International Food Research Journal 23(5): 1849-1856 (2016)
Journal homepage: *http://www.ifrj.upm.edu.my*

Mini Review

Application of nanomaterials in the development of biosensors for food safety and quality control

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Article history

Received: 27 March 2015
Received in revised form: 7 January 2016
Accepted: 21 January 2016

Keywords

Nanotechnology
Nanomaterial
Biosensors
Food safety and quality control

Abstract

Nanotechnology contribute to significant impacts in every way in our daily life. Recently, the application of nanotechnology in biosensors has been a trend in developing a highly sensitive, selective, quick response, inexpensive, high volume production, great reliability and miniaturized sensors. High demands on the production of rapid sensors for food safety and quality control purposes are increasingly become the interest for researchers all over the world. This is because, in food sector, the quality of a certain product is based on their periodic chemical and microbilogical analysis. The uses of nanomaterials in biosensors are very promising because they mediate current flow. Surface modification of the electrode based on various nanomaterials including nanoparticle, nanofiber, nanowire and nanotube significantly increase the performance of the biosensor. Ultimately, this implementation will enhance the sensor's sensitivity and stability. This review explores the previous research and development work on nanomaterials-based sensors for food applications.

Introduction

Biosensor is a well-known rapid alternative detection method which implements the use of biological components that will react with a target molecule or sample. Transducer is needed to translate this reaction into a measurable or detectable signal (Maragos and Thompson, 1999). Fabrication of biological components such as an antibody, enzyme, nucleic acid (DNA), cell structure or tissue onto the surface of transducer is a very important step in developing a biosensor (Monosik *et al*., 2012). Prior to fabrication of biological components, the surface of electrode are recommended to be modified to enhance the attachment of bioreceptor element and subsequently maximize the current, resulting from the signal produced. Enzymatic biosensor, genosensor and immunosensor can be divided into several type of transduction process or energy transfer, which is electrochemical, calorimetric, acoustic, thermal, optical, piezoelectric, electromagnetic and mechanical. However, electrochemical transducer is regconized as the most sensitive and selective analytical method (Ahammad *et al*., 2009).

Biosensors have a wide range of application, namely in health diagnostics, medical engineering, environmental analysis, food safety and quality control and detection of toxic metabolites. Advancement of biosensors in recent years is because that they provide rapid, real-time, reduce labour, time and cost for analysis. Besides, biosensors can be made into a portable instrument which can be operated on-site. This method proposes a real-time basis without pre-treatment of the sample prior to analysis and therefore, reducing the dependence on expensive and centralized laboratory-based method. The application of nanomaterials in modification of electrode will enhance the electronic signal transduction especially for biological recognition events (Wang *et al*., 2006).

Food quality control is very crucial for food industries as well as for consumer. The food production and preservation are the most crucial process to ensure the safety of the food. Chemical and microbial deterioration, insect infestation and pathogenic contamination are some of the factors that needs to be prevented (Viswanathan and Radecki, 2008). Foodborne illness causes a major burden for consumers and traders in terms of their economy, health and confidence on the food safety. The most common foodborne pathogens that can be found in seafood and meat are *Salmonella* spp., *Campylobacter* spp., *Shigella* spp., *Clostridium* spp. and *Listeria monocytogenes* (Carter, 2005). In addition, the exposure of mycotoxins such as aflatoxin, ochratoxin, fumasinin and citrinin in food or agricultural products is hazardous to human as they are potent carcinogens. Aside from detection of

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harmful contaminating microorganisms, biosensors tend to be designed as analyser for common food components such as glutamate, lactose, glucose (dextrose), sucrose, lactate, galactose, cholesterol, choline, glutamine ethanol, hydrogen peroxide, and starch (Terry *et al*., 2005). Biosensor is a rapid detection method that offer many advantages over conventional methods such as liquid chromatography (LC), high performance liquid chromatography (HPLC), thin-layer chromatography (TLC), enzyme-linked immunosorbent assay (ELISA) (Espinosa-Calderon *et al*., 2011).

