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Graphene-based nanomaterials as antimicrobial surface coatings: A parallel approach to restrain the expansion of COVID-19

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ABSTRACT

The recently emerged severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) has become a significant and topmost global health challenge of today. SARS-CoV-2 can propagate through several direct or indirect means resulting in its exponential spread in short times. Consequently, finding new research based real-world and feasible solutions to interrupt the spread of pathogenic microorganisms is indispensable. It has been established that this virus can survive on a variety of available surfaces ranging from a few hours to a few days, which has increased the risk of COVID-19 spread to large populations. Currently, available surface disinfectant chemicals provide only a temporary solution and are not recommended to be used in the long run due to their toxicity and irritation. Apart from the urgent development of vaccine and antiviral drugs, there is also a need to design and develop surface disinfectant antiviral coatings for long-term applications even for new variants. The unique physicochemical properties of graphene-based nanomaterials (GBNs) have been widely investigated for antimicrobial applications. However, the research work for their use in antimicrobial surface coatings is minimal. This perspective enlightens the scope of using GBNs as antimicrobial/antiviral surface coatings to reduce the spread of transmittable microorganisms, precisely, SARS-CoV-2. This study attempts to demonstrate the synergistic effect of GBNs and metallic nanoparticles (MNPs), for their potential antiviral applications in the development of surface disinfectant coatings. Some proposed mechanisms for the antiviral activity of the graphene family against SARS-CoV-2 has also been explained. It is anticipated that this study will potentially lead to new insights and future trends to develop a framework for further investigation on this research area of pivotal importance to minimize the transmission of current and any future viral outbreaks.

1. Introduction

Despite the tremendous amount of research in virology and the development of hundreds of vaccines, viruses are still the leading cause of infectious diseases in humans [1,2]. It has been found in previous studies that infectious viruses have significantly threatened human health globally and are mostly responsible for both acute and chronic infectious diseases. There has been a sustained multidirectional research switch towards developing and exploring activities in various directions to reduce the horrific and lethal attacks of this new pandemic, "Coronavirus Disease 2019″ (COVID-19). One of the most common transmission routes for SARS-CoV-2 is through various viral contaminated surfaces, which humans frequently expose in everyday life. Touching our mouth, nose, or eyes after touching a surface or object with a virus on it may lead to COVID-19. So, it is imperative to disinfect the surfaces by inactivating the virus immediately to stop its spread [3]. Although many cleaning and disinfectant solutions like 62–71% ethanol, 0.5% hydrogen peroxide, or 0.1 sodium hypochlorite are available in the market, these are temporary solutions and have many reported side effects [4]. Therefore, the remedial solutions in antiviral coatings with long-term durability with low toxicity should be considered [5].

As compared to conventional disinfection and killing mechanisms,

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nanotechnology is a better alternative solution to shrink the COVID-19 spread, particularly in healthcare facilities and public places [6,7]. Graphene-based nanomaterials (GBNs) can be used to develop efficient diagnostic devices, effective personal protective equipment (PPE), 3D printed medical and engineering components and surface protective coatings for SARS-CoV-2. Antiviral nano-coatings can be used to destabilize, disinfect and minimize the survival time on different surfaces [8–10]. From the quantitative analysis of published research articles using the SCOPUS database for searching specific keywords for graphene and antimicrobial activity, as shown in Fig. 1(B), it can be found that there is an incremental rise in this research area, especially in the last few years. From the year 2010, graphene compounds got more importance for investigating applications as antimicrobial agents, primarily focusing on bacteria and viruses [11].

Graphene and its derivatives both show a wide range of antimicrobial activity including various types of viruses [9,12–14]. Among these materials, graphene oxide (GO), with the highest negative charge, show a higher affinity for positively charged viruses. It has been investigated that lipid bilayer of feline coronavirus can be adsorbed on GO and reduced GO (rGO) surfaces by electrostatic interactions and hydrogen bonding [15]. Later on, this binding destroyed the viral membrane, thus confirming the GO efficacy against viruses [12,16]. The negatively charged antiviral materials such as heparin, drugs, and heparan sulfate have also been used to modify the GO surface by conjugation [17], which resulted into the increased affinity of GO with positively charged residues of the viruses [18]. Similarly, modified rGO with sulfate derivatives can wipe out herpes virus strains and orthopox virus [19].

Zen Graphene Solutions Ltd. (dealing with graphene applications) has proposed a composite ink using GO and Ag nanoparticles, as an effective virucide that can eradicate the earlier strains of coronavirus by its application on N95 masks and other fabrics for frontline workers [20]. A company known as “G6 Materials” is working on development of air filtration system by incorporating graphene that can be used in offices and warehouses to kill viruses [21]. Another technology using self-sterilized laser-induced graphene (LIG) has been developed for water filters that eliminate viruses and bacteria in water and the same can be employed on masks and heating or air-conditioning system for air filtration to reduce the risk of COVID-19 [22].

A study conducted by Hanaa et al. [23] and Kim et al. [24] suggested that GO can target the mRNA of the hepatitis C virus (HCV) to block the replication of HCV gene. Both GO, and rGO show antiviral properties due to their distinctive single-layer configuration, sharp edges and negatively charged surfaces, whereas, graphite (Gt) and graphite oxide (GtO), show negligible antiviral activity. So, it can be concluded that the presence of various surface functional groups, nanosheet structure and sharp edges all are important features to exhibit antiviral properties [23,25]. It is an important research area and the pushing requirement in the current scenario to explore and design efficient antiviral coatings that can disrupt and impede the transmission of these infectious viruses [5].

Innocenzi et al. [26] suggested in his perspective that carbon-based materials along with polyphenol and Ag-NPs composites show good antiviral properties based on single oxygen emission and virus capture mechanism, in which GO interacts with, heparan sulfate of virus and destroy its envelope. Zhong et al. [27] reported using few-layer graphene in face masks coating due to its superhydrophobic properties, which inhibit the adhering of virus-containing aerosols. However, it needs laser treatment and sunlight to make it reusable. Other researchers have explored the use of GBNs in nano-vaccines as drug delivery agents, biosensors for diagnostic purposes and antiviral nanocomposites [8,26–31]. Srivastava et al. [27] proposed many graphene family applications in the current pandemic, including graphene-based composites for 3D printing masks and as surface coating materials. Some authors have proposed few mechanisms to demonstrate the extraordinary antimicrobial properties of GBNs [9,16,26,32].

Material safety data do not allow the use of virucidal chemicals as disinfectant sprays due to their toxicity and other adverse effects. It may cause health issues for the skin and lungs depending upon concentration and duration of exposure [33]. The most popular disinfectants used for cleaning and fogging the surfaces are chemicals like sodium hypochlorite and hypochlorous acid, which show corrosive nature for various metallic surfaces and residue is also left behind the surface. Continuous use of these chemicals causes mild to severe irritation to the skin, throat and eyes and are not acceptable for hands cleaning or fogging in the long run [34–36].

