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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 sp^2 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 SP_3 to SP_2, 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 stocking 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 $–NH_2$ 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 reseptors, 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 to 10 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 grarphene, 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

B_1, B_2, G_1, and G_2 (*76*). *Aflatoxin* B_1 is believed to have the highest role to produce cancer of the liver among all other groups of *aflatoxins* (*77*). *Aflatoxin* M_1 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 B_1 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

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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REFERENCES

1. Georgakilas V, Otyepka M, Bourlinos AB, Chandra V, Kim N, Kemp KC, Hobza P, Zboril R, Kim KS. Functionalization of graphene: Covalent and non-covalent approaches, derivatives and applications. Chem Rev. 2012;112(11):6156–214. https://doi.org/10.1021/cr3000412.

2. Lee C, Wei X, Kysar JW, Hone J. Measurement of the elastic properties and intrinsic strength of monolayer graphene. Science (80). 2008;321(5887):385–8. https://doi.org/10.1126/science.1157996.

3. Schedin F, Geim AK, Morozov S V., Hill EW, Blake P, Katsnelson MI, Novoselov KS. Detection of

individual gas molecules adsorbed on graphene. Nat Mater. 2007;6(9):652–5. https://doi.org/10.1038/nmat1967.

4.Malhotra BD, Srivastava S, Ali MA, Singh C. Nanomaterial-based biosensors for food toxin detection. Appl Biochem Biotechnol. 2014;174(3):880–96. https://doi.org/10.1007/s12010-014-0993-0.

5.Pourmadadi M, Shayeh JS, Omidi M, Yazdian F, Alebouyeh M, Tayebi L. A glassy carbon electrode modified with reduced graphene oxide and gold nanoparticles for electrochemical aptasensing of lipopolysaccharides from *Escherichia coli* bacteria. Microchim Acta. 2019. https://doi.org/10.1007/s00604-019-3957-9.

6.Pourmadadi M, Shayeh JS, Arjmand S, Omidi M, Fatemi F. An electrochemical sandwich immunosensor of vascular endothelial growth factor based on reduced graphene oxide/gold nanoparticle composites. Microchem J. 2020;105476.

7.Wang Z, Dai Z. Carbon nanomaterial-based electrochemical biosensors: an overview. Nanoscale. 2015;7(15):6420–31.

8.Chimene D, Alge DL, Gaharwar AK. Two-Dimensional Nanomaterials for Biomedical Applications: Emerging Trends and Future Prospects. Adv Mater. 2015;27(45):7261–84. https://doi.org/10.1002/adma.201502422.

9.Kazemi S, Pourmadadi M, Yazdian F, Ghadami A. The synthesis and characterization of targeted delivery curcumin using chitosan-magnetite-reduced graphene oxide as nano-carrier. Int J Biol Macromol. 2021;186:554–62. https://doi.org/10.1016/j.ijbiomac.2021.06.184.

10.Zhao Z, Bai P, Du W, Liu B, Pan D, Das R, Liu C, Guo Z. An overview of graphene and its derivatives reinforced metal matrix composites: Preparation, properties and applications. Carbon N Y. 2020;170:302–26. https://doi.org/10.1016/j.carbon.2020.08.040.

11.Carbone M, Gorton L, Antiochia R. An overview of the latest graphene-based sensors for glucose detection: The effects of graphene defects. Electroanalysis. 2015;27(1):16–31. https://doi.org/10.1002/elan.201400409.

12.El-Desoky HS, Khalifa A, Abdel-Galeil MM. An advanced and facile synthesized graphene/magnetic Fe_3O_4 nanoparticles platform for subnanomolar voltammetric determination of antipsychotic olanzapine drug in human plasma. J Electrochem Soc. 2020;167(6):067527. https://doi.org/10.1149/1945-

7111/ab8366.

13.Liang B, Wang J, Zhang S, Liang X, Huang H, Huang D, Zhou W, Guo J. Hybrid of Co-doped SnO2 and graphene sheets as anode material with enhanced lithium storage properties. Appl Surf Sci. 2020;533:147447. https://doi.org/10.1016/j.apsusc.2020.147447.

14.Tabar FS, Pourmadadi M, Rashedi H, Yazdian F. Design of Electrochemical Nanobiosensor in the Diagnosis of Prostate Specific Antigen (PSA) Using Nanostructures. In: 27th National and 5th International Iranian Conference of Biomedical Engineering, ICBME 2020. IEEE; 2020. p. 35–40. https://doi.org/10.1109/ICBME51989.2020.9319418.

15.Fathi S, Saber R, Adabi M, Rasouli R, Douraghi M, Morshedi M, Farid-Majidi R. Novel competitive voltammetric aptasensor based on electrospun carbon nanofibers-gold nanoparticles modified graphite electrode for *Salmonella enterica* serovar detection. Biointerface Res Appl Chem. 2021;11(2):8702–15. https://doi.org/10.33263/BRIAC112.87028715.

16.Dinani HS, Pourmadadi M, Rashedi H, Yazdian F. Fabrication of nanomaterial-based biosensor for measurement of a microRNA involved in cancer. In: 27th National and 5th International Iranian Conference of Biomedical Engineering, ICBME 2020. IEEE; 2020. 47–54. https://doi.org/10.1109/ICBME51989.2020.9319450.

17.Manasa G, Mascarenhas RJ, Bhakta AK, Mekhalif Z. Nano-graphene-platelet/Brilliant-green composite coated carbon paste electrode interface for electrocatalytic oxidation of flavanone Hesperidin. Microchem J. 2021;160:105768. https://doi.org/10.1016/j.microc.2020.105768.

