Graphene-based biosensors for disease theranostics: Development, applications, and recent advancements

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Abstract: Graphene, owing to its unique chemical structure and extraordinary chemical, electrical, thermal, optical, and mechanical properties, has opened up a new vista of applications, specifically as novel sensing platforms. The last decade has seen an extensive exploration of graphene and graphene-based materials either alone or modified with nanoparticles and polymers for the fabrication of nanoscale biosensors. These biosensors displayed excellent conductivity, high sensitivity, and selectivity, good accuracy, and precision, rapid detection with low detection limits as well as long-term stability. The unmatched properties of graphene and graphene-based materials have been applied for the detection of a number of chemical and biological molecules successfully for the diagnosis of a variety of diseases, pathogens, and biomarkers of the diseases. This review is aimed to cover the fabrication methods, functionalization techniques, and biomedical applications along with the recent advancements in the field of development of graphene-based biosensors. Recent clinical trials and patents as well as market trends and opportunities associated with graphene-based biosensors are also summarized. The application of graphene-based biosensors in the detection of SARS-CoV-2 causing COVID-19 is also reviewed.

Keywords: graphene, biosensors, fabrication, functionalization, application, SARS-CoV-2, COVID-19, detection

1 Introduction

Diagnosis of diseases and their biomarkers requires accurate and highly sensitive methods and to achieve it, a number of conventional and novel methods are available [1,2]. Conventional methods include polymerase chain reaction, lateral flow immunosassay, electrochemical methods, DNA sequencing and microarrays and fluorescence microarray, and enzyme-linked immunosorbent assay (ELISA) techniques [3,4]. These techniques however require highly precise instruments, costly reagents, complicated sample preparation steps, and tedious quantification methods in order to achieve accurate and sensitive detection [5,6]. In addition, these techniques have limitations when it comes to detecting the disease in real-time. Novel methods include the use of sensors that are comparatively inexpensive, simple, and highly specific techniques for the detection of target biomolecules. These sensors can be used in real-time to monitor and diagnose diseases and therefore have broad clinical applications [7–10]. The added advantages associated with sensors are their use in detecting the diseases at an early stage and requires minimal invasive methods.

These sensors are fabricated using nanomaterials that further improve their chemical and electrical properties and therefore their sensitivity [10]. A number of nanomaterials are being used to fabricate these sensors, and graphene and graphene-based nanomaterials have shown exceptional properties with enhanced signal
Recent studies concerning graphene-based biosensors including the application in the COVID-19 testing along with the clinical trials and patents are also summarized.

2 Development of graphene-based biosensors

2.1 Fabrication methods

There exists an industrial revolution with respect to graphene and its utilization into several products around us including drugs and their delivery, medical devices, as well as many products and devices that improve the quality of life. Generally, graphene is produced using two main strategies and each strategy includes different fabrication methods. In a top-down strategy, graphene derivatives can be obtained from any carbon source such as graphite flakes or powder that is exfoliated mechanically or electrochemically and is subjected to chemical oxidation–reduction reactions. For instance, scotch tape techniques, sonication in a liquid phase, Hummers method, Brodie methods, etc., are the top-down techniques used in the fabrication of graphene derivatives. In contrast, the bottom-up strategy depends on the synthesis of graphene layers from the carbon atom bases and involves numerous fabrication methods such as chemical vapor deposition, epitaxial growth, thermal pyrolysis, etc. All these fabrication techniques have their own advantages and disadvantages [33–37]. Fabrication is a critical step in the development of graphene-based biosensors as it can influence the nature of graphene. Graphene and its derivatives are known to be excellent supporting materials that have been exploited extensively in the development of biosensors. A typical biosensor consists of two layers of receptors and transducers that are attached to each other, as shown in Figure 1.

![Figure 1: A typical biosensor device with graphene layer as a transducer and biomolecules as a receptor.](image-url)
The upper layer is made of receptors that are composed of biomolecules such as enzymes, proteins, DNA, or antibodies with specific biorecognition to the targeted analytes. These receptor molecules that are attached to the transducer layer are very sensitive to physicochemical changes upon biomolecular interactions and are capable to convert them into measurable signals \[38-40\]. Herein, graphene and its derivatives work as transducers for these signals that can be detected electrochemically, optically, or thermally. Therefore, graphene-based biosensors are fabricated according to the detection technique, and electrochemical biosensors are more commonly employed in comparison to optical biosensors \[41,42\].

In the electrochemical biosensors, the designed sensors usually consist of a three-electrode system: working electrode, counter electrode also known as the auxiliary electrode, and the reference electrode. These electrodes are coated with graphene-based nanomaterials using fabrication methodologies including coating, direct growth methods, direct deposition methods, and printing-based methods. Direct coating is the most commonly employed technique for the fabrication of biosensors due to its simplicity, cost-effectiveness, and it does not require any specific instrument \[40\]. Usually, a solution/gel containing graphene or graphene derivatives is used to coat the surface of electrodes using methods such as drop-casting, dip coating, spin coating, and blade coating. Each coating technique has its advantages and disadvantages, and therefore, the choice of the technique depends on the desired film properties such as thickness, uniformity, and surface area. Generally, the direct coating method is the first choice for biosensors; however, it involves multistep fabrication, material wasting, and is a time-consuming process \[43-47\]. Direct deposition-based methods involve direct deposition of graphene and other nanomaterials on the electrode surface by dipping the electrodes in a solution containing graphene and applying an electrical voltage to control the coating. The electrospraying technique involves the spinning of a nozzle jet containing the graphene nanomaterial onto the electrode surface. The electrospray deposition method is similar to electrospraying deposition; however, it offers control of droplet size, charge, and speed, and therefore, is utilized in industrial-scale fabrication with high precision \[48-51\].