This perspective mainly focuses on the use of GBNs for antiviral surface coatings at high-risk areas in hospitals, schools, and public places where the risk of spread is very high. After reviewing the previously proposed mechanisms for antibacterial properties of GBNs, a novel surface coating has been proposed in the current perspective using GBNs, metallic nanoparticles (MNPs) and some reported non-ionic polymeric materials. The synergistic antimicrobial effect of these materials can be applied to develop robust antiviral coatings that can be applied in a spray or paint on many surfaces. Previously, the role of GBNs in antiviral surface coatings with various proposed mechanisms and future trends has not been reviewed as an independent topic.

Although vaccines against infectious diseases are estimated to have saved at least 23 million lives between 2011 and 2020 but it still needs much more research to fight for new mutagenic and emerging zoonotic infectious diseases [37]. For the COVID-19, several vaccines have also been launched using global resources. Among these, Pfizer-BioNTech by Pfizer, Inc., and BioNTech, Sputnik-V by Gamaleya Research Institute Russia, Moderna by Moderna TX, Inc; Johnson & Johnson’s vaccine by

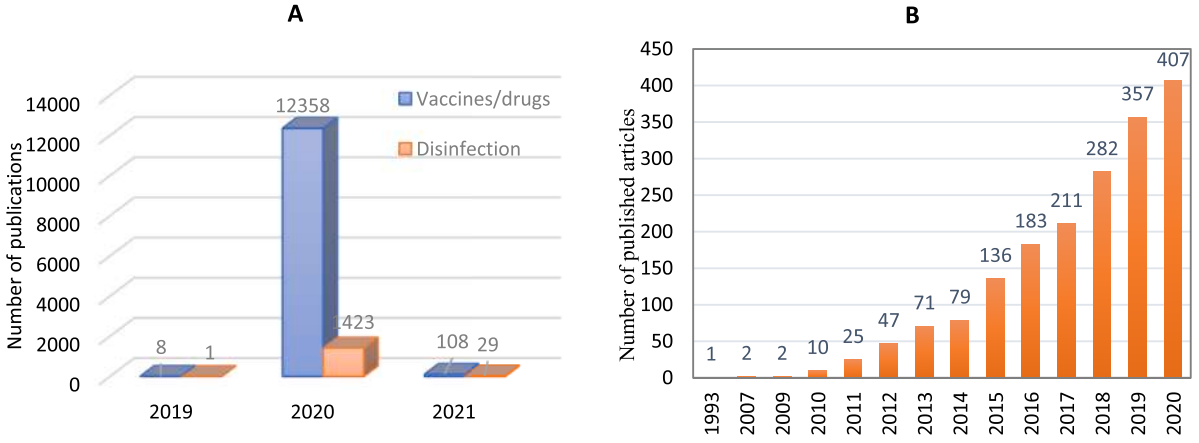

Fig. 1. (A) Comparison of research work on SARS-CoV-2 for vaccines/drugs and surface disinfection/killing procedures [60,61] (B) Number of research articles for antimicrobial activity of graphene [11].

Janssen Pharmaceuticals Companies of Johnson & Johnson and Oxford AstraZeneca Vaccine developed by AstraZeneca and University of Oxford are the major vaccines which have been used globally [38–40]. However, even after a successful launch of these vaccines still these are not 100% effective and breakthrough infections can be expected [38]. In future no one knows when and where the next pandemic may emerge, so to cope with such types of dilemmas, necessary protocols and pre-planning are necessary and for that purpose, the scientific community should work on developing such virucidal surface coatings.

Some global facts and data of SARS CoV-1, Middle East Respiratory Syndrome Coronavirus (MERS-CoV), and current SARS CoV-2 have been compared in Table 1, which shows many common elements.

It has been proved now that this virus infects the human host cells through the angiotensin-converting enzyme-2 (ACE-2), resulting in COVID-19 disease [57]. The current SARS-CoV-2 shows stronger transmission capacity than the previous SARS virus, but its pathogenicity is similar to MERS-COV [58]. The infectious disease caused by SARS-CoV-2 virus is not only a severe threat for humans with high-risk factors, but it also crippled the world economy of many countries, thus generating a dramatic economic impact for overall interruption of economic activities [57,59].

Table 1
Epidemiological characteristics of three coronavirus diseases.

Disease typeParameters	Severe acute respiratory syndrome (SARS CoV-1)	Middle East respiratory syndrome (MERS)	Severe acute respiratory syndrome 2 (SARS CoV-2)	References
Reporting year	2002	2012	2019	[41–43]
Origin country	China	Saudi Arabia	China	[41,44, 45]
Transmission and source	human to human	human to human infected camel to human	human to human	[42,46, 47]
Transmission through	respiratory secretions	respiratory secretions	respiratory secretions virus contaminated surface touch	[42,45, 48]
Total countries affected	32	27	217 ↑	[30,49, 50]
Confirmed cases	8098	2494, 2519	54,075,995 ↑	[45,46, 51]
Fatality ratio	14–15%	34.4% - 35%	3.4 ↑	[44,50, 52]
Total deaths reported	813	858	1231,017 ↑	[46,50, 51]
Symptoms	fever (>38 °C), chills and rigors headache, malaise, and muscle pain	fever, cough, and shortness of breath, septic shock, Pneumonia, multiple organ failure, or asymptomatic	Fever, dry cough, tiredness, difficulty breathing, chest pain, sore throat, loss of taste and smell, or asymptomatic	[42,46,47, 53]
Prevention	isolation, tracing, quarantine 10 days, screening, disinfection of aircraft and cruise vessels	avoiding contact with camels and sick persons	frequent hand wash, social distance, avoid touching surfaces and face, cover mouth and nose, stay home, quarantine 14 days	[42,46,47, 54]
Treatment (Vaccines)	>200 under development Still no vaccine in market	[40,41,55,56]		

This study aims to highlight the use of nanomaterials, specifically GBNs as surface coatings, disinfectants, or alternative sanitizing materials to prevent the spread of microbial infections, mainly from SARS-Cov-2. It also maps possible solutions using variously studied and potential GBNs based hybrid organic surface coatings to destabilize and inactivate the coronavirus's receptors. The current study will help to design safe and human-friendly virus resisting coatings to counteract the transmission of the COVID-19 and other infectious diseases and contribute to preventing any future viral outbursts. It will undoubtedly enable the readers to weigh up such alternative solutions that are yet to be disclosed. This perspective is an effort to draw the attention of researchers, virologists, chemists, and overall scientific bodies toward developing GBNs antimicrobial surface coatings on practical grounds to ease the remediation of current and any future viral diseases and to augment this innovative approach.

Since the evolution of SARS-CoV-2, in December 2019, there was a flood of publications on hot topics related to safety measures, vaccines and other impacts on society, but very few papers have been found in the literature for the work done on disinfection and killing mechanisms of the virus on various surfaces as shown in Fig. 1(A) [60,61].

2. Propagation and stability of COVID-19 on various surfaces

All viruses, including COVID-19, need a living host for replication. It can stay on most surfaces and enter the host body on contact with these surfaces, which is an alarming situation for frequently touched surfaces to increase COVID-19 spread. The work done by Kampf et al. [62] indicates that this virus can stay for up to 9 days at temperatures above 30 °C on some surfaces. The new research explained the variable survival time of SARS-CoV-2 on various plastic, metals, and wood surfaces. Data from different sources have been compared in Fig. 2, showing the virus's stability on different surfaces using almost similar experimental conditions like temperature and concentration and exposure time as reported by few authors [3,62,63].

