Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Carbon-based biosensors: Next-generation diagnostic tool for target-specific detection of SARS-CoV-2 (COVID-19)

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ABSTRACT

Severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) was declared a global pandemic in 2020. Having rapidly spread around the globe, with the emergence of new variants, there is a crucial need to develop diagnostic kits for its rapid detection. Since it validated accuracy and reliability, the reverse transcription polymerase chain reaction (RT-PCR) test has been declared the gold standard for disease detection. However, despite its reliability, the requirement of specialized facilities, reagents, and duration of a PCR run limits its usage for rapid detection. There is thus a continuous increase in the design and development of rapid, point-of-care (PoC), and cost-effective diagnostic kits. In this review, we discuss the potential of carbon-based biosensors for target-specific detection of coronavirus disease 19 (COVID-19) and present an overview of investigation within the timeframe of the last four years (2019–2022), which have developed novel platforms using carbon nanomaterial-based approaches for viral detection. The approaches discussed offer rapid, accurate, and cost-effective strategies for COVID-19 detection for healthcare personnel and research workers.

1. Introduction

Viral infections are causing severe health issues throughout the globe. The ongoing global pandemic was initiated by SARS-CoV-2. This virus has infected millions around the globe. In China, medical cases of patients with peculiar pneumonia-like infections were reported around December 2019. Most of the cases were related to seafood markets located in the Jianghan District of Wuhan. The unusual cause of this pneumonia-like disease led to an investigation of the causative agent. Samples from the lower respiratory collected from the infected individuals were taken for sequencing. Sequence identification placed the novel infectious agent under the β-coronavirus genus. Sequence matches were 85% and 80% identical to bat-SL-CoVZC45 and SARS Coronavirus, respectively. Initially, the sequenced virus was named 2019-nCoV, and later it was renamed SARS-CoV-2. Despite strict measures to quarantine and restrict movement between China and other countries, the virus had already traveled to 53 countries by 29th February 2020. After its first reported case in 2019, on 11th March 2020, the WHO gave the respiratory disease the name COVID-19 and announced it as a global pandemic [1–3]. Previously, in the year 2002, the first Betacoronavirus crossed the species barrier and caused SARS, naming the causative agent SARS-CoV. Later, in September 2012, Saudi Arabia reported the outbreak of a similar respiratory syndrome, MERS-CoV. These epidemics were curtailed as a result of extensive surveillance and quarantine yet may continue to be endemic in the epicenters of origin. Reportedly, very few cases of SARS-CoV have been reported;

Abbreviations: (+ve) ssRNA, Positive single-strand RNA; AuNPs, Gold nanoparticles; BAL, Broncho-alveolar lavage; cDNA, complementary DNA; CNFs, Carbon nanofibers; CNMs, Carbon nanomaterials; CNTs, Carbon nanotubes; COVID-19, Coronavirus disease 19; DNA, Deoxyribonucleic acid; E protein, Envelope protein; ELISA, Enzyme-linked immune sorbent assay; FBG, Fiber Bragg grating; FET, Field-effect transistors; G-FET, Graphene-FET; GR, Graphene; hACE2, human angiotensin-converting enzyme 2; HCoVs, human coronaviruses; IgG, Immunoglobulin G; IgM, Immunoglobulin M; LFA, Lateral flow assay; LI-G-FET, laser-induced Graphene-FET; M protein, Membrane protein; MERS, Middle East Respiratory Syndrome; MIP, Molecularly imprinted polymer; NIR, Near Infrared; ORF, Open reading frame; OTC, Over the counter; PBASE, 1-Pyrenebutanoic acid succinimidyl ester; PMO, Phosphorodiamidate morpholino; PoC, Point-of-care; RdRp, RNA dependent RNA polymerase; RNA, Ribonucleic acid; ROS, Reactive oxygen species; RT-PCR, Reverse transcription polymerase chain reaction; S protein, Spike protein; SARS, Severe acute respiratory syndrome; SARS-CoV-2, Severe acute respiratory syndrome-coronavirus-2; SWCNTs, Single-walled Carbon nanotubes; TMPRSS2, TM protease serine 2; UTR, Un-translated region; VOCs, Variants of concern.

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however, MERS-CoV continues to be an endemic risk factor for the elderly in the Arabian Peninsula [4]. SARS-CoV-2 is the first virus of the family *Coronaviridae* to cause a pandemic and 7th to infect humans. It is transmitted as aerosols by infected individuals in presymptomatic, asymptomatic, and symptomatic conditions. The respiratory droplets from an infected individual can also infect normal individuals via fomite (inanimate) transmission. Some other methods of transmission, such as feco-oral, ocular, and transmission during pregnancy and breastfeeding, are under investigation. The disease ranges from asymptomatic to symptomatic and, in many cases, can be life-threatening (usually in cases with associated comorbidities). To date, the symptoms can be clustered into respiratory, musculoskeletal, neurological, and digestive symptoms. Extensive damage occurs in the respiratory system in two phases, an early phase, and a late phase. The early phase is distinguished by direct viral damage coupled with increased virus replication. Subsequently, the late phase is characterized by an immune response triggered against the infected host cells. The migrated immune cells at the infected sites release cytokines, which may lead to systemic inflammatory syndromes. This condition of prolonged circulating inflammatory cytokines is termed a cytokine storm. Risk of fatality increases in patients with high cytokine storm response [5]. Despite the metrics of high infection and transmissibility, the disease fatality is approximately 1.5% of the infected individuals. The percentage may amount to approximately 10% if the limitations of testing and challenges to attribute the cause of death are taken into consideration. SARS-CoV-2 has a low transmission ratio (R_0), where R_0 is the rate of viral reproduction, used as a measure of viral transmissibility. Based on R_0, viruses are grouped under the death zone and panic zone. SARS-CoV-2 has a medium transmissibility and mortality rate. Viruses are classified as having a higher mortality rate in the Death Zone and a medium transmission and mortality rate in the Panic Zone. SARS-CoV-2 has a medium transmissibility and mortality rate, thus categorizing it as a panic zone virus [6]. Many reviews have been published providing insight into novel nanomaterials for COVID-19 detection [7], and surveillance. Besides sensing, several drug compounds are continuously being developed and discovered to inhibit the spread of these viral particles in the host body [8–10].

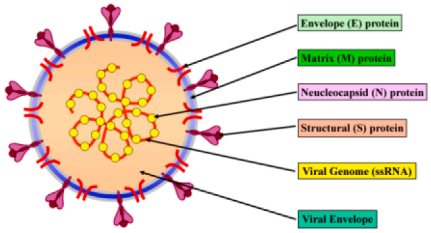
In this review, we provide insight into CNMs in SARS-CoV-2 identification [11]. Initially, a brief discussion on the classification and properties of SARS-CoV-2, followed by a discussion on CNMs-based biosensors for SARS-CoV-2, the crux of this review, is presented.

