Review Article

Recent Advances in Carbon Nanotube Devices for the Application in Food Allergen Detection

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ABSTRACT

Carbon nanotubes are cylindrical shaped structures comprising single or multi layers of graphene sheets which have distinct characteristics of high conductivity, high flexibility, and high stability across a range of scenarios. These carbon nanotube properties provide distinct sensitivity and surface area to manufacture extremely efficient biosensors. Carbon nanotube biosensors detect low concentrations of a target analyte with the aid of specific antibodies or biomolecules immobilized on the carbon nanotube surface, allowing for rapid and accurate diagnostic results. Carbon nanotube-based biosensors have been studied to detect allergens due to the rapid and accurate results provided for identifying allergenic substances in many contexts, including food safety and prevention of subject exposure to allergenic substances, which could potentially trigger allergic reactions. This review will focus on the applications of carbon nanotubes in the development of sensitive biosensors for peanut, wheat, lysozyme, and kidney bean allergens, and will summarize the performance of each biosensor as well as their limitations and promising future applications of carbon nanotubes for allergen detection.

Keywords: Biosensors, Carbon-Nanotubes, Allergens

INTRODUCTION

Food allergies are a significant concern that adversely affects the lives of a large and growing population. It is reported that approximately 8% of children in the United States possess food allergies (1). Food allergens such as specific proteins or antigens can trigger hypersensitivity syndrome, which can cause hyperimmune reactions resulting in adverse health effects and even death (2). Food industries have attempted to prevent these reactions by implementing thorough manufacturing practices to control hazards and ensure critical points. Despite these cautions, cases of food allergen contamination have still increased annually (3). This growing number of contamination cases has created an increasing demand for biosensors that can reliably detect food pollutants in real time to ensure the safety of processed foods. The Market for biosensors was $27.5B USD in 2021 and is projected to expand to $49.6B USD by 2030 (4).

Biosensors detect biological analytes such as metabolites, DNA, viruses, and microorganisms (5) and have applications in many fields, such as medical, environmental, and food safety. A biosensor consists of

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Received April 27, 2025; **Accepted** May 26, 2025
https://doi.org/10.70251/HYJR2348.336671

a template that forms the sensor shape, a sensor receptor that contains the biomaterials, and a signal converter that relays the binding of biomaterials into an easy-to-understand signal. The success of a biosensor depends on its ability to effectively convert biomaterial binding to an easily interpretable signal. In this regard, choosing a proper bioreceptor and receptor transducer device is essential for making a successful biosensor.

Electrochemical biosensors convert biological signals into electrochemical signals. These biosensors are widely studied and utilized in multiple applications owing to their excellent selectivity, high sensitivity, and simultaneous detection capability in the reaction between target materials and biosensor receptors. Carbon is viewed as a potential electrical material because it is inexpensive and has biofriendly properties. Carbon nanotubes (CNTs) are cylinders in which single or multiple graphene sheets are rolled up to form a hexagonal tube (Figure 1). CNTs are composed of either single-walled carbon nanotubes (SWCNTs) or multi-walled carbon nanotubes (MWCNTs), with SWCNTs having a higher thermal conductivity, a smaller diameter, and a higher current transfer capacity (6). Due to their difference in diameter, SWCNTs are applied to smaller semiconductors, while MWCNTs are applied to larger semiconductors. Each carbon atom in a CNT has a lone pair of electrons, allowing CNTs to transfer electrons derived from an electrical signal as a response by the sensor system. CNTs are typically applied as electrodes in biosensors or used as electron transfer mediators (7). Two electrodes in a biosensor are connected by CNTs immobilized onto the sensor device either through drop casting or dielectrophoresis methods that supply voltage to the anode (8).

The high sensitivity of CNT allows them to be used in devices that detect small amounts of analytes. These sensors relay their signals in four ways: electrochemical, optical, semiconductor, and calorimetry (9). Among these, electrochemical signals are the most widely used due to their excellent sensor capacity, small size, low manufacturing cost, and wide detection range (9). Since CNTs can detect small amounts of harmful microorganisms and food allergens, much work has been done to apply such devices in the food safety industry. Specifically, CNT based electrochemical biosensors have label free high sensitivity detection owing to its high electrical conductivity (10).