It has been suggested that various surfaces in hospitals and public places should be adequately disinfected so that the virus cannot stay activated for more extended periods. Researchers concluded through laser beam guidance experiments that a sneeze could produce almost 100,000 particles in an experiment at Toho University in Japan. These particles can be a potential carrier of thousands of viruses that can be categorized into large vs small droplets or sometimes called droplets vs aerosols [64]. It was observed that lighter aerosols might be airborne for a few hours whereas heavy droplets can fall on the various available surface causing the spread of infection. The pieces of evidence concluded that the disease had been worsened to a pandemic due to the high infection rate occurring via 'airborne' aerosols [54,65].

Viruses are more stable on plastic and stainless steel (SS) than on cardboard and copper and the viable virus was detected up to 72 h when exposed to these surfaces. However, Goel et al. [66] has shown that virus is stable in the airborne for up to 3 h but stability increases up to 7 days on surgical masks or stainless-steel surfaces and for up to 4 days on smooth surfaces such as glass, plastic or currency notes [67]. The shortest survival time is observed on copper, where the half-life of the virus is 46 min as compared to plastic, where its half-life is 6 h and 49 min [68]. The virus activity is also dependent upon the environmental conditions and surface temperature. Generally, it has been noted that it is more active at lower temperature and colder surfaces as compared to high temperature and hotter surfaces [69].

3. Antiviral features of graphene derivatives, GO and rGO

Graphene oxide (GO) is the oxidized derivative of a graphene molecule, obtained by acid oxidation of graphite, i.e., it contains oxygen functional groups (hydroxyl, carboxyl, carbonyl, and epoxy) [70,71].

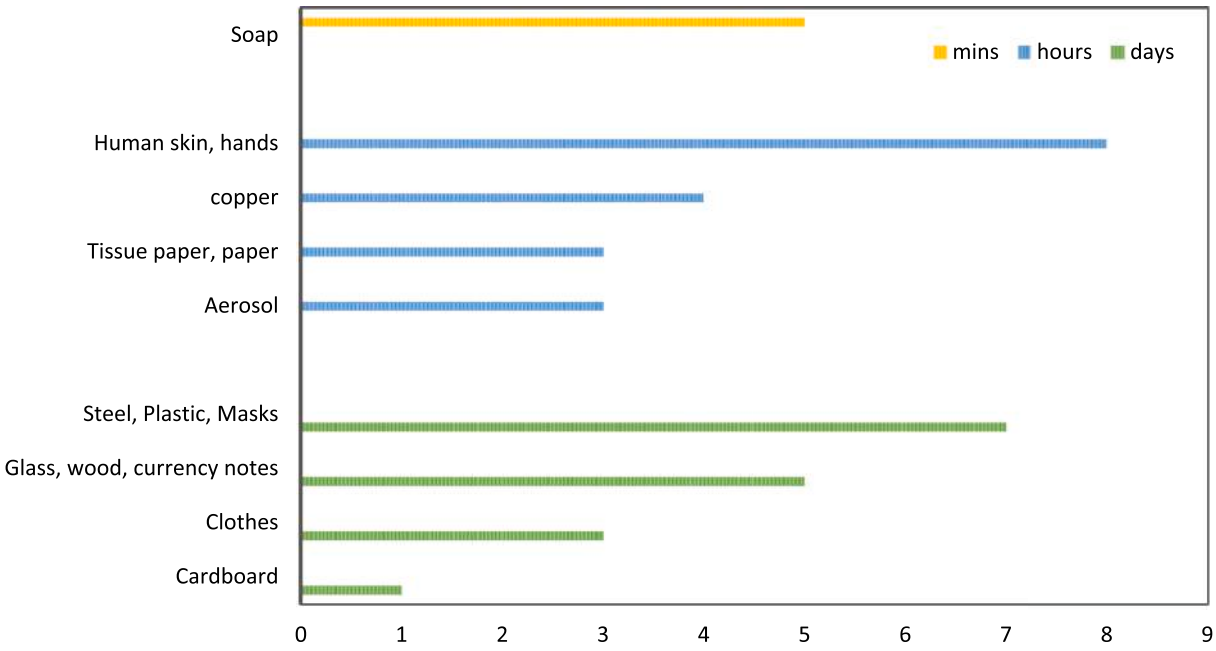

Fig. 2. Comparison of the stability of SARS CoV-2 on various surfaces at ambient temperature.

The reduction of GO through the thermal process produces rGO, with lesser oxygen content due to reduced number of functional groups but higher number of electroactive sites as compared to GO [72]. Graphene derivatives showing Unique physico-chemical properties of graphene derivatives (GO and rGO), which are effective for antiviral applications are summarized below:

(i) Lateral Size: The adsorption, dispersion, and sharp edges are hugely affected by the particle size; these properties are, in turn, pivotal in microorganism's physicochemical interaction [73]. The larger the lateral size, the stronger the adsorption ability, which is attributed to the higher surface energies. An investigative study stated that antimicrobial effects were stronger in association with larger-sized GO sheets than with smaller sheets [74]. For example, GO sheet size was found to influence its antimicrobial activity in suspension due to the capacity of larger sheets to completely wrap around the cells and isolate them from their environment [75]. Significant differences were found in the antiviral activities among GO and rGO, which may be attributed to their different nanosheet structures as shown in Fig. 3 (a-b). Mostly GO appears to bigger sized sheets as compared to rGO but it also depends on adopted synthetic method and post treatment.

(ii) Number of Layers: Increase of number of graphene layers increase the thickness, causing a weakened "nano knife" effect, decreased dispersibility, and increased aggregation tendency, resulting in reduced contact between GBNs and microorganisms [74,77]. Both the edges and surface of graphene derivatives play key roles in antimicrobial activity [28,74].

(iii) Cytotoxicity due to negatively charged groups: GO and rGO both contain groups of negative charge elements which are responsible for their antiviral characteristics. However, GO has the highest negative charge, which can have a higher affinity for positively charged viruses [32]. Subsequently, the binding of these graphene-derived materials with viruses can destroy the viral membrane. These groups include mainly hydroxyl, carbonyl and carboxyl, which result into redox reactions between the graphene oxide layer and the viruses due to the physico-chemical process which act as an important parameter in destroying the viruses. It should be noted that GO and rGO have an intrinsic ability to adsorb charged lipids and destroy membranes after association with their aromatic plane [10]. This property has been used to explain GO activity against feline corona- virus [16]. It has been reported that lipid bilayer of feline coronavirus is adsorbed on the surface of GO and rGO through hydrogen bonding and electrostatic interactions. These electrostatic interaction between the