2. Classifying SARS-CoV-2

At the beginning of the 21st century, in November 2002, the first inter-species CoV infection was reported in China, following which, in 2012, MERS-CoV infection was reported with Saudi Arabia as the epicenter. The third virus of the *Coronaviridae* family, SARS-CoV-2, the causative agent of COVID-19, shares many similarities with the previous two CoVs. Like the others, it has also crossed the species barrier and infected humans. On a phylogenetic basis of classification, SARS-CoV-2 belongs to the Betacoronavirus, a genus under the subfamily *Orthocoronavirinae*, belonging to the family *Coronaviridae* under the order *Nidovirales*. The family *Coronaviridae* has four genera of coronaviruses, namely alphaCoV, betaCoV, deltaCoV, and gammaCoV. The genera betaCoV is further divided into five lineages [12]. Genome sequencing has shown that all the CoVs have a zoonotic origin. The natural reservoir of alphaCoVs and betaCoVs are both bats and rodents, while the deltaCoVs and gammaCoVs infect mammals and avian species. However, these viruses may mutate and cross species-specific barriers [5,13]. Earlier, the CoVs weren't well documented, with only a few HCoVs identified in the 1960s. It wasn't until the SARS-CoV epidemic in 2002 which led to CoVs identification and research. The clinical attributes of SARS-CoV-2 mediated COVID-19 disease is similar to the SARS-CoV infection. Based on complete genomic phylogenetic analysis, SARS-CoV-2 clusters more closely with bat CoV and SARS-CoV than HCoVs and MERS-CoV. Genetic recombination and mutation may have aided in the transmission of SARS-CoV-2. SARS-CoV and MERS-CoV were traced to have evolved by genetic arrangements, originating from civets and camels, respectively [13]. SARS-CoV-2 exhibits genetic recombination of bat CoVs, thus showing origin in bats. Furthermore, natural selection may have aided in its transmissibility either directly to humans or via undocumented intermediate hosts.

3. Morphology and genome structure of SARS-CoV-2

SARS-CoV-2 has a morphological and genomic similarity with the other coronaviruses, having an outer corona-like (crown) appearance and a (+ve) ssRNA (Fig. 1) [14]. The 29 Kb genomic RNA of SARS-CoV-2, the average genome sizes of SARS-CoV (28 Kb) and MERS-CoV (30 Kb), are associated with the nucleocapsid protein (NP). The genomic sequence of SARS-CoV-2 has sequence harmony with different CoVs like SARS-CoV and bat CoV. Additionally, the genomic arrangement is homologous and shared between the Betacoronaviruses [4]. The RNA sequence has its 5′ end capped while the 3′ end has a poly-A tail. The sequence has the following arrangement from 5′ to 3′ termini: replicase (ORF1)-S-E-M-N. A variable number of ORFs between conserved genes, regulatory elements, and 5′ UTR and 3′ UTR are present in the genome. Structurally, the viral RNA is enclosed in a lipid membrane with embedded E, S, and M proteins. The S proteins play a crucial role in SARS-CoV-2 pathogenicity and decorate the viral envelope akin to the sun's corona. It is a trimeric glycoprotein projection of the lipid membrane with S1 and S2 subunits. The S2 subunit is further divided into HR1 and HR2. The S1 subunit interacts and binds with the hACE2. The ACE2 protein is ubiquitously present across various cell and tissue types. It is present in the lungs, kidney, retina, heart, and uteroplacental tissue. The SARS-CoV-2-S1/hACE2 interacts with an affinity higher than the SARS-CoV S1 subunit. Interaction with the S1 subunit renders the ACE2 receptor inactive to convert angiotensin I (Ang I) and angiotensin II (Ang II) into their active forms. The S1 is primed and activated by the TMPRSS2 and undergoes a structural conformational change [15]. A polybasic cleavage site, the target of furin proteases, is present uniquely only in SARS-CoV-2. This furin cleavage site is located between S1 and S2, at which the host serine protease cleaves. This feature increases the pathogenicity of SARS-CoV-2. The S2 subunit facilitates viral entry by conformational change and interaction between HR1 and HR2. This brings the viral envelope and host cell membrane to close proximity for fusion. M protein is present in abundance and spans the viral membrane. It maintains the virus shape and facilitates the budding of virions and the formation of new CoV-2 particles from the host cell membrane [16]. The E protein is a CoV transmembrane hydrophobic protein rich in leucine and valine amino acids. The four structural CoV proteins interact during the assembly of viral particles.



Protein	Subunits	Weight	Functionality
S-Protein	S1 and S2	~ 150 kDa	Attachment, fusion and infection of a host cell.
E-Protein	--	~ 8 -12 kDa	Formation of the viral envelope. Association and maturation of the virus.
N-Protein	NTD and CTD	~ 15.4 kDa (NTD) and ~ 28.7 kDa (CTD)	Formation of Virion.
M-Protein	--	~ 25 - 30 kDa	Shaping of the viral envelope.

Fig. 1. Structure of SARS-COV-2 along with its viral proteins and their functionality.

The high structural homology is shared between SARS-CoV-2 E and M proteins and bat CoV E and M proteins. This could have facilitated a cross-species barrier to transmission and infection [5]. Viruses with an RNA genome are prone to mutations. Mutations enhance the virulence and transmission of virus particles, enhancing their adaptability to new hosts. In the COVID-19 pandemic, the SARS-CoV-2 RNA genome underwent multiple mutations, giving rise to new variants. Depending on the infection rates globally, few are considered VOCs by the WHO [17]. Currently, five VOCs have been reported by WHO, namely:

- A new SARS-CoV-2 VOC was reported in December 2020 in the UK. Formerly termed GR/501Y.V1, it is the alpha variant (or B.1.1.7 lineage).
- South Africa reported two novel VOCs, named Beta (B.1.351) and Omicron (B.1.1.529 in December 2020 and November 2021, respectively.
- The Gamma (P.1) variant was reported in January 2021 in Brazil.
- India reported the Delta (B.1.617.2) variant in December 2020. This VOC was responsible for the deadly second wave of the COVID-19 pandemic.

Mutations are a roadblock to surveillance and treatment. Extensive biosensing, along with sequencing, is important following the event of a novel virus outbreak.

