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Carbon-based nanomaterials: Promising antiviral agents to combat COVID-19 in the microbial resistant era

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Abstract

Therapeutic options for the highly pathogenic human Severe Acute Respiratory Syndrome-Coronavirus 2 (SARS-CoV-2) causing the current pandemic Coronavirus disease (COVID-19) are urgently needed. COVID-19 is associated with viral pneumonia and acute respiratory distress syndrome causing significant morbidity and mortality. The proposed treatments for COVID-19, such as hydroxychloroquine, remdesivir and lopinavir/ritonavir, have shown little or no effect in the clinic. Additionally, bacterial and fungal pathogens contribute to the SARS-CoV-2 mediated pneumonia disease complex. The antibiotic resistance in pneumonia treatment is increasing at an alarming rate. Therefore, carbon-based nanomaterials (CBNs), such as fullerene, carbon dots, graphene, and their derivatives constitute a promising alternative due to their wide-spectrum antimicrobial activity, biocompatibility, biodegradability and capacity to induce tissue regeneration. Furthermore, the antimicrobial mode of action is mainly physical (e.g. membrane distortion), which is characterized by a low risk of antimicrobial resistance. In this review, we evaluated the literature on the antiviral activity and broad-spectrum antimicrobial properties of CBNs. CBNs had antiviral activity against 12 enveloped positive-sense single-stranded RNA viruses similar to SARS-CoV-2. CBNs with low or no toxicity to the humans are promising therapeutics against COVID-19 pneumonia complex with other viruses, bacteria and fungi, including those that are multidrug-resistant.

Keywords: COVID-19, SARS-CoV-2, carbon-based nanomaterials, antiviral properties, pneumonia

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1. Introduction

History has repeatedly manifested that pathogens cause disastrous effects on human beings. Thus, the recent outbreak of the novel Severe Acute Respiratory Syndrome-Coronavirus 2 (SARS-CoV-2), which causes Coronavirus disease 2019 (COVID-19), spread to more than 200 countries, is a clear example. The current confirmed global COVID-19 cases and deaths are 46,597,299 and 1,201,162, respectively as of November 2, 2020 [1]. Nevertheless, experts have suggested that many more undetected or uninformed cases exist [2], especially in developing countries. COVID-19 continues to spread globally, threatening to collapse the health system of many developed countries.

SARS-CoV-2 is an enveloped positive-sense, single-stranded RNA virus [3–5]. Its origin to this date remain enigmatic, however multiple hypotheses have been postulated thus far [6]. However, the host tropism/adaptation pattern raised questions concerning the origin of SARS-CoV-2 [7]. SARS-CoV-2 is the seventh coronavirus known to have infected humans and only the third one causing severe pneumonia [8,9], an infection of the lungs caused by bacteria, viruses or fungi [10,11]. Viral pneumonias may be complicated by secondary bacterial infections, with symptoms of bacterial pneumonia [12]. Thus, co-infection can be caused by viruses in the setting of community-acquired bacterial pneumonia [13–15]. Co-infection of COVID-19 patients is seen with the most common type of bacterial pneumonia caused by *Streptococcus pneumoniae*; which normally resides in the upper respiratory tract, and can prove to be fatal [16]. There is a great concern about the rapid spread of new pathogens, such as SARS-CoV-2 that can coexist with a broad range of other types of clinically relevant pathogens, including those which are multidrug-resistant. Therefore, the co-infection of SARS-CoV-2 with other viruses, bacteria, or fungi constitutes a real life-threatening to humans during the approaching cold season. In this regard, several drugs and treatments have been proposed which include remdesivir, hydroxychloroquine, lopinavir/ritonavir, interferon β-1a**,** tocilizumab, favipiravir, plitidepsin, convalescent plasma infusions and monoclonal antibodies, among many others [17]. However, presently, there is no effective treatment for COVID-19 [18,19]. Furthermore, antibiotic resistance in bacterial pneumonia treatment is a wide-spread problem [20–22]. Therefore, in the quest to finding therapeutics for COVID-19, carbon-based nanomaterials (CBNs) are emerging as promising options that have shown potent antiviral activity against enveloped positive-sense single-stranded RNA viruses [23], which are similar to SARS-CoV-2, and showed low to no toxicity in humans [24–29]. Besides, they exert an effective biocidal action against a broad spectrum of bacteria, viruses and fungi, including multidrug-resistant strains [30–32]. These CBNs are mainly composed of carbon, an essential element in the human body [33], are thus biodegradable, biocompatible and can induce tissue regeneration [34–38]. Moreover, the development of CBNs as new antiviral agents is possible because they possess a high surface area that allows its functionalization or interaction with biocompatible polymers which further enhance their biocompatibility and therapeutic efficacy. Research in this area is still in its early stage, though it is predicted to grow exponentially due to the grave consequences caused by the current pandemic. The increasing number of multidrug-resistant pathogens announced by the World Health Organization constitutes another real threat to humanity in this microbial resistance era [39]. Therefore, there is an urgent need to find new alternative antimicrobial strategies to curb the menace of drug-resistance, thus providing a long-lasting treatment for the COVID-19 disease. This review addresses the application of these broad-spectrum CBNs as antimicrobial agents by analyzing a large number of antiviral studies performed so far with CBNs against enveloped positive-sense single-stranded RNA viruses, looking at their toxicity and biodegradability, and deciphering how they could defeat microbial