Subsequently, printing-based fabrication methods were introduced with the revolution of 3D printers. Printing techniques are very attractive because of many reasons including large-scale fabrication, are cost-saving, and require low temperatures and solution ink. These methods fit properly with graphene-based biosensors and therefore are exploited extensively in the development of various biosensors \[52-55\]. In this method, the ink solution is prepared and sprayed using various techniques including screen-printing, inkjet printing, nozzle-jet printing, and laser scribing printing. Generally, these printing techniques are similar to each other in principle with small variations; for instance, screen printing is used for a thicker nanofilm and is commonly employed for industrial-scale fabrication due to its simplicity and utilization of prefabricated mesh-covered frames to allow the transfer of the desired pattern to the electrode surface. Direct spray of ink solution can be achieved using inkjet printing that exhibits more controlled drop size and high-resolution pattern, while nozzle-jet printing is similar to inkjet printing with the addition of external pressure. Therefore, nozzle-jet printing is suitable for more viscous ink solutions. Meanwhile, laser scribing printing is an advanced technique with high fabrication flexibility for many substrates and supporting materials as well as small size sensors. However, printing using instrumental techniques require special handling skills and need preparation of ink solution critically.

Another method of fabrication of biosensors is the direct growth of nanomaterials on the surface of electrodes. This method gained more attention recently due to several controllable parameters during fabrication such as time, temperature, pH, pressure, and concentration. This strategy depends on the recruitment of several experimental environments to catalyze the nanomaterial growth on the electrode surface via numerous techniques such as thermal, hydrothermal decomposition, anodization, and chemical decomposition \[56,57\]. However, limited attention to these methods is given for the fabrication of graphene-based biosensors as it involves limited direct growth to the electrode surface.

### 2.2 Functionalization methods

Due to its unique electro- and thermomechanical properties, applications of graphene are practically limitless. It showed excellent potential in transforming many areas ranging from electronics to healthcare such as touch screens, sensors, biofunctionalized graphene, quantum dots, novel drug delivery, nanodevices for DNA sequencing, etc. \[58\]. The unique molecular structure of graphene characterized by sp²-hybridized carbons, large specific-surface area \(2,630 \text{ m}^2 \text{g}^{-1}\) as well as strong van der Waal cohesive forces render graphene to agglomerate easily and prevent its uniform dispersion \[59\]. Moreover, graphene sheets have poor water solubility because of the strong π–π interactions between the sheets \[60\]. To address this, the functionalization of graphene is
performed in order to redesign its electronic, physical, and chemical properties [61]. To date, functionalization is the only effective technique that helps in reducing the cohesive forces between graphene sheets and thus prevents its agglomeration without losing its inherent properties [62].

Functionalization involves the process of adding new functions, characteristics, potentials, or properties to graphene by changing its surface chemistry [63]. Functionalization modifies inert graphene sheets and is exceptionally effective in fabricating sensors that have huge biomedical, electrochemical, and diagnostic applications [64]. Functionalization of pristine graphene through the covalent and noncovalent functionalization provides a multitude of chemically activated, soluble, hybrid graphene [65] surfaces. Covalent functionalization forms a stable covalent bond, whereas the noncovalent functionalization is formed through hydrogen bonds, π–π interactions, and van der Waals interactions [66].

2.2.1 Covalent functionalization

Covalent functionalization of graphene involves rehybridization of sp² C-atoms into the sp³-hybridized tetrahedral configuration, chiefly at the edge [67]. This process of covalent surface modifications is also known as chemisorption (grafting) of molecules on graphene lattice [68]. This rehybridization of the π-conjugated carbon network often forms hybrid-graphene materials with chromophores or polymers that enhance the dispersibility of graphene [69]. Figure 2 illustrates different covalent functionalization techniques employed in the development of graphene-based biosensors.

2.2.1.1 Free radical addition (FRA)

Free radicals are extremely reactive uncharged chemical species that contain an unpaired electron. They can react readily with the compounds containing multiple bonds to produce another radical, which reacts further and goes on [70]. FRA can be achieved through one or more synthetic approaches such as aryl diazonium salts [71], peroxides, Bergman cyclization [72], and the Kolbe–Schmitt reaction [73]. Among these, aryl diazonium salts are the most studied method for stabilizing graphene layer with enormous applications in the development of semiconducting nanomaterials, atom transfer radical polymerization, coupling reactions through click chemistry, grafting of

Figure 2: Covalent functionalization of graphene sheets through different synthetic protocols.
heterostructures, and tuning of electrical conductivity [74]. The delocalized \( \pi \)-electrons of the graphene cage are shifted to electron-deficient diazonium electrophile and eliminate its \( \text{N}_2 \) molecule thereby forming a highly reactive aryl \( \pi \) radical that eventually reacts with the carbon atom of the graphene lattice and forms a covalent bond [75]. Functionalization through benzoyl peroxide (BPO) is another common approach for the FRA. BPO has been widely utilized as an important organic peroxide initiator because of its easily accessible benzoyloxy radicals after homolytic fission [76,77]. The functionalization of graphene by Bergman cyclization has many advantages including simple steps, superior efficacy, tailored structure, and catalyst-free procedure [78].