The general structure of CNT-based biosensors include CNTs acting as a link between a bioanalytical receptor, and sensor for electrochemical signals (Figure 2). CNT-based biosensors require a particular receptor assembly process to incorporate sensitive receptors such as enzymes and DNA/RNA fragments (5). CNTs can be functionalized by either covalent modification or by adsorption to the CNT hydrophobic surface through van der Waals interactions. CNT functionalization using a protein as a biosensor acceptor has special considerations to maintain the integrity of the three-dimensional structure of the enzyme. The structure of the enzyme immobilized on CNT reacts very sensitively to temperature, ionic strength, pH, and substrate concentration. Optimal temperature and pH must be maintained in a sensor reaction due to the protein structure.

CNT-based biosensors to detect allergens have made noticeable progress in recent years. Many of the devices

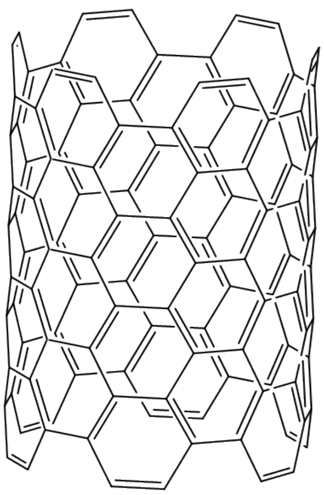

Figure 1. Scheme of single-walled carbon nanotube with positions of carbon atoms and cylindrical assembly.

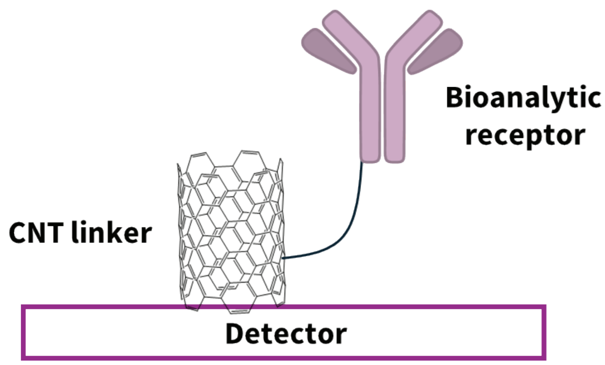

Figure 2. General schematic of CNT-based biosensor with CNT utilized as a link between bioanalytic receptor and signal detector.

developed have extremely low detection limits, showing promise to be applicable in a commercial setting. In this review, we will discuss the devices created to detect allergens from wheat, lysozymes, peanuts, and kidney beans.

WHEAT

Wheat gluten allergies are known to affect 1% of the population (11), with the main allergen component of gluten being the gliadin protein (12). To tackle this issue, researchers have employed electrochemical cell sensors, a new type of biosensor that takes advantage of the rapid developments in microelectronics (13). Construction of these sensors using living cells has been widely used for detection in the food safety industries. Recent developments have allowed researchers to utilize 3D printing when constructing electrochemical cell sensors. 3D printing has the potential of being able to prepare hundreds of cell sensors quickly and accurately eliminating human error and decreasing the cost of production (14).

Allergic reactions occur in vivo when the mast cells are attached to the free surface of tissue and organ degranulation (15). After being ingested and digested, allergen proteins first enter the intestine for absorption, so the small intestine is the primary organ for allergic reactions. To mimic this surface structure, 3D printing technology was used to prepare a small intestinal structure of microvillus.

