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A short review on RT-PCR and graphene-based materials in COVID detection

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ABSTRACT

The global pandemic of COVID-19 made all the earth's inhabitants acknowledge the importance of health care and human life. All the countries allocate millions and millions on military and defense, which are in vain today as real soldiers are doctors and health workers waging a war face to face with the invisible Coronavirus. Researchers and companies in Chemistry, Pharmacy, and Biomedical sciences collaborate with technology to fight against COVID-19 and support bringing analyzers for detection and vaccines for eradication. Graphene, a wonder material, is still playing a lead role and exhibiting its multiple astonishing properties. Graphene-enhanced batteries, sensors, biosensors, and photo detectors are available in the market A great deal of research is carried out by many researchers. The present review paper focuses on the use of Graphene-based materials in the COVID pandemic.

1. Introduction

The coronavirus strain was initially found in December 2019, and it started to cause severe respiratory illness [1]. In a later stage, it was observed as a severe respiratory-related disease as SARS-CoV-2 [2]. In March 2020, WHO declared COVID-19 as a pandemic and requested research organizations all over the world to make diagnostic tests to put up a great fight against the virus and its spread [3]. The name Graphene was first given to a 2-D sp2-hybridized carbon. Graphene was considered the basic structure for many allotropes, which can also be transferred to three-dimensional Graphene [4,5]. Longer range Pi configuration of Graphene can yield extraordinary results in mechanical, electrical, and thermal properties. This process has been a recent area of research. So far, graphene research has been carried out utilizing a single layer. Still, in the year 2004, Manchester university co-workers tried to isolate samples of 2-layer graphene into single-layer Graphene. In recent times, Graphene and graphene-related materials (GRM) have gained more attention among researchers and 2 academicians because of their significant properties, including good conductivity, high surface area, electrochemical, and piezoelectric properties, etc. [6,7]. From analyzing specific literature reviews, it was found that Graphene and GRM act as the best material to be used for fighting against the COVID pandemic [8,9].

A proper analysis using Graphene and various graphene sources can fight against the virus by destroying it. Graphene and GRM support possibly eliminating the virus by controlling the spread and transmission of COVID through graphene biosensing. Specific upcoming Graphene-based diagnosis techniques have helped in detecting covid they are.

- LAMP - Loop-mediated isothermal amplification method.
- RTRPA - Reverse transcription recombinase polymerase amplification.
- NASBA- Nucleic acid sequence-based amplification.
- ELISA- Enzyme-linked immunosorbent assay.

All the above-mentioned diagnosis methods used protein biomarkers ad nuclei acid for bacteria and virus diagnosis. Fig. 1 illustrates the possible Graphene and GRM-based application used for combating COVID-19 spread which all mainly used nucleic acid and protein biomarkers for viral and bacterial diagnosis (see Fig. 2).

2. History of Graphene

It is essential to know about graphene history before starting research related to Graphene. Single-layer Graphene was initially

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Fig. 1. Possible Graphene and GRM-based application used for combating the COVID-19 spread.

property of graphite continues to attract both the technologist and the scientist as well. The S, Px, and Py atomic orbitals on each carbon hybridize to form strong covalent SP^2 bonds, giving rise to 120° C-C-C bond angles and the familiar chicken-wire-like layers.

3. Chemistry involved in Graphene

Graphite is a material with a rich chemical property because it is used as a reducing agent or an oxidizer. The chemistry involved in graphite directly influences its electronic structure as a result. This has both ionization potential and electron affinity. Many experiments related to graphene mainly focus on increasing the basal planes, chemical species or in intercalation etc. The GIC, known as the Graphite Intercalation Compound [10–12], will be mainly found in the layered compound. GIC can be used for exhibiting staging through which the layers of graphitic found between the adjacent intercalants will vary in a controlled fashion. The spacing between the interlayer may increase from 0.33 nm to 3.3. This is in native graphite, but in GIC, it will increase up to 1 nm. The raised interlayer spacing of Graphite Intercalation Compound will also make a considerable reduction between the adjacent sheet through which possible route to single layer Graphene will be created [10-12].

