Detection of Microorganisms Using Graphene-Based Nanobiosensors

Running title: Graphene as Nanobiosensor

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SUMMARY

Having an insight into graphene and graphene derivatives such as graphene oxide, reduced graphene oxide, and graphene quantum dots structures is necessary since it can help scientists to suspect the possible properties and features that using these carbon materials in preparation of a nanocomposite could bring out. In recent years, graphene and its derivatives are attractive with extensive applications in biosensors due to fascinating properties, such as high surface area, optical and magnetic properties, and high elasticity for the detection of microorganisms can be modified with some other materials such as macromolecules, oxide metals, and metals to improve the electrochemical behavior of the biosensor, and also can be modified with some other materials such as macromolecules, oxide metals, and metals to improve the electrochemical behavior of the biosensor.

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In this review paper, biosensors design strategies based on graphene and its derivatives (graphene-based nanocomposites in biosensors) are introduced. Then their application for the detection of microorganisms including Prions, Viroids, viral and bacterial cells as well as fungi, protozoa, microbial toxins, and even microbial-derived antibiotics are reviewed.

Key words: graphene, graphene oxide, reduced graphene oxide, graphene quantum dots, microorganism detection, nanobiosensors

INTRODUCTION

Graphene is a mono-layered carbons atoms (Fig. 1), arranged in a honeycomb lattice. each of these carbons participates in three intralayer sp² or sigma (σ) bonds with its three neighbor carbon atoms (1). Although the intrinsic strength of a graphene layer is in consequence of these bonds, which are known as covalent bonds, this strength is still limited by the presence of defects and grain boundaries (2). In addition to mono-layer graphene Bi-, few- and multi-layer graphene are possible as well. 1 to <10 layer graphene is distinguished as a 2D crystal while a structure consists of a higher number of graphene layers is considered as a 3D thin film (3). Interlayer pi (π) bonds that are involved both between two graphene layers and between graphene and other molecules are usually weaker than sigma bonds and are responsible for electrical and thermal conductivities and functional group attachments (4). Graphene oxide (GO) is a nanomaterial from the graphene family that is obtained by the chemical peeling of graphite using strong oxidizing materials (5). GO can be modified with some other materials such as macromolecules, oxide metals, and metals to improve the electrochemical behavior of the biosensor (6). Recently, graphene-based nanocomposites in sensitive sensors have received more attention (7). Functional groups such as hydroxyl and epoxy, are present in the base plate, as well as carboxyl, carbonyl, and phenol at the GO edge. Compared to graphene, GO shows different optical, electrical, and electrochemical behavior due to its oxygen-containing structure due to oxidation (8). This carbon nanomaterial has been considered as a promised material in biotechnology (6). FTIR analysis has much information about the structure of GO shows an absorption band at 3360 and 1040 cm⁻¹ correspond to OH and C-O groups, respectively. Furthermore, an absorption peak at 1710 cm⁻¹ is related to C=O functional groups that can react with other biomaterials' functional groups such as aptamer chains. By reduction of GO sheets and C=O groups, the electronic structure of carbon atoms also changes from SP³ to SP², and therefore the magnitude of C=C bonds increased (9). In this review paper, we focus on the
biosensors design strategies based on graphene and its derivatives due to their attractive properties (graphene-based nanocomposites in biosensors) for microorganisms Detection by different strategies.

Fig. 1.

GRAPHENE-BASED NANOCOMPOSITES IN BIOSENSORS

Fascinating properties of graphene, such as high surface area, optical and magnetic properties, and high elasticity, make it an appropriate basic structure for preparing graphene-based nanocomposites (10). Depending on the number of graphene layers, the absence or presence of defects, the materials which are in combination with graphene, and what kind of assembly methods are used, several nanocomposites with different features, electrochemical properties, and applications have been prepared (11).

