# Laser-Induced Graphene-Functionalized Field-Effect Transistor-Based Biosensing: A Potent Candidate for COVID-19 Detection

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**Abstract—Speedy and on-time detection of coronavirus disease 2019 (COVID-19) is of high importance to control the pandemic effectively and stop its disastrous consequences. A widely available, reliable, label-free, and rapid test that can recognize tiny amounts of specific biomarkers might be the solution. Nanobiosensors are one of the most attractive candidates for this purpose. Integration of graphene with biosensingdevices shifts the performance of these systems to an incomparable level. Between the various arrangements using this wonder material, field-effect transistors (FETs) display a precise detection even in complex samples. The emergence of pioneering biosensors for detecting a wide range of diseases especially COVID-19 created the incentive to prepare a review of the recent graphene-FET biosensing platforms. However, the graphene fabrication and transfer to the surface of the device is an imperative factor for researchers to take into account. Therefore, we also reviewed the common methods of manufacturing graphene for biosensing applications and discuss their advantages and disadvantages. One of the most recent synthesizing techniques - laser-induced graphene (LIG) - is attractingattention owing to its extraordinarybenefits which are thoroughly explained in this article. Finally, a conclusion highlighting the current challenges is presented.**

**Index Terms—COVID-19, FET biosensors, graphene, LIG, SARS-CoV-2.**

#### I. INTRODUCTION

OVER 120 million people have been infected by<br>COVID-19 and the death toll has reached 3 million in 1 year by March 2021 [1]. It has a high reproduction number, and superfast transmission rate in comparison to the other emerging viral diseases such as SARS and Ebola. Thus it caused an unprecedented health crisis in the past 200 years compared to any other pandemic [2]. This lethal and fastspreading infection is caused by a new strand of coronaviruses named Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) [3]. This crown-like single-stranded RNA virus is a member of the *Coronaviridae* subfamily. Its genome encodes a polyprotein, non-structural, structural, and accessory

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proteins [4]. The majority of the non-structural proteins are necessary for the replication of the virus, whereas the structural ones like the membrane (M), envelope (E), and nucleocapsid (N) proteins are vital for the assembly process [5]. The spike (S) protein is responsible for the cell membrane fusion as it binds to the receptor molecules on the host cell. It links to the angiotensin-converting enzyme 2 (ACE2) and facilitates cell entry [6]. Between the aforementioned molecules in the structure of SARS-CoV-2, E protein which plays critical roles during the life cycle of the virus, is the most antigenic one which can act as an important target in COVID-19 biosensing, vaccine, and drug discovery [7]. Since this lethal malady is highly contagious and can transmit from symptomatic or asymptomatic individuals, it is essential to identify it in its early phases in order to curb its transmission rate and control it more efficiently [8]. The most widespread detection method is Reverse Transcriptase Polymerase Chain Reaction (RT-PCR), which identifies the existence of SARS-CoV-2's genetic material in nasopharyngeal swab samples [9]. It has disadvantages like being time-consuming and expensive. Additionally, it cannot spot the virus during the incubation period and after the inception of symptoms [10]. Since it takes time to vaccinate the majority of the people all around the globe, an alternate technique is needed to enable facile management of the disease and relax the strict quarantine rules [11]. Moreover, this new methodology should be accessible, easy-to-use, affordable, and speedy to expedite massive on-site testing, especially in airports and public places [12]–[14]. Biosensing platforms based on field-effect transistors (BioFETs) are one of the most appropriate choices for coronavirus detection as they follow electro-analysis of charged biomolecules like virus-related biomarkers [15]. Attributable to their advantages like being compact, rapid, label-free, real-time, simple to fabricate and use, reliable, and compatible with state-of-the-art micro-and nanofabrication technologies, they have the potential to replace the presently used diagnostic methods [16]. Furthermore, a FET-based biosensor can be used for multiplexed detection of COVID-19 related biomarkers in human biofluids without or with minimal sample preparation steps [17]. However, it is necessary to choose a suitable substrate for covering the channel region of a FET device in order to have an optimized surface functionalization and biorecognition element (BRE) immobilization process [18]. One of the commonly used and