4. Current conventional methods used in SARS-CoV-2 diagnostics

Curtailing the ongoing infection and re-infection is an important aspect of bringing the pandemic to an end. To achieve the same, prevention of viral shedding is important to contain the pandemic. Additionally, the duration of COVID-19 infection differs between a normal individual and an individual with associated comorbidities. The diagnostic tools [18] can be classified under nucleic acid, protein, and antibody-based assays. Imaging techniques also aid in diagnosing the disease's severity. Patients with COVID-19 symptoms must undergo a diagnostic test, while asymptomatic individuals should get tested in the event of possible virus exposure. It enables the identification of infected individuals, their quarantine, and treatment subject to the severity of the disease. Multiple diagnostic assays are available for COVID-19 diagnosis [19], of which nucleotide detection is the most standard and sought-after assay. Liv and their group, in the year 2021 [20], developed an electrochemical immunosensing platform for diagnosing COVID-19. This platform is made up of gold (Au)- clusters, cysteamine (CysAm), and glutaraldehyde (GluAl). In 2022, Muduganti and their group investigated silica nanoparticles (SiNPs) coated with porphyrinoids for COVID-19 detection [21].

4.1. Reverse-transcription polymerase chain reaction (RT-PCR)

RT-PCR is the gold standard test for COVID-19 [22]. In the process, the SARS-CoV-2 RNA genome is reverse-transcribed into cDNA. The cDNA is then taken for a PCR cycle with sequence-specific primers. The presence of SARS-CoV-2 is interpreted by the C_t value, which is the cyclic threshold value. A low value usually corresponds to a high viral load, while a high value corresponds to a low viral titer. The test is sensitive and specific, giving results for both symptomatic and asymptomatic individuals [23]. The test can analyze RNA sequences from BAL, saliva, nasal, and stool samples. In patients with comorbidities, the RT-PCR of anal-fecal swabs tests positive for weeks, despite a negative nasopharyngeal swab [24]. RT-PCR serves as a viral sequence-specific diagnostic tool. The overall workflow takes approximately two days to give a quantitative report. However, the overall process requires a qualified skillset, sensitive reagents, good quality nucleic acid extraction, expensive tools, and a high turn-around time. Despite the sequence specificity, the test may give false negatives due to evolving RNA sequence variations [25]. Therefore, in addition to RT-PCR tests, the availability of rapid, sensitive, portable assays as biological sensors has an important role to play in COVID-19 and other disease diagnostics.

4.2. ELISA and other serological assays

RT-PCR-based COVID-19 has a high sensitivity, yet it has limitations in understanding disease progression, immune responses, and past infections. Thus, assays based on immunological interaction, such as ELISA and LFA, are commonly used as serological assays. Quantitative and/or qualitative data may be from the presence of antibodies and antigens in response to COVID-19 infection. Detecting the presence of IgM and IgG antibodies gives an idea of the stage (early/primary/prior) of infection. The SARS-CoV-2 antigens, namely the S protein domains, S1, S2, and the NP, are used in ELISA-based quantitative and LFA-based qualitative detection. ELISA-based assays are highly sensitive and can analyze multiple samples at a feasible turn-around time [26]. Yet, there are limitations such as reagent cost, skilled manpower, and cost of the equipment. At the same time, despite giving a qualitative assessment of infection, the LFAs are portable, require less expertise in handling, and are cost-effective. The availability of commercial serological test kits has been instrumental in testing and field surveillance. However, its limitations are that test kits may give variable results with a variable range of specificity and sensitivity, depending upon the sensitivity of the biorecognition elements [27].

4.3. Computed tomography (CT) scanning

Computed tomography is a diagnosis giving an approximation of disease progression stage and severity. Initial CT scan results from the first cases of SARS-CoV-2 in China reported a severely organized pneumonia-like pattern of pulmonary tissue damage. CT scans are non-invasive and use X-ray radiation to take cross-sectional images at various angles. The images are then compiled and studied by radiologists for possible disease diagnosis. A chest CT scan for COVID-19 shows a consolidated lung with solid or liquid mass deposition in the tissues. In addition to RT-PCR as a standard reference test, CT scan findings give improvised diagnosis and confirmation in the event of a false negative RT-PCR report [28]. CT scans are highly reliable and sensitive in comparison to RT-PCR. Few studies have shown that an initial CT scan gives positive reports of a COVID-19 infection while obtaining a negative RT-PCR report. Later stages provide a positive RT-PCR report. In the recovery stage, improved CT scan results of the lungs are obtained before obtaining a negative RT-PCR report. These findings imply the importance of CT scan over RT-PCR. Yet, overlapping results from CT scan in different pathogenic infections such as SARS-CoV and H1N1 reduce its diagnostic specificity [29].

5. Designing biosensor for SARS-CoV-2

During the innovation and design of a novel system, for specific detection of targets, such as viruses (in reference to the context of the paper), information must be sought about markers present on the targeted virus. Information on the physical attributes of the marker plays a key role in sensor design (Fig. 2). Various detection methods have been formulated that target one or more of the viral surface proteins, nucleoproteins, and/or the viral genome [11,30]. In carbon nanomaterials, the superficial surface area available not only increases the quantity of immobilized bioactive molecules but also enhances the qualitative aspects such as electrical conductivity, responsiveness, and reaction sites. The excellent performance capabilities of the sensor are achieved by unique identification elements which target the analyte to be identified [5]. Herein the subsequent sections, we have discussed recent advancements in carbon nanomaterials, wherein they are surface decorated by different structural modifications and biorecognition units, which include antibodies, molecularly imprinted polymers, and

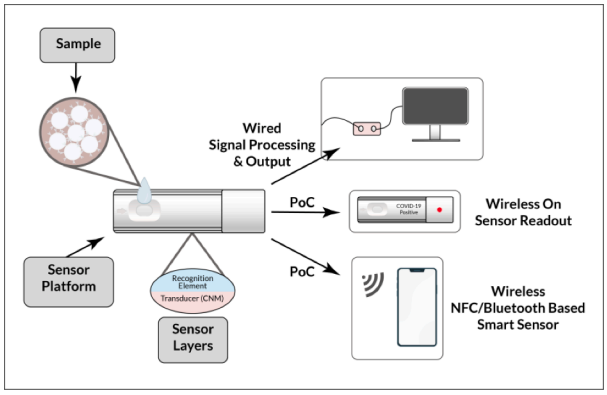


Fig. 2. Graphical representation of the working mechanism of SARS-CoV-2 sensor.

aptamers. Such carbon nanomaterial systems have been employed in various areas of biomedical research for diagnostics and pathogen detection.