2.2.1.2 Nucleophilic addition

The nucleophilic addition reaction is widely employed in the functionalization of pristine graphene sheets due to its electron acceptor properties [79]. Recently, a soluble charm-bracelet-type poly(N-vinylcarbazole) functionalized graphene sheet has been developed by the reaction of carbanion intermediate of poly(N-vinylcarbazole) and graphene [80]. In another study, covalent modification of rGO was performed through the nucleophilic addition reaction using nitrogen anions, formed by sodium hydride. The \( \pi-\pi \) interaction and “polymer wrapping” effect between the polymers and graphene resulted in improved dispersion of graphene [81].

2.2.1.3 Cycloaddition reaction

The cycloaddition reaction involves the formation of a new ring by the \( \sigma \) bonds through the reaction of two \( \pi \)-electron systems [82]. The aromatic properties of graphene is exploited through a number of cycloaddition reactions involving 1,3 dipolar cycloadditions, \( [2 + 2] \) cycloadditions, \( [2 + 1] \) cycloadditions, and Diels–Alder reaction [83–86]. Diels–Alder reaction is one of the promising methods for the modification of pristine graphene because of its click-type procedure, high efficiency, versatility, and efficiency [87]. Dihydronaphthalene-grafted graphene was designed using cis-diene and the resulting modified graphene showed a p-type doping effect with improved conductivity that can be used for making transparent electrodes [88].

2.2.1.4 Electrophilic substitution reactions (ESRs)

Electrophilic aromatic substitution reactions are very fascinating versatile organic reactions where an electrophile substitutes the atom attached to the aromatic ring; usually, a hydrogen atom is replaced by an electrophile [89]. This reaction has gained much attention in the field of covalent functionalization of graphene because ESR can introduce a wide range of functional groups on the graphene surface and thus is a useful tool for tailoring the graphene properties [90]. Some of the hitherto reported ESR functionalization includes halogenation [91], nitration [92], sulfonation [93], Friedel–Crafts acylation, alkylation [94] reactions, etc.

2.2.1.5 Addition of chromophores

Organic compounds having extended \( \pi \)-system such as porphyrins [95], phthalocyanines [96], azobenzenes [97], and other chromophores were identified as potential candidates for preparing hybrid graphene nanomaterials due to their superior optical limiting property. Covalently functionalized soluble hybrid porphyrin–graphene was prepared by the reaction of 5,15,20-triphenylporphyrin and GO in \( N,N \)-dimethylformamide (DMF). The amide linkage in the hybrid porphyrin–graphene significantly improved the dispersion and thereby solubility of graphene in organic solvents. Moreover, this donor–acceptor nanohybrid also exhibited superior optical limiting performance due to photoinduced electron and/or energy transfer [98]. Table 1 summarizes different covalent functionalization methods used in the development of graphene-based biosensors.

2.2.2 Noncovalent functionalization

Noncovalent interactions are reversible interactions between the graphene and organic molecules or polymers without disruption of the delocalized \( \pi \)-system of graphene and therefore its electronic properties [99]. Physical forces such as hydrophobic, van der Waals, and electrostatic forces are the major forms of interactions utilized in the noncovalent functionalization [100]. Numerous studies reported the immobilization of proteins, DNA–protein complexes, enzyme–drug complexes, functional nanomaterials, and organic supramolecules using a noncovalent functionalization technique [101]. In recent years, enormous advancements were made in terms of functionalization of graphene through \( \pi-\pi \) stacking with polyaromatic compounds such as naphthalene [102], pyrene, 4-n-octyl-A'-cyanoisobiphenyl, tetrafluoro-tetra-cyanocuoniodimethane [103], 3,4,9,10-perylenetetracarboxylic diimidebisbenzensulfonic acid [104], pyridinium-functionalized porphyrin, [105] etc. Noncovalent immobilization of enzymes on graphene surfaces was also reported to have...
improved biocatalytic efficiency; for instance, immobilization of glucose oxidase and glucoamylase enzyme for one-pot conversion of starch into gluconic acid [106]. Highly stable and water-soluble gold nanoparticles (Au-NPs) of DNA-decorated graphene nanosheets were also reported to be a promising approach to design a 2D-conductance device for DNA sequencing (Figure 3) [107].

3 Biomedical applications of graphene-based biosensors

Traditional sensing methods are expensive, require high-precision equipment and costly reagents, and the majority of reactions are not quantifiable in real-time. Graphene-based sensors are now being used as an alternative method for the identification of disease-related biomolecules and they offer a wide range of biomedical applications. Graphene biosensors are easy-to-use, cost-effective, nontoxic, and are equipped with excellent sensing properties [108]. Generally, a sensor consists of two elements: receptor (linked with target molecule) and transducer (converts chemical information into signals). A graphene or GO-based biosensor acts as a transducer converting the receptor–target molecule interaction into a detectable signal. Bioreceptors such as antibodies, enzymes, or nucleic acid are usually immobilized to the transducers in order to allow target molecules to interact (Figure 4) [12,109].