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resistance. This novel vision could surpass substantially any technological paradigms that currently exist or are under development a part of advanced therapeutics for the treatment of COVID-19.

1. New generation of antimicrobials: carbon-based nanomaterials

To face the ever-increasing range of infections caused by multidrug-resistant microorganisms and viruses [39], the use of antibiotics or alternative antimicrobial agents such as metals in the form of ions or oxides [40], quaternary ammonium compounds [41], peptoids [42], α-peptides [43] and β-peptides [44] present diverse problems including drug resistance after long-term utilization. In this regard, alternative materials such as CBNs with intrinsic broad-spectrum antimicrobial activity [45,46,55,56,47–54] represent a promising option that would probably overcome the microbial resistance problem due to their specific antimicrobial mechanisms. Thus, the antimicrobial action of CBNs, such as graphene is often attributed to a combination of several physical and chemical mechanisms: membrane disruption, entrapment of microorganisms, transfer of electrons and the induction of oxidative stress by reactive oxygen species (ROS) [57,58]. CBNs are increasingly proposed as a new generation of antimicrobials against multidrug-resistant infections because they possess unique properties which includes very high surface area, excellent electrical and thermal conductivity, biocompatibility and the possibility to be combined with engineered polymers for the development of advanced antimicrobial biomaterial composites [30,59–64]. Furthermore, a recent study about the paramount concern of this proposal regarding to the interaction of CBNs with the respiratory system showed that a single exposure of several CBNs (at ~0.3 and 1 μg/cm^2) did not manifest any adverse effects under acute exposure scenarios after 24 h [65]. Regarding the biodegradability of CBNs, it has been demonstrated that the human myeloperoxidase, a peroxide enzyme released by neutrophils, degraded graphene and its derivatives [66,67] leading to biodegradation of graphene-based nanomaterials (GBNs) in the blood after 14 days [68]. Some signals of *in vivo* degradation of graphene were reported in the lungs, liver, kidneys and spleen upon 90 days [69]. Oxidized forms of GBNs have shown higher susceptibility to degradation than the reduced forms [70]. The degradation products of graphene oxide (GO) with different dimensions exhibited no genotoxicity to human lung cells [71]. *In vivo* studies have shown that a concentration of 1.0 mg/kg of small GO particles (148-160 nm) tends to accumulate mainly in the liver with a lower amount in the spleen and lungs, and remain longer in circulation than large ones (556-780 nm)[72], which were mostly present in lungs. However, when the concentration of injected GO, increased 10-folds, the smaller GO particles accumulated in the lungs instead of the liver thereby potentially increasing its efficacy for treatment of pulmonary infections. GO and reduced GO (rGO) of different lateral dimensions (10-800 nm) in a concentration of 1 mg/kg exhibited long blood circulation times of 14 days after intravenous administrated in mice, low uptake by the endothelial reticulum and no pathological variations were detected in the analyzed organs [64]. Biodegradation and cytotoxicity towards different cell lines depended on concentration, exposure time, oxidation degree, lateral size, and cell type [73–76] and could be modified by functionalization [73,77–80]. Smaller and more oxidized GBNs seem to be more cytocompatible than non-oxidized and larger particles [78]. Proteins, such as bovine serum albumin, adsorbed on the GBNs' surfaces, seem to have a protective effect on the hemolytic potential [81–84]. The biocompatibility of CBNs depends on their concentration, oxidation degree, lateral size and dispersibility [85–93]. The inflammation, and other effects on cells and blood components are minimal when a lower concentration of CBNs is applied. Oxidized and smaller GBNs are more

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biocompatible and accessible to biodegradation in the body. CBNs have shown the potential to support the growth, proliferation, and differentiation of stem cells into different tissue lineages [36,94]. These features potentiate the use of CBNs in combination with stem cell therapies for tissue regeneration.