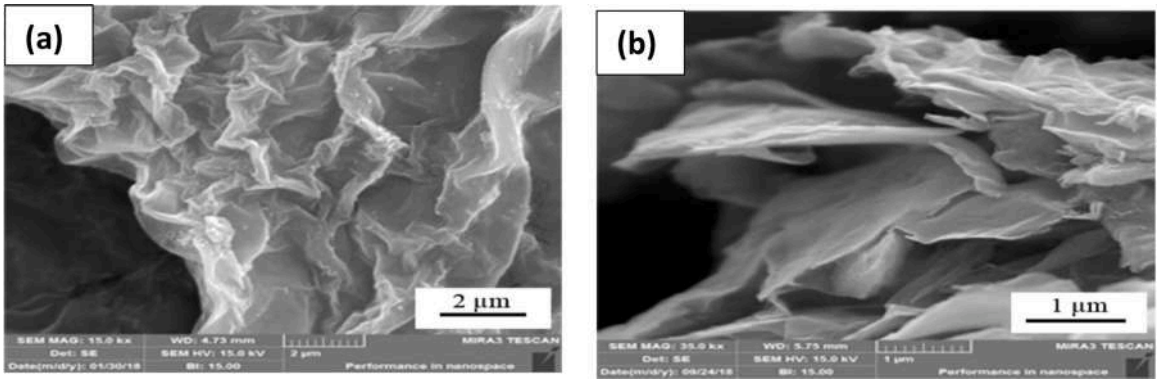

Fig. 3. FESEM images of GO nanosheets (a) and RGO nanosheets (b) [76].

negatively charged groups of GO and the positively charged virus particles blocks viral bindings [78]. Ye et al. [79] reported the antimicrobial and antiviral activity of graphene and its derivatives and its cytotoxicity. Furthermore, the charge density also play a crucial role in the binding and destruction of viruses as it has been demonstrated by Matias Sametband et al. that GO and rGO−SO3 blocked HSV-1 infections, and they believe that the charge density is probably the dominant factor affecting the inhibition properties, and GO has higher charge density as compared to rGO however GO and rGO−SO3 have roughly the same charge density [80].

(iv) Particles Shape (sharp edges): An interesting finding demonstrated that the sharp edges of GO nanowalls (GONWs) and rGO nanowalls (rGNWs) significantly decreased the rate of survival of both bacteria and viruses [81]. Experimental evidences show that the sharp edges of GONWs and rGNWs inactivate the virus before it enters the cells and these sharp edges of graphene layers inactivate the attack of viruses through direct association with outer layer leading to destruction of Viral Morphology and viral function [32]. These sharp edges are actually irregular nano-walls of GO and rGO layers, which may also develop due to the presence of dangling bonds, representing at least two times greater reactivity in comparison with the basal plane [78,82].

(v) Surface Modifications/surface functionalization: Modification of graphene's surface or edge characteristics via covalent and non-covalent modulation has been found to play a key role in preventing particle agglomeration and, consequently, influencing their antimicrobial activities [82,83]. It has been found that graphene-based surfaces both GO and rGO can improve the antiviral surfaces and coatings due to their antifouling properties for microorganisms including corona virus when used with different functional materials (i.e. silver nanoparticles (Ag NPs), metal oxides and polymers) [78]. Similarly, rGO modified with sulfate derivatives effectively ex- terminate herpesvirus strains, swine fever, and orthopoxvirus [12,19]. Functionalized graphene materials can prevent cellular viral infections based on the interaction between negatively charged functional groups which imitate the cell surface heparan sulfate [19]. It has been reported that the flake sizes of around 300 nm along with around 10% functionalization could provide the optimum performance against viruses [18].

(vi) Agglomeration and Dispersion: Agglomeration weakens the dispersibility and adsorption capacity, which alters blade efficacy and consequently reduces their interaction with the microorganisms [74]. However, these properties differ among the different forms of graphene, with GO dispersion exhibiting the most potent antimicrobial activity followed by rGO, graphite (Gt), and graphite oxide, successively [84]. The proper dispersion of GO results in thin sheets of this nanomaterial which is capable of easily wrapping the viruses and bacteria, whereas rGO exhibits aggregate formation and reduced impact when it is not fully exfoliated [74].

4. Graphene and MNPs as antimicrobial agents in surface coatings

Essential interventions to block the spreading of the pathogens through various means include proper diagnosis coverage, digital contact tracing, disinfection, sanitization, social distancing, and population immunization against the pathogen. Research on nanomaterials to explore their antiviral activities against viruses by different mechanisms makes them attractive for viral treatments [55]. Nanomaterial has shown antimicrobial properties due to their unique physical and chemical properties, which can be further customized to provide solutions in the current pandemic. These advanced materials can play a crucial role in multiple directions to defeat the pandemic [28,85]. For example, nanosystems with antiviral activity can work on binding and destruction mechanisms directly by surface contact or on receiving any external stimulus [31].

While focussing the current pandemic, it has been found that contaminated surfaces are the second main cause for the virus spread [45]. So, sterilization of frequently used surfaces like doorknobs, elevator buttons, handrails in the public area and hospitals should be done to block the transmission. Currently employed surface disinfectant chemicals like sodium hypochlorite (bleach), hydrogen peroxide or alcohol are temporary measures until the next exposure to the virus and also poisonous and irritants when overused for a prolonged time [36,86]. Novel surface coatings can be formulated and designed by using GBNs, MNPs and some sulfonated polymers to overcome the current challenge. These coatings offer long-term antimicrobial activity against germs when applied or sprayed on frequently touched surfaces [4,13,31]. Based on the graphene family's unique physical and chemical aspects and high surface area, their antimicrobial ability has also been extended to viruses [87,88]. The antimicrobial mechanism of graphene materials is still debatable among the scientific community; however, few possibilities have been explained based on some experimental evidence [89–91].

Three main mechanisms have been proposed for the working of antimicrobial surfaces, which include (i) anti-biofouling mechanism, (ii) release killing mechanism, and (iii) contact killing mechanism, as shown in Fig. 4. Anti-biofouling mechanism repels microbes and prevents them from adhering to the surface which is due to customized super-hydrophobic properties in case of GBNs. In release killing mechanism, antimicrobial agents and reactive oxygen species are produced near the surface which causes cytoplasmic efflux, decreases metabolism, affects lipid membrane, induces oxidative stress and finally causes microbial death. In the contact killing mechanism, sharp edges or nano-knives of GBNs due to their single or few layer structure play their role in the near-surface to kill the incoming microbes and instantly damaging the microbial agents if adhered to the surface [25,66,92].

It has been reported that the antimicrobial action of copper nanoparticles is due to their oxidative behavior and the solubility properties of its oxides [93]. It was observed by Sametband et al. [80] that even small concentrations of partially reduced sulfonated GO (rGO-SO3) and GO could deactivate herpes simplex virus (HSV-1) infection. Some authors have analyzed the interaction between bacteria and GBNs containing surfaces [94–97]. Similarly, graphene-WO_2 composite thin film has also been applied in the photo-inhibition of viruses [98]. Another study performed by Hu et al. [99] investigated GO functioned aptamer, a new photocatalyst that destructed the virus proteins capsid and nucleic acids under irradiation. Furthermore, GO, and copper composite materials can be used effectively for the surface disinfection and inactivation of viruses to prevent further propagation [66]. Few graphene-based composite coatings showing antimicrobial activity have been reported by deposition on titanium, stainless steel (SS) [81] and copper (Cu) [100]. The graphene's biocidal activity varies with the surface as for SS, it is 95% and reduced to 34% when Cu is used [101]. From the discussion above, it has become clear that GBNs and some other MNPs find great potential in antiviral applications in various fields, mainly due to the availability of more surface-active sites, excellent chemical stability and conductivity [102,103]. These magnificent features allow these materials to be applied in many other antimicrobial fields such as water disinfection, wound disinfection, dental care, and treatment.