6. Carbon nanomaterials employed in biosensors

The abundance of various carbon allotropes in nature has led to their varied use. Found in various synthetic and natural allotropic states, their sp^2-sp^2 hybridized state finds use in diagnostic devices. The sp^2-sp^2 state gives rise to various allotropes that can be used both for the development of electrochemical and optical biosensors. Carbon allotropes such as CNTs, CNFs, and GR have been used in biosensor fabrication [31]. These allotropes provide metallic, non-metallic, conductor, semiconductor, high aspect, and surface area to volume ratio. Functional groups such as -COOH, -NH_2, -PO_4^{3-} etc., when added, increase the biocompatibility and biosensing efficiency. Oxidized graphene (GO) and reduced graphene (rGO) offer several advantages in comparison to pure GR. GO is water soluble, increasing its interaction with an aqueous biological sample. rGO has a few of its oxygen groups removed, enhancing the conductivity and signal transduction. Recently, GR and CNTs have been used in designing FET. They provide sensitive, label-free, and rapid detection. CNTs provide robust, sensitive fluorescent emission. The interaction of the CNT-tagged biorecognition element with the target analyte gives a measurable optical signal. Moreover, their excellent characteristic of fast electron transfer enables measuring redox activities of CNT-coupled biomolecules. Thus, biofunctionalized CNTs play an important role in the design of biosensors. Additionally, CNT-coated electrodes amplify the signal of interest in electrochemical sensors. CNFs are cylindrical or conical carbon nanomaterials with large surface areas and increased porosity. Photoresistive blocking, adsorption, and chemical cross-linking of biomolecules on CNFs have led to their popularity and use in biosensors. Screen-printed technologies have revolutionized the design of electrochemical sensors, where a thin film/layer of electrode ink is printed on the desired surface/electrode and shape. The printed surface may then be impregnated with analytical elements such as antibodies/receptors for biological detection [32,33]. MIP is another exciting avenue of biosensor design, which are ultra-specific surfaces molded as locks for their target analyte (key). The surfaces are robust, withstanding high pH, temperature, and pressure variations. Limited work exists on this technology regarding CNM-based MIP for SARS-CoV-2 detection [34]. Computational methods and machine learning methodologies provide insights into structural design and correlation with the desired outputs. Such techniques must be incorporated while designing biosensors for biological detection, hence avoiding limitations of performance in relation to sensor design. These aspects were explored in a study that designed CNTs by controlling three parameters, namely, alignment, roughness, and thickness. Two different CNT sensors were designed with anti-SARS-CoV-2-IgM antibodies and were fabricated as resistive sensors. The study demonstrated optimized and efficient detection at the early stages of COVID-19 infection [35]. Furthermore, the stability of the bound biorecognition moiety may also depend on the CNMs configuration, as has been shown in a recent study. Herein this study [36], different peptides against the SARS-CoV-2 RBD were identified, and their binding affinities with two CNMs, graphene, and CNT, were observed. It was observed that specific sites of the peptides upon binding with the linker molecule lead to variation in sensor efficiency. Further, specific peptide-linker molecule bonds affect and differ between the two CNM sensing chemistry [36]. These results must be kept in view while designing sensors with different identifying biological elements between different sensing platforms.

7. Categorizing of carbon nanomaterial-based biosensors for SARS-CoV-2 detection

7.1. Carbon nanomaterials in field-effect transistor (FET) biosensors

Field-effect transistors are based on measuring the fluctuation in the voltage across the semiconductor material. They are an important class of sensors developed for rapid and accurate detection of SARS-CoV-2. In a typical FET biosensor [37], the identification molecule is usually surface immobilized via cross-linking, embedding, and physical or chemical binding, which undergoes conjugation with the target analyte. As a result, potential difference as an electrical signal is produced, measured, and amplified by the FET. These sensors are fabricated with novel nanostructures and biological sensing probes. In designing the sensors, FET may work as the transducing element or be coupled with transducers. In the COVID-19 pandemic, carbon nanomaterials (Table 1) used in the fabrication of Bio-FET (Fig. 3), have been discussed below.

7.1.1. graphene

Graphene has excellent mechanical and electrical properties, which makes it a suitable candidate for surface decoration with various materials to enhance the detection and sensitivity in numerous sample types. In response to the COVID-19 pandemic, numerous groups have conducted research on designing G-FET. Recently, researchers have designed G-FET biosensors with surface-decorated AuNPs. The sensor exhibited high sensitivity towards trace amounts of the RdRp gene by the use of artificially constructed uncharged complementary oligomeric probes, PMO immobilized on the AuNP surface. When samples spiked with both SARS-CoV and SARS-CoV-2 RdRp genes were tested with the sensor, specificity towards the SAR-CoV-2 sequence was confirmed, implying sensor efficiency. Furthermore, standardization was done using RT-PCR as a reference in comparison to the sensor to assess the device's reliability in undiluted clinical samples. Subsequent to RNA extraction, the device detects the viral genome in a span of 2 min, with high reliability and specificity, a PCR, and pre-amplification-free detection [38]. Similarly, ELISA based system using antigen immobilization for targeting SARS-CoV-2 antibodies is another important perspective for the detection and identification of asymptomatic and symptomatic individuals. Such an approach was exploited by researchers in designing G-FET with immobilized S1 protein, which could detect as low as 150 antibodies from serum samples within a time of 2 min, similar to the previously discussed study. The sensor exhibited rapid detection and identification of infected individuals, thus enabling their rapid isolation, hence curtailing the disease communicability [39]. Cui et al. have developed an LI-G-FET with a porous graphene structure embedded with the spike protein antibody. Interaction of the immobilized antibody with the spike protein produces a measurable electrical signal. The advantages of the usage of laser for sensor fabrication help in rapid industrial scale-up, with porous reef-like structure enhancing the antibody and analyte interaction and preventing antibody flush out during a sample surge, thus providing stability [40]. Seo et al. also developed a similar diagnostic platform utilizing anti-S protein antibodies immobilized on the graphene sheet surface with PBASE as the

Table 1
Carbon nanomaterials used in Bio-FET sensors.