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competent materials is graphene which offers several benefits as a transducer in BioFETs [19]. It has outstanding electrical and thermal conductivity, a large surface-to-volume ratio, high capacitance, low contact resistance, and adjustable ambipolar field-effect behaviors [20]. The superior electrical conductivity of graphene accelerates the electron shuttle and thus minimizes the response time of the biosensor [21]. Besides, graphenebased FETs (GFETs) are highly scalable, miniaturized, and do not require sophisticated laboratory equipment and personnel [22]. Therefore, they are broadly utilized for pointof-care (POC) measurement of various biomarkers such as viral and bacterial particles, oligonucleotides, oncobiomarkers, hormones, and other biomolecules [20]. POC application of BioGFET demands a suitable method to develop its miniaturized parts like source, drain, and gate terminals, and also the graphene channel with the immobilized BREs on top of it [23], [24]. Conventionally, chemical vapor deposition (CVD) [25], mask photolithography techniques [26] along proper etching and transfer processes are used for this purpose [27]. Despite the homogenous, impermeable, pure, and well-structured final product of the CVD, several toxic gases are produced as the by-products which are the unwanted consequences of this expensive procedure. Additionally, it easily gets affected by the changes of related parameters [28]. These multiple-step methodologies increase the fabrication cost of the device and make the device labor-intensive which hinder the frequent use of the BioGFET in large scale viral and bacterial detection. Therefore, there is an urgent need for an alternate technique to prepare BioGFET in a rapid and facile manner. Laser-induced graphene (LIG) is a strong candidate due to its ability to quickly generate different graphene-based patterns in single step [29], [30]. Additionally, LIGs have successfully been functionalized with different BREs such as antibodies, nucleic acids, and aptamers, which is a crucial part of any viral detection. Therefore, it is considered an efficient strategy in constructing GFET biosensors especially for novel coronavirus detection. This review article discusses different techniques of synthesizing graphene including topdown methodologies (mechanical and chemical exfoliation), bottom-up approaches (Epitaxial growth (EG) and CVD), and the most recent technology of LIG for being used in the structure of GFET-based biosensing devices. It also reviews the recently proposed biosensors for detecting various biomolecules such as, viruses, bacteria, nucleic acids, oncobiomarkers, hormones, etc.

#### II. GRAPHENE SYNTHESIS

Theoretically, graphene is an infinite two-dimensional monolayer of the honeycomb structure of *sp*<sup>2</sup> hybridized carbon atoms [31]. Geim and Novoselov first discovered graphene in 2004 during a repeated peeling-off exfoliation experiment on graphite [32], as shown in Fig. 1. Graphite material is composed of graphene sheets connected by weak Van der forces [33]. Exfoliation and cleavage processes can separate these sheets apart. Although, this first discovered graphene method is a time-consuming process but paved the way for the application of other exfoliation processes [34]. Till to date, a large number of graphene synthesis methods have been



Fig. 1. Schematic of the conventional fabrication methods of graphene fabrication. (Reproduced with permission from [35]).

proposed that can be categories into top-bottom and bottomup approaches, as depicted in Fig. 1. The first approach involves the split-up of graphite material into graphene layers, and the second involves building-up graphene layers on a substrate [36].

### A. Top-Down Approach

1) Mechanical Exfoliation: Mechanical exfoliation follows the application of a suitable mechanical process on a piece of high-quality graphite to separate weakly attached single or few layers of graphene one by one. These processes typically include cleavage during the scotch tape, lathe-like, ball-milling experiments where shear force separates the sheets like threeball mill, dry and wet ball milling experiments, etc. [37]. This technique is low-yielding, time-consuming, and challenging to scale-up for the industry. After each cleavage process conductivity of the remaining sample decreases. For example, after 12 hours of mechanical exfoliation by a three-rollmill experiment, a sample of few graphene layers exhibited 7500 S/m conductivity than the original graphite's conductivity of 25000 S/m before the start of the process [38].

2) Chemical Exfoliation: Instead of using mechanical processes, different chemical methods have successfully been employed on graphite for its large-scale exfoliation into graphene sheets, like Brodie, Staudenmaier, and Hummer's methods, etc. [39]. It is one of the low-cost methods. However, the use of strong acids and oxidizers during chemical exfoliation processes creates defects in produced  $sp<sup>2</sup>$  hybridized sheets of the graphene. Therefore rather than pristine graphene, these methods provide graphene oxide (GO) sheets that exhibit low conductivity. Hummer's method is one of the most popular methods used for graphene production and many types of graphene have been seen like three-dimensional graphene, sponge-like graphene, etc. [40]. But this method requires a subsequent reduction method to restore the graphene's conductivity, called reduced graphene oxide (RGO) [41]. Different methodologies have been employed to reduce GO like chemical reduction, thermal, plasma, and solvothermal reduction [42]. The application of different reduction methods reports different electrical conductivities. For example, the chemical reduction of Zn/HCl reported 15,000 S/m conductivity [43], whereas thermal treatment of  $C_2H_2$  at 1000 $\rm ^{\circ}C$ 

exhibited 143,000 S/m conductivity [44]. Moreover, the use of RGO as active material in a device demands a suitable experiment-design that involves a patterning process as well [40].

#### B. Bottom-Up Approach

1) Epitaxial Growth (EG) of Graphene: Another method that retains high crystallinity is the epitaxial growth (EG) of graphene on silicon carbide (SiC) substrate. In this process, carbon atoms rearrange under high vacuum and form mono or multilayers of high-quality graphene over the substrate [45], [46]. This method is common for the preparation of a miniaturized semiconductor-based electrical biosensor. This process exhibits very low sheet resistance  $(0.43 \Omega/sq)$  [47] and it is very suitable for fundamental study on the laboratory scale. However, SiC substrate cost, high temperature >1000 $°C$ , size of the substrate, transfer of graphene to another substrate, and control over graphene thickness pose challenges in device preparation. Various other substrates have also been reported using epitaxial growth techniques like Pt, Ru, TiC, and Cu [48].