18.Singh S, Tuteja SK, Sillu D, Deep A, Suri CR. Gold nanoparticles-reduced graphene oxide based electrochemical immunosensor for the cardiac biomarker myoglobin. Microchim Acta. 2016;183(5):1729–38. https://doi.org/10.1007/s00604-016-1803-x.

19.Lee J, Kim J, Kim S, Min DH. Biosensors based on graphene oxide and its biomedical application. Adv Drug Deliv Rev. 2016;105:275–87. https://doi.org/10.1016/j.addr.2016.06.001.

20.Nandini S, Nalini S, Reddy MBM, Suresh GS, Melo JS, Niranjana P, Sanetuntikul J, Shanmugam S. Synthesis of one-dimensional gold nanostructures and the electrochemical application of the nanohybrid containing functionalized graphene oxide for cholesterol biosensing. Bioelectrochem. 2016;110:79–90.

https://doi.org/10.1016/j.bioelechem.2016.03.006.

21.Luo X, Morrin A, Killard AJ, Smyth MR. Application of nanoparticles in electrochemical sensors and biosensors. Electroanal. 2006. 18:319–26.
https://doi.org/10.1002/elan.200503415.

22.Doria G, Conde J, Veigas B, Giestas L, Almeida C, Assunção M, Rosa J, Baptista PV. Noble metal nanoparticles for biosensing applications. Sensors. 2012;12(2):1657–87.
https://doi.org/10.3390/s120201657.

23.Govindhan M, Amiri M, Chen A. Au nanoparticle/graphene nanocomposite as a platform for the sensitive detection of NADH in human urine. Biosens Bioelectron. 2015;66:474–80.
https://doi.org/10.1016/j.bios.2014.12.012.

24.Lv W, Guo M, Liang MH, Jin FM, Cui L, Zhi L, Yang QH. Graphene-DNA hybrids: Self-assembly and electrochemical detection performance. J Mater Chem. 2010;20(32):6668–73.
https://doi.org/10.1039/c0jm01066a.

25.Gao F, Zhang X, Gao F, Cai X, Zheng M, Jiang S,Wang Q. Application of graphene-pyrenebutyric acid nanocomposite as probe oligonucleotide immobilization platform in a DNA biosensor. Mater Sci Eng C. 2013;33(7):3851–7.
https://doi.org/10.1016/j.msec.2013.05.022.

26.Bhardwaj N, Bhardwaj SK, Mehta J, Mohanta GC, Deep A. Bacteriophage immobilized graphene electrodes for impedimetric sensing of bacteria (*Staphylococcus arlettae*). Anal Biochem. 2016;505:18–25.
https://doi.org/10.1016/j.ab.2016.04.008.

27.Cui Y, Kim SN, Jones SE, Wissler LL, Naik RR, McAlpine MC. Chemical functionalization of graphene enabled by phage displayed peptides. Nano Lett. 2010;10(11):4559–65.
https://doi.org/10.1021/nl102564d.

28.Morales MA, Halpern JM. Guide to selecting a biorecognition element for biosensors. Bioconjug Chem. 2018;29(10):3231–9. https://doi.org/10.1021/acs.bioconjchem.8b00592.

29.Martinkova P, Kostelnik A, Valek T, Pohanka M. Main streams in the construction of biosensors and their applications. Int J Electrochem Sci. 2017;12(8):7386–403.
https://doi.org/10.20964/2017.08.02.

30.Zhang CC, Steele AD, Lindquist S, Lodish HF. Prion protein is expressed on long-term repopulating hematopoietic stem cells and its important for their self-renewal. Proc Natl Acad Sci U S A.

2006;103(7):2184–9.
https://doi.org/10.1073/pnas.0510577103.
31.Woerman AL, Patel S, Kazmi SA, Oehler A, Lee J, Mordes DA, Olson SH, Pruiner SB. Kinetics of α-synuclein prions preceding neuropathological inclusions in multiple system atrophy. PLoS Pathog. 2020;16(2):e1008222.
https://doi.org/10.1371/journal.ppat.1008222.
32.Liu L, Xia N, Zhang J, Mao W, Wu Y, Ge X. A graphene oxide-based fluorescent platform for selective detection of amyloid-β oligomers. Anal Methods. 2015;7(20):8727–32.
https://doi.org/10.1039/c5ay02018b.
33.Zhao J, Chang W, Liu L, Xing X, Zhang C, Meng H, Gopinath SCB, Lakshmipriya T, Chen Y, Liu Y. Graphene oxide-gold nanoparticle-aptamer complexed probe for detecting amyloid beta oligomer by ELISA-based immunoassay. J Immunol Methods. 2021;489:112942.
https://doi.org/10.1016/j.jim.2020.112942.
34.Bahamonde JP, Nguyen HN, Fanourakis SK, Rodrigues DF. Recent advances in graphene‐based biosensor technology with applications in life sciences. J Nanobiotechnol. 2018;1–17.
https://doi.org/10.1186/s12951-018-0400-z.
35.Suvarnaphaet P, Pechprasarn S. Graphene-based materials for biosensors: A review. Sensors (Switzerland). 2017;17(10):2161. https://doi.org/10.3390/s17102161.
36.Lou Z, Wan J, Zhang X, Zhang H, Zhou X, Cheng S, Gu N.. Quick and sensitive SPR detection of prion disease-associated isoform (PrPSc) based on its self-assembling behavior on bare gold film and specific interactions with aptamer-graphene oxide (AGO). Colloids Surf. B. 2017;157:31–9.
https://doi.org/10.1016/j.colsurfb.2017.05.058.
37.Gilad S, Meiri E, Yogev Y, Benjamin S, Lebanony D, Yerushalmi N, Benjamin H, Kushnir M, Cholakh H, Melamed N, Bentwich Z, Hod M, Goren Y, Chajut A. Serum microRNAs are promising novel biomarkers. PLoS One. 2008;3(9):e3148.
https://doi.org/10.1371/journal.pone.0003148.
38.Low SS, Loh HS, Boey JS, Khiew PS, Chiu WS, Tan MTT. Sensitivity enhancement of graphene/zinc oxide nanocomposite-based electrochemical impedance genosensor for single stranded RNA detection. Biosens Bioelectron. 2017;94:365–73.
https://doi.org/10.1016/j.bios.2017.02.038.
39.Malecka K, Stachyra A, Góra-Sochacka A, Sirko A, Zagórski-Ostoja W, Radecka H, Radecki J.