5. GBNs based antimicrobial surface coatings

Keeping in view the discussion in Section 3 about the stability of the coronavirus on various surfaces, it becomes imperative to work on long-lasting antiviral surface coatings that could minimize the spread of SARS-CoV-2. Developing surface protective coatings with excellent antiviral properties for diverse surfaces can help to break the chain of COVID-19. Graphene-based surface coatings with proven antimicrobial

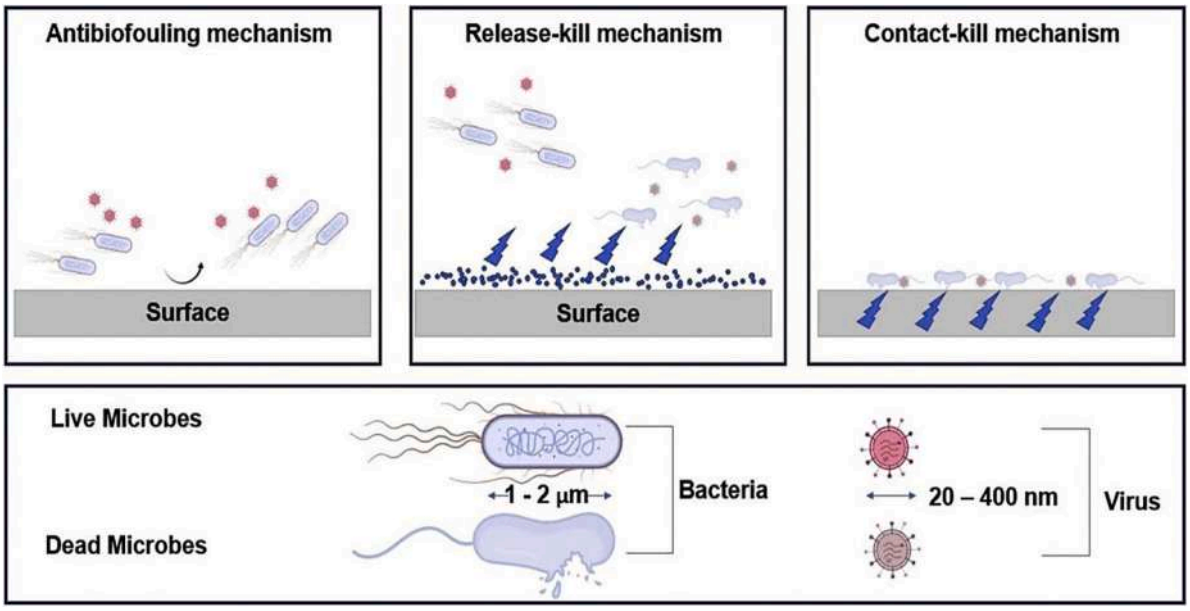

Fig. 4. Three principal mechanisms of antimicrobial materials on various surfaces [66].

metals can play a vital role to inactivate or reduce the survival time of SARS-CoV-2 on many surfaces. Some reported composites include single-layer graphene (SLG)/multilayer graphene (MLG)-copper composite, SLG/MLG-silver composite, GO-copper composite, GO-silver composite and many other combinations [16,104,105]. High-performance coatings can be developed by varying the concentration of metal nanoparticles to effectively destabilize and minimize the survival time of SARS-CoV-2 on high contact surfaces, i.e., medical and surgical equipment, PPEs and hospital door handles, etc. [8,106].

Xu et al. [107] used poly m-aminophenol (PmAP)-deposited graphene oxide (GO) coatings, fabricated on metal surfaces which work both for microbial and corrosion prevention. GO, and its derivatives have been extensively studied for their antimicrobial properties based on their higher surface area and unique physical and chemical properties. Its unique characteristics, like single-layer structure and negative surface charge, are mainly responsible for antiviral effects [29,108,109]. From the previous study, it has been found that the graphene family shows significant antiviral activity for a broad range of viruses belonging to different virus families, as shown in Table 2. From this data, it can be found that graphene can be used as independent material or in composite form using MNPs and some selected polymers for antiviral applications.

Issues of poor solubility and processing of GO hinder the fabrication of antimicrobial coatings because of strong interplanar interactions. To alleviate this problem, polymer matrices like polyethylene glycol or amine containing molecules are incorporated in graphene for surface modifications [25,123]. Other metallic nanomaterials such as Au, Ag, Cu, Pd, Pt,Ni, TiO_2, ZnO, MnO2, Fe_3O_4, Co_3O_4, carbon radicals (•C), and quantum dots are also reinforced into graphene-based compounds to improve functionalization and synergistic antimicrobial effects [124–126].

A study conducted by Ye et al. [29] uncovered the role of ionic character of GO for its antiviral action against two RNA viruses namely, PEDV (porcine epidemic diarrhea virus, a member of corona family) and PRV (pseudorabies virus). Polymer composites of GO with poly (diallyldimethylammonium chloride) (GO-PDDA), and polyvinylpyrrolidone (GO-PVP) have been investigated for antiviral activity. It was observed that GO-PVP shows antiviral activity due to nonionic nature of PVP, whereas cationic PDDA shows no antiviral activity. It has been proved through this study that GO shows antiviral properties against both RNA and DNA viruses which were later confirmed by Western blot analysis, plaque formation and indirect immunofluorescence assays. As shown in Fig. 5 (A & B) that in the absence of GO, the cell cultures show typical cytopathic effects whereas on GO treated cultures, the plaque formation unit (pfu) has been drastically reduced from 5×10^7 pfu/mL to 2.5×10^5 pfu/mL which also confirmed the antiviral nature of GO [29]. It was also concluded that these materials' broad-spectrum antiviral activity also depends on their concentration and time of exposure. Based on the current discussion, it can be summarized that both GO and rGO practice almost same antiviral mechanism, which may be credited to their negative surface charge and nanosheet structure [26,31]. The mechanism of cell membrane rupture through charge transfer has been explained well by Li et al. [127] as shown in Fig. 5(C). Membrane destruction of bacterial cells can be observed for cells seeded onto graphene-Cu and graphene-Ge except for graphene-SiO_2, where no antibacterial activity was seen, which shows

Table 2
Comparison of antiviral activity of the graphene family members against a broad range of viruses.