In 2003 individual scientists tried with KC8 (potassium intercalated graphite) potassium intercalation compound using a different type of solvents such as alcohol. Still, it just produced a thickness of around 30 layers with a tendency of scrolling under high-powered sonication. The interlayered spacing in GIC can be increased by.

thermal shock, which helps produce an expanded form of graphite, which is now used as a starting material for many techniques, including nanoribbon synthesis.

4. Graphene in tackling COVID-19 pandemic

4.1. Recent explorations of Graphene

In 2019, the Chemist James Tour of Rice University team [13] transformed laser-induced Graphene into self-sterilizing filters that can kill pathogens in the air by passing small pulses of electricity. Rice university Lab produced pristine Graphene from waste food, plastic, and other materials. This green process can facilitate the environmental impact of concrete and other building materials. A transparent graphene photodetector was designed to capture objects far from the camera lens. This device is used in biological

discovered in the year 1947 by P.R. Wallace. The name Graphene was initially originated in the year 1986 introduced by chemist Hanns-Peter Boehm. Graphene is the superficial layer that can be found in graphite. The significant honeycomb properties of Graphene are not a newer one to known. Naturally, available graphite was discovered almost 500 years ago. In middle age, graphite was used for making instruments like how we are making pencils in the present time. Such a significant graphite property has made it an ideal material used for several purposes like a dry lubricant, etc. Having an excellent conductor of electricity and thermal power is used in electrodes in industries for blasting furnaces. Graphite is also used in carbon fiber-reinforced composite. The demand for graphite reached almost 1 million tons worldwide. The material
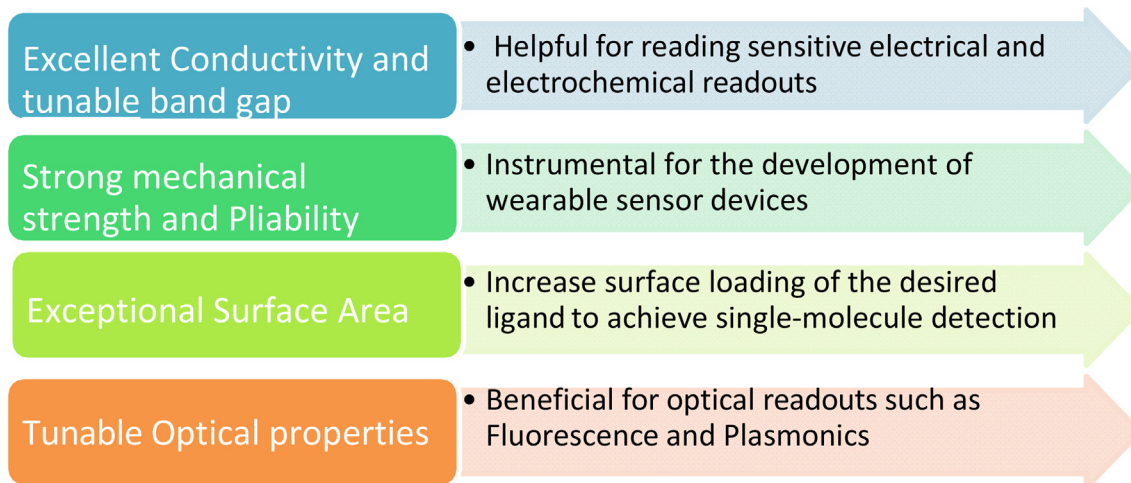

Fig. 2. Application of Graphene in Biosensing.

imaging and robots. To selectively identify dead strains of bacteria, Boston college Researchers used a Graphene sheet to track electronic signals inherent in physical structures. Graphene is used to increase the sensitivity of devices used in diagnosing and monitoring HIV.

4.2. Potential applications

Conventional LFP batteries (Lithium iron phosphate) enhanced with Graphene allowed the batteries to charge much faster than Lithium-ion batteries; they are lightweight and high capacity. Huawei's Mate 20X smartphone [14] uses graphene film cooling technology, Graphene -reinforced foam covers for a noisy component in its 2019F-150 & Mustang cars by FORD. Cardea (present San Diego Nanomedical Diagnostics) developed Graphene-based sensors for real-time detection of small molecules. DGIST (Daegu Gyeongbuk Institute of Science and Technology) fabricated electrodes using nickel–cobalt sulfide nanoflakes on sulfur- 5 doped graphene, which helps to increase the high discharge capacity and long shelf life of a battery.