2. Antiviral properties of carbon-based materials

In this section, we analyze the antiviral properties of CBNs with different carbon-based structures (Figure 1), such as fullerene, carbon dots, graphene, and derivatives against 12 enveloped positive-sense single-stranded RNA viruses, such as SARS-CoV-2.

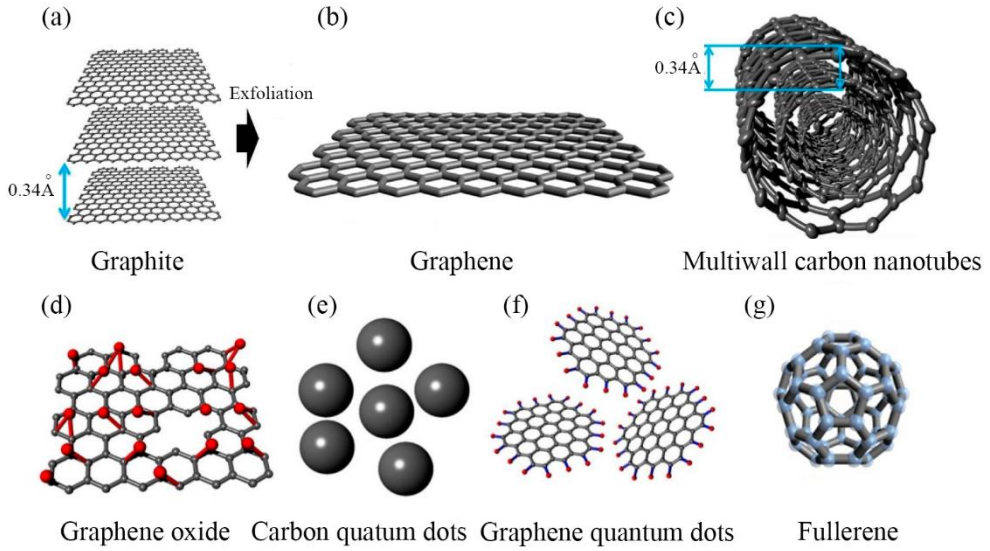

Figure 1. Main carbon-based structures studied against enveloped positive-sense single-stranded RNA viruses. *Adapted from Elsevier* [95] and MDPI [96].

2.1. Fullerene and derivatives

Fullerene is a zero-dimensional allotrope of CBNs with antiradical and, antioxidant properties [97,98]. Due to the high hydrophobicity character of pristine fullerene, antiviral fullerene derivatives can be synthesized to produce hydrophilic drugs that can be easily dispersed in aqueous media and can inhibit viral entry, modifying its morphology and functions, and blocking the viral replication [31]. Studies on fullerenes as antiviral agents started in 1993 on HIV-1 infections to model the interaction between derivatives of this CBNs and HIV-1 protease [99]. In that study, compound 1 showed effective *in vitro* antiviral activity. In 1997, nonderivatized fullerene (buckminsterfullerene) showed *in vitro* antiviral activity against another 2 enveloped positive-sense single-stranded RNA viruses similar to SARS-CoV-2: the Moloney murine leukemia virus (M-MuLV) and the simian immunodeficiency virus (SIV) [100]. However, a study performed in 2003 tested a series of fullerene derivatives (13 in total, compounds 1-13) against HIV-1 and HIV-2. The results showed that some of these CBNs (6 (*trans-2*), 7 (*trans-3*) 8 (*trans-4*), 9 (*equatorial*), 12 (*trans-2*)) exhibited potent antiviral activity against HIV-1 but not HIV-2 in the low micromolar concentration range [101]. Nonetheless, cationic fullerene derivatives showed broader anti-HIV properties [102]. In 2007, chlorofullerene was developed as a precursor for the straightforward synthesis of antiviral fullerene derivatives against HIV with high solubility in water [103]. A later

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study in 2011 showed that derivatives of C70-fullerene (i.e., the fullerene molecule consisting of 70 carbon atoms) exhibited high water-solubility and virucidal activity against HIV and influenza virus [27]. More recently, a new series of fullerene derivatives (Figure 2) have shown potential inhibition of hepatitis C virus (HCV) NS5B polymerase and HCV NS3/4 protease [104].