Immobilisation strategy is the most important part in developing a biosensor. This is to ensure a stable bond occur between the sensor surface and the biosensing element without interrupting the biological activity of biocomponent and the compound to be detected (Salam, 2010). The common methods for the attachment of biological components to solid surfaces are physical adsorption, covalent coupling or cross-linking and entrapment in a gel network. This review will highlight the application of nanomaterials , which are namely nanoparticles, nanofibers, nanowire and nanotubes as the modification of sensor's surface as well as their contribution towards food safety and quality assurance.

Nanomaterials

Nanomaterials display excellent properties like present unique physical and chemical features. Besides that, other features such as mini size effect, quantum size effect, macro-quantum tunnel effect and surface effect, makes it the material of choice in designing novel sensing systems. Figure 1 shows example of nanomaterials, which are commonly use in developing a sensitive biosensor. Any particle can be classified according to their size either coarse, fine or ultrafine (nano) which cover a range of 2500 to 10000 nm, 100 to 2500 nm and 1 to 100 nm, respectively. Nanomaterials have few advantageous physicochemical characteristic including their small size structures which play a crucial role in fabrication of biosensor. Meanwhile, nanotechnology refers to the manipulation or creation of functional system at molecular scale, mainly incorporating the use of nanomaterials (Jianrong *et al*., 2004).

Zhao *et al.* (2010) briefly described four general techniques for fabrication of nanomaterials into biosensors namely wet chemical route (chemical solution deposition), hydrothermal synthesis, vapour-liquid-solid method and sol-gel process. Those techniques are suitable for the synthesis of different types of nanomaterials. Compared to conventional processing and gas phase synthesis, wet

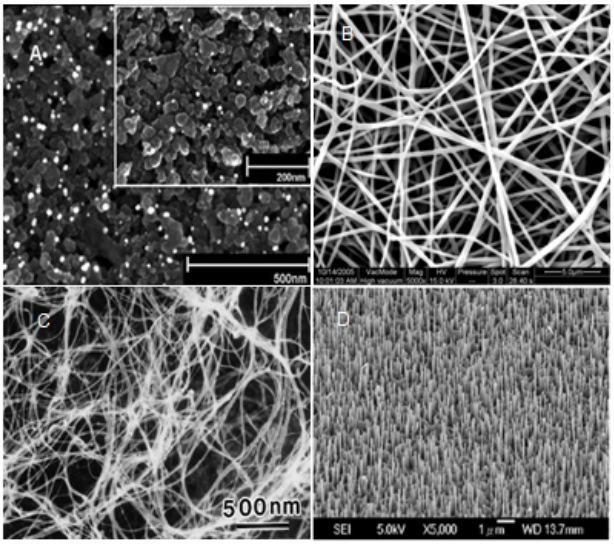

Figure 1. SEM images of various nanomaterials including
(A) Gold nanoparticles (Bernalte *et al.*, 2012)
(B) Nanofibers (Emalco, 2010)
(C) Carbon nanotubes (NanoLab, 2012)
(D) ZnO nanowire (Chao *et al*., 2010)

chemical route is a better alternative in producing nanostructures related to commercial application (Martinez *et al*., 2014) . This is because, it offers many advantages such as environmental friendly, economical, easy to handle, simple and requires small energy input (Van den Rul *et al*., 2006). Ku *et al*. (2008) used a simple wet-chemical route to synthesize ZnO nanowire-layered basic zinc acetate (LBZA)/ ZnO nanoparticle (NP) composite films. ZnO nanowire (NW) was first produced by using an aqueous chemical bath deposition (CBD) before evolution of NPs among ZnO NWs by another base-free CBD. Meanwhile, Hu *et al.* (2010) synthesized a narrow size distribution of multiferroic bismuth ferrite ($BiFeO_2$) nanoparticles (average diameters ranged from 4 to 30 nm) via a wet chemical route . Starting materials of bismuth nitrate and iron nitrate were used together with chelating agents of excess tartaric acid and citric acid at low temperature.