Researchers developed a novel electrochemical biosensor utilizing 3D printed microvillus with a mast cell sensor to detect the presence of wheat gliadin (16). The 3D-bioprinted microvillus structure was able to emulate the small intestine to allow for gliadin antibody-sensitized mast cells to adhere to the structure. To improve sensitivity, self-assembled flower-like copper oxide nanosheets (FCONp) and Hydrazide functionalized MWCNTs (MWCNT-CDH) were dispersed in the 3D printed hydrogel to provide conductivity and improve cell detection and adhesion. Experiments showed that the method yielded a sensor in a good linear concentration range with excellent stability and reproducibility with an LOD of 0.036 ng/ml and a range of 0.1-0.8ng/mL while allowing for high throughput preparation.

LYSOZYME

Lysozymes are enzymes that are found in many natural substances, with the most common being egg whites of hens. Around 31% of children with food allergies are allergic to eggs (17). While most allergic reactions to egg whites aren't anaphylaxis, egg allergies still cause rashes, hives, nasal congestion, and vomiting.

Inkjet printing has gained prevalence in areas such as disposable sensors and flexible electronics. The on-demand printability of the devices creates potential for disposable testing kits and POC diagnostic tools. Researchers created a biosensor for detecting lysosomes through inkjet printing with a DNA aptamer and CNT mixture (18). The strong binding affinity of single-stranded DNA onto SWCNT through π-π stacking allowed researchers to immobilize aptamers onto an electrode through inkjet printing. After the ink was deposited onto the electrode, the researchers used electrochemical impedance spectroscopy to detect lysosomes. The biosensor was highly sensitive, with a LOD of 90 ng/mL, range of 2 fmol/L - 10 nmol/L, reproducible, had a rapid response to lysosomes and can be used as a low-cost flexible disposable sensor for lysosomes.

The bio-sensor takes advantage of aptamers which are selected through a process known as Systematic Evolution of Ligands by Exponential enrichment (SELEX). These aptamers are used for the selective detection of a broad range of analytes including proteins, peptides, amino acids, drugs, metal ions, and even whole cells (19). They are versatile in biosensing due to the high affinity and efficiency to the target analyte, chemical and thermal stability, resistance to harsh environmental conditions, long shelf-life, mass producibility at low-cost, and reusability.

PEANUT

The peanut protein Arachis hypogaea 6 (Ara h 6) is one of the most serious food allergens that contributes to food-related, life-threatening problems worldwide (20). Around 1 in every 50 adults possess varying degrees of a peanut allergy. For some, the faintest trace of a peanut can cause a hypersensitivity anaphylaxis reaction (21). Previously, researchers had used ELISA as a means to detect peanut traces in food, however, this method is expensive and not reusable (22). Recently, biosensors have been seen as a more effective way to detect ara h 6 (peanut traces) due to their ability to integrate molecular biology and information technology into the sensor. Researchers began developing a new biosensor for Ara h 6 using SWCNT as a bridge between gold wafers and the antibodies PBASE and pAB which are capable of detecting the Ara h 6 protein (23). The biosensor takes advantage of π-π stacking interactions and the adsorption of PBASE onto the SWCNT to manufacture the sensor. This new biosensor had a LOD of 10 pg/ml, a range of 1-1000 ng/mL, and the biosensor values intensified when placed in the presence of food containing peanut traces. This new sensor is a cheaper, reproducible alternative to the former ELISA method.

When designing biosensors as alternatives for the ELISA method, researchers faced challenges determining the ideal concentration of SWCNTs to maximize electrical conductivity. In order to determine ideal concentration, the resistance of the biosensor was studied for differing concentrations of SWCNTs on the biosensor. Researchers found resistance to increase substantially when the concentration was lower than 0.5 g/L, and that there was no significant detectable difference between 1 g/L and 5 g/L concentrations of SWCNT. Based on this, researchers determined 1 g/L to be the optimal concentration for electrical conductivity within the biosensor.