4.3. Graphene-based biosensors

EIS (Electrochemical impedance spectroscopy) uses Faraday's law for characterizing the chemical property through which it can be implemented in electrical measurement. A small oscillating EIS voltage with varied frequency is applied to the electrochemical cell and electrical signal recorder. The electrical signal recorder is used for obtaining information related to charging transfer and diffusion, which occurs on the electrode's surface. A graphene-based impedimetric biosensor is employed for detecting the COVID-19 virus. A glassy carbon electrode was modified using graphene, which helps in detecting the HIV gene with a detection limit of $2.4 \times 10-14$ M. Graphene and gold nanoparticles rGO were used for detecting the hepatitis B virus with the lowest detection limit. For detecting diarrhea, virus rGO and nanocomposite were combined along with gold nanoparticles. But certain drawbacks were found in impedimetric biosensors because it was the one that is consuming more time.

4.4. The benefits of applying Graphene to biosensor applications

It was found that single-layer graphene can be used as a biosensing application due to the following properties [15–18].

- Single-layer Graphene is having high mechanical strength and a larger surface area.
- Single-layer Graphene is having good thermal conductivity and superior elasticity.
- Single-layer Graphene can be processed at room temperature to attain the Quantum Hall effect.
- Single-layer Graphene at high room temperature has significant electron mobility.

The physical and chemical properties of Graphene allow for the enhancement and development of biosensors with significant performance. Graphene is a material with less production cost and causes fewer hazards to the environment, making it an attractive material to select. A chemical functionalization process should occur through which Graphene can be used for biosensor application to enable a biocompatible surface. Functionalization of Graphene can occur either in covalent or in non-covalent bonding of smaller molecules, which is having the property of providing compatibility for immobilization of the biological receptor.

Researchers at the University of Illinois Chicago have successfully used graphene to detect the SARS-CoV-2 virus in laboratory experiments. Graphene is a single-atom-thick material made up of carbon. Carbon atoms are bound by chemical bonds whose elasticity and movement can produce resonant vibrations, also known as phonons, which can be very accurately measured. When a molecule like a SARS-CoV-2 molecule interacts with graphene, it changes these resonant vibrations in a very specific and quantifiable way. Researchers include that graphene atomic level sensors can help in the detection of COVID and cancer.

4.5. *Recent Graphene-based biosensors for covid detection* [40]

See Table 1.

5. Covid Detection

There are two broad categories in COVID detection, FDA-approved Reverse Transcriptase Real-Time Polymerase Chain Reaction (RT-PCR) and nucleic acid hybridization strategies to identify viral RNA. The second category of tests focuses on the detection of antibodies. 5.1 RT-PCR: RT-PCR test is used first developed and deployed worldwide for testing COVID-19 Virus [19–23]. For this test, a unique 6-inch cotton swab will be inserted into the patient's nostrils and throat. A swab of a person affected with COVID 19 contains a mixture of human cells, microbes, and virus particles. DNA is known as the hereditary material found in humans, which is the genetic material that has the tendency to pass information about the previous generation who belongs to a particular family. RNA is a similar material to DNA found in viruses like COVID, MERS, SARS, and also in HIV. DNA has a double twisted strand, but RNA has only one strand in it. The RNA is surrounded by nucleocapsid protein, where the virus starts to develop. The genes found in SARS CoV2 can direct the proteins to replicate the virus inside the human cell. Fig. 3. illustrates the overview of the testing procedure for SARS-CoV-2 through RT-PCR testing (see Fig. 4).

The procedure for the COVID-19 test is done in the following manner:

a) The test swab collected from the patient affected by COVID-19
b) Deactivating the virus by heating
c) Extracting RNA
d) Transferring the extracted RNA to a PCR plate (C-D)
e) Where DNA synthesis takes place in RT

The detection of the COVID-19 virus can also be done through high-through out or by sample barcoding. In the DNA sequencing, the viral particles will be inactivated after being subjected to the heating process. The samples after inactivation will be used for the downstream RT-PCR diagnosis process.

Table 1
Recent graphene-based biosensors.