Normally graphene tends to agglomerate through van der Waals and π-π stacking bonds, so various methods have been proposed to solve this problem (12). It has recently been shown that hybridizing metal nanoparticles with graphene plates is electrically conductive and improves the heat of graphene. Hybridization also prevents aggregation by creating gaps between graphene plates (13). Gold nanoparticles with unique properties have a great potential to hybridize with graphene and create a new structure with many applications in electrochemistry (14). In electrochemical sensors, gold nanoparticles can increase be sensitive to the sensor (15). Nanoparticles play the role of catalyst in electron transfer between the analyte and the electrode surface (16). On the other hand, graphene itself plays an important role in increasing the speed of electron transfer. The presence of oxygen groups on graphene layers has a great effect on the adsorption and surface desorption of chemical reaction products from the surface of graphene electrodes (17). Adsorbed products often slow down the electrochemical reaction for highly sensitive compounds to oxygenated groups (18). The graphene oxide layer, which has oxide edges, is placed vertically or obliquely between the electrode surface and the active center of the biomarker, without the protein changing its structure (19). Graphene oxide binds biosensor depth to the electrode, Studies have shown that gold graphene nanohybrid biosensor biosensor increases biocompatibility and measurement sensitivity (20). Some of these nanocomposites are described in this review. using other carbon nanomaterials in composition with graphene is a good way to increase its novel properties due to their synergistic effects and make the new more sufficient composite in comparison to each of the carbon nanomaterials separately more sufficient composite in comparison to each of the carbon nanomaterials separately. These properties are including electrochemical activity, electrical conductivity, large surface
area, ease of functionalization, and biocompatibility (21). Variation in structure and compatibility in chemical properties make different carbon nanomaterials such as graphene, carbon nanotubes, fullerene, nanodiamonds, etc. appropriate hybrid partners for each other to form different possibilities of bindings to various recognition agents in a biosensor system (22). An example of this effective synergistic cooperation is shown by Wu (23) in which the effect of the weight ratio of GO to CNT on tensile strength of PEC (polyelectrolyte complex) membranes at 3% by mass filler loadings, 0:0, 0:1, 1:0 refers to PEC, PEC/CNT, and PEC/GO, respectively, that approved the hybridization of GO and CNT has improved the tensile strength of the initial nanocomposite membrane two times higher than each of them.

Metal nanoparticles such as Au-, Pt-, Pd-, Ag- and Li-nanoparticles as well as their oxide and sulfide compounds, are highly used in combination with graphene to form a favorable nanocomposite for use in different types of biosensors. Due to their free electrons, metal nanoparticles can absorb visible and ultraviolet light, and therefore are applicative in many optical biosensors using surface plasmon resonance effect (24). The adequate catalytic properties of metal nanoparticles make them ideal for electrochemical biosensors (25). On the other hand, high surface area and high mechanical strength, electrostatic adsorption of biomolecules are general properties of metal nanoparticles that are beneficial to the biosensor structure (26). As Govindhan (27) reported, Au/RGO/ glassy carbon electrode(GCE) makes a more significant anodic peak in the cyclic voltammograms compared to both Au/GCE and RGO/GCE, approving that these two have a better electrochemical feature in collaboration with each other.

After designing an efficient scaffold, there have to be agents to recognize the target microorganism or its product. Choosing an appropriate agent is of high importance since it has direct influences on the results of all evaluation criteria of a biosensor such as the limit of detection (LOD), the linear range of detection, detection time, selectivity, reliability, and reproducibility. Biorecognition elements provides specificity, selective and strong affinity to the targets (28). They may be natural, such as enzymes, antibodies, and nucleic acids, or pseudo-natural, such as aptamer, or synthetic, such as molecularly printed polymers (MIPs). Nucleic acids, peptides, proteins, antibodies, and phages are more or less used as biorecognition elements to detect microorganisms or their products. Criteria of selection of the type of recognition element and methods of their immobilization on graphene could offer ideas to be used in the manufacture of other biosensors with different target elements as well (29). DNA-Graphene hybrids are mainly manufactured by ultrasonication-driven self-assembly process (30). These DNA molecules are supposed to match a certain sequence of the organism's genome specifically. Zhang (31)
has manufactured a graphene-pyrenebutyric acid nanocomposite by ultrasonication and covalently immobilized amino-modified oligonucleotides on the nanocomposite through linkage with carboxylic groups of PBA.