Virus family	Target Virus name	Nucleic acid	Viral envelope	GBNs used	Ref
Coronaviridae	PEDV	RNA	Enveloped	GO-PVP, C-dots	[29,110]
Coronaviridae	FCoV	RNA	Enveloped	GO-AgNPs	[16]
Leviviridae	MS2 bacteriophage	RNA	Non- Enveloped	GO, fullerene	[111–114]
Birnaviridae	IBDV	RNA	Non- Enveloped	GO-AgNPs	[16]
Arteriviridae	PRRSV	RNA	Enveloped	Go-AgNPs, C-dots	[115,116]
Rhabdoviridae	VSV,	RNA	Enveloped	GO	[117]
Retroviridae	HIV,	RNA	Enveloped	Graphene, Fullerene	[118,119]
Reoviridae	NDRV,	RNA	Non- enveloped	GO/HY	[120]
Pneumoviridae	RSV	RNA	Enveloped	GO, Fullerene	[121,122]
Herpesviridae	SuHV-1 (PRV)	DNA	Enveloped	GO, C-dots	[29,115]

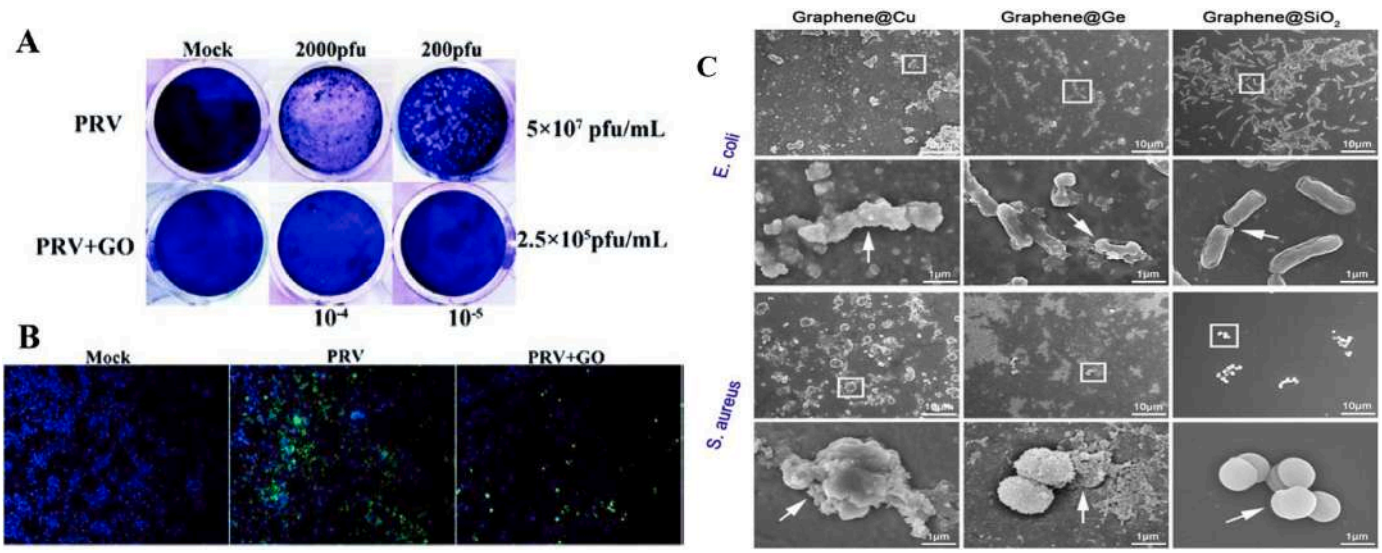

Fig. 5. (A) Anti-viral and antibacterial activity of GO (A) plaque-reduction assay for PRV infected cells with and without using GO (B) Indirect immunofluorescence assay of PRV infected cells in the presence and absence of GO [29], (C) SEM images showing the destruction of *E. coli* and *S. aureus* grown onto Cu, Ge and SiO_2 deposited graphene films [127]. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

that not all graphene composites can damage the bacteria.

Palestino et al. [31] studied the antiviral activity of the graphene family and found the order as: GO ≈ rGO > GO-PVP > Graphite Oxide (GtO). The authors have also established a linkage of nanosheet structure with the antiviral activity of these materials. It was found that graphite had no nanosheet structure, so it does not exhibit antiviral properties, whereas GtO, GO, and rGO show antiviral properties due to their nanosheet structures. Due to GO nanosheets, amphiphilic properties, partial hydrophobic surfaces and some hydrophilic spots show more interaction to the microorganisms. These properties can also be tuned using polymeric material and MNPs to get more chances for surface modification to design better bactericidal coatings [23,90,128].

It can be concluded that negatively charged layered GO having sharp edges can play a vital role in virus interaction followed by inactivation. There are electrostatic interactions that become effective, resulting in disruption of the outer protein layer of viruses releasing RNA and the same phenomenon works for antiviral applications in surface coatings.

6. Mechanism of action of GBNs against viruses

Various proposed mechanisms have explained the antimicrobial activity of GBNs. Mainly three mechanisms are extensively reported in the literature: production of reactive oxygen species (ROS), oxidative stress at the membrane, and phospholipids [84,129,130]. Some other reported mechanisms are the physical interaction of graphene's sharp edges with microbial membranes, photo-thermal ablation, and mechanical wrapping, leading to cell lysis [131,132]. There are three stages of antimicrobial action of graphene (i) deposition of virus onto the graphene material, (ii) membrane stress by direct contact with sharp nanosheets, and (iii) superoxide anion-independent oxidation [133]. Some researchers mentioned two main mechanisms for the germicidal nature of graphene sheets, i.e. physical disruption [81,134] and oxidative stress [135,136]. Previous studies have demonstrated that GBNs can penetrate the cell membrane for physical disruption and rupture [134,137]. The study conducted by Akhavan and Ghaderi [81] concluded that when GO nanosheets were coated on stainless steel, destruction of *E. coli or S. aureus* membranes occurred mainly due to the direct contact of the bacteria with extremely sharp edges of the nanowalls [137]. So, sharp edges play a significant role in the mechanical damage of bacteria cells as smooth surfaces cannot physically disrupt the cell membrane.

The oxidative stress mechanism generates ROS, which can damage the macromolecules like DNA and RNA, protein, and lipid bilayer of microorganisms. In the first step of the mechanism, virus envelope damage starts from the GO's interactions with the outer wall, which can lead to excessive ROS generation and virus inactivation [131]. Physicochemical features and alterations in GO sheets, e.g., sheet size and attached functional groups, may also have a diverse effect when employed on a surface. The bactericidal activity of GBNs has been criticized by Hegeb et al. due to unclarified effect of the physicochemical features like shape, size, hydrophilicity, roughness and functionality of these materials on their antibacterial activity, but the same can be justified based on variations in the physicochemical properties for individual samples [23].