2) Chemical Vapor Deposition (CVD): The other technique that allows the successful transfer of generated high-quality graphene from one substrate to another is the carbon's CVD. This process follows the diffusion of thermally decomposed carbon atoms from hydrocarbons into the metal surface placed inside a furnace tube's controlled environment. The graphene formation occurs when metal substrate containing metalcarbon solid solution cools down and allows carbon atoms to precipitate and segregate over the surface [49]. Controlling various CVD parameters like growth temperature, duration, and changing metal substrate and types of hydrocarbons, different graphene layers, and their sizes have been seen. CVD is the most commonly used method nowadays for the fabrication of GFET biosensors [50]. The graphene sheet is grown on a metal substrate (commonly Cu or Ni) by CVD and transfer to another separate substrate. TABLE I summarises conventional methods of graphene fabrication and their electrical performances.

Preparation of functional GFET demands an efficient manufacturing technique for creating its various parts, including graphene-based FET channel, source, drain, and gate terminals. CVD is the most extensively used technique for the fabrication of GFET based biosensors because produced graphene offers high conductivity and surface area by produced defectfree  $sp<sup>2</sup>$  hybridized carbon structure. In a typical GFET device fabrication by CVD, few graphene layers are first produced on a metallic planar substrate (for example, Cu or Ni), transfer to another substrate, followed by the generation of source, drain, and gate electrodes by photolithography. The photolithography technique is an advanced form of lithography commonly employed to develop small-scale electrodes, which calls for photoresist, patterning the photoresist, and controlled etching process. For example, Wu *et al.* used CVD to develop few graphene layers and coated the graphene with a photoresist for the photolithography process. It followed careful attachment of a tiny featured-mask over the photoresist surface and exposure to ultraviolet light for some time. Multiple etching and metal coating processes were applied in a careful sequence to develop multiple contacts with few tenths of nanometer-wide graphene [51]. J Tu *et al.* synthesized graphene thin film over the copper foil by CVD, capped with thermally grown  $SiO<sub>2</sub>$ and polymethylmethacrylate (PMMA), followed by etching copper foil and PMMA by acid and acetone, respectively [52]. After transferring graphene to  $SiO<sub>2</sub>$  substrate, standard photolithography was employed two times to pattern the graphene channel region and a few nanometer source and drain contacts, as shown in Fig. 2(a). Prepared GFET biosensor detected mercury contaminants based on single-stranded DNA aptamer. Following similar one-time photolithography Z Gao *et al.* developed in plan two gate Au-electrodes on graphene and source and drain on the surface of  $SiO<sub>2</sub>$  and used the design as GFET based DNA biosensor [53]. A similar approach is being followed by W Yue *et al.* to prepare GFET based biosensor for the detection of binding-kinetics of DNA hybridization [54]. There are many other reports about the use of this technique to develop GFET based biosensor [55]–[57].

Although photolithography is a very suitable bottom-up technique of the graphene synthesis but involved careful etching of the metallic substrate during the transfer process, coating of photoresist, and photolithography techniques make the whole process lengthy and complex. Moreover, produced defect-free CVD graphene is difficult to functionalize, which is sometimes required for specific biosensing applications.

Therefore, an alternative method is earnestly required to generate channel material more straightforwardly than CVD, which can be further functionalized easily for GFET related biosensing applications.

#### C. Laser-Induced Graphene (LIG)

The LIG technique is a recently discovered strategy for generating patterned graphene with several advantages. It is simple, fast, environmentally-friendly, and produced material can easily be functionalized and decorated, as shown in Fig. 2(b). Since its first discovery in 2014 [69], different types of. graphene materials have been produced and employed as active materials in many biosensing platforms. The detailed process involves surface interaction between laser and substrate material of some carbon source which triggers localized heating or thermal ablation and photochemical reactions. As a result, temperature in a tiny surface area increases. These reactions derive decomposition of the surface carbon, and as a result,  $sp<sup>3</sup>$ hybridized carbon molecules of the source rearrange into  $sp^2$ hybridized carbon of graphene with some gas molecules' evolution. The lowest sheet resistance of  $\sim$ 6  $\Omega$ /sq has been seen by one of the LIG materials [70]. This localized surface heat process is also known as laser irradiation, engraving, and laser writing. Different lasering parameters like laser carrier speed, pulse width, wavelength, and power of the used laser light can control the heat. Surface-generated heat plays a vital role in the formation of graphene structures. Hence, many morphologies of different thicknesses like hierarchical porous graphene, fibrous graphene, graphene sheets, and their different physiochemical properties along with surface functionalization have