A bacteriophage is a virus that recognizes its specific receptors on bacteria and archaea. Phages can infect these cells and, through different steps, replicate themselves within them. With the aid of immobilized peptides or proteins on their surface, phages can bind to a vast range of molecules. Bhardwaj (32) has been succeeded to covalently immobilize bacteriophages specific to bacteria *Staphylococcus arlettae* on a pre-carboxylated graphene surface amid binds between these carboxyl groups on graphene and –NH₂ groups on the bacteriophages head. In another way, bacteriophages are useful in the phage display method to carry a certain gene and represent the peptide belong to this gene on their surface. This method helps to find the correlation between specific genotypes and their unknown phenotypes plus finding peptides that can bind with a particular target and could be used as recognition elements in biosensors (33). aptamers and different types of receptors, such as enzymes and antibodies, and other biorecognition elements can be immobilized through covalent and non-covalent bonds (28). Immobilization is done either chemically or physically by either interacting or trapping receptors. Immobilization is one of the most difficult steps in designing a sensor. The choice of the appropriate method for immobilization depends on the nature and physicochemical conditions of the transducers and receptors (29). Entrapment, microencapsulation, sol-gel technique and adsorption belong to physical immobilization and is mostly used for sensors that have enzyme receptors. Another method is chemical immobilization, which is usually based on creating a chemical bond between the functional groups on the surface of the transducers and the receptors (34). It usually occurs through cross-linking chemical reagents such as glyoxal, hexamethylenediamine, glutaraldehyde, carbodiimide, etc. Cross-linking is part of the covalent binding that is usually accomplished by activating amine and carboxyl functional groups, which results in strong, highly stable, and effective binding. pure graphene, as mentioned, can prepare a charged region for the adsorption of any charged molecules or metal ions as an interaction in an empty defect. graphene derivatives are synthesized by their oxide components due to the synthesis of large amounts of epoxy, hydroxyl, and carboxyl groups at the edges and surfaces. The active region (functionalized region) of graphene is able to directly binding to heteroatoms, nanoparticles (NPS), enzymes, antigens, proteins, antibodies, DNA, and other specific molecules (28). Graphene can also increase sensitivity and LOD of biosensor device by improving the charge or electron transfer between graphene and biomolecules due to its extraordinary properties (35).
DETECTION OF MICROORGANISMS

The direct and indirect effects of microorganisms and their products on human health are of great concern for both governments and, societies globally. Since microorganisms are spread in many areas such as air, water, soil, foods, plants, and animal bodies and considering that many of these organisms' existences or a certain amount of concentrations are beneficial or even vital for human life, it is necessary to distinguish harmful microorganisms from safe ones and determine the concentration of them in different types of given samples. Today, many fields of research, such as environment, food safety, and health care, are working on developing new methods and more efficient devices for such a purpose. Among all, the design and fabrication of more cost-effective biosensors with better selectivity, sensibilities, and stabilities are of particular importance. As a consequence of graphene's excellent properties, which have been discussed formerly in this article, it is evident that this nanostructure is a great candidate for being used in the field of biosensing. Following is a review of graphene-based biosensors for the detection of each group of microorganisms and microbial products.

DETECTION OF PRIONS

Prions are misfolded proteins that can cause several neurogenic diseases in humans and other animals. The main reason for the misfolding of the structure of proteins and their conversion to prions is not clear. But this abnormal three-dimensional structure causes infections, protein diseases, and protein collapses (36). Prions caused by the aggregation of abnormal proteins are called amyloids, which are the main cause of diseases such as Alzheimer's and Parkinson's (37). A GO-based fluorescent biosensor for the selective measuring of amyloid-b oligomers concentration indicated by Lin Liu et al. (Fig. 2) (38). Jing Zhao et al. developed a complex of graphene oxide-gold nanoparticle-aptamer for amyloid-beta oligomer detection using ELISA-based immunoassay. They applied a sandwich aptamer-Aβ oligomer-antibody to help to achieve the detection at 50 pM (39). Zhichao Lou et al. reported SPR detection of prion disease-associated isoform (PrPSc) applying aptamer-graphene oxide and the results showed that good linearity at concentrations over a range of 0.001–1 ng/mL (40). Hong Lin Zhuang et al. fabricated resonance energy transfer a sensitive biosensor for prion protein by using graphene oxide and aptamer beacon and the results demonstrated a range of 10.2–78.8 μg/mL with a detection limit as low as 0.309 μg/mL and with high selectivity (41). Yanli Zhou et al. fabricated an Au-vertical graphene/carbon cloth electrode for applying poly(thymine)-templated copper nanoparticles as probes for Ultrasensitive
detection of amyloid-beta and this biosensor showed a low detection limit of about 3.5 pM, excellent specificity with great stability (42).