Electrochemical genosensor based on disc and screen printed gold electrodes for detection of specific DNA and RNA sequences derived from Avian Influenza Virus H5N1. Sensors Actuators, B Chem. 2016;224:290–7.
https://doi.org/10.1016/j.snb.2015.10.044.
40.Yuan C, Fang J, Duan Q, Yan Q, Guo J, Yuan T, Yi G. Two-layer three-dimensional DNA walker with highly integrated entropy-driven and enzyme-powered reactions for HIV detection. Biosens Bioelectron. 2019;133:243–9.
https://doi.org/10.1016/j.bios.2019.03.015.
41.Omatola CA, Onoja BA, Agama J. Detection of Hepatitis B Surface Antigen among Febrile Patients in Ankpa, Kogi State, Nigeria. J Trop Med. 2020;2020.
https://doi.org/10.1155/2020/5136785.
42.Biasotto G, Costa JPC, Costa PI, Zaghete MA. ZnO nanorods-gold nanoparticle-based biosensor for detecting hepatitis C. Appl Phys A Mater Sci Process. 2019;125(12):1–7.
https://doi.org/10.1007/s00339-019-3128-1.
43.Silva J, Fernandes C, Marques A, Maria AT, Correia C, Tuna ML, Chasqueira MJ, Paixão P. Evaluation of saliva pools method for detection of congenital human cytomegalovirus infection. J Virol Methods. 2020;275:113759.
https://doi.org/10.1016/j.jviromet.2019.113759.
44. Kelishadi M, Kelishadi M, Ahmadi A, Javid N, Ashrafi GH, Tabarraei A., Frequency of Human Herpesvirus 6 (HHV-6) in Pterygium Using Real-Time PCR Based on SYBR-Green I Fluorescence. Med Lab J. 2019;13(2):16–22.
https://doi.org/10.29252/mlj.13.2.16.
45.Chekin F, Bagga K, Subramanian P, Jijie R, Singh SK, Kurungot S, Boukherroub R, Szunerits S. Nucleic aptamer modified porous reduced graphene oxide/MoS2 based electrodes for viral detection: Application to human papillomavirus (HPV). Sensors Actuators, B Chem. 2018;262:991–1000.
https://doi.org/10.1016/j.snb.2018.02.065.
46.Hasan MA, Esther ACM, Dey A, Mukhopadhyay AK. A review on coronavirus survivability on material ' s surfaces : present research scenarios , technologies and future directions. Surface Eng. 2020; 36(12). https://doi.org/10.1080/02670844.2020.1833277.
47.Wang Y, Ping J, Ye Z, Wu J, Ying Y. Impedimetric immunosensor based on gold nanoparticles modified graphene paper for label-free detection of *Escherichia coli* O157: H7. Biosens Bioelectron.

2013;49:492–8.
https://doi.org/10.1016/j.bios.2013.05.061.
48.Muniandy S, Teh SJ, Appaturi JN, Thong KL, Lai CW, Ibrahim F, Leo BF. A reduced graphene oxide-titanium dioxide nanocomposite based electrochemical aptasensor for rapid and sensitive detection of *Salmonella enterica*. Bioelectrochemistry. 2019;127:136–44.
https://doi.org/10.1016/j.bioelechem.2019.02.005.
49.Singh C, Ali MA, Reddy V, Singh D, Kim CG, Sumana G, Malhotra BD. Biofunctionalized graphene oxide wrapped carbon nanotubes enabled microfluidic immunochip for bacterial cells detection. Sensors Actuators, B Chem. 2018;255:2495–503. https://doi.org/10.1016/j.snb.2017.09.054.
50.Chang J, Mao S, Zhang Y, Cui S, Zhou G. Nanoscale graphene oxide for rapid detection of *Escherichia coli*. Nanoscale*, 2013, 5, 3620–6. https://doi.org/10.1039/c3nr00141e.*
51.Dehghani Z, Mohammadnejad J, Hosseini M, Bakhshi B, Rezayan AH. Whole cell FRET immunosensor based on graphene oxide and graphene dot for *Campylobacter jejuni* detection. Food Chem. 2020;309:125690.
https://doi.org/10.1016/j.foodchem.2019.125690.
52.Pandey A, Gurbuz Y, Ozguz V, Niazi JH, Qureshi A. Graphene-interfaced electrical biosensor for label-free and sensitive detection of foodborne pathogenic *E. coli* O157:H7. Biosens Bioelectron. 2017;91:225–31.
https://doi.org/10.1016/j.bios.2016.12.041.
53.Hernández R, Vallés C, Benito AM, Maser WK, Xavier Rius F, Riu J. Graphene-based potentiometric biosensor for the immediate detection of living bacteria. Biosens Bioelectron. 2014;54:553–7.
https://doi.org/10.1016/j.bios.2013.11.053.
54.Kendrick B. Fungi : ecological importance and impact on humans. Wiley Online Library, Columbia B. 2011; 1–5. https://doi.org/10.1002/9780470015902.a0000369.pub2.
55.Bennett JW, Inamdar AA. Are some fungal volatile organic compounds (VOCs) mycotoxins? Toxins (Basel). 2015;7(9):3785–804. https://doi.org/10.3390/toxins7093785.
56.Costa CP, Silva DG, Rudnitskaya A, Almeida A. Shedding light on Aspergillus niger volatile exometabolome. Nat Publ Gr. 2016; (June):1–13. https://doi.org/10.1038/srep27441.
57.Singh R, Hong S, Jang J. Label-free detection of influenza viruses using a reduced graphene oxide-based electrochemical immunosensor integrated with a microfluidic platform. Sci Rep. 2017;7(1):1–11.