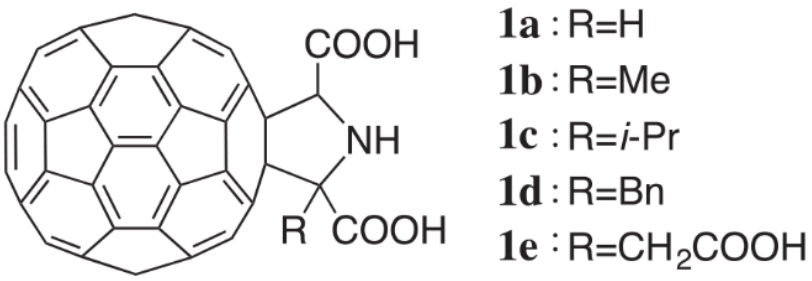

Figure 2. Chemical structure of fullerene derivatives 1a-1e. *Reprinted with permission from Elsevier* [104]

Recently, tridecafullerenes appended with up to 360 1,2- mannobiose showed outstanding antiviral activity against Zika virus (ZIKV) and Dengue virus (DENV)[25]. Examples of antiviral studies performed with fullerenes and their derivatives are summarized in Table 1.

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Table 1. Studies analyzing the antiviral properties of carbon-based nanomaterials (fullerene, carbon dots, graphene and derivatives) against 12 enveloped positive-sense single-stranded RNA viruses similar to SARS-CoV-2

Fullerene and derivatives	**Toxicity**	**Antiviral**	**Tested viruses**	**Tested cell line/inhibition**	**Year**	**Ref.**
Fullerene derivatives (Compound 1 and 2)	None (compound 1) Not tested (compound 2)	Yes	HIV-1	HIV-1 protease	1993	[99]
Nonderivatized fullerene (buckminsterfullerene)	Not tested	Yes	SIV and M-MuLV	MT-2 (for SIV) and M-MuLV reverse transcriptase inhibition	1997	[100]
Bis-functionalized fullerene derivatives bearing two or more solubilizing chains	High (derivatives 2-13, *in vitro*) Low (derivative 1, *in vitro*)	Yes (HIV-1) No (HIV-2)	HIV-1 and HIV-2	CEM	2003	[101]
Cationic fullerene derivatives	Low (*in vitro*)	Yes	HIV-1 and HIV-2	CEM	2005	[102]
Chlorofullerene	Low (*in vitro*)	Yes	HIV-1 and HIV-2	CEM	2007	[103]
Polycarboxylic derivatives of C70-fullerene	Low *(in vitro* and *in vivo*)	Yes	HIV-1 and HIV-2	CEM	2011	[27]
Fulllerene derivatives (1a, 1b, 1c, 1d, 1e)	Not tested None (1a, *in vitro*, reported in [26])	Yes	HCV	NS5B polymerase and HCV NS3/4A protease	2016	[104]
Tridecafullerenes appended with up to 360 1,2-mannobiosides	None (*in vitro*)	Yes	ZIKV and DENV	Jurkat	2019	[25]
Carbon dots and derivatives	**Toxicity**	**Antiviral**	**Tested viruses**	**Tested cell line/animal model/inhibition**	**Year**	**Ref.**
Carbon dots	Low (*in vitro*)	Yes	PRRSV	MARC-145	2016	[54]
Boronic acid-attributed carbon quantum dots	None (*in vitro*)	Yes	HIV-1	MT-4/HIV-1 and MOLT-4	2016	[52]
Functional carbon quantum dots	None (*in vitro*)	Yes	HCoV	Huh-7	2019	[23]
Benzoxazine monomer derived carbon dots	None (*in vitro*)	Yes	JEV, ZIKV and DENV	BHK-21 (for JEV) and Vero (for ZIKV and DENV)	2019	[105]
Glycyrrhizic-acid-based carbon dots	None (*in vitro*)	Yes	PEDV, PRRSV	Vero (for PEDV), MARC-145 (for PRRSV)	2020	[55]
Graphene and derivatives	**Toxicity**	**Antiviral**	**Tested viruses**	**Tested cell line/animal model/inhibition**	**Year**	**Ref.**
Graphene	Not tested	Yes	HIV-1	Essential target proteins (HIVVpr, Nef and Gag)	2014	[106]
Pristine MWCNT, ox-MWCNT and drug-conjugated MWCNT-C-CHI36	Low (ox-MWCNT, *in vitro*) Low (MWCNT-C-CHI36, *in vitro*) High (MWCNT, *in vitro*)	Yes (ox-MWCNT) Yes (MWCNT-C-CHI36) No (MWCNT)	HIV-1	MT-4	2015	[107]
GO, rGO, GO/PVP, GO/PDDA, Gt, GtO	None (GO *in vitro at* ≤ 6 $\mu g/mL$) The rest CBNs were not tested	No (GO/PDDA and Gt) Yes (the rest CBNs)	PEDV	Vero	2015	[29]
GO-AgNPs	Very low to none (*in vitro*)	Yes	FCoV	fcwf-4	2016	[108]
GO-AgNPs	None (*in vitro at* ≤ 4 $\mu g/mL$)	Yes	PRRSV, PEDV	MARC-145(for PRRSV) and Vero (for PEDV)	2018	[109]
Water-soluble GQD and drug-conjugated GQD	Low to High (*in vitro*)	Yes	HIV-1	MT-4	2018	[110]