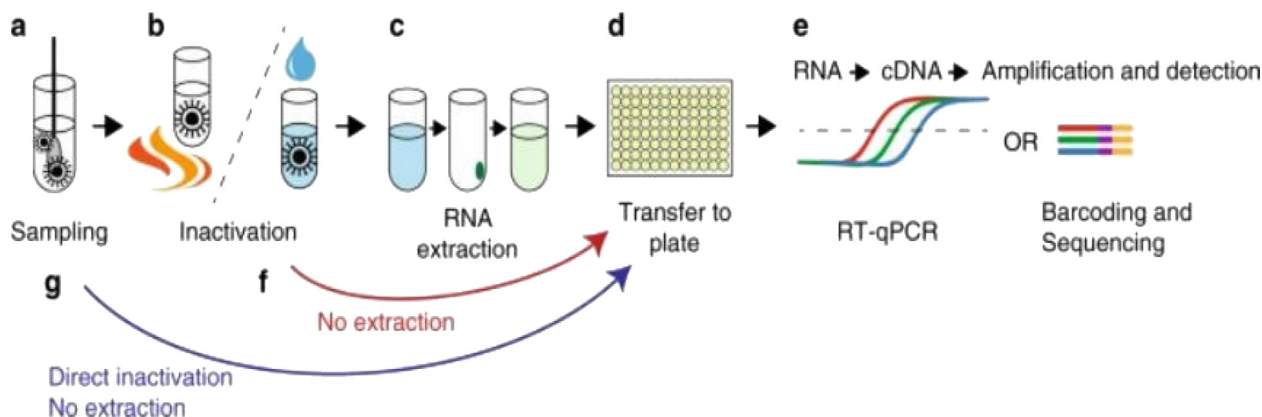| S. No.	Detected Element	Sensing material and sensor type
1	SARS-CoV-2 spike protein	Graphene (Field Effect Transistor [FET] sensor)
2	Influenza Virus and 2009 -nCoV	MXene – Graphene (FET sensor)
3	SARS-CoV-2	Graphene (nanoresonator sensors)
4	SARS-CoV-2	BK7/Au/PtSe2/Graphene (surface plasmon resonance [SPR] biosensor)

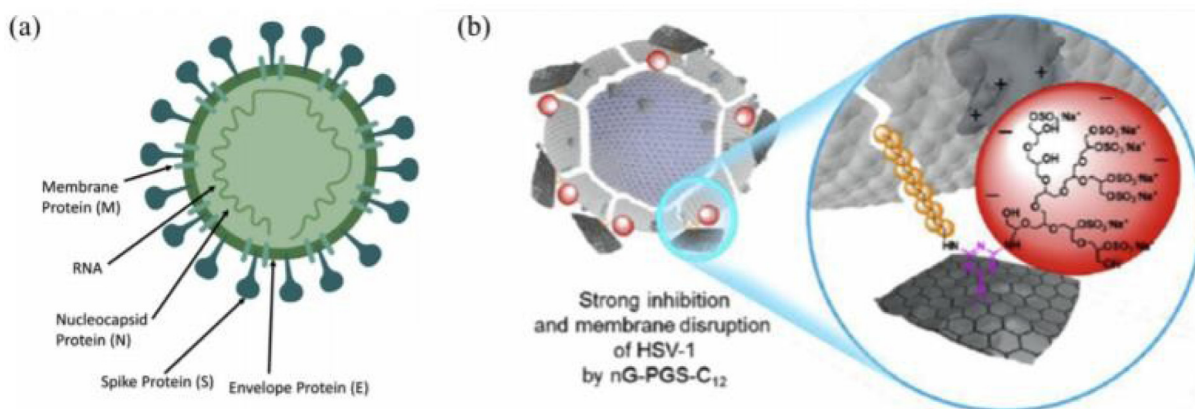**Fig. 3.** Schematic overview of SARS-CoV-2 RT-PCR testing procedure.

Fig. 4. COVID-19 transmission process and its transmission pattern.