Fig. 2.

DETECTION OF VIROIDS

Viroids are classified as single-stranded RNA with no protein covering (43). Many viruses, such as HIV, Epstein Barr, Human Cytomegalovirus, Ebola, Human Herpesvirus, Hepatitis C, or Dengue, could encode unique viral miRNAs that are critical to transcription mechanisms of gene expression and viral replication (44). MiRNAs are non-coding sequences of 20-25 nucleotides (45). Therefore, the diagnosis of viroids and miRNAs is of great importance in clinical diagnoses. As shown (Fig. 3), Sze Shin Low et al. (46) fabricate a graphene/ZnO/PSE-modified electrochemical impedance genosensor for detecting Coconut cadang-cadang viroid, that has enhanced sensitivity properties. Kamila Malecka et al. developed an electrochemical genosensor using screen-printed gold electrodes for specific DNA and RNA sequences derived from Avian Influenza Virus H5N1 this method was able to ~280-mer RNA sequences detection (47).

Fig. 3.

DETECTION OF VIRAL CELLS

Since viruses, especially viruses such as HIV (48), Hepatitis A, Hepatitis B (49) and C (50), Human Cytomegalovirus (51), Ebola (52), Human Herpesvirus (53), and so on, cause many diseases in humans, animals, and plants, the diagnosis of viruses is clinically crucial. Krongkaew Navakul proposed a novel approach for the diagnosis of dengue viruses and antibody screening using an electrochemical biosensor based on graphene polymer (54). Xin Jin et al. reported a reduced graphene oxide-based field-effect transistor for immunodetection of Ebola virus with a limit of detection as low as 2.4 pg/mL (55). Fereshteh Chekin et al. fabricated electrodes for human papillomavirus (HPV) based on porous reduced graphene oxide/MoS2 they achieved a detection limit of 0.1 ng/mL (1.75 pM) for HPV16 L1 (56). Renu Singh et al. developed an electrochemical immunosensor integrated with a microfluidic platform applying a reduced graphene oxide for influenza virus detection that exhibited good selectivity and an enhanced detection limit of 0.5 PFU/mL, a great linearly with H1N1 virus concentration within the range of 1 to 104 PFU/mL ($R^2 = 0.99$) (57).
DETECTION OF BACTERIAL CELLS

A bacterium could be observed as a whole cell while it may be active or inactive. In many cases, it is important to distinguish between these two. For instance, to evaluate a particular antibacterial treatment, it is necessary to compare the concentration of the bacterial population within the sample before and after the treatment. In the case of detecting a whole-cell, it is more common to use an antibody (58), or aptamer (59), which is specific to a certain antigen on the bacterium’s surface (Fig. 4), or use a phage which the bacterium of interest is the host of it (32). Shalini Muniandy et al. (48) fabricated an electrochemical aptasensor based on a reduced graphene oxide-titanium dioxide nanocomposite for detection of Salmonella enterica and the optimized aptasensor showed high sensitivity with a wide detection range \((10^8 \text{ to } 10 \text{ cfu/mL})\), and also a low detection limit of 10 cfu/mL for Salmonella bacteria. Chandan Singh et al. (49) developed a microfluidic immunochip applying Biofunctionalized graphene oxide for Salmonella bacteria detection microfluidics biosensor showed low detection limit as 0.376 cfu/mL. Jingbo Chang et al. (50) reported ultrasonic-assisted self-assembly of monolayer graphene oxide by a high affinity to Escherichia coli bacteria detection with a concentration as low as 10 colony-forming units (CFU) per mL that demonstrate highly sensitive and selective field-effect transistor. Zahra Dehghani et al. fabricated a graphene oxide and graphene dot-based FRET for immunosensing of Campylobacter jejuni and the results showed a good Limit of detection for this bacterial sensor about 10 CFU/mL (60). Ashish Pandey et al. reported a Graphene-based electrical biosensor for pathogenic E. coli O157:H7 detection in food and this biosensor demonstrated a sensitivity as low as 10–100 cells/mL (61). Rafael Hernández et al. reported a potentiometric biosensor for living bacteria detection based on graphene, and the biosensor could detect a single CFU/mL of Staphylococcus aureus with a very low time of detection (62).

Fig. 4.