				Process	<b>Electronic Properties</b>	Ref
Synthesis Methods		Advantages	Disadvantages		Conductivity S/m <b>Or</b> Sheet resistance $\Omega$ /sq <b>Or</b> resistance $\Omega$	
Top-to-bottom	Mechanical Exfoliation	Graphene layers formation.	Large scale production, time-consuming.	Peel of	$\sim$ 2m $\Omega$ at Vg=0	$[32]$
				Wet ball milling	1200 S/m	$[58]$
				Three-roll-mill	7500 S/m	$[38]$
	Chemical Exfoliation	Large scale production.	Surface oxygen, reduction method.	GO reduction by Zn/HCl.	15,000 S/m	$[43]$
				GO reduction by $NH3$ - BH <sub>3</sub>	20,300 S/m	$[59]$
				GO reduction under $H2$ at 1000°C for 1h.	76,000 S/m	[60]
				GO reduction under $C_2H_2$ at 1000°C for 30 min.	143,000 S/m	$[44]$
Bottom-to-top	Epitaxial Growth	High-quality graphene is suitable for device fabrication.	Transfer method, expensive SiC substrate.	Graphene on SiC	$1200 \Omega/sq$	[61]
				Graphene on SiC	3 $\Omega$ /sq	$[62]$
				Graphene on SiC	$0.43 \Omega/sq$	$[47]$
	Chemical Vapor Deposition	High-quality graphene is suitable for device fabrication.	Transfer method	Plasma CVD	2200 $\Omega$ /sq	[63]
				CVD graphene on Cu	1200 $\Omega$ /sq	[64]
				CVD graphene on Cu	120 $\Omega$ /sq	[65]
	Laser induced graphene	Pattern graphene, single step.	Low resolution.	LIG	50 $\Omega$ /sq	[66]
				LIG	$30 \Omega/sq$	[67]
				LIG	$10 \Omega/sq$	[68]

TABLE I GRAPHENE SYNTHESIS METHODS

been seen [78], [79]. Furthermore, graphene patterns of different feature sizes and resolutions have been prepared. Owing to mask-free patterning, non-toxic nature, and process flexibility, it has attracted attention. Developed laser graphene-based architectures have been used in a wide range of advanced graphene-based devices as active materials [78], [80]. This method has also been employed in the biosensor field to design a wide variety of in-plan graphene-based electrodes for various biosensing applications [81], [82]. For example, Guo *et al.* reported successful LIG-based FET preparation in which only back gate was fabricated using laser writing technique over graphene oxide [76]. He *et al.* reported the preparation of source, drain, and top gates using laser writing over graphene oxide surface [77]. On-chip application of small channel material of LIG demands successful formation of source

and drain for complete preparation of a FET-based biosensor which demands more research in the field. Numerous studies are focusing on designing tailored detection systems employing specific BRE-functionalized LIG. For instance, A R. Cardoso *et al.* successfully employed LIG on the surface of polyimide to fabricate LIG-based active area of working, counter, and a reference electrode in a one-step engraving process, followed by molecularly imprinted polymerization [71]. Electrochemical polymerization and incubation processes were used along with the electrodes' passivation to obtain a molecularly-imprinted polymer. This imprinted polymerization was followed by electropolymerization of the functional monomer in chloramphenicol's presence. The detection process followed electrochemical test performed in a buffer solution after removing chloramphenicol, and enhanced







Fig. 3. (a) Schematic representation of the workflow employed for the LIG electrodes' preparation and functionalization for the detection of chloramphenicol [71]. Illustration of Thrombin detection by aptamer modified LIG by (b) COOH groups and (c) nanoparticle plus polymer. (d) Depiction of anti-Salmonella antibody attachment with LIG for the detection of Salmonella. Illustration of detection of biogenic amine by (e) diamine oxidase modification of LIG. (f) schematic depiction of bottom-gate GO FET (top) and scheme of the experimental procedures for LIG-based FET (bottom). Reproduced with permissions from [71]–[77].

removal is noticed compared with other carbon electrodes, as shown in Fig. 3(a). C. Fenzl *et al.* reported successful LIG functionalization of the aptamer for thrombin detection. A pyrene butyric acid-treated LIG electrode resulted in the formation of COOH-group on the surface of porous LIG, which attracted aptamers and hence thrombin as shown in Fig. 3(b) [72]. As a result, aptamer-functionalized LIG electrode of porous morphology blocked (Fe(CN)<sub>6</sub>)<sup>4−</sup> ions' electrochemical activity when electrochemically tested in  $K_3(Fe(CN)_6)$  added buffer solution of phosphorous. Hence, a reduced redox current is reported. This aptamer-labeling

electrochemical biosensing system successfully detected a small amount of 1 pM in a buffer solution and 5 pM in the serum's complex matrix. A. K. Yagati *et al.* reported thrombin detection with an improved low limit of detection of 0.12 pM in phosphorous buffer solution. In which the surface of the pores of interdigitated LIG electrodes was chemically modified with polymer-contained nanoparticles after the attachment of COOH-groups, as shown in Fig. 3(c) [73]. This interdigitated design of modified LIG electrodes showed a linear current-concentration response of 0.01 to 1000 nM and an exceptionally low detection limit because of the quick