Besides that, other technique for fabrication of nanomaterials into biosensors is done via hydrothermal synthesis. It is a process which involves heating the metal salt aqueous solutions to create metal oxide crystals by the equilibrium reaction that changes with temperature (Adschiri *et al*., 2001). The process can be done in a certain temperature range according to the metal salt namely magnetite nanoparticles at 70°C (Daou *et al*., 2006), crystalline zinc oxide (ZnO) nanoparticles at 120°C (Baruwati *et al.*, 2006), titanium dioxide (TiO_2) nanowire at 200°C (Zhang *et al*., 2002), metal oxide nanoparticles at a range of 250 to 400°C (Adschiri *et al*., 2001) and tin dioxide (SnO_2) at 300°C (Chiu and Yeh, 2007).

Nanoparticles

Nanoparticles can be defined by their size and diameter. Nanoclusters have at least one dimension between 1 and 10 nm and a narrow size distribution. Nanopowders consist of agglomerates of ultrafine particles, nanoparticles, or nanoclusters. Nanometer-sized single crystals, or single-domain ultrafine particles, are often referred to as nanocrystals. The use of functional nanoparticles in the construction of biosensor subsequently enhances the attachment of biological molecules such as peptides, proteins and nucleic acid to assist in the detection of various analyte thus amplify the signals (Jianrong *et al*., 2004). Various types of nanoparticles have been used in biosensors including metal, semiconductor, oxide and even composite nanoparticles. Gold, silver and zinc are the most popular metal nanoparticles in constructing a biosensor. The application of metal nanoparticles can catalyse biochemical reactions due to their high catalytic abilities for many organic reactions (Crooks *et al.*, 2001). Metal nanoparticles are also regarded to assist in the electron transfer and can be applied as an electrochemical label for most biological components while maintaining their biological activities (Bhattacharya and Mukherjee, 2008). Magnetic nanoparticles are another type of nanoparticles which are usually prepared in either single domain or super-paramagnetic (Fe_3O_4), gregite (Fe_3S_4) and maghemite (y-Fe_2O_3) (Jianrong *et al*., 2004). It can be used to disperse and enhance the detection of analyte.

Next, by using a non-competitive immunoassay format, Sun and co-workers (2008) reported a work on development of an electrochemical enzyme immunosensor for aflatoxin B_1 based on bio-electrocatalytic reaction of an imidazolium cation room-temperature ionic liquid (RTIL) and gold nanoparticles, fabricated on glassy carbon electrode. In this work, RTIL and [EMIm][BF4] was primarily immobilized on the surface of electrode through titania sol and nafion film prior to attachment of antibody onto the nanogold. The presence of nafion and sol gel help to sustain the steadiness of the modified electrode due to cation-exchange process between RTILs and prevent from leak. Results showed that combination of RTILs and gold nanoparticles offer condusive environment for the electron transmission between the immobilized HRP-anti-AFB_1 and the base electrode as the separation of anodic and cathodic peak potentials (ΔEp) was 50 mV, which represents a fast electron transfer reaction. Furthermore, RTILs have unique physicochemical properties such as high ionic conductivity, wide electrochemical windows, good chemical and thermal stability, and negligible vapour pressure hence make them suitable as the supporting electrolyte or the modifier in electroanalysis (Guo *et al*., 2014).

Meanwhile, Gomathi *et al.* (2011) fabricated a novel chitosan nanofiber/gold nanoparticle composite to improve the performance of a cholesterol sensor. This matrices layer had high surface areas which were suitable for excellent enzyme loading where it provides biocompatible micro-environment to aid in the stability of enzyme as well as preservation of its bioactivity. The ITO/CSNF-AuNPs/Chox cholesterol biosensor showed high sensitivity (1.02 μA/μM), great reproducibility (R.S.D. = 4.2%) and shorter response time of approximately 5 s with a linear response to cholesterol in the range of 1-45 μM. Other conventional method such as reverse-phase high performance liquid chromatography (RP-HPLC) took around 10 minutes for a direct determination of cholesterol in food samples in the range of 0.03 to 10 μgL^{-1} (Daneshfar *et al*., 2009).