3.1 Detection of microbes

Antibodies, nucleic acids, proteins, and enzymes are immobilized to graphene biosensors using various
processes, which can be identified using spectroscopic methods [15]. Attachment of antibodies to the graphene surface is mainly applied for the detection of infectious diseases caused by viruses and bacteria. Table 2 summarizes the studies on graphene-based biosensors used for the detection of microbes including bacteria and viruses. Graphene biosensors have been successfully applied for the detection of Ebola virus [110,111], Escherichia coli [112], and Zika virus [113], whereas graphene biosensors modified with silver and gold nanoparticles were developed for the detection of Salmonella typhimurium [114], hepatitis-C virus (HCV) [115,116], and avian influenza virus H7 [117]. Modifications of graphene biosensors have further been proven to be effective in the detection of diseases as dendrimers, polymers, and cyclodextrin modifications could all be used to diagnose celiac disease [118], human immunodeficiency virus (HIV) [119,120], and cholera toxin [121].

Different types of antibodies are immobilized on the graphene surface and are being used for the detection of target molecules. For instance, PAC1 is used for the diagnosis of cardiovascular diseases, anti-GHRL and anti-PYY antibodies for hormone detection, an anti-tTG antibody for celiac diseases, an anti-HCV antibody for Hepatitis C virus, anti-CT for cholera toxin, anti-rotavirus antibodies for rotavirus detection, monoclonal antibodies (H5N1, H1N1, H7) for avian influenza virus H7 and influenza A virus, anti-žika NS1 antibody for žika virus, anti-S. typhimurium antibody for S. typhimurium bacteria, and anti-E. coli O157:H7 antibody for the detection of E. coli. Moreover, the Dengue virus and rotavirus have also been identified using antibodies immobilized to GO biosensors [122–124]. Rotavirus and G2 monoclonal antibodies were used in these techniques that bind to the graphene nanomaterial using a carbodiimide-assisted amidation reaction and an electrostatic bond. Another promising breakthrough was achieved when graphene quantum dots were prepared that were successfully applied for the highly sensitive detection of hepatitis B and adenovirus [125–127].

### 3.2 Detection of nucleic acids and genes

The DNA-based graphene biosensor helps in the detection of various types of biomarkers (DNA, RNA, small molecules, proteins), viruses, and genes using electrochemical and fluorescent detection techniques (Table 3). In the electrochemical approach, the immobilization of DNA is achieved using covalent bonds, π–π interactions, or EDC/NHS chemistry on the surface of graphene biosensors. Electrochemical signals are generated when DNA is hybridized or oxidized. Differential pulse voltammetry, CV, and electrochemical impedance spectroscopy were used to quantify voltage and current shifts triggered by a number...
of factors, such as conductivity changes or electron deple-
tion caused by oxidation or hybridization. In the fluo-
rescence approach, the immobilization of DNA can be
achieved through π–π interaction (direct adsorption of the DNA probe
on the biosensor). This is based on the hybridization of two
single-stranded DNA (ssDNA); one strand being fluo-
rescently labeled while the other is the complementary DNA
to the target DNA.

### 3.3 Detection of enzymes and other molecules

Similarly, a number of enzymes and related biomolecules
such as horseradish peroxidase, laccase, glucose, and
bilirubin oxidase were also immobilized onto the graphene
biosensor via covalent bonding, physical entrapment,
1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride/
N-hydroxy sulfo succinimide (EDC/NHS) chemistry, and adsorp-
tion methods. These types of sensors are based on two
main mechanisms: catalytic properties of the enzymes
and enzyme activity inhibition/moderation. Different
molecules such as phenols (2,6-dimethoxyphenol), hydrogen
peroxide, caffeic acid, glucose, bilirubin, and 17β-estradiol
have been detected by enzyme-immobilized graphene biosen-
sors (Table 4).

### 3.4 Detection of severe acute respiratory
syndrome-coronavirus-2 (SARS-CoV-2)

Recently, graphene biosensors were applied in the devel-
opment of point-of-care (POC) testing (POCT) devices for
the detection of coronavirus disease-19 (COVID-19), an
ongoing pandemic the world is suffering from. The rapid
and sensitive detection of SARS-CoV-2, the causative
pathogen of COVID-19, is an ongoing global challenge
that is associated with large-scale diagnosis in order to
downregulate its spread within the communities. It demands
early identification of infection in presymptomatic and
asymptomatic individuals [162, 163]. There remains an urgent
need for a rapid and precise diagnostic method for the
detection and screening of the disease. The graphene-
based biosensors have attracted much attention in the out-
brake owing to their extraordinary properties [164] and
have emerged as a successful application tool to detect
### Table 3: Graphene-based biosensors for the detection of nucleic acids and genes