change in impedimetric capacitance calculated in an electrolyte of buffer solution. Furthermore, this study showed a comparison between the label and label-free functionalization of LIG. This approach to quantifying the concentration of thrombin in blood serum was found amazingly effective compared with other expensive thrombin detection methods that can help in the early detection of related human diseases. R. R. S. Soares *et al.* showed successful loading of anti-Salmonella antibody on LIG material by chemical treatment and reported low-limit detection of Salmonella in chicken broth (as shown in Fig. 3(d)) [74]. Lacquer passivation was used to cover LIG strand and expose circular shape active area for the chemical loading of anti-Salmonella antibody. The modified electrode as a working electrode in a threeelectrode electrochemical cell exhibited an electron transfer rate of 0.0146 cm/s and detection of a low concentration of 13 cfu mL−<sup>1</sup> of Salmonella in chicken broth. These results of the LIG-based Salmonella biosensor are comparable to other sensors prepared by laborious and expensive CVD and inkjet printing methods. D.C. Vanegas *et al.* reported the preparation of a LIG-based biogenic amine biosensor prepared by diamine oxidase functionalization due to electrodeposition of copper nano-composite on the surface of LIG pores (as shown in Fig.  $3(e)$ ). This biosensor detected a low biogenic amine concentration of 11.6  $\mu$ M in fish paste samples subjected to fermentation with lactic acid bacteria.

These successful research studies exhibit the immense potential of LIG-based biosensors for diagnostic applications. They can be a potent candidate for early detection of the COVID-19. Seo *et al.* fabricated GFET to detect novel coronavirus successfully using antibody attachment within the graphene channel [17]. GFET biosensors are suffering from high cost because they involved complex and expensive fabrication techniques for designing their separate parts (e.g., photolithography and CVD, etc.). In the COVID-19 scenario considering the execution of many daily tests, the biosensor cost is a significant concern. Therefore, the low-cost LIG material's fabrication in small features makes this technique an ideal choice for the biosensors' preparation for COVID-19. However, on-chip application of small channel material of LIG demands successful formation of source and drain for complete preparation of a FET-based biosensor which requires more research in the field.

## III. FET BIOSENSORS FOR DETECTING BIOMOLECULES

Biosensors based on FET have been evolving in recent decades [83]. They are being used for detecting a wide range of biomolecules sensitively and selectively [84]. Since FETs are sensitive to surface charge and most of the biological particles are charged in physiological circumstances, they are desirable for conducting a rapid, real-time, and labelfree identification of numerous biological analytes such as viruses [16]. For this purpose, their surfaces must be modified with biocompatible and conductive materials to be desirable for detecting a biological element and also amplify the generated electrical signal [85]. Graphene is one of the suitable candidates which can act as an efficient interface between the electrical and biological departments. Thus, GFETs are attracting ever-increasing attention in the field of early-stage diagnosis [22]. As it is summarized in TABLE II, s variety of target biomolecules such as viruses, nucleic acids, oncobiomarkers, hormones, etc. have been studied using GFET devices functionalized with specific bioreceptors (antibody, DNA, RNA, aptamer, etc.) [86]. A list of the most recent research works can be seen in Table II. The detection plan of these systems can be easily adopted in designing GFET-based devices for early recognition of COVID-19-related biomarkers (whole virus, antigen, antibody, RNA).

# A. Virus

As discussed previously, GFET-based sensing systems are one of the beneficial alternatives for the early detection of viral infections like the ongoing universal pandemic [22], [87], [88]. There are four strategies for identifying viruses without using labels. In the first approach, the whole virus is being studied using virus-specific antibodies as the capturing probes. While the target in the second approach is viral antigens like its surface proteins and the probe is the antibodies against them. The third approach targets the viral genomic material utilizing its complementary nucleotides and the fourth is designed to capture the antibodies produced in the host body as a response to the virus employing the matching antigens as BREs [89]–[91]. Overall, the sensing scheme is founded on measuring the electrical characteristics of the GFET's gate after the occurrence of biological reactions [86]. Recently, several studies have focused on detecting viruses such as COVID-19, Encephalitis Virus (JEV), Avian Influenza Virus (AIV), Vesicular Stomatitis Indiana Virus (VSV), Rotavirus, Human Papillomavirus (HPV), and Ebola exploiting GFET. For example, an innovative GFET-based immunosensing system was developed for the early identification of SARS-CoV-2 in nasopharyngeal swab samples. The graphene-modified surface of the sensor was functionalized by antibodies against the spike protein of the virus. The LOD of this device was reported 2.42  $\times$  10<sup>2</sup> copies/mL in biological specimens which is a promising achievement in early detection of this viral infection [17]. Or In a recent study, a GFET was utilized to sense the interaction between COVID-19 spike protein S1- and its specific antibody in less than two minutes. They reached a limit of detection of 0.2 pM [92]. Another example of GFET biosensor for virus detection can be seen in Roberts *et al.* study (See Fig. 4). They designed a miniaturized and easy-to-use device for recognizing JEV and AIV. The  $Si/SiO<sub>2</sub>$  surface of the FET was coated with graphene which itself was decorated with carboxy groups. These functional groups facilitated the immobilization of virus-specific antibodies through covalent bonding. Because of the interaction between the antibody and the target antigen, a variation in the resistance occurred and LODs down to 1 fM and 10 fM for JEV and AIV were recorded, respectively [93]. Chen and colleagues constructed an aptamer-modified GFET by Micro-electromechanical system (MEMS) for detecting influenza virus (IV). This system was able to sense as low as 1 ng/ml of the target analyte [94].