Generally, two approaches have been investigated to understand the inactivation mechanism of enveloped viruses, which include physical interaction and incorporation of additional features. Physical interaction is based on the interaction between the outer S-protein layer of a virus and the surface of the nanosystem to inhibit the virus activity directly. The antiviral function of GBNs is dependent upon their physicochemical properties. On the other hand, the second approach focuses on developing additional features on nanosystems' surface to kill the virus completely. Synergised action of both methods can be sufficient to fight against SARS-CoV-2. The first approach is preferable as various functional groups can be introduced in graphene oxide, binding with these viruses and inactivating them. For binding and entering of viruses into the host cell, the virus must first interact with the host cell surface receptors. It has been reported by Song et al. [15] that GO was able to efficiently destroy the coating proteins available on corona viruses and extracting the viral RNA in an aqueous environment due to the presence of hydroxyls, epoxides, diols, ketones, or carboxylic functional groups located on its surface. Furthermore, viral envelope glycoproteins, heparan sulfate proteoglycans and chondroitin sulfate proteoglycans function as the cell surface receptors and the functionalized GBNs having negatively charged functional groups can mimic cell surface heparan sulfate [19]. Still, these efforts are mainly restricted to proof-of-concept scenarios; however, some limited reports proposed the application of nanosystems on surfaces, personal protection equipment, and water purification. Based on these mechanisms, it has been proposed that graphene-based nanomaterials can be effectively used for antiviral applications [26,31,138].

A schematic representation is given in Fig. 6(A) depicts various graphene derivatives' possible interactions with SARS-CoV-2.

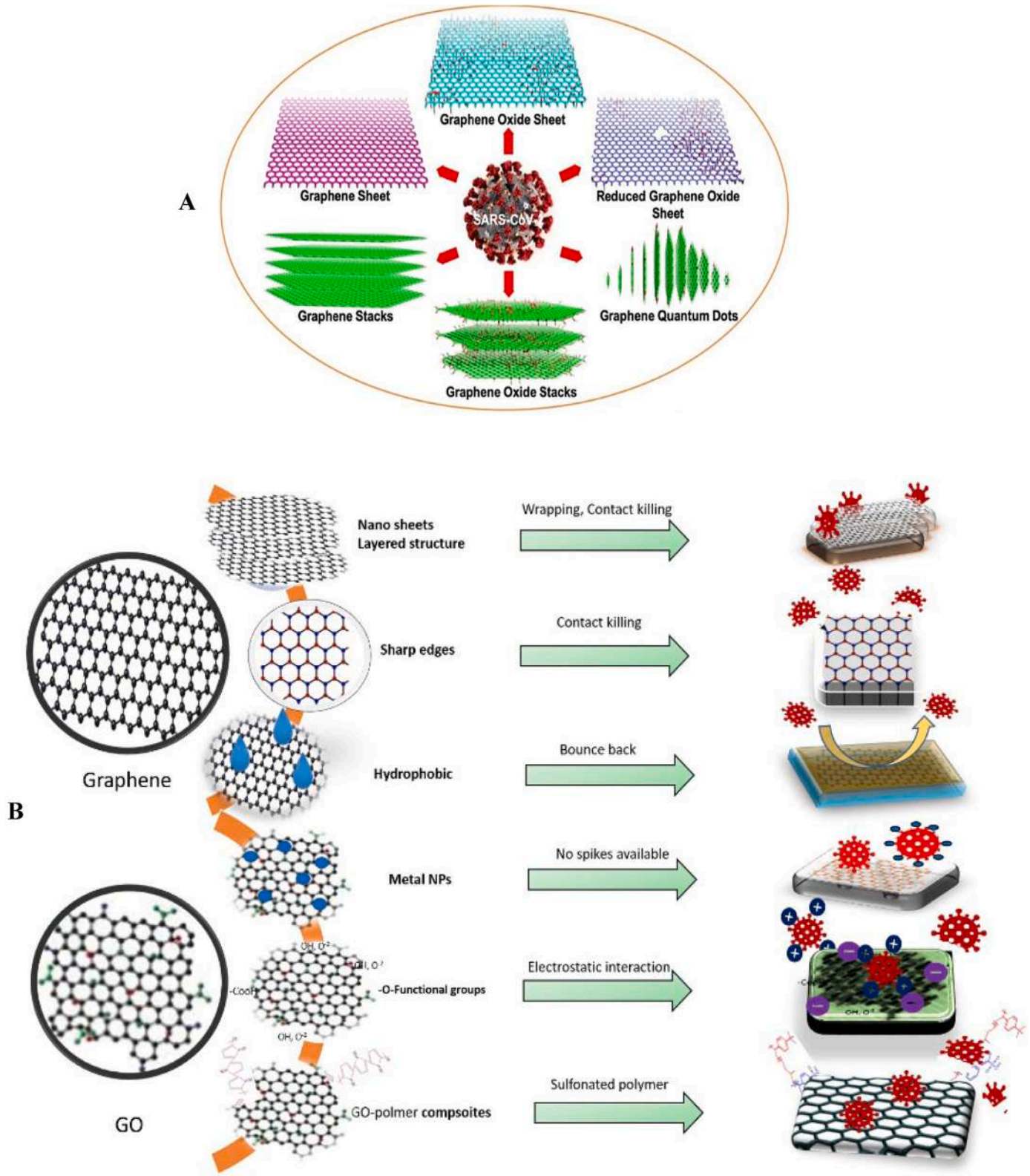

Fig. 6. (A) Various reported graphene derivatives for SARS-CoV-2 inactivation [12] (B) Schematic illustration showing antiviral properties and inactivation mechanisms of SARS-CoV-2 on GBNs coated surfaces.

Identification of interaction between GBNs and viruses can help develop versatile graphene-based products in nanoparticles, nano surface cleaner wipes, nano-spray, nano-drugs, PPEs, and air filters to encounter COVID-19 [12]. The electroconductive properties of graphene and its derivatives also play an essential role in their interaction with many biomolecules. Another molecular mechanism between the S-protein of SARS-CoV-2 and nanoelectromechanical materials such as graphene and graphene-derivatives can be proposed to block virus interaction with human Angiotensin-converting enzyme (ACE2) [12]. GBNs can be applied as antiviral surface coatings either themselves or in combination

with metal nanoparticles or polymers. The various mechanisms that have been reported or proposed in the literature include oxidative stress, contact killing, bounce back mechanism, electrostatic interactions, polymer contact killing, wrap killing, ionic interactions. All these mechanisms have been demonstrated in schematic form in Fig. 6(B), which shows the interaction and inactivation/killing of SARS-CoV-2 viruses on graphene-based coatings. These coatings can be applied on various surfaces like face masks, doorknobs, handrails, and elevator buttons etc. Such type of potential graphene-based virucidal approach can be proved as an economical and efficient remedy against COVID-19.

7. Current progress and way forward

Unfortunately, fully 100%, effective antiviral drug and vaccine is not available, and this situation has triggered the scientific community to sought out any alternative critical potential interventions to fight against COVID-19. According to a WHO's statistical surveillance report, following the prevention and control guidelines for using surface disinfectants, hand sanitizer and personal protective equipment (PPE) can result in a 30% reduction in healthcare-associated infections [86]. Smart and modern technologies, especially exploiting emerging nanotechnology, could help manage the current and future outbreaks. As already discussed, GBNs, particularly GO and rGO exhibit antimicrobial properties, but due to agglomeration problems, metal ions/oxides in the form of nanoparticles (NPs) have been incorporated to modify the surface properties and antimicrobial action.