| Target | Immunosensor design | biosensor | Detection methods | Detection limit | References |
|--------|---------------------|-----------|-------------------|----------------|------------|
| HIV-1 gene | RNA hybridization | DNA | Fluorescence detection | 0.00625 nM | [136] |
| ssDNA | ssDNA | GO | Fluorescence detection | 15 nM | [137] |
| ssDNA | ssDNA | GO | Electrochemical detection | 16.3 nM | [138] |
| ssDNA | ssDNA | GO | Fluorescence detection | 600 nM and 200 nM | [139,140] |
| ssDNA | ssDNA | GO | Electrochemical detection | 3.2 × 10⁻³ M | [141] |
| ssDNA | ssDNA | GO | Fluorescence detection | 2.02 nM | [142] |
| ssDNA | ssDNA | GO | Electrochemical detection | 4.28 × 10⁻¹⁰ M and 1.58 × 10⁻¹⁰ M | [143,144] |
| ssDNA | ssDNA | GO | Electrochemical detection | 600 nM and 200 nM | [145] |
| ssDNA | ssDNA | GO | Electrical | 600 nM | [146] |
| ssDNA | ssDNA | GO | Electrical | 600 nM | [147] |
| ssDNA | ssDNA | GO | Electrical | 600 nM | [148] |
| ssDNA | ssDNA | GO | Electrical | 600 nM | [149] |
| ssDNA | ssDNA | GO | Electrical | 600 nM | [150] |

COVID-19. Antibody-conjugated graphene sheets could rapidly detect target viral proteins and were used for large-scale screening and development of biosensors [165]. The available conventional technology such as RT-PCR is time-consuming, labor-intensive, and scarcely available in remote areas. These POC biosensors are typically low-cost, user-friendly, and have remarkable potential as medical diagnostics [166]. These biosensors are now a future diagnostic approach for COVID-19 as clinical diagnostics [167] owing to their accuracy, affordability, and portability [168]. The introduction of nanomaterials unquestionably enhanced the performance of graphene biosensors, and the sensing abilities of these biosensors are of a peerless level of ultrasensitivity [169]. Table 5 summarizes the graphene-based biosensors developed so far in the point-of-care testing (POCT) for COVID-19.

The breakthrough in the discovery of graphene-based biosensors for the diagnosis of COVID-19 was made when a graphene-based electrochemical biosensor coupled with an electrical readout setup was developed, which was selectively able to detect the SARS-CoV-2 genetic material [162]. The biosensor was made selective by integrating thiol-modified antisense oligonucleotides (ssDNA), which were specific for the N-gene (nucleocapsid phosphoprotein) of SARS-CoV-2. The sensitivity of the detector was further enhanced when the thiol-modified ssDNA-capped gold nanoparticles (AuNPs) were applied on the gold electrode. Similarly, a wireless graphene-based telemedicine platform known as the SARS-CoV-2 RapidPlex was developed, which could successfully detect the nucleocapsid protein (NP) as well as specific immunoglobulins against the spike protein (S1) (S1-IgG and S1-IgM), in both blood and saliva of the patients [163]. The detector consisted of laser-engraved graphene sensor arrays that were proved to be a highly convenient, rapid, accurate, and stage-specific tool for the detection of the virus. 1-Pyrene butyric acid (PBA) was utilized as the linker to attach the receptors onto the graphene surface.

In another study, the SARS-CoV-2 spike protein antibody was immobilized onto the fabricated graphene-based device using 1-pyrene butyric acid N-hydroxysuccinimide ester (PBASE) as an interface coupling agent [170]. The developed biosensor showed an excellent LOD of 1 fg mL⁻¹ of the viral spike protein. The sensitivity of the biosensor was assessed using a control experiment that showed that the spike protein was essential for specific binding with the viral antigen. The selectivity was confirmed when the developed COVID-19 FET did not exhibit any response to MERS-CoV spike proteins. Recently, another graphene-based FET device was developed as a portable bifunctional electrical detector through either nucleic acid hybridization
A number of patents were granted worldwide on graphene-based biosensors owing to a much expected attraction of scientists worldwide. In the following years on graphene-based biosensors owing to a much expected attraction of scientists worldwide. In 2013, a US patent was granted in which the graphene biosensor was described that helped to detect biological molecules. This led to an increase in the number of patents in the graphene field. The change in conductivity in the prepared sensor measured the biological molecule.

### Table 4: Graphene-based biosensors for the detection of enzymes and other molecules

| Target with enzymes                  | Immunosensor design                                                                 | Detection methods                      | Detection limit                  | Refs |
|-------------------------------------|--------------------------------------------------------------------------------------|----------------------------------------|----------------------------------|------|
| 17β-Estradiol with laccase          | GO–rhodium nanoparticles                                                            | Electrochemical detection              | 0.9–11 pM                       | [151]|
| Catechol with laccase               | Graphene/cellulose microfiber                                                       | Amperometric detection                 | 0.085–209.7 µM and 0.01–12 mM   | [152]|
| Hydrogen peroxide with horseradish  | 3D graphene/methylene blue-carbon nanotubes and calcium carbonate (CaCO3) microspheres encapsulated with graphene capsule | Electrochemical detection              | 0.2 µM to 1.1 mM and 0.01–12 mM | [153,154]|
| Peroxidase                          | Molybdenum disulfide and graphene quantum dots                                      | Electrochemical detection              | 0.38–100 µM                      | [155]|
| Caffeic acid with laccase           | 3D graphene and 3D GO and polyaniline                                              | Electrochemical detection              | 0.3–6 mM and 0.07–1.10 mM       | [156,157]|
| Glucose with glucose oxidase        | 3D graphene and 3D GO and polyaniline                                              | Electrochemical detection              | 0.2 µM to 1.1 mM and 0.01–12 mM | [153,154]|
| L-Lactic acid                       | Lactate dehydrogenase immobilized on the active graphene surface using Nafion, chitosan, and glutaraldehyde | Electrochemical detection              | 7.5 mM                           | [158]|
| Acetaminophen, Epinephrine, tyrosine| l-Aspartic acid modified CVD graphene electrode                                     | Electrochemical                        | 0.011, 0.006, 0.31 µM respectively | [159]|
| Lung cancer biomarker (CD59)        | GO–NP embodied complex                                                              | Electrochemical                        | 1 fg mL⁻¹                        | [160]|
| Glucose oxidase                     | CVD-graphene on SiO2/Si substrate followed by deposition of Nafion                  | Electrochemical                        | 124.19 µM                        | [161]|