5.1. The objective of Covid-19 tests

The main aim of the COVD-19 test is to detect at which part the viral genome is found in the affected patient. The N gene uses nucleocapsid protein to carry the direction for the virus. But this is not enough for detecting the RNA in the sample of the patient. The RT-PCR process boost to make more copies of the N-gene segment. The single-strand DNA helps in detecting the region of the N-gene from the sample of the patient. After witnessing the single-strand DNA, copy the RNA, which can also be termed as c DNA or complementary DNA. The DNA copying process of RNA will take place in a cyclic process. In each cycle, it tries to copy more viral RNA. The PCR (Polymerase chain reaction) will produce nearly 30 cycles of copying almost one million of viral RNA copies by DNA. The virus will be detected by PCR process after 30 to 45 cycles of DNA copying of viral RNA. Results of swabs vary. A negative effect can be observed if there is no virus RNA in the swab.

RT - PCR identifies tiny amounts of the SARS-CoV2 virus even in small quantities, but the method takes at least 3 h, including step-wise preparation of Viral RNA for analysis. Since the early diagnosis of Covid-19 for slowing down its spread, there is a need to develop a more accurate, fast, and straightforward diagnostic test. Few kinds of research developed a field-effect transistor-based biosensor that can detect SARS-CoV2 in a nasopharyngeal swab from a patient with Covid-19 in less than a minute. Researchers reported in ACS Nano field-effect transistor consisting of a graphene sheet with electronic conductance. This process avoids sampling preparation.

5.2. Paper-based electrochemical sensor of Graphene to detect COVID

The University of Illinois reported a rapid, ultra-sensitive test that can detect covid in less than 5 min. Graphene-based electrochemical biosensor consists of two components, a probe to detect the presence of viral RNA and a platform to measure an electrical readout. In order to create a conductive platform, a filter paper was coated with a layer of graphene nanoplatelets. In the readout, the electrical signal of a gold electrode with a predefined design was placed on the top of the graphene as a contact pad. As RNA-based RT-PCR screens for the presence of N- gene on SARS CoV-2 virus, the paper-based electrochemical sensor of graphene is designed with an antisense oligonucleotide (ASOs) probe to target two N- genes. Thus, ensuring the reliability of the sensor if one N-gene undergoes mutation. The probe helps in the hybridization of viral RNA and causes a change in sensor electrical response. The gold nanoparticles accelerate the electron transfer which results in an increase in the output signal when broadcasted over the sensor platform. The sensor was able to differentiate the viral load and also showed an increase in voltage of positive samples indicating the presence of viral genetic material within five minutes.

5.3. Interaction of graphene materials with the virus

Graphene has adequate inhibition capacity. Initially, the 1st evidence to prove that Graphene has a significant antivirus effect was showcased in 2012. When sulphate enriched employment and heparin drugs are combined with Graphene, they act as good

antivirals. rGO sulphate derivative synthesized to thermal power was found to have antiviral capacity against African virus [24]. When rGO functioned with DPG (dendritic polyglycerol) and sulfation, it serves as an antivirus to detect different forms of orthopoxviral strain [25]. The GO flakes can be confronted and wrapped with micro-organisms by subjecting them to a carbon blanket. Interestingly, the SARS-CoV-2 Spike S1 protein receptor-binding domain has recently been demonstrated to interact with heparin and change conformation [26].

The GO-based sensing method used in the textile industry is demonstrated in Fig. 5. [27–33]. In the future, research must be conducted on how protective clothing can be used for controlling the virus spread. GO films act as a significant element that helps in developing a breathable layer in clothing fabrics. Graphene has efficient water permeation, which is significantly superior compared to other materials and technologies, and this character helps in excluding viruses, bacteria, etc. (see Fig. 6).

Graphene facemask can be recycled through heating or by using the photocatalysis method. In graphene facemask, the protective effect is due to the layer of hydrophobic. Without that, micro-organisms would have crept into the protective effect of the graphene mask. The coating in graphene is done by using titanium oxide and by silver nitrate.

Graphene is used in filters and textiles for sterilizing the virus that comes in contact. After coming in contact with graphene, the virus can be denatured by subjecting it to mild heat at 56 °C for about thirty minutes.