Principle	Carbon Nanomaterial	Recognition Element	Analyte Target	Sample Type	Duration of Detection	Limit of Detection	Point of Care	Ref
Field Effect Transistor	Graphene	Morpholino	RdRp gene	Serum Throat Swab	2 min	3.99 fM 2.29 fM	No	[38]
		S1 protein	Anti-S1-Ab	Serum	15 min	14 aM		[39]
		Antibody	Spike protein	Serum	15 min	1 pg/mL		[40]
		Antibody	S1 protein	Spiked PBS Sample	Real-time	pM		[41]
		S1 protein	Anti-S1-RBD-Ab			0.2 pM		
		Anti-S-Ab	Spike protein	Nasal swab		2.42×10^2 copies/mL		[42]
	Carbon nanotubes	Anti-S-Ab	Spike protein	Spiked PBS Sample		4.12 fg/mL		[43]
		Anti-S-Ab Anti-N-Ab	Spike protein Nucleocapsid protein	Nasal swab	Real-time (?)	0.55 fg/mL 0.016 fg/mL		[44]

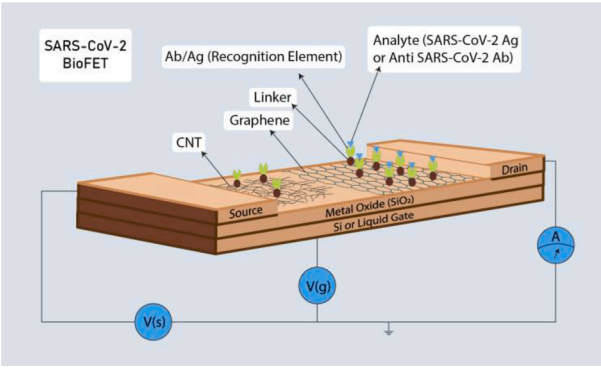


Fig. 3. Basic working principle of carbon nanomaterial-based Bio-FET sensors.

coupling agent. The designed sensor could detect nasopharyngeal samples directly without the need for sample preprocessing or amplification [42]. Such graphene-based BioFETs demonstrate robust platforms, which can be upscaled and promoted for COVID-19 diagnosis.

7.1.2. Carbon nanotubes

Carbon nanotubes have also exhibited significant progress in the fabrication of diagnostics. Besides G-FETs, studies have explored CNT-FET for the detection of SARS-CoV-2. Using antibody functionalization, a sensor platform was developed with CNT-FET, where the sensor specificity and cross-reactivity were checked for the antigens of MERS-CoV, SARS-CoV, and SARS-CoV-2. In the sensor, SWCNTs were prepared and used with antibody functionalization. The antibody immobilization was carried out by using the interface coupling compound PBASE. Furthermore, to check the CNT-FET-Ab and target Ag binding, AuNPs conjugated SARS-CoV-2 S1 antigens were prepared and utilized to check for the sensor resolution. The pH during the measurement also plays a crucial function, as demonstrated in this study, where SARS-CoV-2 S1 antigen carries a net positive charge in comparison to other coronaviruses at neutral pH. In the experiment discussed here, the researchers not only designed a sensor with high specificity against the spike protein of SARS-CoV-2 but also demonstrated the importance of the sample pH during immunological interactions [43]. Another study developed BioFET with semiconducting SWCNTs, which were surface functionalized with a single antibody specific against both SARS-CoV-2 S and N protein antigens. The SWCNTs used were semiconducting, and placed between interdigitated gold electrodes on the SiO_2 surface using nanopure water for liquid gating. Upon the introduction of the negatively charged S and positively charged N antigens, the voltage shift increases towards positive values. However, the SWCNT-S-Ab complex detecting S antigen demonstrates superior sensitivity and discriminates between positive and negative samples efficiently over the SWCNT-N-Ab complex against N antigen [44].

7.2. Carbon nanomaterials in electrochemical sensors

Electrochemical sensors are chemical sensors based on the use of electrodes for signal transduction. In the design of electrochemical sensors, they are composed of WE, RE, and CE as transducers, along with a receptor for analyte identification and sensor circuit. Electrochemical indicators are an additional component of electrochemical sensors. They are used as a visual confirmation or optical signals of an electrochemical reaction. In one study, a redox indicator complex of MB-CD is used to detect the SARS-CoV-2 sequence. Changes in the electroactivity of the MB-CD interaction with the oligonucleotide probe undergoing hybridization with the SARS-CoV-2 sequence are measured by DPV (Fig. 4). In the process, the indicator discriminates between single and double-stranded oligonucleotide sequences. Hence, in electrochemical measurements, novelty can be applied at ~~the~~ varied levels of sensor components [45]. Such interventions have been described in the sub-sections below. Additionally, Table 2 provides a summary of the various carbon nanomaterials used in electrochemical sensors for SARS-CoV-2 detection. We discuss the recent trend in screen printing electrodes utilizing CNMs for designing electrochemical sensors under SPEs and other methods for the fabrication of electrodes with CNMs under nanostructured electrodes.

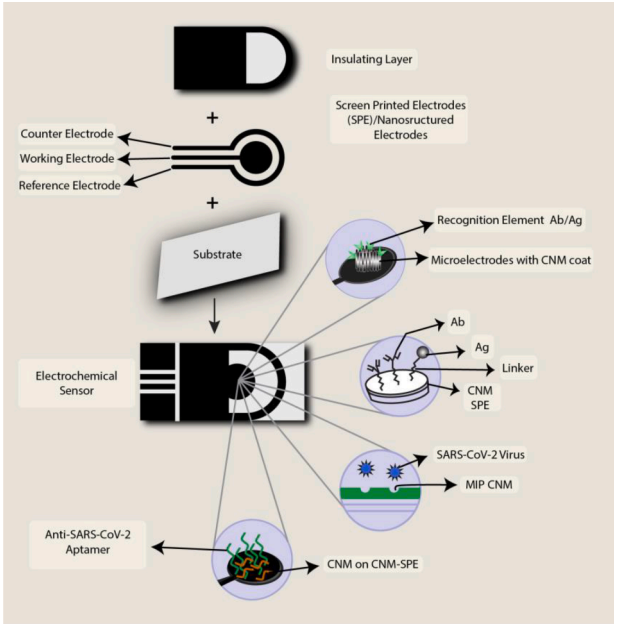


Fig. 4. Flow diagram of SARS-CoV-2 detection via carbon nanomaterials.

Table 2
Carbon nanomaterials utilized as electrochemical sensors.