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2.2. Carbon dots and derivatives

Carbon dots (CDs), also known as carbon quantum dots (CQDs) or Cdots, are other members of the CBNs family with small dimensions up to 10 nm in diameter, which are cost-effective and environmentally inert. It possesses the chemical functionality of an organic molecule, show very high surface-to-volume ratio, and can be homogeneously dispersed in water [24,111]. Many CDs are explored in several fields, such as chemical sensing, bioimaging, and electrocatalysis, as well as for other applications [53,112]. In the context of antiviral mechanisms, CDs could inhibit viral replication by activating the interferon response for the porcine reproductive and respiratory syndrome virus (PRRSV) [54] (see Table 1). Furthermore, CDs conjugated with carboxyl phenylboronic acid (CBBA) prevented the entry of HIV-1 viruses into cells by suppressing syncytium (Figure 3) [52].

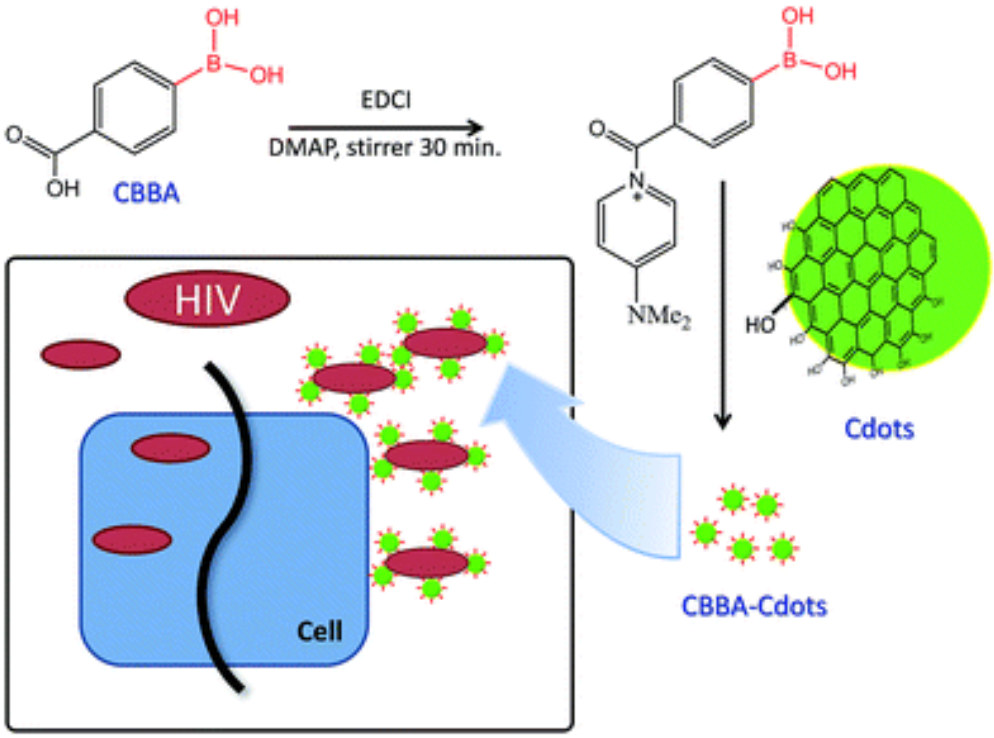

Figure 3. Schematic illustration of conjugating carboxyl phenylboronic acid (CBBA) on Cdots (CBBA-Cdots) and different mechanisms of inhibition entry - *Published by The Royal Society of Chemistry (RSC)* [52].