DETECTION OF FUNGI

Because of their elaborate genetic makeup and metabolism, Fungi are considered geological microorganisms (63). In addition, a group of microorganisms plays an important role in the environment, agriculture, forestry, and human health. In ecology, fungi play a role as a biosphere balance (64). Fungi are the main source of antibiotic production, and among the many species of fungi, Aspergillus spp has attracted the most attention (65). For example, there are several fungi plant pathogens, which can cost billions of dollars a year for crop damage. Fungi also affect humans by causing disease by contaminating and spoiling food (66). Xingpu Qi fabricated an electrochemical biosensor by impedance methods based
on applying graphene- Au nanoparticles for *Aphanomyces Invadans* detection (67). As discussed graphene and graphene derivatives such as GO, reduced GO, and graphene quantum dots nanocomposites are promised nanomaterials that can be used for fungi detection.

DETECTION OF PROTOZOA

A group of single-celled eukaryotes that may have free-living or parasitic (67). Some *protozoa* have a two-phase life cycle, alternating between proliferative stages (such as *trophozoites*) and dormant cysts. Historically, *protozoa* have been categorized as single-celled species, distinct from phototaxis, single-celled photosynthetic organisms (*algae*) that are called primitive plants. In both classes, the rank of *phylum* was commonly granted under the *Protista* kingdom (68). As Sona Jain has reported, *Cryptosporidium parvum* oocyst directed assembly of gold nanoparticles and GO treated with citrate and DNA-capped AuNPs by thiolated DNA (69). Jen It Wong *et al.* prepared graphene by using the chemical vapor deposition-grown method and fabricated an electrical biosensor for *Cryptosporidium parvum* oocysts detection that results showed a high sensor sensitivity of 25 oocysts per milliliter solution and with good specificity (70).

DETECTION OF MICROBIAL TOXINS

Besides the whole body of a microorganism, its secondary metabolites could lead to unwanted consequences from human point of view, name as food spoilage, water contamination, and as a result, occurring diseases. These concerns are the main reasons for seeking new and effective methods for detecting these hazards. One of the main groups of microbial toxins is those produced by *Fungi*. *Mycotoxins* are a range of fungal toxins, which can contaminate raw and processed foods during different steps of preparation (71). As a very stable compound and the most occurring *mycotoxin*, *ochratoxin A* is produced by *Aspergillus ochraceous, Aspergillus carbonarius, and Penicillium verrucosum* (72).

This toxin may be present in many daily consumed food products such as cereals, coffees, spices, or drinks like beer and wine (73). Structural similarity between *Ochratoxin A* and the amino acid phenylalanine leads to inhibitory effects on some enzymes and subsequently results in impairments in protein synthesis. *Ochratoxin A* may dispose of several cell types to apoptosis or, on the other hand, may cause tumors in human (74). *Aflatoxin* is a vastly presented *mycotoxin* produced by *Aspergillus flavus* in both herbal and animal food products, which can cause mutations, developmental malformations, cancer, and chronic liver disease (75). This group of mycotoxins consists of four main subgroups name aflatoxin
Aflatoxin B₁ is believed to have the highest role to produce cancer of the liver among all other groups of aflatoxins (77). Aflatoxin M₁ is another subgroup that is mostly known to be presented in dairy products and even breast milk of lactating mothers (78). Although the toxicity of Aflatoxin M1 is ten times less than the B₁ subgroup, consuming this toxin at a very early age may cause impaired growth, especially in infants, who are much more vulnerable to any harm (79). Zearalenone is a Fusarium species produced by mycotoxin, which is most likely to exist in cereal grains and animal feeds (80). This mycotoxin is mainly known because of its xenoestrogenicity, which means having a similar structure to estrogen and, therefore, has a great affinity to attach the estrogen receptors. This activity has been shown to cause reproductive disorders like the low quality of semen and unbalance of hormones in mice and be carcinogenic in human, developing endometrial cancer or breast cancer (81).

Besides fungi, numerous other microbial cells are capable of producing harmful toxins. Microcystin is produced by cyanobacteria and contaminates water. This toxin can promote cancer, especially liver tumors, during its inhibition effect on certain protein phosphatase activities (82).