Nanofiber

Nanofiber (NF) thin film is suitable as a detecting interface for chemical and biochemical sensor applications. Nanofiber film is made from Poly-Lactic Acid-Co-Glycolic Acid (PLAGA) polymer with the diameters of approximately 500 nm (Kwoun *et al*., 2001). Nanofiber can be produced by interfacial polymerization, electrospinning, and forcespinning. Carbon nanofiber (CNF) is widely used in application of biosensor. CNFs are cylindrical nanostructures which consist of diverse stacking arrangements of grapheme sheets or layers, arranged as stacked plate, ribbon or herringbone (Huang *et al*., 2010). Furthermore, there is no difference between CNF and CNT (carbon nanotube) in terms of their mechanical strength and electrical properties as their size and graphite ordering are well organized. However, CNFs are distinctive because their whole surface area can be functionalized via treatment of nitric acid or electrochemical oxidation which subsequently increases the surface-active groups-to-volume ratio by creating a range of oxygen containing groups without changing the main structure (Huang *et al.*, 2010). Electrospun nanofiber can be prepared by elecrospinning which refers to a formation process of fiber with sub-micron diameter that relies on electrical rather than mechanical forces (Li *et al.*, 2006).

Recently, there are many researches that developed glucose sensor which incorporated various types of nanomaterials. The mechanism of glucose sensor with the presence of glucose oxidase enzyme is shown in Figure 2. The reduction and oxidation of

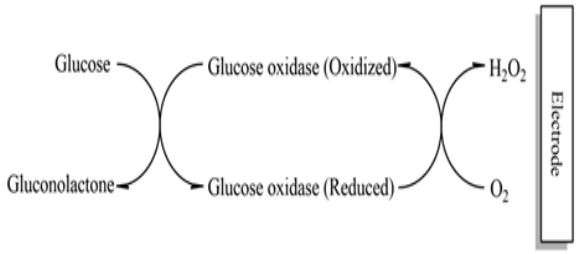

Figure 2. The mechanism of glucose sensor

this enzyme will convert oxygen to hydrogen peroxide and ultimately being detected by the electrode. Vamvakaki *et al.* (2006) studied the potential of three different types of carbon nanofiber namely LHT, HTE and GFE (graphitized) as the immobilization matrixes for biomolecules (enzyme or protein) as well as transmission of the electrochemical signal as transducer, for improvement of glucose biosensor. In this work, the performances of these three types of carbon nanofiber were compared to carbon nanotube (single walled) and also graphite powder matrix. It was shown that all different types of carbon nanofibers had comparable and very low electrical resistivity meanwhile single-walled carbon nanotube showed a large non-Faradaic current, with a large ΔEredox value. In addition, GFE nanofibers were found to contain low concentration of basic groups which made it the strongest immobilization environment. This is because, the amount of basic functional group is related to the strength of interaction with the negatively charged external surface of the protein (Vamvakaki *et al.*, 2006).

Nanowire

Nanowires (NW) are referred to as metallic or semiconducting particles that consist of a high aspect ratio with cross-sectional diameters less than 1 μm and lengths as long as tens of microns (Peng and Qin, 2011). A label-free, sensitive and real-time detection of a wide range of chemical and biological species can be developed in array-based screening and *in vivo* diagnostics due to the small size and capability of these semiconductor nanowires (Jianrong *et al*., 2004). Few examples of nanowires are boron-doped silicon nanowires (SiNWs) and ZnO nanowire (Cui *et al*., 2001; Yeh *et al*., 2009).