The antiviral activity of functionalized CDs produced from 4-aminophenylboronic acid against human coronavirus (HCoV) infections has been recently demonstrated in human Huh-7 liver cells [23]. The study showed inhibition of the viral entry and effects at the replication steps which was ascribed to the interaction of the CBNs' functional groups with the viral entry receptor DPP4 (see Figure 4).

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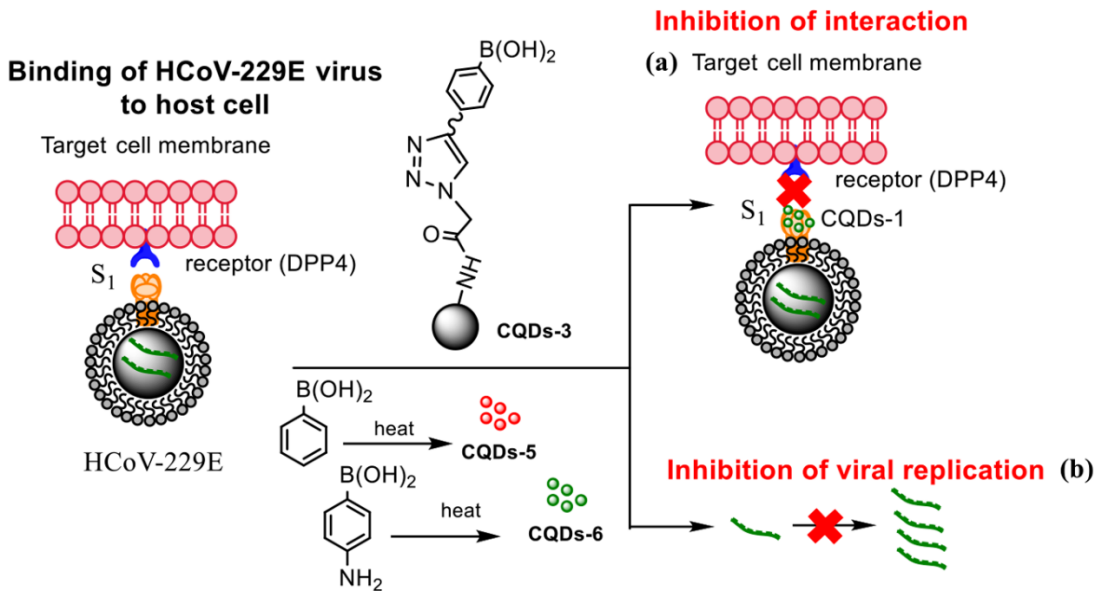

Figure 4. Influence of carbon quantum dots (CQDs) on the binding of HCoV229E virus to cells: (a) inhibition of protein S receptor interaction (b) inhibition of viral replication. *Reprinted with permission from*[23]. *Copyright (2019) American Chemical Society. Further permissions related to the material excerpted should be directed to the ACS.*

Benzoxazine monomer-derived carbon dots (BZM-CDs) were effective against the Japanese encephalitis virus (JEV), ZIKV, and DENV [105]. The antiviral activity of highly biocompatible glycyrrhizic-acid-based carbon dots (Gly-CDs) synthesized from glycyrrhizic acid was demonstrated against large-enveloped RNA viruses by using the porcine epidemic diarrhea virus (PEDV) as a viral model of the coronavirus (CoVs) [55]. This virus belongs to the *alphacoronavirus* genus and it cannot be transmitted to humans [113].

2.3. Graphene and related carbon-based nanomaterials

Graphene and its oxidated form, GO, are 2D CBNs with excellent physical and biological properties that can be used successfully to detect, and capture viruses, by destroying their surface proteins, and extracting their RNA by bioreduction [114] (see Table 1). The high binding affinity of graphene to the essential target proteins HIV Vpr, Nef, and Gag during HIV infections was first reported in 2014 [106]. The antiviral activity of carbon nanofibers (CNFs) was reported using a non-enveloped double-stranded DNA viral model [115]. However, the antiviral capacity of CNFs has never been tested against an enveloped virus belonging to the same Baltimore group [116] such as SARS-CoV-2. Functionalized multiwall carbon nanotubes (MWCNT), another type of carbon-based filamentous nanomaterials, exhibited potent viral inhibition against HIV [107]. In this study, the effect of hydrophilicity and dispersibility of the nanomaterials showed to be the key to control the antiviral activity of MWCNT-based nanomaterials. Carboxylated MWCNT (ox-MWCNT sample) and drug-conjugated MWCNT (MWCNT-C-CHI36) showed high antiviral activity contrary to pristine MWCNT which exhibited high cytotoxicity. Evaluation of the antiviral capacity of GO and rGO against PEDV showed significant viral inhibition by inactivating the virus before entering the cell (Figure 5) [29].