Fig. 4. (A) Illustration of modifying the graphene surface with virusspecific antibody including, exfoliated graphene, EDC-NHS activated surface which contains carboxylic groups, and antibody-immobilization via its amine groups, (B) SEM images graphene and graphene-Ab. (Reconstructed from [93]).

In another study, a GFET-based immunosensor was fabricated for VSV detection. The graphene was functionalized with antibodies for capturing the target viral particles using 1-pyrenebutanoic acid succinimidyl ester (PASE) as the linker. This molecule is commonly employed on graphene-based surfaces, since it attaches via  $\pi$ - $\pi$  interactions and covalent linkage to the graphene substrate and antibody's primary amine, respectively. This device was successful enough to identify down to 47.8 aM of the virus [95]. Pant and coworkers designed an rGO-modified FET biosensing platform for Rotavirus recognition. The employment of graphene on the sensor's surface enabled an efficient antibody immobilization through a linking molecule. Pyrene-NHS which is commonly used in functionalizing graphene-based surfaces was utilized to link the probes to the surface of the sensing site. After the generation of the antibody-antigen complex, a change in conductance was recorded which validated the success of detection [96]. The detection of HPV has also been studied by rGO-FET biosensors. Aspermair *et al.* modified the surface with RNA aptamers using pyrene and reached a LOD of 1.75 nM in saliva samples [97]. The next example study is done by Chen *et al.* for detecting the Ebola virus. They fabricated an immunosensor based on rGO-FET and identified down to 1 nM of Ebola glycoprotein in PBS, human serum, and plasma samples [98]. These achievements represent the applicability of graphene-modified FET biosensors for virus detection diagnosis especially COVID-19.

#### B. Bacteria

The severe disorders caused by toxins released from different strains of bacteria can lead to lethal consequences [99]. The



Fig. 5. Configuration of GFET biosensor for detection of E. coli bacteria. (Reconstructed from [104]).

postponement of the therapeutic process after the initiation of toxicity symptoms may result in the worsening of the patient's health status [100]. Therefore, it is imperative to identify the infection in its early stages in order to prevent the progress and control it [101]. The currently used techniques for this purpose like polymerase chain reaction (PCR) and colony-counting methodology are time-consuming, expensive, not sensitive, and selective enough [102]. GFETs are capable of conquering these limitations since they are highly scalable, accurate, reliable, economical, and simple. For instance, a pioneering GFET-based immunosensor was developed for bacteria detection to control food and water sanitary. Bacteria-specific antibodies were immobilized using PBASE which could successfully recognize the target biomolecules. Furthermore, they integrated the biosensor with a signal display device to show the variations of the bias current *I*ds which increased after the attachment of the bacteria [103]. As it can be seen in Fig. 5, another GFET immunosensing platform was developed for Escherichia coli O157:H7 detection. It was reported that the conductance of the sensor's surface increases after the target antigen attaches to the surface probes. Because of the p-type operation of the GFET and the concentration of the negativelycharged bacteria on the sensing region, an increase in the conductance occurred which augmented the current [104]. Thakur and coworkers reported the use of an rGO-functionalized FET immunosensor for label-free identification of *E.coli* bacteria. A thin layer of  $Al_2O_3$  was coated on top of the rGO in order to facilitate easy immobilization of antibody-anchored gold nanoparticles. Attributable to the negatively-charged nature of the target species, the density of charge carriers on the rGO layer increased which enabled rapid (50 s) quantification of *E. coli* in river water samples [105]. The success of these works validate the adoptability of their strategies in redesigning these devices for recognizing COVID-19 in biological fluids. For example, by replacing the bacteria-specific antibodies by SARS-CoV-2-selective antibodies, antigens, oligonucleotides, or aptamers, innovative platforms can be fabricated for early detection of this disastrous viral infection.

#### C. Nucleic Acid

Swift and reliable nucleic acid quantification are vital for biomedical diagnostics which can replace the conventional



Fig. 6. Fabrication of GFETs on flat (A) and crumpled graphene (B), (C) SEM image of crumpled graphene (The scale bar is 500 nm). (Reconstructed from [110]).

screening techniques [106], [107]. Owing to the superior biocompatibility, high conductivity, chemical, and mechanical stamina, and large available surface area, graphenemodified bioFETs are considered promising methods for single-molecule detection of nucleic acids [108]. By functionalizing the surface of these miniaturized devices with complementary oligonucleotides or aptamers, the target biomolecules can be captured readily [109]. In recent years, several studies have highlighted the importance of designing such platforms for DNA/RNA measurement. As an example, Hwang *et al.* employed a unique GFET for nucleic acid identification. Fig. 6 illustrates that a malformed single layer of graphene in the channel region was employed which demonstrated superior performance in comparison to conventional flat designs. It could sense down to 600 zM and 20 aM of the target in the buffer and human serum specimens. It was reported that the novel configuration of the graphene resulted in ultrasensitive detection owing to the reduction in charge screening at the indented sites. Additionally, it displayed an extraordinary shift in the source-drain current which shows the capability of this structure in detecting small biomolecules in millimeter-scale devices [110]. The other example is a biosensing platform for RNA detection based on graphene-modified FET. This device demonstrated superior performance and identified down to 0.1 fM of the analyte. It also differentiated the target RNA from other molecules efficaciously [111]. If the probes of theses developed devices are replaced with COVID-19-specific biorecognition elements like the complementary RNA of this virus or the related aptamer, it can be detected at its incubation time. In this way, the infection can be controlled and better treated.