Principle	Carbon Nanomaterial	Recognition Element	Analyte Target	Sample Type	Duration of Detection	Limit of Detection	Point of Care	Ref.
Nanostructured Electrochemical Sensor	Graphene	Antibody	Spike protein	Serum	1 hr	2.9 ng/mL	Yes	[46]
		Spike protein	Anti-S-Ab	Serum	10 min	500 pg/mL		[47]
		Nucleocapsid protein	Anti-N-Ab	and saliva		(N protein)		
		Antibody (CRP and N protein)	CRP and N protein			250 ng/mL (Anti-SARS-CoV-2 IgG and IgM) 50 ng/mL (CRP)		
	Reduced Graphene oxide	Antibody	S1 RBD Site	Saliva	N/A	2.4 ng/mL	No	[48]
		Nucleocapsid protein	Anti-N-Ab	Serum	Within secs	2.5 fM		[49]
	Graphene oxide	A molecular imprint of SARS-CoV-2	SARS-CoV-2 whole particle	Aquatic	< 1 min	11.32 fg/mL		[34]
		Nucleocapsid protein	Anti-N-Ab	Serum	Real-time (?)	~1.0 fg/mL		[50]
		Anti-N-Ab	Nucleocapsid protein	Nasal swab		3.99 ag/mL		
	Graphite	Anti-S-Ab	Spike protein	Saliva	20 min	pg/mL		[51]
	Carbon nanotubes	N/A	Reactive oxygen species	Sputum	Real-time	N/A		[52]
Screen Printed Electrochemical Sensor	Graphene	Anti-S-Ab	Spike protein	Buffer	45 min	20 μg/mL		[53]
	Graphene oxide	Anti-S-Ab	Spike protein	Biological/ non-biological	1 min	1.68×10^{-22} μg/mL	Yes	[54]
	Carbon	Anti-S-Ab	Spike protein	Saliva	30 min	19 ng/mL	No	[55]
		Anti-N-Ab	Nucleocapsid protein			8 ng/mL		
		Anti-S-Ab	Spike protein	Buffer	10 min	1.065 fg/mL	Yes	[56]
	Graphene/ Carbon	Anti-S-Ab	Spike protein	Nasal swab	5 min	0.25 fg/mL	No	[57]
	Carbon/ carbon nanofibres	Aptamer	S1 RBD Site	Saliva	40 min	7.0 pM	No	[58]

7.2.1. Nanostructured electrodes

Nanostructuring the electrode is an important avenue of research in nano-electrochemistry. The reduced size leads to an increase in surface area, and resolution and enables researchers to control the electrode properties at the nanoscale. The unique properties at the nanoscale have led to multiple biological applications in recent times [59].

7.2.1.1. Graphene. Beduk et al. have recently developed a three-electrode system using laser-scribed graphene. The electrodes are scribed on a polyimide surface with a CO_2 laser, followed by decorating the WE with AuNSs. The surface was then coated with an anti-S1 RBD antibody, followed by SARS-CoV-2 detection in spiked and clinical samples. The sensor was fabricated into a PoC device using a portable potentiostat, connected to a smartphone with customized software for signal readability. The overall time from sample input to a readable signal output takes an hour, which is efficient and maybe home-based and avoids the need to visit a diagnostic lab. Such intelligent diagnostics designed by combining analytical, molecular, and nanomaterial-based approaches with rapid, specific, and PoC systems are promising candidates for industrial large-scale production, commercialization, and consumer use [46]. Another study has similarly devised a laser-engraved graphene 4-WE system, with wireless technology, as a ready-to-use portable device. The device is multiplexed, for targeting various COVID-19 disease markers, thus identifying the stage of infection. The biomarkers targeted are NP for viral infection, circulating S1-IgG and S1-IgM in the event of an immune response, and CRP as a measure of SARS-CoV-2 mediated pneumonia and inflammation. The graphene surface is functionalized using S1 antigen (for IgG and IgM), anti-NP antibody, and anti-CRP antibody. Electrochemical characterizations were done for the developed sensor, which exhibited its efficiency in terms of diagnosing positive and negative RT-PCR-confirmed samples [47].

7.2.1.2. Graphene oxide. Graphene oxide and reduced graphene oxide (rGO) provide a large surface area, with numerous sites for material functionalization with oxygen-rich sites and biomolecule immobilization. In a recent study, rGO was used to coat a glassy carbon electrode (GCE as WE), followed by anti-S1 antibody conjugation on the GC-rGO-EDC—NHS coupled surface, thus targeting the S1-RBD antigen of SARS-CoV-2. EIS and CV were employed to assay the detection profile [48]. A novelty introduced in another study was the spatial arrangement of electrodes, wherein vertical working microelectrodes with bio-recognition elements were synthesized. The system increased the overall working and detection surface area. The electrodes were manufactured with AuNPs as a base, followed by layering with rGO sheets. The rGO layer provided the sensor surface to be modified with EDC—NHS coupling chemistry, subsequent to which SARS-CoV-2 N protein was attached to the rGO coated WE surface, which upon interaction with the anti-N protein antibody in clinical samples led to dielectric changes, measured via EIS, giving a signal output of positive infection [49]. Another study developed a dual immunosensor platform, which can detect SARS-CoV-2 antigen and anti-SARS-CoV-2 antibody simultaneously. This was done via surface decoration of a GCE with GO and Au nanocomposites, by simultaneous reduction of both the nanomaterials on the GCE surface in an aqueous solution. This modified GCE is used as the WE and further functionalized with SARS-CoV-2 antigen and anti-SARS-CoV-2 antibody. The antibody-fabricated immunosensor detects SARS-CoV-2 antigen in patient swab samples, while the antigen fabricated immunosensor detects antibody level in patient serum samples. DPV, EIS and CV were used for electrochemical characterization of the sensors, which showed very low limit of detection accurately, and can be fabricated for PoC sensor systems [50]. MIPs are potential candidates against biorecognition elements. They provide robust surfaces, with long shelf life and identification of target molecules, overcoming the limitation posed by biomolecules. In a recent investigation, MIPs were used to fabricate the surface of a sensor electrode. MIPs acted as artificial biorecognition elements, with cavities designed to fit the target analyte, SARS-CoV-2 particles. In the study, graphene oxide flakes were processed and imprinted with SARS-CoV-2. The SARS-CoV-2 particles were washed off, leaving the imprinted template. Then a GCE was surface modified with the MIP, such that enhanced detection, response time, and resolution were achieved, with a label-free detection [34].

7.2.1.3. Graphite. Graphite is a CNM with multiple sheet layers in comparison to graphene, which has a single graphite sheet. Hence, graphite also has the potency to be explored in sensor design. A study using graphite was conducted by Adeel et al., wherein they utilized graphitic carbon foil as the WE, surface functionalized with an anti-spike protein antibody to detect the SARS-CoV-2 antigen. Detections were carried out in buffer and artificially prepared human saliva samples with optimized analytical and sensitivity studies, implying the lowest antigen detection possible in comparison to the detection levels established clinically [51].