https://doi.org/10.1038/srep42771.

58.Qi X, Chen T, Lu D, Chen B. Graphene-Au nanoparticle based electrochemical immunosensor for fish pathogen Aphanomyces invadans detection. Fuller Nanotub Car N. 2017;25(1):12–6. https://doi.org/10.1080/1536383X.2016.1239080.

59.Simfukwe J, Mapasha RE, Braun A, Diale M. Biopatterning of keratinocytes in aqueous two-phase systems as a potential tool for skin tissue engineering. MRS Adv. 2017;357(May):1–8. https://doi.org/10.1557/adv.201.

60.Vejarano R, Siche R, Tesfaye W. Evaluation of biological contaminants in foods by hyperspectral imaging: A review. Int J Food Prop. 2017;20(sup2):1264–97. https://doi.org/10.1080/10942912.2017.1338729.

61.Kabak B, Dobson ADW, Var I. Strategies to prevent mycotoxin contamination of food and animal feed: A review. Crit Rev Food Sci Nutr. 2006;46(8):593–619. https://doi.org/10.1080/10408390500436185.

62.Zheng Y, Wang Y, Yang X. Aptamer-based colorimetric biosensing of dopamine using unmodified gold nanoparticles. Sensors Actuators, B Chem. 2011;156(1):95–9. https://doi.org/10.1016/j.snb.2011.03.077.

63.Turner NW, Subrahmanyam S, Piletsky SA. Analytical methods for determination of mycotoxins: A review. Anal Chim Acta. 2009;632(2):168–80. https://doi.org/10.1016/j.aca.2008.11.010.

64.Marin S, Ramos AJ, Cano-Sancho G, Sanchis V. Mycotoxins: Occurrence, toxicology, and exposure assessment. Food Chem Toxicol. 2013;60:218–37.

65.Yu J, Chang PK, Ehrlich KC, Cary JW, Bhatnagar D, Cleveland TE, Payne CA, Linz JE, Woloshuk CP, Bennett JW. Clustered Pathway Genes in Aflatoxin Biosynthesis. Appl Environ Microbiol. 2004;70(3):1253–62. https://doi.org/10.1128/AEM.70.3.1253-1262.2004.

66.Polychronaki N, West RM, Turner PC, Amra H, Abdel-Wahhab M, Mykkänen H, El-Nezami H. A longitudinal assessment of aflatoxin M_1 excretion in breast milk of selected Egyptian mothers. Food Chem Toxicol. 2007;45(7):1210–5. https://doi.org/10.1016/j.fct.2007.01.001.

67.Zinedine A, Soriano JM, Moltó JC, Mañes J. Review on the toxicity, occurrence, metabolism, detoxification, regulations and intake of zearalenone: An oestrogenic mycotoxin. Food Chem Toxicol.