4 Patents and clinical trials on graphene-based biosensors

A number of patents were granted worldwide on graphene-based biosensors and the methods of manufacturing the first patent was granted in the year 2010, a few years after the discovery of graphene in which the configuration of graphene biosensor was described [177]. This device consisted of two electrodes, three layered structures (conductive, insulating, and graphene), and a superconducting dopant island. The graphene layer was adsorbed to the dopant island and observing the voltage response of the dopant island. Subsequently, in 2011, a worldwide patent was granted for the graphene-based biosensor showing ultra-low detection limits in the range of 0.1–1 fg mL⁻¹. The biosensor was fabricated using PBASE in accordance and was exposed to ssDNA probe or antigen–antibody protein interaction [178]. The development of graphene-based biosensor showed ultra-low detection limits in the range of 0.1–1 fg mL⁻¹, and was able to detect the pathogen in real samples. The biosensor was fabricated using PBASE in accordance and was exposed to ssDNA probe or antigen–antibody protein interaction [178].
### Table 5: Portable biosensors in POCT for COVID-19

| Type of graphene biosensor | Technology/nanotechnology used | Time of detection | Limit of detection (LOD) | Outcomes of the study | Reference |
|----------------------------|--------------------------------|-------------------|--------------------------|-----------------------|-----------|
| Quantitative paper-based electrochemical sensor chip | AuNPs capped with highly specific antisense oligonucleotides (ssDNA) aimed to target nucleocapsid phosphoprotein (N-gene) | Less than 5 min | Significant amplification in the output signal in the presence of SARS-CoV-2-RNA; Sensitivity: 231 (copies µL⁻¹); LOD: 6.9 copies µL⁻¹ | The developed sensor showed almost 100% accuracy, specificity, and sensitivity | [162] |
| Multiplexed, portable, wireless electrochemical biosensor | Laser engraved graphene electrodes | — | — | | |
| FET-based biosensor | Graphene sheets of the FET were coated with specific SARS-CoV-2 monoclonal antibodies against S-protein | — | FET device could detect SARS-CoV-2; LOD (in the culture medium): 1.6 × 10³ PFU mL⁻¹; LOD (in clinical samples): 2.42 × 10² copies mL⁻¹ | Successful fabrication of the FET biosensor as a highly sensitive immunological diagnostic method | [170] |
| A novel biosensor based on bioelectric recognition assay | Human chimeric spike S1 antibody immobilized on membrane-engineered mammalian cells | Ultra-rapid manner (3 min) | 1 fg mL⁻¹ | Configured biosensor could be applied as a ready-to-use tool in the mass screening of SARS-CoV-2 antigens. No sample processing step is required | [171] |
| CRISPR-based assay | Based on CRISPR complex (Cas12a/gRNA) attached to a fluorescent probe that could detect targeted amplicons produced by RT-PCR | — | LOD of 2 copies per sample | Results demonstrated rapid analytical sensitivity and robust diagnostic performance to improve the current COVID-19 screening. Functionality validated by detecting SARS-CoV-2 N-protein; a promising platform for POCT | [172] |
| Simple immunosensor integrated onto a microfluidic chip | Utilizes the dual-labeled magnetic nanobeads immunomagnetic enrichment and signal amplification | Rapid quantification (<1 h) of SARS-CoV-2 antigen in whole serum samples | Rapid detection of SARS-CoV-2 protein biomarkers; highly sensitive; less than 50 µL serum sample required. LOD of the clinical specimen was 200 copies mL⁻¹ | Both in silico analysis and actual testing showed high selectivity and high specificity | [173] |
| Ultrasensitive electrochemical detection using calixarene functionalized GO | Based on super sandwich type recognition strategy; practical detection of SARS-CoV-2 RNA using electrochemical Smartphone without amplification of nucleic acids and reverse transcription | — | | | |
| Electrochemical immunosensor | Usage of magnetic beads as immunological chain support with secondary antibodies coupled with alkaline phosphatase used as an immunological label; applied for the detection of both S- and N-protein of SARS-CoV-2 | 30 min | The analytical features of the electrochemical immunoassay were assessed using N- and S-protein standard solutions with untreated saliva; detection limit: 19 and 8 ng mL⁻¹, respectively, for S- and N-proteins | An analytical tool with high potentiality for market entry for SARS-CoV-2 detection in untreated saliva | [175] |