An example of toxins produced by bacteria is a polypeptide called the cholera toxin of the bacterium Vibrio cholerae. At first, this toxin binds to the ganglioside Gm1 of the target cell membrane and continues a process that leads to activation of adenylate cyclase and promotes secretion of water and ions into the intestinal lumen, ended with severe diarrhea (83). Drinking sewage-contaminated water or consuming crops cultivated with this water are some ways of vibrio transmission into the human body (84). Enterotoxins are another group of bacterial toxins produced by Staphylococcus aureus. These toxins are made of protein and are mostly heat-stable. Enterotoxin Type B, as an example, is produced by Staphylococcus aureus and can cause diarrhea as a result of consuming contaminated foods, which are very varied from milk and cheese to ham and sausages. Contamination can be due to the bacterium’s favorable growth temperatures in processing steps (85). Botulinum is another bacterial toxin that is produced by the bacterium Clostridium botulinum and causes food poisoning in addition to its possibility of being used as a bioterrorism tool. To prevent from deadly results of consuming this neurotoxin, very accurate methods are needed to detect the toxin on the scale of nanograms especially in canned food products (86).

DETECTION OF MICROBIAL-DERIVED ANTIBIOTICS

The consumption of food products such as meat, milk, honey, and vegetables or pharmaceutical products containing antibiotics causes accumulation of this metabolite in the human body, which could
lead to different types of diseases. An example is chloramphenicol as an antibiotic against gram-positive and gram-negative bacteria, which also is used as a veterinary drug and even water disinfectant due to its low cost and effectiveness, but may cause potential side effects such as the development of plastic anemia, a blood disorder, and the failure of bone marrow to produce blood cells mainly because of its toxic transformation by-products (57). By disrupting mitochondrial iron metabolism, chloramphenicol causes problems with the iron-sulfur clusters (FeS) of the electron transport chain, especially depletion of ATP, which probably leads to tumorigenesis (87). It has been shown that chloramphenicol residues have long persistence of at least 35 days after the end of the treatment in animals' tissues (88).

Metronidazole has antibacterial and anti-inflammatory effects, which are making this antibiotic part of protozoal diseases treatments, but extreme usage of it in the long term may be genotoxic, carcinogenic, and mutagenic (89).

Neomycin is an aminoglycoside antibiotic found in eardrops with ototoxicity properties, which causes hearing impairment or loss by inducing auditory hair cell apoptosis (90). Although Bleomycin has an essential advantage as an antitumor antibiotic in many anticancer drugs, over usage of it may have a toxic effect on the lung, which leads to pulmonary dysfunction and, subsequently, death (91).

Oxytetracycline, which is being widely used in dermatology and veterinary medicine, can decrease melanocyte viability during phototoxicity. The extent of this effect is relative to the drug dosage (92). Streptomycin has been broadly used in veterinary drugs and pesticides to control different groups of microorganisms. A high amount of this antibiotic in food products is the potential to cause ototoxicity and nephrotoxicity (93).

In Table 1, a comprehensive list of graphene derivative materials which have been applied as a biosensor for detection of prions, viroids, viruses, bacterial cells, fungi, protozoa microbial toxins, microbially derived antibiotics are listed.

CONCLUSIONS

In this review, we represented a comprehensive our point of view about intrinsic properties of graphene and graphene derivatives application in microorganisms detection applying graphene based nanobiosensors. Recently graphene has become well-known 2D nanomaterials and graphene derivatives such as GO, reduced GO, and graphene quantum dots nanocomposites have fascinating properties, such as high surface area, optical and magnetic properties, and high elasticity makes it an appropriate basic structure for preparing several graphene-based nanocomposites. They would be scaffolds to immobilize biomolecules and create highly selective biosensors. Based on recent studies
A literature review shows that, among several detection methods employed grapheme based microorganisms nanobiosensors, the most common is electrochemical due to its simple, high sensitivity in a rapid assay. Due to these attractive properties and features that cause applying these carbon structures in biosensors for the detection of microorganisms (such as prions, viroids, viral cells, bacterial cells, protozoa, microbial toxins, fungi, microbial-derived antibiotics, etc.).

**CONFLICT OF INTEREST**

The authors declared that there is no conflict of interest.

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**AUTHORS’ CONTRIBUTION**

Mehrab Pourmadadi and Sara Hojjati drafted the manuscript, performed a search of articles, and collected the data. Fatemeh Yazdian and Kianoush Khosravi-Darani designed the work, performed discussion, and performed data interpretation as well as critical revision and final approval of the version to be published.