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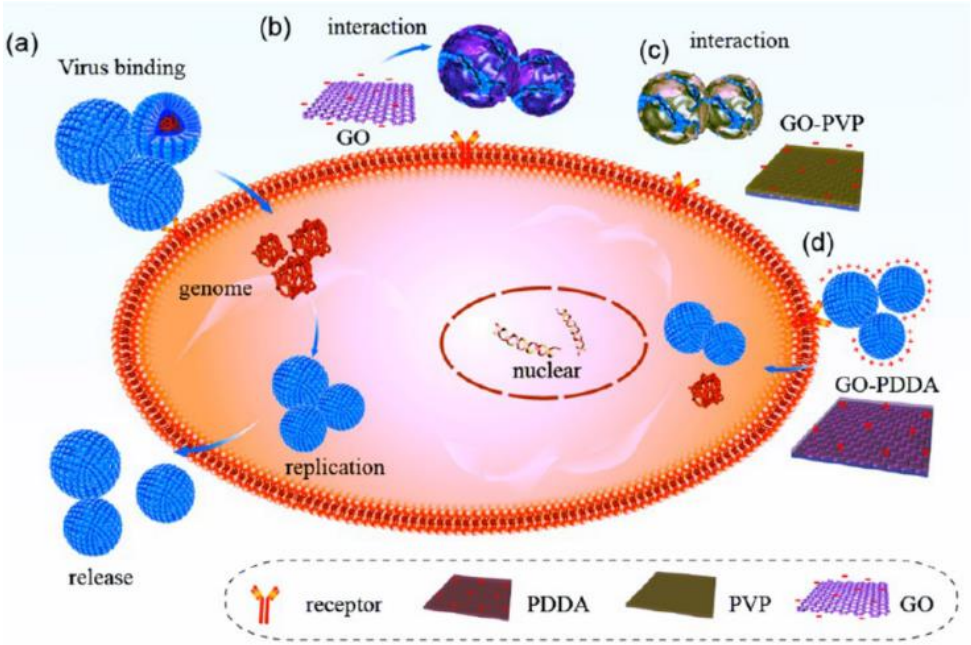

Figure 5. Possible antiviral mechanisms of graphene oxide (GO): (a) Infection initiation: virus binding by interaction with cell receptors; (b) Interaction of negatively charged GO nanosheet with the positively charged viruses, producing virus damage and infection inhibition; (c) GO conjugated with nonionic PVP blocked infection but GO with cationic PDDA did not (d). *Reprinted with permission from* [29]. *Copyright (2015) American Chemical Society.*

It is reported that, the sharp edges of the GO or rGO nanosheet inactivated the virus by physical disruption of its biological structure through direct interaction which resulted in the outflow of intracellular metabolites [29]. Since rGO and GO exhibited a similar viral inhibition capacity, the surface functional groups present in these CBNs may play a minor role. The antiviral activity was concentration and incubation time-dependent. Therefore, the potent antiviral activity of both GO and rGO can be attributed to the negative charge and single nanosheet-layer structure. GO and rGO nanomaterials possess similar negative charge which favors the electrostatic interaction with the positive charge of the virus [31]. GO has also shown potent antiviral activity with nonionic polyvinyl pyrrolidone (PVP) in contrast to combination with the cationic poly(diallyl dimethyl ammonium chloride) (PDDA) (see Figure 5) [29]. On the other hand, graphite (Gt) showed no viral inhibition and graphite oxide (GtO) exhibited weaker viral inhibition than monolayer GO and rGO, which suggests that the nanosheet form plays a very important role in the antiviral activity. The combination of silver nanoparticles (AgNPs) with GO inhibited the infectivity of enveloped feline coronavirus (FCoV) by 25% compared to 16% for GO [108]. AgNPs are well-known alternative antiviral agents that interact with cell surface receptors and blocks of the virus entry into the host cells [117]. In the same research line, GO-AgNPs nanocomposites showed better viral inhibition capacity than AgNPs or GO using a PRRSV pattern on the replication of virus [109]. This advanced nanocomposite prevents PRRSV from entering the host cells (~59.2% inhibition) and improves the production of interferon-α (IFN-α) and ISGs, which directly block the proliferation of PRRSV. Water-soluble graphene quantum dots (GQD) synthesized from MWCNT through oxidation and exfoliation with and without conjugated antiretroviral agents exhibited efficient viral inhibition of the HIV [110]. Therefore, all these results confirm the potential utilization of CBNs in the fight against viruses such as SARS-CoV-2.