(Continued)
| Type of graphene biosensor          | Technology/nanotechnology used                                                                 | Time of detection | Limit of detection (LOD)                                                        | Outcomes of the study                                                                 | Reference |
|-----------------------------------|--------------------------------------------------------------------------------------------------|-------------------|---------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|-----------|
| Voltammetric genosensor           | Hexathia-18-crown-6 (HT18C6) modified with Ag + ions used as redox probe; carbon paste electrode (CPE) coated with HT18C6-Ag and modified further using chitosan and PAMAM dendrimer-coated silicon quantum dots (SiQDs-PAMAM) | —                 | Concentration range: 1.0 pM to 8.0 nM; LOD: 0.3 pM. Against SARS-CoV-2 RdRP | Detection of SARS-CoV-2 in human sputum                                              | [176]     |
| Label-free paper-based electrochemical biosensor | Immunosensor designed for the detection of immunoglobulin against SARS-CoV-2 with high specificity and sensitivity | —                 | —                                                                               | Opens new possibilities for diagnosing COVID-19                                     | [177]     |
| FET                               | Portable bifunctional electrical detector based on graphene FET for the detection of SARS-CoV-2 via nucleic acid hybridization or Ag–Ab interaction | Exhibited rapid detection speed (~10 min for nucleic acid detection and ~5 min for immunoassay) | —                                                                               | Efficient and accurate tool for high-throughput point of care testing of COVID-19 | [178]     |
graphene electrode, the voltage might be applied and the concentration was measured via the electric current response. In the same year, another graphene-based biosensor device received the patented status where the graphene nanosheet modified with chitosan were coated on glassy carbon electrodes and were used as sensing agents for polynucleotide and mutation detection using voltammetry [182]. Another breakthrough in the research on graphene-based biosensors came when a patent on FET was developed for the detection of biomolecular samples [183]. This sensor consisted of a graphene layer (semiconductor material) bound with ligand-binding protein that helped to detect protein-based substances.

Another graphene-based FET biosensor device was developed by modifying their edges with functional groups that helped in detecting the sample using an analyzer by measuring the change in the current generated by the sample connected to the functional group. Thin-film graphic layers made up of many graphene layers were added to a base in the desired pattern and altered for better electrode connection [184]. A novel strategy for the detection of samples present in food was developed using electrochemical DNA graphene or biochip biosensor. Their detection was based on the mechanism of DNA (present in the sample)—redox (ruthenium hexamine molecule) electrostatic complex, and their subsequent nonspecific adsorption on the graphene surface. The developed biosensor was able to detect meat samples using isothermal amplification of DNA and electrochemical detection via square wave voltammetry [185]. Two Chinese patents were granted recently in the years 2018 and 2019 using graphene-based biosensors for the detection of guanine ribose [186] and cancer marker microRNA [187] using 3D graphene biosensors. These biosensors consisted of a graphene layer over a glass substrate where indium tin oxide was arranged on the sides of the sensor. This 3D biosensor showed a number of advantages including ease of operation and use, improved specificity at lower operating voltages, and even great safety. Very recently, a graphene biosensor was designed and manufactured using GNP ink, which was deposited on the surface of the substrate to provide at least one sensing layer. This layer was processed via photonic curing to generate a graphene electrode vertically arranged graphene sheets and used for plasma treatment for functionalization, which was used for the identification of biological molecules [188].

Various nongraphene biosensors are being tested in clinical trials for the identification of various diseases, but graphene-based biosensors are only a few. A new approach based on the photoelectrochemical immunosensor employed graphene quantum dots in conjunction with Si nanowires for the early detection of myocardial infarction was under clinical trial but is not yet recruiting [189]. They aimed to examine 100 people above the age of 18 who complained of chest pain to see if they had an acute myocardial infarction.

5 Biomedical graphene biosensors – market trends, opportunities, and future perspectives

Graphene often heralded as the wonder material is an advanced 2D carbon-based material of the 21st century having exceptional properties of light-weightedness, high strength, super flexibility, excellent superconductivity, and having a paramount potential in improving the human life quality. Today, biosensors have made a huge impact in various biosensing platforms as electrochemical biosensors, fluorescent biosensors, and graphene biosensors for enzymatic biosensing and immunosensing applications. The superconductive material graphene is a low-cost ideal material for the construction of sensors and biosensors-based devices for biomedical applications [190]. Graphene is considered one of the most advanced materials in human healthcare as biosensing and diagnostic tool and this has accelerated medical diagnosing to new dimensions. It has successfully revolutionized our understanding of the treatment of deadly diseases through its derivatives and hybrids-based biosensors and their march toward the marketplace is at a high pace and more of these are expected to come into the market in the next decade as high-end diagnostic tools in POCT [191].

As research and development are growing each year, biosensors are becoming a commercial reality. But, before becoming a commercially significant device, the graphene-based biosensors are bound to face initial and primary challenges such as quality control, scalability, and durability issues that should be resolved transparently. The intrinsic properties of graphene, their integration with other functional materials, fabrication of devices, and processing steps need close considerations [192]. The market forecast reports on biosensors were shared for understanding more about their market trends and opportunities. The Biomedical Sensor Market - Forecast Report (2021–2026) stated that these biosensors adjusted themselves according to the genetic make-up of each patient and are programmed to send alarm signals when anomalies or unexpected body readings are registered in real-time [193]. Biomedical sensor devices greatly improved early detection of health problems by measuring blood pressure remotely, detection of toxins
in blood, analysis of glucose, lactate, and glutamine in aqueous media, and analysis of other complex biological media.