Hong *et al.* (2011) patented a novel invention of a nanowire biosensor for detecting food additive monosodium glutamate (MSG) with a receptor having capability to bind at the target substance and directly immobilized on the nanowire matrix. MSG is an amino acid that is normally used to enhance the flavour of foods. However, high levels of glutamate will develop toxicity due to overstimulation and calcium saturating in nerve cells (Parthasarathy, 2014). The oxidation of glutamate occurred with the presence of catalyse enzyme (glutamate oxidase) to aid the reaction. This reaction made it separated into by-products of α-ketoglutarate, hydrogen peroxide and ammonia. The deposition of electrode can be done either by thermal evaporator, E-beam evaporator or sputter (de Barros *et al*., 2014). However, prior to that, the integration of nanowire onto the surface of electrode was optimized with the presence of slippery molecular layer which will enhance the binding ability towards the substrate. In addition, in this work, glutaraldehyde acted as a linker that connects the functional group (amine group/carboxyl group/thiol group) to the nanowire and the surface of solid substrate.

Nanotube

Nanotube is a nanometer scale of tubular structure namely carbon nanotube, silicon nanotube, boron nitride, inorganic nanotube and membrane nanotube. Carbon nanotube is widely been used in biosensor application as compared to others. Carbon nanotube (CNT) can be referred to as a hexagonal network or a rolled up tube that consist of carbon atoms sheets with a diameter of approximately ten nanometers (Veetil and Ye, 2007). It can be simply known as a layer of graphite rolled-up into a cylinder. CNTs have advantages over their small size, great strength, good electrical and thermal conductivity and high specific surface area (Sinha *et al*., 2006). Two types of CNT which are single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs) have excellent mechanical and conductive properties for producing ultrasensitive biosensors. Their conductive properties are due to high actuating stresses, low driving voltages and high energy densities (Veetil and Ye, 2007). MWCNTs are made up of several concentric cylinders of graphite sheets, with about 3.4 Å gaps in the middle of the layers and commonly have 2 to 100 nm diameter, larger compared to the SWCNTs which are usually about 0.2 to 2 nm in diameter.

Furthermore, CNTs need to be functionalized prior to application in biosensor due to limited solubility in aqueous media. Therefore, solubilization and functionalization will modify the surface of nanotube by changing it from hydrophobic to hydrophilic which may be done by chemical, electrochemical, thermal, plasma oxidation treatment, adsorption, electrostatic interaction or covalent bonding of different molecules (Akiladevi and Basak, 2010; Huang *et al*., 2004). Although treatment of CNTs with nitric acid and sulphuric acid mixtures under

Table 1. Development of various type of biosensor with nanomaterial based for food analysis

Electrode	Nanomaterial	Analyte	LOD	References
Micro-comb	AET/AuNP	Aflatoxin B_1	0.10 ng mL^{-1}	Liu *et al.*, 2006
ITO	CH/TiO2	Ochratoxin A	10 ng mL^{-1}	Khan and Dhayal, 2008
ITO	CH/AuNP	Cholestrol	-	Gomathi *et al.*, 2011
Glassy carbon	Nafion/RTIL/TiO2/AuNP	Aflatoxin B_1	-	Sun *et al.*, 2008
ITO	CH/CeO2	Mycotoxin	0.25 ng dL^{-1}	Kaushik *et al.*, 2009
Screen Printed Carbon	Superparamagnetic nanoparticles	Aflatoxin M_1	0.25 µg L^{-1}	Paniel *et al.*, 2010
Glassy carbon	CH/AuNP	Aflatoxin B_1	0.20 ng mL^{-1}	Masoomi *et al.*, 2013
Glassy carbon	MWCNT/Nafion	*Escherichia coli*	10 – 104 cfu mL^{-1}	Cheng *et al.*, 2008
FET	SWCNT	*Salmonella infantis*	100 cfu mL^{-1}	Villamizar *et al.*, 2008
Glassy carbon	Bismuth nano-film (BiNF)	*E. coli*	100 cfu mL^{-1}	Zhang *et al.*, 2007
Slide glass printed with graphite paste	Peptide nanotubes (PNTs)	*E. coli*	-	Cho *et al.*, 2008
Screen printed electrode	A composite of agarose doped gold nanoparticles	*Vibrio parahaemolyticus*	7.374 × 10^4 cfu mL^{-1}	Zhao *et al.*, 2007
Gold electrode	MWCNT	Sterigmatocystin	0.13 µM	Yao *et al.*, 2006
Glassy carbon	Au nanoparticles	Xanthine and hypoxanthine	-	Cubukcu *et al.*, 2007
Glassy carbon	MWCNT/ Polyvinyl Sulfonic Acid	Folic acid	-	Unnikrishnan *et al.*, 2011
Glassy carbon	Platinum nanoparticles/N-GSS	Glucose	1 µM	Meng *et al.*, 2015

