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#### **Review Article**

## Nanomaterial-based sensors for detection of foodborne bacterial pathogens and toxins as well as pork adulteration in meat products



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#### ABSTRACT

Food safety draws considerable attention in the modern pace of the world owing to rapidchanging food recipes and food habits. Foodborne illnesses associated with pathogens, toxins, and other contaminants pose serious threat to human health. Besides, a large amount of money is spent on both analyses and control measures, which causes significant loss to the food industry. Conventional detection methods for bacterial pathogens and toxins are time consuming and laborious, requiring certain sophisticated instruments and trained personnel. In recent years, nanotechnology has emerged as a promising field for solving food safety issues in terms of detecting contaminants, enabling controlled release of preservatives to extend the shelf life of foods, and improving food-packaging strategies. Nanomaterials including metal oxide and metal nanoparticles, carbon nanotubes, and quantum dots are gaining a prominent role in the design of sensors and biosensors for food analysis. In this review, various nanomaterial-based sensors reported in the literature for detection of several foodborne bacterial pathogens and toxins are summarized highlighting their principles, advantages, and limitations in terms of simplicity, sensitivity, and multiplexing capability. In addition, the application through a noncross-linking method without the need for any surface modification is also presented for detection of pork adulteration in meat products.

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#### 1. Introduction

Foodborne diseases are caused by consuming foods or beverages contaminated by bacteria, viruses, and parasites. The worldwide statistics on foodborne diseases published for

2011—2012 by the Centers for Disease Control and Prevention reported a total of 1632 outbreaks, 29,112 affected patients, 1750 hospitalizations, and 68 deaths [1]. Some of the various bacterial pathogens that cause foodborne diseases and eventual death are Salmonella (31%), Listeria (28%), Campylobacter (5%), and Escherichia coli O157:H7 (3%) [1,2]. Likewise, the trend

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of foodborne disease outbreaks in Taiwan between 1991 and 2010 reported by the Food and Drug Administration of Department of Health in Taiwan indicated 4284 outbreaks and 82,342 cases, with annual average number of 285 outbreaks during 2001–2010 being substantially greater than that of 143 during 1991–2000 [3]. The three most common foodborne pathogens responsible for these outbreaks in Taiwan include Vibrio parahaemolyticus, Staphylococcus aureus, and Bacillus cereus [3]. The spread of foodborne disease due to pathogens and toxins causes a substantial loss to the food industry because a large amount of money will be spent on analyzing and identifying preventive measures for food protection [4,5]. Thus, the development of a rapid, sensitive, specific, and cost-effective analytical method is of great importance for detection of microbial contaminants.

Conventional methods for detecting pathogens include microscopy-, nucleic acid-, and immunoassay-based techniques. The microscopy-based methods require a large amount of sample, long incubation time, and tedious culture preparations [6]. However, the discovery of DNA and development of polymerase chain reaction (PCR) have led microbiologists to target genes and proteins instead of the microorganism itself. Although the PCR-based techniques and several other molecular diagnostic methods such as rapid-PCR, ligand chain reaction, checkerboard hybridization, ligase chain reaction, ribotyping, and pulsed-field gel electrophoresis are highly sensitive and selective, they require undamaged DNA, experienced personnel, and expensive equipment as well as reagents, thus making the overall cost of detection high enough to prevent wide-scale application, especially in developing nations and point-of-care scenario [6,7]. The immunoassays involving targeting of specific proteins or carbohydrate moieties to pathogens include enzymelinked immunosorbent assays (ELISAs) and Western blot analyses, both of which are sensitive and can provide molecular fingerprints of the pathogen. Despite their sensitivity, both ELISA and PCR require extensive sample preparation and long readout time, which can delay the pathogen detection and immediate preventive action toward the infected patients [4,6-8].

Similarly, the toxins secreted by bacteria can induce cytotoxicity by altering the physiological activity and integrity of the plasma membrane [6,7,9]. For example, the Shiga toxin secreted by E. coli O157:H7 can inhibit protein synthesis and activate apoptosis and necrosis, whereas listeriolysin O secreted by Listeria monocytogenes can create pores for subsequent disruption of the phospholipid bilayer and eventual cell lysis [6,7,9,10]. Although toxins are not usually transmitted through infected individuals, they are able to cause significant devastating effects on organs and tissues. Besides, the toxins can remain in the food, environmental, and clinical samples even after the death of their corresponding pathogens. Therefore, prompt screening of toxins is highly essential to minimize intoxication. Similar to pathogens, the existing detection methods for toxins include ELISA, Western blots, surface plasmon resonance (SPR) biosensors, antibody microarrays, and antibody-coated polystyrene microbeads, all of which are sensitive and possess multiplexing capability [2,4-7,9]. However, these methods are time consuming and laborious, besides requiring homogeneous or purified

samples. Furthermore, as these methods are performed in the fluorometric or spectrophotometric mode, the number of samples screened can be limited [2,4–7,9]. Nevertheless, these limitations can be overcome by some other toxin detection techniques such as liquid chromatography-mass spectrometry and multidimensional protein identification [6]. Yet, the requirement of sophisticated instrumentation as well as lack of portability and user friendliness still limits their wide-scale application. Thus, the development of an advanced detection method with nanomaterials as a platform is crucial.

Meatball is a special type of restructured and pulverized meat product, which is popular in many Asian and European countries [11,12]. Pork has been identified as a potential adulterant in beef and chicken meatballs because of cheaper cost [11,13]. Moreover, from the religious and health point of view, the mixing of pork or pork-related products in food raises serious concerns due to violation of Kosher and Halal food laws [12–14]. In addition, consumption of food products with pork adulteration has been reported to cause allergic reactions [10-12], and consumption of these foods at high levels can cause accumulation of cholesterol and saturated fats in the human body, resulting in chronic diseases such as diabetes and cardiovascular disease [11-14]. Thus, the development of a sensitive and selective analytical method is imperative for detection of pork adulteration in meatball preparations.

Recent developments in the field of nanotechnology offer many technological advances for detection of foodborne pathogens and toxins as well as adulteration among meat formulations [2,4-6,9,11-14]. However, most of the published articles have mainly dealt with the theranostic application of nanomaterials for cancer detection and treatment [15]. Owing to the presence of unique properties in nanoscale materials, the sensing devices can be designed to enhance sensitivity, reduce detection time, and enable multiplexing capability [16-18]. Compared with their bulk counterparts, the nanosized materials (1-100 nm) possess large surface area, exhibit quantum confinement effects, as well as enhance surface reactivity, electrical conductivity, and magnetic properties [6,18]. Most importantly, the properties of nanomaterials can be tailored by changing the size, shape, composition, and modifying the nanomaterial surface with appropriate functionalization. In view of this, the electronic, spectroscopic, light-scattering, and conductive properties can be modified by engineering the structural parameters of nanomaterials including size, composition, self-assembling, and binding [2-6,16-18]. In addition, the groundbreaking developments in surface patterning techniques have paved the way for generating nanoscale arrays for pathogen-targeting ligands, which can drastically improve the accuracy of analytical techniques associated with detection of food-related toxins [6,16,17]. In addition, the application of nanoparticles as sensors in conjugation with affinity ligands, antibodies, as well as the existing novel detection techniques has led to improved sensitivity for simultaneous detection of multiple toxins [2,4-6,16-18]. Several nanomaterials commonly used for detection of foodborne pathogens and toxins include gold nanoparticles (GNPs), gold nanorods, magnetic nanoparticles (MNPs), quantum dots (QDs), silver nanoparticles (SNPs), and silica nanoparticles [2,4,5]. Detection of foodborne pathogens

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