A few studies have reported the antiviral activity of fully dispersed GO sheets and found that GBNs can prevent the entry of DNA virus (herpesvirus) and RNA virus (coronavirus) in their target cells [29,80,139]. Various inorganic MNPs (Ag, Au, Zn, Ge, Si, Cu) have been successfully used to enhance the antimicrobial effect of GBNs due to their photocatalytic properties and production of ROS [140]. It has been suggested that GO-based virucidal mist spray can be used to clean any object's surface and even for sanitization of the human body. The mist spray can be formulated as nasal or mouth spray to block the S-protein of SARS-CoV-2, especially at the entry points. Interestingly, graphene-coated surface cleaner wipes can better disinfect the infected area [12]. A company (GrapheneCA) has formulated a graphene-based antimicrobial coating that can be applied in the form of paints and varnishes to walls and surfaces of public use that are high-risk areas like shopping malls, metro stations, airports and hospitals. The coating will act to inactivate the outer surface of microorganisms on physical contact [141]. Nano-coatings using N9 blue silver and zinc based nano-complexes have been used for their synergistic effect as antimicrobial nano-coatings. These nano-coatings are found very convincing and can work on multiple levels simultaneously as antiviral, antibacterial, antifungal and self-cleaning [142–144]. A company named nanoSeptic introduced a nano touch product using mineral nanocrystals charged by any visible light and proved for the breakdown of organic contaminants, including coronavirus envelope using oxidation reaction [110]. Similarly, another Italian company, Nanotech Surface, presented some self-sterilizing and self-cleaning nanosheets using silver and titanium oxide, which can be used on buildings and other surfaces. The company has claimed that the surface remains effective against pathogenic organisms, including coronavirus, from 6 to 24 months [145].

The antimicrobial properties of graphene sheets depend upon the size, number of layers and oxygen functionality. Due to the presence of reactive surface groups, it can be customized for their antimicrobial activity by decorating with various nanometals and other organic and inorganic compounds having antimicrobial action [89]. It has been found that silver-NPs show antibacterial, antifungal, antiprotozoal, and antiviral activities [146]. These investigations show that GO-Ag-NPs can be used for the fabrication of coatings against SARS-CoV-2. GO sheets play a dual function, first as a supporting and stabilizing media to prevent the agglomeration of Ag-NPs and secondly enhancing the antimicrobial activity due to its inherent functional groups, negative charge and sharp edges [139]. Graphene derivatives with active MNPs such as Au and Ag can interact with cysteine-sensitive molecules active components of SARS-CoV-2 proteins. As graphene also shows excellent optical properties, based on this phenomenon, it is possible to fabricate graphene and MNPs coated masks and special clothes to detect and prevent virus by changing their color. This theory is presented based on recent research showing the sustainability of this virus in the air for more than three hours, and its aerosol particle has supported movement up to eight meters [55].

There are some limitations and challenges while using GBNs in antiviral applications. Nanotoxicity is a major limitation. Although toxicity of graphene is still a matter of debate however, a few researchers have already found the evidence of their toxic effects [147,148]. Immediate use of graphene for antiviral surface coatings looks difficult due to insufficient data and still more research is needed. Graphene based nano materials can cross biological barriers due to their nano size however these are less cytotoxic as compared to other carbon nanomaterials. There are also limitations related to costs and processing of such type of coatings at different surfaces. Furthermore problems associated with accuracy, repeatability, specificity, and sensitivity, can be faced due to the high genetic variability of several viruses [28]. After devising appropriate solutions for toxic concerns such as preparation of hybrid coatings with eco-friendly nanomaterials and MNPs can reduce their minor toxic effects. These materials can be produced in a cost-effective way using waste biomass to further reduce the processing costs [149,150]. GBNs can be combined with green technology using hybrid nanotechnologies for their synthesis, use, storage, recycle and environmental-friendly disposal [28]. Graphene materials possess superior mechanical, thermal and chemical properties and show high resistance to harsh environmental conditions [89,151]. These can be used to develop robust antiviral coatings that can be used at various environmental conditions without affecting their performance and life span. Further experimental work is required in this area that can smoothen the way towards the development of safe and cheaper antiviral coatings.

The investigation of the antiviral properties and utilization of carbon nanomaterials or GBNs is yet a new research field, and further study is required to evaluate the potential benefits in this area. However, carbon nanomaterials can be used to make antiviral systems having reduced toxicity and human friendly. A complete assessment of cytotoxicity is the main problem for allowing the development of antiviral applications for such type of materials. The current pandemic has already taught us some important lessons to consider in terms of future antiviral material development. Due to the evolution of unpredictable bacterial and viral infections, there is a dire need to highlight the future research areas for a precise assessment of the antimicrobial activity of the different forms of graphene-based materials for targeted applications. There is an urgent requirement to identify and develop possible treatments and approaches to block S-protein or exterminate the SARS-CoV-2 after its access to the human body. Current analysis suggested the possible use of GBNs to combat the spread of this disease through contaminated surfaces, specifically focussing the antiviral surface coatings.

8. Concluding remarks

Scientists and researchers worldwide are racing against the time to develop a highly effective and accessible vaccine or any antiviral medicine for the treatment of COVID-19. It has been noted that very recently, few vaccines showing good effectivity against SARS-CoV-2 have been disclosed by some companies. However, there are still many challenging issues such as scalability, manufacturing costs, accessibility, intellectual and regulatory property issues, cytotoxicity, and environmental impacts before these vaccines can be safely marketed. It has been shown in the current perspective that GBNs find great potential as antimicrobial agents against pathogens. Among these materials, GO, and rGO showed excellent antimicrobial activity in combination with other organic and

inorganic moieties. Virus-contaminated surfaces are the second major source of transmission. Hence, there is a strong demand for long-lasting and robust antiviral coatings that can be sprayed or painted on frequently used surfaces in hospitals, homes and public areas to restrain viral transmission. It has been proposed that GBNs and some activated MNPs can be used as long-lasting antiviral coatings to obstruct the spread of the current pandemic. These universal virucidal coatings can be applied for wide range of applications, not only against the coronavirus platforms but also against any other future viruses and microbial agents. Similarly, self-disinfecting and reusable PPEs can also be smartly designed using these advanced materials, which may include face masks, protective clothing, and gloves.

Further research in this field is required to understand and find more possible mechanisms of action of GBNs having antiviral characteristics. New nanomaterials can be explored for the development of multipurpose efficient disinfection systems that can be applied on permanent basis having self-reactivation mechanisms. It has been proposed that new flexible and reusable GO incorporated cotton fabrics can be designed as a new antimicrobial material for many medical-related applications. Similarly, for antiviral drugs and vaccines, nano-vaccines can be developed where graphene material act as inactive Virus-Like Particles (VLPs). Multifunctional antimicrobial GBNs can prove as ideal products for environmental pollution protection, wastewater treatment, food safety and any other application where control of bacteria, fungi, and viruses is required. So, it is the pressing need of time to identify the long-term and short-term approaches that can be fully employed to cope with the current and any future unforeseen outbreak. Multidirectional long-term actions are required before the emergence of any new biological threat in the form of a pandemic in future that can affect the people globally and make it challenging to create a bio-secure environment.

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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