SWCNT: Single-walled carbon nanotube ITO: Indium-tin-oxide CH: Chitosan
MWCNT: Multi-walled carbon nanotube AET: 2-aminoethane thiol FET: Field-effect transistor
TiO_2: Titanium dioxide nanoparticles RTIL: Room temperature ionic liquid AuNP: Gold Nanoparticle
CeO_2: Cerium oxide nanaoparticles N-GSS: Nitrogen doped graphene nanoscrolls

oxidative conditions will allow the end and tips to open and functional groups like carboxylic acid will be added, however, this acid treatment normally will shorten the tube length and subsequently lessens the high aspect ratio of SWCNTs (Veetil and Ye, 2007).

Lin *et al.* (2004) utilized carbon nanotube (CNT) nanoelectrode ensembles (NEEs) for the development of selective glucose biosensors. Immobilization via carbodiimide chemistry of glucose oxidase enzyme on CNT NEEs was successful done by formation of amide linkages between their amine residues and carboxylic acid groups on the CNT tips. The selective detection of glucose is contributed by the catalytic reduction of hydrogen peroxide from the enzymatic reaction of glucose oxidase upon the glucose and oxygen on CNT NEEs.

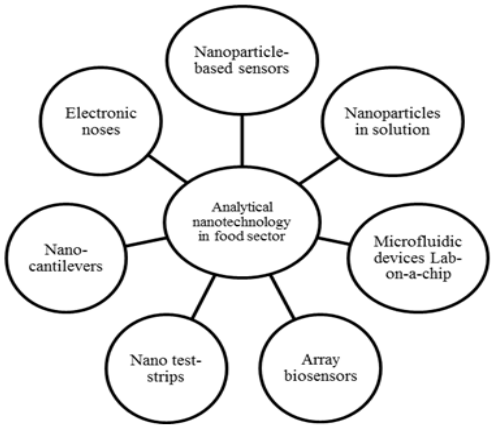

Figure 3. Nano-based analytical approaches. (Valdes *et al.*, 2009)

Application of nanotechnology in food analysis

The integral characteristic of biosensors mainly their selectivity, specificity and adaptability, produce an excellent and suitable detection method which can be applied in food industry especially in food safety and quality control. Furthermore, possible usage of biosensor includes confirmation of pesticide residue, analysis of analyte concentrations such as glucose, sucrose, alcohol, cholesterol, toxin, bacteria and others which are important in food quality and acceptability. According to Serna-cock and Perenguez-verdugo (2011), biosensors have been adapted for the assessment of food quality and composition, predominantly in plant and animal food products which may be affected during postharvest and processing. Moreover, the sensory properties and chemical composition of food are vastly depend on factors such as suitable handling practice, optimal conditions and temperature of storage, exposure to sunlight and oxygen level. Improper management of these factors will cause undesirable changes to the food. The integration of nanotechnology in food safety and quality control is summarised in Figure 3 and Table 1. Chen *et al*. (2014) postulated that nanoscale sensors will be extremely important tools for food quality and safety revolution in the next 5 to 10 years. Furthermore, the authorities should take action to guarantee confidence in the delivery of safer food to consumers and to improve food safety in the

supply chain.

Conclusion

Increasing demands for food require the woe of innovative technologies to ensure the safety of food from farm to plate. Therefore, the applications of nanosensor for food hazards should be further explored. It can be referred to self-contained measurement elements, which are capable of detecting specific chemical compounds in biological samples. Nanomaterials incorporated into various biosensors can play a vital role in assuring food safety in producing a rapid and sensitive detection method due to their excellent properties. Ultimately, the detection procedures are made simpler and easier especially for on-site performance with minimal cost and expertise.

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