2007;45(1):1–18.
https://doi.org/10.1016/j.fct.2006.07.030.
68.Nakamura Y, Imai I, Yamaguchi A, Tuji A. Molecular phylogeny of the widely distributed marine protists, phaeodaria (Rhizaria, Cercozoa). Ann Anat. 2015;166(3):363–73.
https://doi.org/10.1016/j.protis.2015.05.004.
69.Jain S, Costa Melo TG, Dolabella SS, Liu J. Current and emerging tools for detecting protozoan cysts and oocysts in water. TrAC, Trends Anal Chem. 2019;121:115695.
https://doi.org/10.1016/j.trac.2019.115695.
70.Broeck D Vanden, Horvath C, De Wolf MJS. *Vibrio cholerae*: cholera toxin. Int J Biochem Cell Biol. 2007;39(10):1771–5.
71.Sapsford KE, Taitt CR, Loo N, Ligler FS. Biosensor detection of botulinum toxoid a and staphylococcal enterotoxin B in food. Appl Environ Microbiol. 2005;71(9):5590–2.
https://doi.org/10.1128/AEM.71.9.5590-5592.2005.
72.Zhang Y, Shao Y, Gao N, Gao Y, Chu W, Li S, Wang Y, Xu S. Kinetics and by-products formation of chloramphenicol (CAP) using chlorination and photocatalytic oxidation. Chem Eng J. 2018;333:85–91.
https://doi.org/10.1016/j.cej.2017.09.094.
73.L. Elliott R. Antibiotic overusage causes mitochondrial dysfunction which may promote tumorigenesis. J Cancer Treat Res. 2017;5(4):62. https://doi.org/10.11648/j.jctr.20170504.11.
74.Rejtharová M, Rejthar L, Bureš J, Vernerová E, Hera A. Persistence of chloramphenicol residues in chicken muscle tissue after a therapeutic dose administration. Food Addit Contam Part A. 2017;34(4):547–51. https://doi.org/10.1080/19440049.2016.1253113.
75.Della Latta V, Cecchettini A, Del Ry S, Morales MA. Bleomycin in the setting of lung fibrosis induction: From biological mechanisms to counteractions. Pharmacol Res. 2015;97:122–30.
https://doi.org/10.1016/j.phrs.2015.04.012.
76.Rok J, Wrześniok D, Beberok A, Otręba M, Delijewski M, Buszman E. Phototoxic effect of oxytetracycline on normal human melanocytes. Toxicol Vitr. 2018;48:26–32.
https://doi.org/10.1016/j.tiv.2017.12.008.
77.Emrani AS, Danesh NM, Lavaee P, Ramezani M, Abnous K, Taghdisi SM. Colorimetric and fluorescence quenching aptasensors for detection of streptomycin in blood serum and milk based on double-stranded DNA and gold nanoparticles. Food Chem. 2016;190:115–21.
https://doi.org/10.1016/j.foodchem.2015.05.079.

78.Laurenti M, Paez-perez M, Algarra M, Alonso-cristobal P, Lopez-cabarcos E, Mendez-gonzalez D, Rubio-Retama J. Enhancement of the upconversion emission by visible to near infrared fluorescent graphene quantum dots for miRNA Detection. ACS Appl. Mater. Interfaces 2016, 8, 20, 12644–12651 https://doi.org/10.1021/acsami.6b02361.

79.Wang Z, Shang K, Dong J, Cheng Z, Ai S. Electrochemical immunoassay for subgroup J of avian leukosis viruses using a glassy carbon electrode modified with a film of poly (3-thiophene boronic acid), gold nanoparticles , graphene and immobilized antibody. *Microchim Acta*, 2012, 179, 227–34. https://doi.org/10.1007/s00604-012-0874-6.

80.Liu F, Choi KS, Park TJ, Lee SY, Seo TS. Graphene-based electrochemical biosensor for pathogenic virus detection. Biochip J. 2011;5(2):123–8. https://doi.org/10.1007/s13206-011-5204-2.

81.Huang J, Xie Z, Xie Z, Luo S, Xie L, Huang L, Fan Q, Zhang Y, Wang S, Zeng T. Silver nanoparticles coated graphene electrochemical sensor for the ultrasensitive analysis of avian influenza virus H7. Anal Chim Acta. 2016;913:121–7. https://doi.org/10.1016/j.aca.2016.01.050.

82.Liu F, Kim YH, Cheon DS, Seo TS. Micropatterned reduced graphene oxide based field-effect transistor for real-time virus detection. Sensors Actuators, B Chem. 2013;186:252–7. https://doi.org/10.1016/j.snb.2013.05.097.

83.Song Z, Wang X, Zhu G, Nian Q, Zhou H, Yang D, Qin C, Tang R. Virus capture and destruction by label-free graphene oxide for detection and disinfection applications. Small, 2014;11(9) 1171–6. https://doi.org/10.1002/smll.201401706.

84.Wen J, Li W, Li J, Tao B, Xu Y, Li H, Lu A., Sun S. Study on rolling circle amplification of Ebola virus and fluorescence detection based on graphene oxide. Sensors Actuators, B Chem. 2016;227:655–9. https://doi.org/10.1016/j.snb.2016.01.036.

85.Zhao F, Cao L, Liang Y, Wu Z, Chen Z, Zeng R. Label-free amperometric immunosensor based on graphene oxide and ferrocene-chitosan nanocomposites for detection of Hepatis B virus antigen. J Biomed Nanotechnol. 2017;13(10):1300–8. https://doi.org/10.1166/jbn.2017.2415.

86.Ono T, Oe T, Kanai Y, Ikuta T, Ohno Y, Maehashi K*,* Inoue K, Watanabe Y, Nakakita SC, Suzuki Y, Kawahara T, Matsumoto K. *Glycan-functionalized graphene-FETs toward selective de*tection of human-infectious avian influenza virus. Jpn J Appl Phys. 2017;56(3):30302. https://doi.org/10.7567/JJAP.56.030302.

87.Zhang Z, Yu HW, Wan GC, Jiang JH, Wang N, Liu ZY, Chang D, Pan HZ. A label-free electrochemical biosensor based on a reduced graphene oxide and indole-5-carboxylic acid nanocomposite for the detection of *Klebsiella pneumoniae*. J AOAC Int. 2019;100(2):548–52. https://doi.org/10.5740/jaoacint.16-0251.

88.Yue H, He Y, Fan E, Wang L, Lu S, Fu Z. Label-free electrochemiluminescent biosensor for rapid and sensitive detection of *Pseudomonas aeruginosa* using phage as highly specific recognition agent. Biosens Bioelectron. 2017;94:429–32. https://doi.org/10.1016/j.bios.2017.03.033.