Similarly, the Frost and Sullivan Analysis Report (2018–2023) on biosensor market forecast expected growth in the biosensor market at a 12% compound annual growth rate during 2018–2023 from $17.7 billion in 2018 to reach up to $31.2 billion by the end of 2023 [194]. The key application areas and segments marked by them included point-of-care home diagnostics in the healthcare system, food, water, and air quality monitoring, agriculture and security, etc. Wearable biosensors for the noninvasive monitoring of heart rate, breathing rate, glucose, disease diagnostics, and detection are also mentioned in their report. The US National Institute of Health would encourage and fund biosensor-related research activities. The nano-biosensors have promising applications in the biomedical sector and diagnostics because of their improved specificity and selectivity. Other than the upward market trend of nano-biosensors and wearable biosensors, the digestible biosensors, silicon photonic biosensors, implantable biosensors, and touch-based in-vehicle sensors are also having promising future in this area of biosensing [194].

The Graphene Market and 2D Materials Assessment Report (2021–2031) forecasted for 18 key applications areas and the movement of graphene biosensors from the laboratory to the commercial market in the coming decades. According to this assessment report, the forecast was made that the graphene market will continue to grow from <$100 m in 2020 up to $700 m by 2031. This success will not be immediate or overnight and will be because of continued efforts by global research and commercialization efforts. In this regard, nanoplatoletes are closest to commercial success, and progress toward their standardization, safety, legislation, quantification is progressive and quite evident [195]. Very recently, a Graphene Research Hub was launched in 2017 as a collaborative platform in academia and industry at the University of Adelaide with the aim of enabling the development of a sustainable graphene-based industry in Australia [196].

There is a race to the commercialization of graphene-based biosensors and bringing them to market will change the way diseases are diagnosed and treatment is delivered. Patients will receive the test results fast, the diagnosis will be at the site in minutes, and treatment could begin immediately thus saving valuable and precious time [197]. For instance, very recently, researchers from the University of Illinois, Grainger College of Engineering, developed rapid, ultrasensitive testing using a paper-based graphene electrochemical biosensor that can detect the COVID-19 virus in less than 5 min [162].

The understanding of physics and nanotechnology goes hand-in-hand and it holds potential in improving the quality, cost of biological testing, speed, and capability to analyze the resulting complex data. Biosensors enable biotechnicians to control the precise biological data [198]. Based on their pharmaceutical applications, the biomedical biosensors’ market has been segmented as cardiac care, pain management, drug discovery, diagnostics, and genomics. The global market trends and opportunities show that wearable biosensors are in an upward trend, especially in diabetics and cardiovascular diseases [199–201].

6 Conclusions and future perspectives

A vast variety of graphene-based nanomaterials consisting of pristine graphene, GO, rGO, and graphene quantum dots are used for the preparation of graphene-based biosensors. These biosensors showed a plethora of applications in biomedical and nonbiomedical fields for the detection of target molecules. The uncountable unique properties of graphene including mobility, large surface area, transparency, nontoxicity, tensile strength, and superior electrical and thermal conductivity allowed the functionalization and immobilization steps leading to the development of sensitive and accurate biosensors. Graphene-based materials can be employed and integrated into designing biosensing platforms resulting in the development of ultrasensitive biosensors, which can be utilized for the early detection of many infectious and noninfectious diseases. As the research on graphene-based nanomaterials is growing, the excellent and unmatched potential of these materials is being revealed for specific purposes. The added advantages of graphene-based biosensors include cost-effectiveness, miniaturization, accuracy, and reproducibility, which showed promising market potential and large-scale commercialization of these biosensors as an alternative to conventional sensing techniques. However, there are several drawbacks associated with graphene-based technology that cannot be ignored. For instance, graphene, owing to its ability to adsorb non-target molecules may result in giving false-positive detection, especially if noncovalent functionalization methods are used. On the other hand, covalent functionalization methods are complex, and precise conditional control is required to achieve it. Moreover, the loading of biomolecules onto the graphene surface is affected by external environmental factors such as temperature, pH, salt concentration, as well as the intrinsic properties of graphene.
such as type, molecular weight, surface properties, etc. Indeed, more studies are warranted on graphene and its functionalized derivatives with lesser impurities and improved methods of manufacturing, which would contribute to the growing momentum toward designing new biosensing devices suitable for POCT and improve the quality of the healthcare system worldwide.

Acknowledgements: The authors extend their appreciation to the deanship of Research & Innovation, Ministry of Education in Saudi Arabia for funding this research work through the project with no. ISP20 – 3.

Funding information: This work was funded by the Deanship of Scientific Research, Jazan University, Jazan, Saudi Arabia, under the Jazan University Research Groups Funding Program for the Ministry of Education’s initiative for institutional funding (Project no. ISP20 – 3).

Author contributions: HAA and WA developed the concept of the manuscript, WA, BM, MZH, MA, and SJ conducted the literature survey; WA, BM, MZH, and SJ developed the methodology and performed data curation. WA, HAA, BM, SJ, MZH, and MA wrote the draft manuscript, while MAB, AN, and HAA performed the writing-review and editing. HAA, MAB, and AN supervised the work. All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Conflict of interest: The authors state no conflict of interest.

Data availability statement: The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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