5.4. Limitations and challenges of Graphene

1. The antiviral characteristic of Graphene was described in the past literature but it seems to obtain a positive result from Graphene after using it for treating COVID-19.
2. It is possible when demonstrated the efficacy of GO-hypericin in ducklings infected with the novel duck reovirus.
3. The characteristic of GRM has passed over certain levels of a biological barrier but is still less cytotoxic over macrophages than other nanomaterials of carbon.
4. The Graphene toxicity was found to be a little inefficient due to the wrong combination of dosage exposure route, surface chemistry, etc

5.5. Graphene enacted face mask

The Graphene enhanced facemask was designed with the motive of making it available for all working in all sort of workplaces. Many medical institutions and companies state that hypo-allergic G + facemask will provide quite a lot of advantages
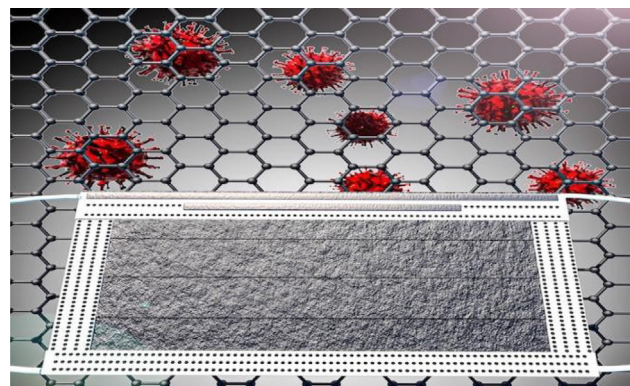

Fig. 6. Graphene Enhanced Face Mask.

for users to protect them from any virus, especially COVID-19. The graphene face mask is designed with enhanced performance, especially in the means of breathing. This mask will make breathing easier after wearing a mask. The G + mask can be washed and used again. This makes it eco-friendly. This is the perfect alternative for use and throws mask surgical mask.

Graphene can be taken as severe material for fighting against any sort of virus and infection in the upcoming decade. The antibiotic material used in graphene masks will lose its effectiveness after overuse and overprescribing. So this issue must be combated in the future by making it more combat to fight infection and disease.

Due to this issue now, researchers are trying to utilize another material to overcome the drawback of graphene. Yet much research has been conducted against graphene to make it impressive for antibacterial and antiviral activities. Research is carried out on the antibacterial and antiviral nature of graphene to know the entire property and nature of graphene and its usage in face masks. Research on the potential impact of the atom thin edges found in graphene bombards the virus cells with the usage of accelerated electrons which is considered a weapon for dealing with a dangerous virus. Research on the negative impact of inhaling microscopic graphene which may lead to lung cancer under study. Great theoretical risk is seen in many publications on graphene face risks but experimental confirmation is lacking.

There are many significant properties found in graphene they are.

1. it can be prepared quickly.
2. it is renewable and reused.

Fig. 5. Usage of Graphene in Textile industry.

3. it has the substantial catalytic property.
4. it has a significant physical and chemical property.
5. it has unimaginable mechanical strength and surface area.
6. it can be obtained at a cheaper cost.
7. it is an abundant material.

5.6. Advantages and disadvantages of using Graphene enacted face masks

Graphene is a thin but conductive two-dimensional sheet of carbon atoms. The mobile electrons can electrostatically trap and inactivate the virus. Microscopic graphene particles possess sharp edges that mechanically damage viruses. Researchers are reporting the negative impacts of graphene face masks. Inhalation of graphene poses a risk to lung tissues. Earlier research results indicate that the graphene nanomaterial aerosols inhaled into the human respiratory tract could easily penetrate the head airways and then transit down to the lower lung airways [34].

6. Conclusion

Graphene and GRM-based material serve as a significant one for healthcare application, and it is significantly considered the primary material in the fields of technology and science [35–39]. Graphene is regarded as an excellent antiviral material used for combating the virus, disease, and infection spread. This present review deals with Graphene and GRM-based material for fighting against virus spread. They act as a dominant material with antiviral coating in the facemask, and it acts as an excellent material for detecting and combating the spread of viruses including the present COVID-19 pandemic. It is a nanomaterial with 1000 s of advantage in it. WHO suggested that graphene facemask can be used by frontline workers, which will surely help combat the deadly virus. The graphene facemask will minimize the risk of transmission of the virus from a COVID-affected patient to a normal person.

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.

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