7.2.1.4. Carbon nanotubes. In the period of SARS-CoV-2 infection, the viral particles harbor themselves in the respiratory system and replicate in the host lung cells. As an inflammatory response, ROS are released. These molecules are electroactive groups, which upon interaction with a conductive surface can give an electrical output as a measure of inflammation. Such a system was designed wherein the sensor transducer could directly interact with the ROS in the sputum or BAL of suspected/infected individuals. MWCNTs were used for the sensor fabrication, wherein the WE was designed with MWCNTs coating the tip of a steel probe. The intensity of ROS generated was detected from infected samples, which gave high CV peaks upon interaction with the MWCNTs, in comparison to CV peaks from uninfected samples. The sensor gives an estimate of lung/respiratory damage, as well as reduces the time and cost it takes for an individual to get a CT scan or MRI diagnostic test [52].

7.2.2. Screen-printed electrodes

SPE is a thin, screen-like layer of material functioning as the electrode. They reduce the cost and time of electrochemical sensor fabrication, maintaining standards of reproducibility and efficiency. Under CNMs, graphene and its derived composites are widely used in SPE fabrication. In most fabrications, the CNMs are used in designing WE (and at times CE or RE). In the continuing pandemic, notable studies have contributed to designing SPE biosensors.

7.2.2.1. Graphene. Graphene has excellent electrical and physical properties which confer numerous advantages for designing biosensors. Mojsoska et al. developed SPE with graphene ink. The surface of the graphene WE were functionalized with a PANHS coupling agent. Anti-SARS-CoV-2 spike antibody was linked to the PBASE-activated graphene surface. Interaction of the antibody with the spike protein, leads to changes in the electrical properties of graphene, giving a measurable electrical signal, which is exploited in terms of EIS and CV, with LOD of 20 μg/mL [53].

7.2.2.2. Graphene oxide. A study developed graphene oxide printed WE which measured voltage differences, which were identified as unique fingerprints for specific glycoproteins, thus targeting the SARS-CoV-2 spike glycoprotein. The novelty of the study lies in creating voltammograms for glycoproteins of various viruses. These voltammograms are specific to individual viruses and also their mutants. The biosensor has GO-8H-EDC—NHS and gold nanostars (GO-8H-EDC—NHS-AuNS), cast on the surface of a GCE, platinum (Pt) electrode and saturated Ag/AgCl electrode as WE, CE, and RE, respectively with KCl as electrolyte. The designed electrode when placed in the sample, gives a voltage specific to the glycoprotein of the biological species present, based on the principle of EIS. The designed system is novel such that it avoids a cumbersome RNA extraction or a biological analyte for detection [54].

7.2.2.3. Carbon. In yet another study, SPE was developed using carbon black for WE, which interacted with anti-SARS-CoV-2 Ab tethered on MB. The WE and CE were printed with graphite ink, while RE was designed with silver ink. Further, the WE was cast and optimized with carbon black to enhance sensor sensitivity. Saliva/sputum was directly transferred to a tube, housing the reagents (with MAb anti-SARS-CoV-2 coated MB and secondary alkaline phosphatase-conjugated anti-SARS-CoV-2 antibody). SARS-CoV-2 containing sample binds with the antibody-coated MBs, which further binds with the secondary antibody. Further steps for detection include a washing step (to remove residual secondary antibodies), followed by resuspension in buffer, the addition of 1-naphthyl phosphate, and drop casting onto the WE. Release of 1-naphthol as an electroactive product by alkaline phosphatase-mediated 1-naphthyl phosphate degradation leads to a voltammetric measurement, as a direct measure of viral presence [55]. In a similar study, anti-S protein antibodies were coupled by EDC—NHS conjugated with electrodeposited PABA, to functionalize the surface of carbon screen printed WE. Measurements were carried out using EIS and CV. The perks of the device lie in its low weight (<200 g), portability, and rapid, accurate testing [56].

7.2.2.4. Composite. Under SPEs, researchers may utilize CNMs in combination with other nanomaterials as a mixture forming nanocomposite or fabricated separately in the same sensor environment. Ehsan et al. have formulated a paper-based (cellulose) sensor with a printed circuit for detecting the COVID-19 virus. On the paper strips, highly conductive hybrid graphene/carbon ink, Ag/AgCl ink, and carbon ink were used to screen print the WE, RE, and CE respectively. An interface of PBASE was applied on the fabricated paper electrode strips for surface immobilization of anti-S antibody. A parallel modification was done with protein A mediating an oriented immobilization of anti-spike IgG on the WE. The sensor is based on the principle of redox EIS using ferro/ferricyanide redox couple and detects the virus in nasopharyngeal samples using portable devices. In the study, important insights were provided on the effects surface activation of nanomaterial has on electrical signals. The authors demonstrated that the activation of the graphene surface with a PBASE linker doesn't significantly affect the redox couple signals and electron transfer efficiencies. However, a noticeable rise in signal was observed with protA immobilization, which reduced basal noise upon antibody-protA binding. The device is selective, in which protA provides a high sensitivity on the basis of the stability it provides to the antibody, and the strips are disposable [57]. In another study, readymade CSPE was surface decorated with CNF and AuNPs. A thiol-terminated aptamer, specific against the RBD protein of SARS-CoV-2 was combined with the AuNPs via an Au-S bond. The sensor could detect the virus in spiked saliva samples by EIS. However, the study shows that the aptasensor shows less sensitivity, with a poor limit of detection in comparison to existing methods and immunosensors [58].

7.3. Carbon nanomaterials in other varied detection methods

7.3.1. Carbon nanoparticles and nanotubes

7.3.1.1. Optical methods. In one study, researchers utilized lanthanide metals praseodymium (Pr), gadolinium (Gd), and ytterbium (Yb) for synthesizing three different carbon nanoparticles from each element. They developed three different agarose matrices containing these lanthanide-doped carbon nanoparticles (CNP)-based (Fig. 5a) as a water surveillance system, which gives distinct photoluminescence upon interaction with bacterial and viral samples. Different pathogenic strains gave optical signatures distinct from each other, which were established as signatures for the three lanthanide metals. The specific optical response was identified for each system against SARS-CoV-2 contaminated water samples. The basis for the fluorescence lies in the surface chemistry of each pathogen, wherein the surface molecules may undergo counterionic–ligand interactions with the LnCNP - agarose matrix. The system is an ideal candidate, along with a few notable others, given the absence of the need for a biological probe which may lose its stability, or the need to extract the viral RNA for genomic detection, which