KIDNEY BEAN LECTINS

Over the past decades, high culinary and industrial uses of kidney beans (Phaseolus vulgaris) have raised concerns to human health and wellness due to their potential toxic and allergenic reactions induced by the presence of lectins (He *et al*., y2018; Sun *et al*., 2019). Kidney bean lectins (KBLs) are the sugar-binding proteins that bind to the surface of epithelial cells to induce gastroenteritis, nausea, diarrhea, and even fatal reactions (Miyake *et al*., 2007; Vasconcelos and Oliveira, 2004). Although the safety guidelines have been recommended regarding kidney beans consumption (Kumar *et al*., 2013), fewer methods could be used for the rapid quantification of lectin in kidney bean-derived foodstuffs, which would be in detriment to food consumption safety.

A newly-designed label-free voltammetric immunosensor combined with one-pot synthesized AuNPs-PEI-MWCNTs nanocomposite and Ab immobilization was successfully developed for KBL detection (24). Compared to sugar-mediated sensors and ELISA, this new approach provided a time-saving, sensitive, and allergenicity-recognizable detection for lectin regarding the safety consumption of kidney beans-derived foodstuffs with a wider linear range of 0.05–100 μg/mL and low LOD of 0.023 μg/mL, as well as good selectivity and interference-resistant ability, acceptable reproducibility, and excellent stability.

Researchers faced challenges during the development of the novel immunosensor involving large van der Waals interactions present when submerging MWCNTs in water. These forces resulted in large groups of MWCNTs forming in aqueous environments preventing their further applications towards biosensors. To address the forces, scientists employed polyelectrolytes, molecules possessing extraordinary water solubility, to serve as dispersers and stabilizers. Specifically, researchers utilized polyethyleneimine (PEI), to prevent MWCNTs from aggregating in water.

CHALLENGES WITHIN IMPLEMENTATION

CNT-based biosensors face significant challenges within real world implementations. The environment necessary for proper CNT and receptor function lead to decreased shelf life. Inkjet lysosome biosensors showed a shelf life of only 21 days at room temperature, although researchers note that shelf life would increase given a colder environment (18). Additionally, while cheaper as an alternative to traditional detection methods such as ELISA (23), the synthesis of CNTs as well as CNT-based biosensors is time consuming and under current conditions, still too expensive for mass production (25). These factors inhibit the full scale implementation of CNTs within the field of biosensing, however, with the novel methods discussed within this review, the eventual full implementation of CNT-based biosensors shows great promise.

CONCLUSION

Overall, carbon nanotubes' high sensitivity and ability to bond with bio receptors have led to the creation of new types of biosensors specifically for allergen detection. Researchers have successfully applied CNTs to create novel biosensors that are cheaper, faster, and more accurate than older biosensors to detect Wheat, Peanut, Lysosome, and KBL allergens. Shown below is a summary of the biosensors discussed within this review along with their accompanying detection mechanisms, LOD, and ranges (Table 1). Gliadin (Wheat) biosensors utilized inkjet printing techniques simulating the small intestine to achieve an LOD of 3.6ng/mL. Ara h 6 (Peanut) was detected with a pAB antibody immobilized to a SWCNT that relayed its signal to a bridge gold wafer and achieved an LOD of 10 pg/mL. Lysosome biosensors used Dna fragments, or aptamers, combined with inkjet printing onto an electrode to achieve a 1.9 fmol/L LOD. One-pot synthesized AuNPs-PEI-MWCNTs nanocomposite and Ab immobilization were used to detect KBL with an LOD of 0.023 μg/mL. CNT-enabled devices show

Table 1. Methods of Detection, LOD, and Range of allergen biosensors that employ CNTs

Analyte	Method Employed	LOD	Range
Gliadin	3D Printing	0.036 ng/ml	0.1-0.8 ng/mL
Lysozymes	Inkjet Printing	90 ng/mL	20 fmo/L-10 nmol/L
Ara h6	SWCNTs linked with Antibodies	10 pg/ml	1-1,000 ng/mL
KBL	Voltammetric Immunosensor	0.023 μg/mL	0.05-100 μg/mL

promise in the allergen detection field, with impressive new developments and advancements coming frequently.

ACKNOWLEDGEMENTS

I would like to graciously acknowledge Dr. Hunter Batchelder for his advice and guidance as a mentor throughout the process of writing this review.

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