89.Mohammadi Z, Abbott P V. On the local applications of antibiotics and antibiotic-based agents in endodontics and dental traumatology. Int Endod J., 2009;555–67. https://doi.org/10.1111/j.1365-2591.2009.01564.x.

90.Wu Y, Chai H. Development of an electrochemical biosensor for rapid detection of foodborne pathogenic bacteria. Int J Electrochem Sci. 2017;12(5):4291–300. https://doi.org/10.20964/2017.05.09.

91.Singh KP, Dhek NS, Nehra A, Ahlawat S, Puri A. Applying graphene oxide nano-film over a polycarbonate nanoporous membrane to monitor E. coli by infrared spectroscopy. Spectrochim Acta - Part A Mol Biomol Spectrosc. 2017;170:14–8. https://doi.org/10.1016/j.saa.2016.06.053.

92.Guo J, Chan EWC, Chen S, Zeng Z. Development of a novel quantum dots and graphene oxide based fret assay for rapid detection of inva gene of Salmonella. Front Microbiol. 2017;8(JAN):8. https://doi.org/10.3389/fmicb.2017.00008.

93.Chen S, Chen X, Zhang L, Gao J, Ma Q. Electrochemiluminescence Detection of Escherichia coli O157 : H7 Based on a Novel Polydopamine Surface Imprinted Polymer Biosensor Electrochemiluminescence Detection of Escherichia coli O157 : H7 Based on a Novel Polydopamine Surface Imprinted Polymer Bio. 2017. https://doi.org/10.1021/acsami.6b12455.

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

Graphene derivative	Materials in composition with graphene	Biorecognition element	Detected material	Detection limit	Linear range	Detection time	Type of biosensor	Ref.
detection of prions								
GO‡	-	FITC-PrP(95–110)	amyloid-b oligomers		0.01-2 μM	1 hour	fluorescent	*(32)*
GO	-	ssDNA	sensitive prion disease-associated isoform	$4.24 \cdot 10^{-5}$nM	0.001-1 ng/mL.	40 min	surface plasmon resonance	(*36*)
detection of viroids								
Graphene	zinc oxide	ssDNA	Coconut Cadang-Cadang Viroid	$4.3 \cdot 10^{-12}$ M	10^{-11}-10^{-6} M	60 min	electrochemical	(*38*)
GQD†	SiO_2 nanoparticles and NaYF4:Yb,Er	ssDNA	miRNA HIV1-miR-Tar-5p	10 fM	above1^{-6} M	-	Fluorescent	(*78*)
detection of viruses								
GO	1-pyrenebutyric acid N-hydroxysuccinimide ester	antibody	Rotavirus	10 PFU	10-10^5 PFU /mL	-	Electrochemical	(*80*)
Graphene	Silver nanoparticles and chitosan	H7-polyclonal antibody	avian influenza virus H7	$1.6 \cdot 10^{-3}$ to 16 ng/mL	30 min	1.6 pg/mL	Electrochemical	(*81*)
Graphene	poly (3-thiophene boronic acid) and nanogold	antibody	avian leukosis viruses	210 tissue culture infective dose /50·mL	527 -3162 infective dose/50·mL	-	Electrochemical	(*79*)

RGO	-	antibody	Rotavirus	10^2 PFU	10-10^5 PFU/mL	-	Field-Effect Transistor	(*82*)
GO	-	-	enteric EV71 and H9N2	-	-	30 min	RT-PCR	(*83*)
GO	-	ssDNA	Ebola virus	1.4 pM	30 fM-3 nM	-	Fluorescent	(*84*)
GO	Ferrocene-Chitosan	antibody	Hepatis B Virus Antigen	0.1 ng/mL	0.1-350 ng/mL	15 min	Amperometric	(*85*)
Graphene	Glycan	-	avian influenza virus	-	-	-	Field-Effect Transistor	(*86*)
RGO††	-	antibody	Influenza Virus H1N1	10^2 PFU	$10–10^6$ PFU/mL	15 min	Electrochemical	(*57*)
RGO	MOS_2	ssDNA	human papillomavirus	0.1 ng/mL	0.2-2 ng/mL	-	Electrochemical	(*45*)
detection of bacterial cells								
RGO	Indole-5-carboxylic acid	ssDNA	*Klebsiella pneumonia*	target DNA down to $3·10^{-11}$ M	10^{-6} -10^{-10} M		electrochemical	(*87*)
Graphene	Carboxyl	A virulent phage named as PaP1	*Pseudomonas aeruginosa*	56 CFU/mL	*P. aeruginosa* $1.4·10^2–10^6$ CFU/mL	30 min	Electrochemilumi nescent	(*88*)
GQD	amino-modified GQDs	Anti-Salmonella antibody	Salmonella & bacterial response to antibiotics	LOD for antibiotics can reach the pM level		30 min	electrochemical	(*95*)
RGO	RGO-Cu(II)	monoclonal antibodies	*Staphylococcus aureus*	4.4 CFU/mL	$10–10^8$ CFU/mL		electrochemical	(*90*)
GO	-	Anti-E. coli β-gal Abs	*E. coli*	10-100 μg/mL	-	-	infrared spectroscopy	(*91*)
GNPs and MG	SiO_2 substrates-Graphene (GNPs and MG)	anti-*E. coli* antibodies	*E. coli* O157:H7	10–100 cells/mL	GNPs $10^2–10^6$ cells/mL, MG $10–10^7$ cells/mL	30 min	Electrical	(*52*)
QDs & GO	-	complementaryt othe *invA* oligo	*Salmonella* specific *invA* gene	4 nM of the *invA* gene	-	20 min	fluorescencereso nanceenergytran sfer	(*92*)