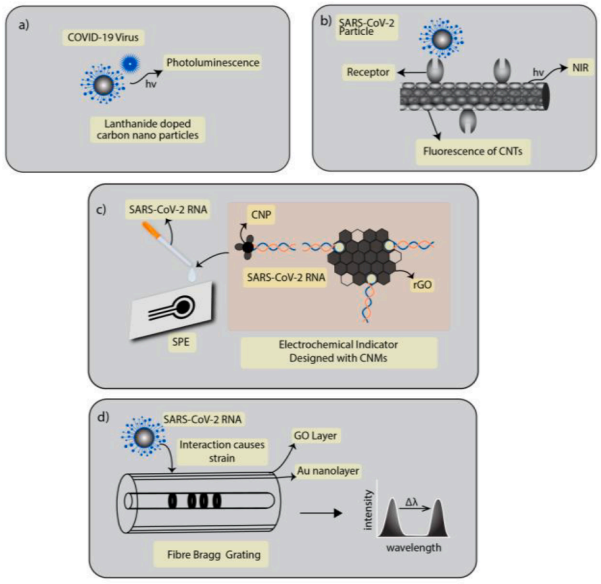


Fig. 5. Detection methods of SARS-CoV-2 via other types of carbon nanomaterials a. Lanthanide doped carbon nanoparticles (Ln-CNPs) b. Single walled Carbon nanotubes (SWCNTs) c. Electrochemical indicator with carbon nanoparticles and nanotubes d. FBG with a graphene oxide layer.

may introduce contaminants, handling errors or increase the duration of detection [60]. In yet another study, carbon-derived SWCNTs were used for their optical properties in SARS-CoV-2 detection (Fig. 5b). The SWCNTs were functionalized with ACE2 receptor protein, which shows specific affinity towards the S-RBD of SARS-CoV-2. The SWCNTs have absorption and emission properties in the NIR, which are not quenched by the bioanalytical moiety. Thus, in the sensor, SARS-CoV-2 S-protein RBD sequence binding with the immobilized ACE2 protein gives a measurable optical signal as output [61].

7.3.2. *Graphene oxide*

7.3.2.1. Electrochemical indicator. In a study using electrochemical sensors with CSPE (Fig. 5c), two unique premixes were prepared which interact in a sandwich configuration with the extracted RNA from SARS-CoV-2. One premix was designed using a capture probe (CP) complexed with gold/iron nanoparticles (CP/Au@Fe_3O_4), while the other premix contains reduced graphene oxide functionalized with p-sulfocalix[8] arene (SCX8), gold, and toluidine blue as the complex Au@SCX8-RGO-TB and further modified with label probe (LP) and auxiliary probe (AP) forming LP-AP/Au@SCX8-RGO-TB nanocomposite. The extracted RNA interacts with the CP, followed by the interaction of the RNA-CP hybrid with the AP-LP hybrid. The mixture is then dropped onto the sensor surface, which is modified into a USB device and gives output in a smartphone. Here TB used is an electrochemical indicator that identifies the hybridization and gives a readable electronic signal measured by DPV [62].

7.3.2.2. Fiber Bragg grating. Optical fibers have been used extensively as biosensors in the health industry. In a study, optical fibers were utilized with FBG (Fig. 5d). In the work, the fiber central core was kept intact while most of the fiber underwent uncladding. Subsequently, in the unclad portion, Au layers were deposited, followed by the deposition of GO for biosensing. The GO surface is activated by EDC—NHS for creating free carboxylic groups (-COOH) and amine groups (-NH_2) for interaction with the SARS-CoV-2 virus. In the optical fiber biosensor, SARS-CoV-2 from the infected samples is trapped at the activated GO platform, thus leading to a change in the refractive index of the sensor. This leads to a light wave reflected from the FBG, known as the evanescent wave, which propagates and interacts with the surface plasmon resonant waves at the Au surface. The wavelength and intensity of light change are observed as a measure of SARS-CoV-2 detection [63].

8. Conclusion and future perspective

A quick observation around us leads to the understanding that there is an ongoing plethora of research in applied sciences and healthcare. There has been a boost of novel diagnostic kits based on nanomaterials science in the COVID-19 pandemic. It is because rapid and accurate detection is the need of the hour. In the review, we have aimed in providing insights into such novel nanomaterial-based diagnostics for SARS-CoV-2. The sensors described have targeted the viral RNA, NP proteins, S proteins, and antibodies. To validate the same, RT-PCR is to be taken as a standard of reference. While upscaling, the production must be closely assessed to ensure batch-to-batch uniformity and nanoparticle stability in various environmental conditions. Many studies test biosensor specificity in spiked PBS or human samples. This limits sensor reproducibility, with the possible challenges of cross-reactivity or bio-fouling in unprocessed clinical samples. Moreover, immunological assays rely on detecting the presence of anti-COVID-19 antibodies. In accordance with the USFDA, these assays, however, may be biased in individuals vaccinated against SARS-CoV-2 and hence aren't reliable. To strategize and improve the biosensor design, two or more nanomaterials may be used on the basis of their characteristics. This enhances the detection and sensor performance. In a research organization, the end product as a biosensor has huge investments in terms of manpower and economy. Such investments shouldn't become a hindrance to the affordability of the device, especially to economically constrained communities without healthcare facilities. Although RT-PCR is deemed the gold standard test for COVID-19, it is surpassed by the numerous advantages of nanomaterial-based PoC devices. Such modifications have led to the development of rapid antigen tests, giving results in 15–20 min, with a price margin of around $1. However, the major limitation of PoC sensors is their complex design in research laboratories, which limits their industrial manufacturing and scalability. In one study, besides developing a diagnostic platform for COVID-19 detection from oral samples, the platform was made portable, with a user-friendly mobile interface, and digitalized with a Bluetooth communication system. Though the study utilized gold-based EDL-FET, it is a model for developing portable, scalable products for commercialization and user-friendliness. This must be the aim while designing novel biosensors, such that it is easily accessible to and interpreted by the users. Thus, such carbon nanomaterial-based sensors must attempt to follow the set standards under RE-ASSURED, thus abiding by the principles laid down by the US FDA. Many such devices are commercially available and used for mass SARS-CoV-2 testing in a home-based setting. It is estimated that in the near future, biosensors fabricated with nanomaterials will gain attraction and become available as OTC and bedside diagnostic devices.

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CRediT authorship contribution statement

Shivam Mishra: Data curation, Visualization, Formal analysis, Writing – original draft. **Bari Aamna:** Conceptualization, Data curation, Visualization, Formal analysis, Validation, Writing – review & editing. **Sagarika Parida:** Visualization, Formal analysis, Validation, Supervision. **Aritra Kumar Dan:** Conceptualization, Visualization, Formal analysis, Validation, Writing – review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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