non-ferrous silica nanoparticles containing hexahydrate tris (2,2-bipyridyl) dichlorothronium (II) hexahydrate were used to detect *E. coli*, which was enhanced by the pure color of the fluorescence signal 1000 times [68]. In another study, an electrochemical immunosuppressant was proposed to identify two pathogenic bacteria, *E. coli* O157: H7 and *Enterobacter sakazakii*. The biosensor consists of carbon arrays with a printed screen and four carbon working electrodes, an integrated carbon counter electrode and an integrated Ag / AgCl reference electrode. Finally, to enhance the sensitivity of the electrode, multi-volt carbon nanotubes / sodium alginate / carboxymethyl chitosan were placed on the electrodes [69]. Also, an experiment based on SPR band associated with antibody binding to silver nanotubes was performed, which showed high speed and good diagnostic power in the diagnosis of *E. coli* [70]. The Piezoelectric biosensors have a high potential for food safety and environmental monitoring, and this high potential provides the basis for combining this method for bacterial detection. To diagnose *E. coli* O157: H7, a Quartz Crystal Biosensor (QCM) based on DNA was presented. In this biosensor, gold nanoparticles combined with foreign avidin coatings combined with target DNA to increase mass. At each stage, electrochemical techniques such as Cyclic Voltammetry (CV) and Electrochemical Impedance Spectroscopy (EIS) were used for detection. The target DNA of *E. coli* O157: H7 was easily detected by this biosensor [71], The researchers provided an amprometric sensitizing safety bar to detect *E. coli* O157: H7 in milk samples. In this immunosorbent, ferrosilicon carboxylic acid (FeDC) was used to enhance the effect of ampetrometric and gold nanoparticles to increase biosensor sensitivity. In addition to *E. coli*, this biosensor can be effective in quickly diagnosing other pathogens [72]. A sensitive electrochemical biosensor for *E. coli* detection was also introduced based on Cu@Au core nanoparticles and Anode-Neutral voltammetry (ASV) [73].

A study was conducted to identify *Mycobacterium avium*. In this study, an ultrasonic emission method based on Surface-Enhanced Raman scattering (SERS) was used in which gold nanoparticles were used as external Raman labels. And a stationary layer of monoclonal antibodies was placed to detect the protein level of microorganisms [74]. A magnetic nanoparticle-based biosensor (EAPM) was developed with an active electric polyamine coating to identify *B. anthracis* in food samples. In this study, EAPM nano biosensor were biologically modified to identify *B. anthracis* spores in lettuce, beef, and milk samples and apply a direct charge transfer to a biosensor. The EAPM nano biosensor is able to detect *B. anthracis* spores even at low concentrations [75]. An adenosine colorimetric biosensor was created based on gold nanoparticles. In this sensor, a combined surface based on the absorption of gold nanoparticles was used to detect DNA. These biosensors are also able to detect target DNA at low concentrations [76].

In a study by monoclonal antibodies)(mAb), Salmonella was studied on gold nanoparticles and the interaction of mAb with Salmonella species. In this study, the spectroscopy technique of Electrochemical Impedance (EIS) was used, which was recognized as an efficient method for diagnosing Salmonella species [53].

Mycotoxins are highly toxic in food and can be very important. This toxin poses a risk to human health through mycotoxin poisoning. In this context, an optical sensor based on the fluorescence pole was proposed. Also, the use of nanomaterials in these biosensors increases productivity, which will increase the detection speed by identifying multiple target analyzes simultaneously [77]. An electrochemical sensor was developed for the microcystin toxin. The toxin, produced by cyanobacteria, binds to the surface of single-walled carbon nanotubes with an anti-MCLR coating and changes the electrical conductivity. This change in conductivity and toxin detection can be easily detected at MCLR concentrations of 0.6 nm[78].

The use of nanobiosensors in the microbial detection in the food industry is listed in Table 1.

Table 1: Summary of nanobiosensors used to microbial detection.

Analytes	Nanomaterial used	Biosensor Type	reference
Brucella	silica	Opticale	[66]
S. typhimurium	Au NPs	Opticale	[67]
E. coli O157: H7	silica	Opticale	[68]
E. coli O157: H7	Ag NPs	Electrochemical	[69]
E. coli O157: H7	Ag NPs	Opticale	[70]
E. coli O157: H7	Au NPs	Piezoelectric	[71]
E. coli O157: H7	Au NPs	Amperometric	[72]
E. coli O157: H7	Cu @ Au NPs	Electrochemical	[73]
Mycobacterium avium	Au NPs	Optical	[74]
B. anthracis	Electrically active polyaniline coated magnetic (EAPM) NPs	Mechanical	[75]
Salmonella	Au NPs	Electrochemical	[53]
Myco toxins		Opticale	[77]
Cyanobacteria toxin	carbon nanotubes	electrochemical	[78]

Conclusion

This chapter reviews biosensors and their functionality. As mentioned, biosensors include electrochemical, optical, piezoelectric and thermal, that depending on their properties, they can help identify pathogens in the sample by evaluating them. By combining nanomaterials with these biosensors, their capability increases several times, so that they detect microbes with high speed and accuracy. According to studies, gold nanoparticles can be used in all three biosensors. The use of gold nanoparticles in optical biosensors helps to identify *S. typhimurium* and *Mycobacterium avium* and its use in electrochemical and piezoelectric biosensors identifies *E. coli* O157: H7 in food. By integrating silver nanoparticles into the electrochemical and optical biosensors, *E. coli* O157: H7 in food can be detected. Also, the use of silica in the optical biosensor to identify Brucella can be effective. By combining several nanomaterials in biosensors or other biological systems, it may be possible to identify a group of pathogens in food.

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