GQD	nitrogen-doped GQD	*E. coli* polyclonal antibody	*E. coli* O157:H7	8 CFU/mL	10-10^7 CFU/mL	2 h	ECL	(*93*)
detection of fungi								
Graphene	Gold nanoparticles and cysteamine	Antibody (anti-mycelium)	*Aphanomyces Invadans*	309 ng/mL	0.2-4 mg/mL	90 min	Electrochemical	(*58*)
detection of microbial toxins								
Graphene	-	Antibody	microcystin-LR	0.05 µg/L	0.05-20 µg/L	-	electrochemical	(*36*)
RGO	Gold nanoparticles	ssDNA	Endotoxin	1 fg.mL^{-1}	0.9 pg/mL	30 min	electrochemical	(*6*)
detection of microbially derived antibiotics								

† 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.

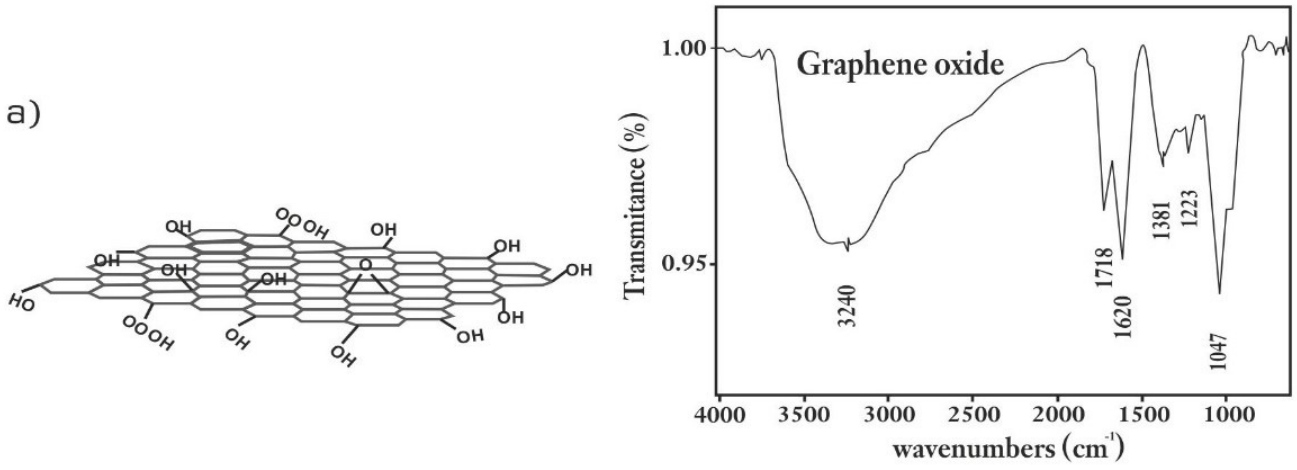

Fig. 3. Structure of GO (left) and its FTIR spectrum (right). Reproduced from Ref. (*1*) with permission

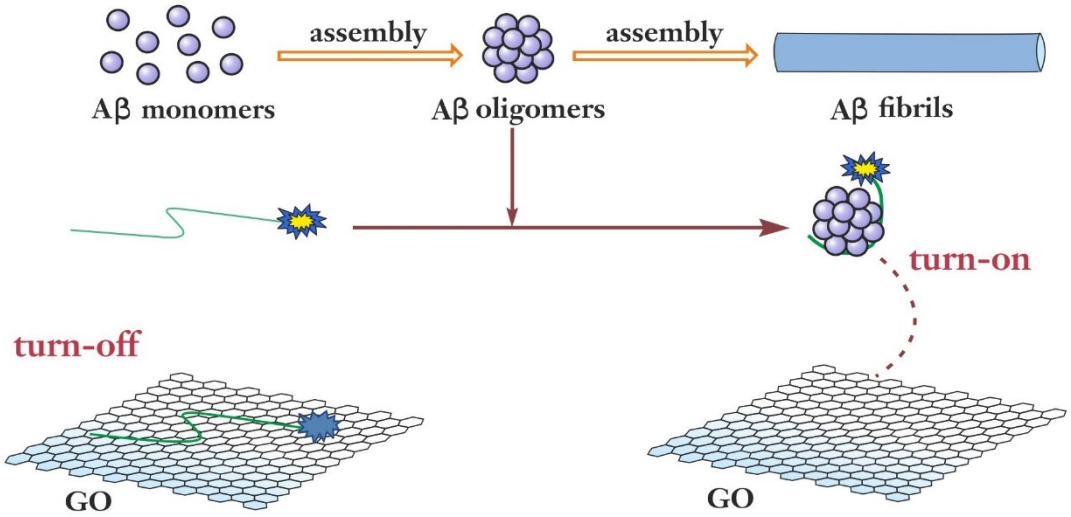

Fig. 2. Schematic illustration of the GO-based fluorescent biosensor. Reproduced from Ref. (*32*) with permission