#### D. Cancer Biomarker

Ultra-sensitive measurement of cancer-associated biomolecules in biological fluids such as proteins, enzymes, nucleic acids, and exosomes is essential in the early detection and monitoring of cancer [112]. Although the sample containing these tiny molecules is complex and their concentration is very low especially in early-stage cancer patients, nanobioelectronic devices have the potential to sense them accurately [113]. The advancement of GFET in recent years paved the way for the emergence of pioneering nano biosensors for accomplishing this goal. As an instance, a novel GFET device was introduced for detecting prostate-specific antigen (PSA). This aptasensor was modified with polyethylene glycol (PEG) in order to ease the DNA aptamers' immobilization process and reached a LOD of 1 nM (See Fig. 7) [114]. Alpha-fetoprotein (AFP) – known as the hepatocellular carcinoma (HCC) biomarker - is the other biomolecule that has been measured by a tailor-made GFET device. The anti-AFP antibodies were immobilized on the surface of the graphene through PBASE and detected as low as 12.9 ng.mL<sup>-1</sup> and 0.1 ng.mL<sup> $-1$ </sup> of the target analyte in HCC patients' serum and buffer, respectively [115]. Zhou and colleagues introduced an accurate and easy-to-use GFET device for recognizing carcinoembryonic antigen (CEA). The surface functionalization was done using nano-denatured bovine serum albumin (nanodBSA) to both facilitate the anti-CEA immobilization on the EDC and sulfo-NHS-activated graphene channel and protect the sensing site against contamination. This arrangement could detect 337.58 fg mL<sup>-1</sup> of the target and validated the functionality of this surface modification strategy in designing biosensing systems [116]. A GFET biosensor was arranged for recognizing human carbonic anhydrase 1 (CA1) using RNA aptamers as capturing probes. PBASE was the linking molecule for aptamer immobilization which bonded with graphene through  $\pi$ - $\pi$  interactions. This methodology was capable of detecting low concentrations (70 *pM*) of the target oncobiomarker which in could act as an alternative technique in the early detection of diseases related to variations in CA1 level [117]. A similar strategy was used to functionalize the surface of a GFET with anti-CD63 antibodies for detecting exosomes. They integrated the sensor with a microfluidic channel and left a section of the graphene surface uncovered to become exposed to the sample. In the presence of exosomes, *V*g shifted with time which represented the detection of target biomolecules [118]. Such simple and accurate devices have the potential to substitute the conventional screening techniques for the early detection of critical disorders like cancer and critical infectious diseases.

## E. Hormone

Measuring the level of hormones is critical in the prevention, detection, and monitoring of some diseases [119], [120]. During the last few years, GFET biosensors have also been used for this purpose. For instance, a GFET-based immunosensor was fabricated to detect thyroid-stimulating hormone (TSH) in a complicated biological sample. The surface modification was done using polyethylene glycol (PEG) and TSH-specific antibody fragments. This arrangement was able to detect as low as  $10 \times 10^{-15}$  M in serum specimens [121]. Another graphenebased electrolyte-gated FET biosensor for hormone detection was used for quantifying Human Chorionic Gonadotrophin



# TABLE II RECENTLY DEVELOPED GFET-BASED BIOSENSORS



Fig. 7. (A) Schematic of a GFET device for sensing the biological analyte. (B) The image of a chip-based system on which a PDMS microfluidic channel is mounted. (Reconstructed from [114]).

(hCG). The surface was decorated with pyrene-NHS to maintain the  $sp<sup>2</sup>$  structure and easily immobilize the antibodies. The reported LOD was  $\sim$ 1 pg.mL<sup>-1</sup> which demonstrates the potential of graphene-based POC devices for medical applications [122]. In another study, a GFET-based cortisol sensing platform was constructed that can sense down to 10 pg/ml of the target in human tears. This real-time detection was integrated with a smartphone in order to ease the process. The reliability, repeatability, and biocompatibility of this design were validated using live rabbit and human pilot tests [123]. Fig. 8 presents anti-diuretic hormone (ADH) detection utilizing a GFET-based aptasensor. As a result of the captured target peptides by the immobilized aptamers, the density of charge carriers changed which showed the occurrence of detection, and a LOD of 3.55 ag/mL was recorded [124]. By substituting the biorecognition elements of these tailored devices, it is feasible to design a specific biosensing system for spotting SARS-CoV-2-related biomarkers.