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Table 1. Graphene derivative materials applied for biosensor for detection of prions, viroids, viruses, bacterial cells, fungi, protozoa microbial toxins, microbially derived antibiotics

<table>
<thead>
<tr>
<th>Graphene derivative</th>
<th>Materials in composition with graphene</th>
<th>Biorecognition element</th>
<th>Detected material</th>
<th>Detection limit</th>
<th>Linear range</th>
<th>Detection time</th>
<th>Type of biosensor</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>detection of prions</td>
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<tr>
<td>GO‡</td>
<td>FITC-PrP(95–110)</td>
<td>amyloid-b oligomers</td>
<td>0.01-2 µM</td>
<td>1 hour</td>
<td>fluorescent</td>
<td>(32)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GO</td>
<td>ssDNA</td>
<td>sensitive prion disease-associated isoform</td>
<td>4.24·10⁻⁵nM</td>
<td>0.001-1 ng/mL.</td>
<td>40 min</td>
<td>surface plasmon resonance</td>
<td>(36)</td>
<td></td>
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<tr>
<td>detection of viroids</td>
<td></td>
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<tr>
<td>Graphene</td>
<td>zinc oxide</td>
<td>ssDNA</td>
<td>Coconut Cadang-Cadang Viroid</td>
<td>4.3·10⁻¹² M</td>
<td>10⁻¹¹-10⁻⁶ M</td>
<td>60 min</td>
<td>electrochemical</td>
<td>(38)</td>
</tr>
<tr>
<td>GQD†</td>
<td>SiO₂ nanoparticles and NaYF4·Yb,Er</td>
<td>miRNA HIV1-miR-Tar-5p</td>
<td>10 fM</td>
<td>above1⁶ M</td>
<td>-</td>
<td>Fluorescent</td>
<td>(78)</td>
<td></td>
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<tr>
<td>detection of viruses</td>
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<tr>
<td>GO</td>
<td>1-pyrenebutyric acid N-hydroxysuccinimide ester antibody</td>
<td>Rotavirus</td>
<td>10 PFU</td>
<td>10⁻⁵ PFU/mL</td>
<td>-</td>
<td>Electrochemical</td>
<td>(80)</td>
<td></td>
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<tr>
<td>Graphene</td>
<td>H7-polyclonal antibody Silver nanoparticles and chitosan poly (3-thiophene boronic acid) and nanogold</td>
<td>avian influenza virus H7</td>
<td>1.6·10⁻³ to 16 ng/mL</td>
<td>30 min</td>
<td>1.6 pg/mL</td>
<td>Electrochemical</td>
<td>(81)</td>
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<td>Graphene</td>
<td>antibody</td>
<td>avian leukemia viruses</td>
<td>210 tissue culture infective dose /50-mL</td>
<td>527 -3162 infective dose/50-mL</td>
<td>-</td>
<td>Electrochemical</td>
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<td>Material/Enzyme</td>
<td>Functional Component</td>
<td>Target</td>
<td>Sensitivity</td>
<td>LOD</td>
<td>Detection Method</td>
<td>Reference</td>
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<tr>
<td>RGO</td>
<td>antibody</td>
<td>Rotavirus enteric EV71 and H9N2</td>
<td>$10^2$ PFU</td>
<td>-</td>
<td>Field-Effect Transistor</td>
<td>(82)</td>
<td></td>
<td></td>
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<tr>
<td>GO</td>
<td>-</td>
<td>Ebola virus</td>
<td>$1.4 \text{ pM}$</td>
<td>30 fm-3 nM</td>
<td>RT-PCR</td>
<td>(83)</td>
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<td>GO</td>
<td>ssDNA</td>
<td>Hepatitis B Virus</td>
<td>$0.1 \text{ ng/mL}$</td>
<td>0.1-350 ng/mL</td>
<td>Fluorescent</td>
<td>(84)</td>
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<tr>
<td>GO</td>
<td>antibody</td>
<td>avian influenza virus</td>
<td>-</td>
<td>-</td>
<td>Amperometric</td>
<td>(85)</td>
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<tr>
<td>Graphene</td>
<td>- Glycan</td>
<td>Influenza Virus H1N1</td>
<td>$10^2$ PFU</td>
<td>$10^{-6}$ PFU/mL</td>
<td>Field-Effect Transistor</td>
<td>(86)</td>
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<tr>
<td>RGO††</td>
<td>antibody</td>
<td>human papillomavirus</td>
<td>$0.1 \text{ ng/mL}$</td>
<td>0.2-2 ng/mL</td>
<td>Electrochemical</td>
<td>(57)</td>
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<tr>
<td>RGO</td>
<td>ssDNA</td>
<td>Klebsiella pneumonia target DNA down to $3 \cdot 10^{-11} \text{ M}$</td>
<td>$10^{-6}$-$10^{-10} \text{ M}$</td>
<td>-</td>
<td>electrochemical</td>
<td>(87)</td>
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<tr>
<td>RGO</td>
<td>Indole-5-carboxylic acid ssDNA</td>
<td><em>Pseudomonas aeruginosa</em> 56 CFU/mL</td>
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<td>-</td>
<td>Electrochemiluminescent</td>
<td>(88)</td>
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<tr>
<td>Graphene</td>
<td>Carboxyl</td>
<td>A virulent phage named as PaP1</td>
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<tr>
<td>GQD</td>
<td>amino-modified GQDs Anti-Salmonella antibody</td>
<td>Salmonella &amp; bacterial response to antibiotics</td>
<td>LOD for antibiotics can reach the pM level</td>
<td>-</td>
<td>30 min</td>
<td>electrochemical</td>
<td>(95)</td>
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<tr>
<td>RGO</td>
<td>RGO-Cu(II) monoclonal antibodies</td>
<td><em>Staphylococcus aureus</em> 4.4 CFU/mL</td>
<td>$10^{-6}$-10$^8$ CFU/mL</td>
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<td>electrochemical</td>
<td>(90)</td>
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<td>GO</td>
<td>SiO$_2$ substrates Anti-E. coli β-gal Abs</td>
<td><em>E. coli</em> 10-100 µg/mL</td>
<td>-</td>
<td>-</td>
<td>infrared spectroscopy</td>
<td>(91)</td>
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<tr>
<td>GNPs and MG</td>
<td>anti-<em>E. coli</em> antibodies</td>
<td><em>E. coli O157:H7</em> 10-100 cells/mL</td>
<td>GNPs $10^2$–$10^6$ cells/mL, MG $10^{-6}$ cells/mL</td>
<td>30 min</td>
<td>Electrical</td>
<td>(52)</td>
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<td>QDs &amp; GO</td>
<td>complementarit othe invA oligo</td>
<td><em>Salmonella specific invA gene</em> 4 nM of the invA gene</td>
<td>-</td>
<td>20 min</td>
<td>fluorescencereso nanceenergytran sfer</td>
<td>(92)</td>
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</tbody>
</table>
Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