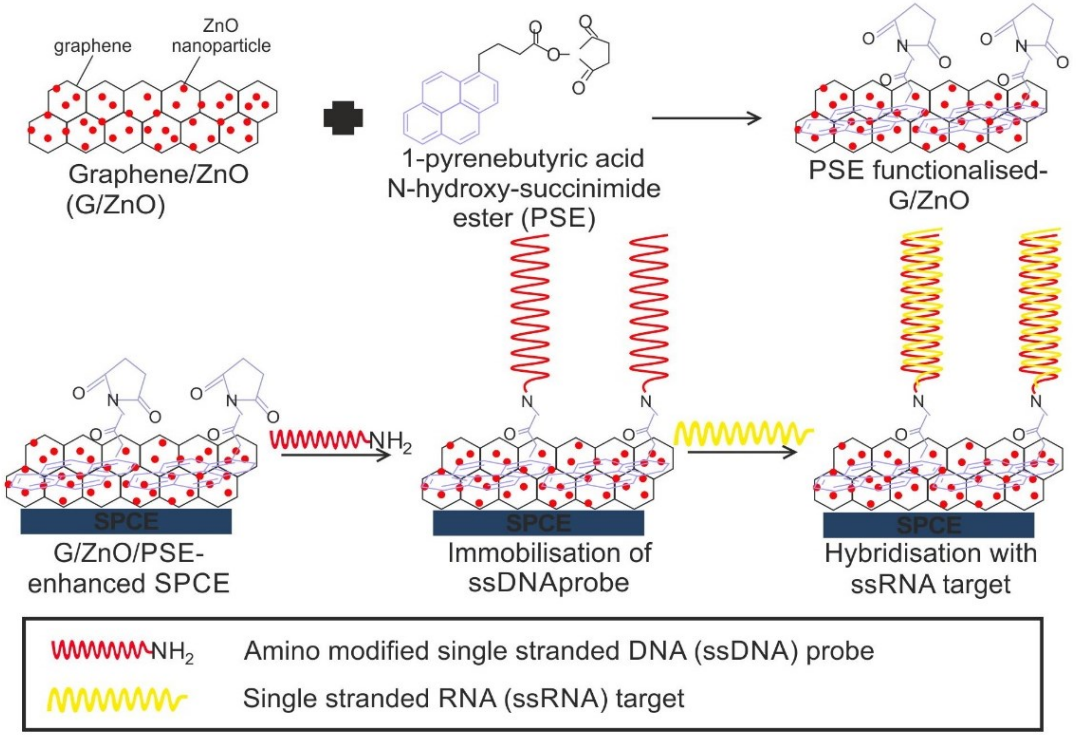

Fig. 3. Schematic diagram for the development of graphene/zinc oxide nanocomposite-modified electrochemical impedance genosensor for ssRNA detection, Reproduced from Ref. (*38*) with permission

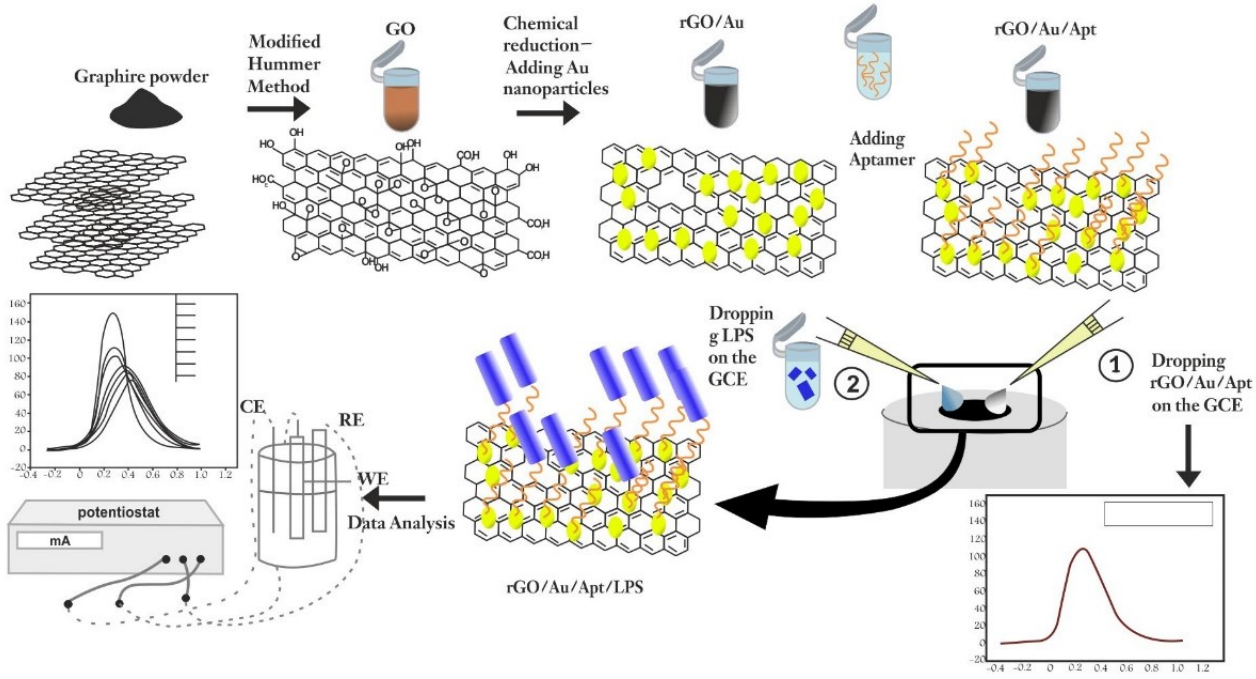

Fig. 4. schematic of electrochemical aptasensing of lipopolysaccharides from *Escherichia coli* bacteria. Reproduced from Ref. (*5*) with permission