# F. Other

GFET sensing devices are being utilized for identifying other types of biomolecules as well. For example, Ferritin which is known as a biomarker for early detection of iron deficiency (ID) has been studied employing a GFET immunosensor. This PBASE-anchored graphene-based sensing system was able to detect as low as 10 fM of the ferritin antigen [125]. The other GFET device was designed by Wang *et al.* to detect biotin. The sensing site of this system was decorated with PBASE cross-linkers to facilitate the attachment of avidin. This immobilization was based on the affinity between the lysine group of avidin and the N-hydroxysuccinimide ester



Fig. 8. Illustration of the surface functionalization process of a GFET biosensor. 1. KOH activation, 2.APTES, and 3. Glutaraldehyde (GA) treatment, and 4. ADH-specific aptamer immobilization. (Reconstructed from [124]).

group of PBASE. After anchoring the probes on the monolayer of graphene and introducing the target-containing sample, the current variations were monitored in a real-time manner. This novel structure was capable of selectively capturing the desired target biomolecules with a 0.37 pM sensitivity [126]. Smell sensors are the other category of sensing platforms designed utilizing GFET technology. The target analyte of these devices is a tiny and lipophilic structure like homovanillic acid, eugenol, and methyl vanillate. In a recent research work, an odorant-binding protein-modified GFET biosensor was introduced by Rozman *et al.* which was able to spot down to 100pM of the target analyte [127]. In another similar study, an rGO-coated FET biosensor was developed for identifying odorants in water-based solutions. Odorant-binding protein 14 (OBP14) was used as the capturing probe that can bind to the hydroxyl group of the target aromatic molecules. Analyzing the electrical measurement results indicated that all the probe proteins were at their optimum functionality such that they can identify the target sensitively and selectively [128]. In a patented work, an odorant biosensor was constructed based on a ligand binding protein-anchored GFET. The use of a lipid bilayer on the first graphene layer of this structure made the immobilization process easier [129]. Zheng and coworkers presented a precise FET biosensor for measuring hydrogen peroxide  $(H_2O_2)$ . They used molybdenum disulfide  $(MoS<sub>2</sub>)$  and rGO to modify the sensing area. This methodology demonstrated a sensitive and selective detection in a complex sample. Additionally, it could directly sense the  $H_2O_2$ produced by cancer cells which is a prominent improvement in monitoring  $H_2O_2$ -associated malfunctions [130]. In another investigation, the possibility of sensitive measurement of lead and potassium ions by a GFET aptasensor was examined. A methylene blue (MB) molecule was attached to the terminal end of the probes. The binding of target ions to the specific

aptamers resulted in a shape change and accordingly increased the proximity of the MB to the surface. as a result, an electron was donated and the concentration of the target analyte was quantified by measuring the current [131].

The success of these novel platforms shows the functionality of GFET biosensors for detecting COVID-19-related biomarkers such as surface proteins (spike (S), nucleocapsid (N), membrane (M), and envelope (E)), viral RNA, and host antibodies. For this purpose, the sensing region of the GFET biosensor should be functionalized with capture probes to identify the target analytes. For example, tailor-made antibodies against the viral antigens, complementary strands of an oligonucleotide against the genomic material of the virus, and selective aptamers can be designed and immobilized on the surface of LIG which has many binding sites. Decorating the graphene surface of these devices with bioreceptors can lead to the detection of COVID-19- specific biomarkers even in the most complex samples.

## IV. CONCLUSION

To conclude, the huge potential of GFET-based biosensors for early detection of different biomarkers, including COVID-19-related biomolecules specified in this review paper. Besides, the importance of choosing an efficient graphene fabrication methodology which is an essential parameter in designing a GFET biosensing device was discussed. Among the diverse methods of graphene fabrication, LIG is an advantageous technique since it provides thermal stability, wide available surface area, high electrical conductivity, and costeffectiveness. These attributions turn it into a popular and appropriate candidate for biosensing applications. Even though these compact, scalable, and ultra-sensitive GFET biosensors are attracting the attention of researchers in the field of early-phase disease detection especially during the past few years, there is still a burdensome track in front of its widespread use in biomedical fields. For instance, synthesizing graphene sheets with uniform properties is arduous and a minor discrepancy in their structure may cause a major change in the performance of the device. Furthermore, the baseline drift in the aqueous milieu is a common issue that these sensing platforms often experience. It complicates the process of response analysis which is not a desirable feature while using a biosensor. Another point that needs to be taken into account is the reusability of the GFET biosensor, particularly when it is utilized as a POC device. For a biosensor to be used over and over again it should minimize the probability of cross-contamination. One simple solution for this problem is cartridge-type GFET biosensors. The disposability of the cartridge and the reusability of the readout system enable using the device several times. However, currently developed GFET Biosystems lack this feature. Thus, constructing such a device would be beneficial in terms of COVID-19 detection, since it can be used numerous times and examine a large number of people. One other point is the sample treatment and delivery to the sensing region which can be done through integrating microfluidics with GFET technology. Innovative designs can be created to easily deliver the sample to be tested with microfluidic-based GFETs. To reach this ultimate goal,

effective collaboration between scientists from different fields of study such as electrical engineering, chemistry, physics, nanotechnology, and medicine is required. All in all, LIG is a unique technique that can tackle the current limitations in graphene fabrication and transfer to the sensing area of a biosensing system, particularly GFETs. Thus LIG-based GFET biosensors hold great potential in sensing COVID-19 specific biomolecules in biological samples. They are expected to be one of the most preferred and widely used detection techniques in the near future after further improvements.

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