<table>
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<th>Detection Method</th>
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<th>Sensitivity</th>
<th>Time</th>
<th>Detection Method</th>
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<td>GQD</td>
<td>E. coli polyclonal antibody</td>
<td>E. coli O157:H7</td>
<td>8 CFU/mL</td>
<td>$10^{-7}$ CFU/mL</td>
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<td>Graphene</td>
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<td>Aphanomyces Invadans</td>
<td>309 ng/mL</td>
<td>0.2-4 mg/mL</td>
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<td>ssDNA</td>
<td>microcystin-LR</td>
<td>0.05 μg/L</td>
<td>0.05-20 μg/L</td>
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</table>

† Graphen quantum dots
‡ Graphene oxide
†† Reduced Graphene Oxide
Figure Legends

Figure 2. (left) Structure of GO and (right) its FTIR spectrum, Reproduced from Ref. (1) with permission.

Figure 2. Schematic illustration of the GO-based fluorescent biosensor, Reproduced from Ref. (32) with permission.

Figure 3. Schematic diagram for the development of graphene/zinc oxide nanocomposite-modified electrochemical impedance genosensor for ssRNA detection, Reproduced from Ref. (38) with permission.

Figure 4. Schematic of electrochemical aptasensing of lipopolysaccharides from Escherichia coli bacteria, Reproduced from Ref. (5) with permission.

Fig. 3. Structure of GO (left) and its FTIR spectrum (right). Reproduced from Ref. (1) with permission
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