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3. Viruses studied with carbon-based nanomaterials

This review analyzes the antiviral studies of CBNs against 12 viruses. In 32 (out of 36) of these studies, antiviral activity was detected (Figure 6).

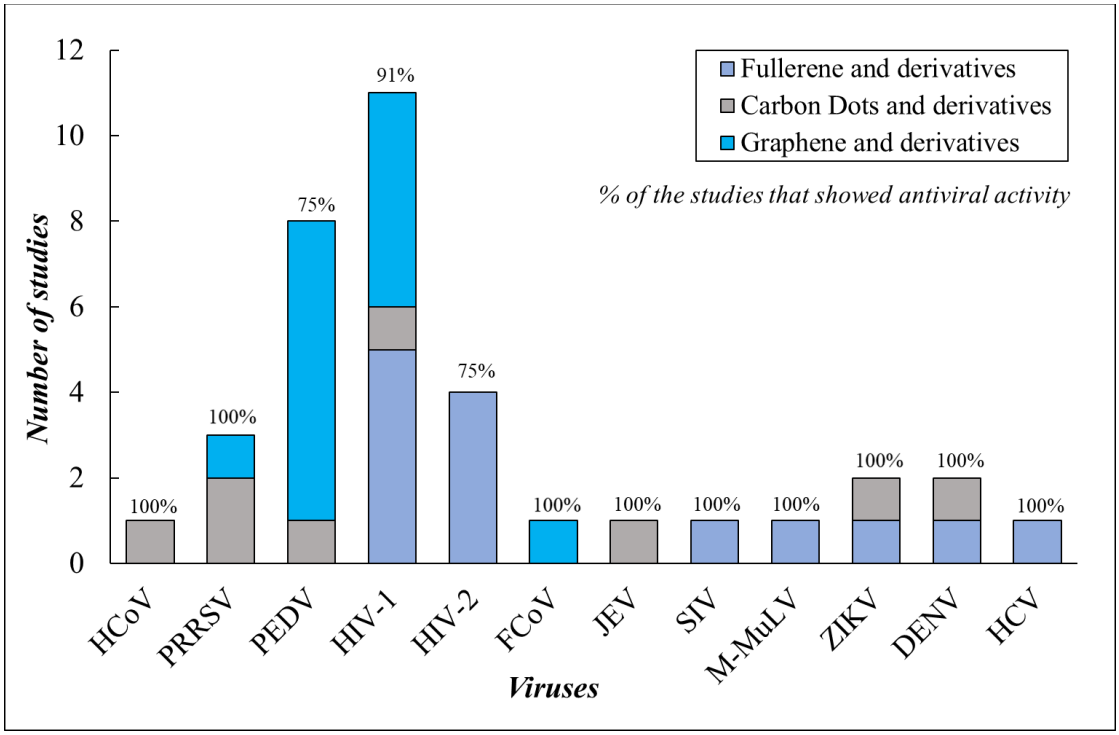

Figure. 6. Studies of the antiviral activity of carbon-based nanomaterials indicating the percentage of studies that showed antiviral activity against 12 enveloped positive-sense single-stranded RNA viruses. The carbon-based nanomaterials were in the form of fullerenes, carbon dots, graphene and derivatives shown in Table 1.

The number of studies is greater than the 19 published papers (Table 1) because several CBNs were studied in some publications. To perform this count of studies, only CBNs with different chemical structural forms were considered. Thus, fullerene and its derivatives showed antiviral activity in 5 studies against HIV-1 [27,99,101–103], 3 studies against HIV-2 [27,101–103] and other viruses such as SIV [100], M-MuLV[100], HCV[104], ZIKV[25] and DENV[25]. However, one study out of the four with the fullerene and its derivatives showed no viral inhibition against HIV-2 (see Table 1). CQDs and their derivatives exhibited antiviral activity against HCoV[23], HIV-1[52], JEV[105], ZIKV[105], DENV[105], PEDV[55] and two studies against PRRSV[54,55]. Graphene and related CBNs have shown antiviral activity against HIV-1 in four studies out of five [106,107,110], five studies against PEDV out of seven [29,109], and one study against FCoV[108] and PRRSV[109]. All these viruses are enveloped positive-sense single-stranded RNA viruses like SARS-CoV2 (IV Baltimore group [116]). Therefore, the antiviral properties of CBNs have been tested against a wide range of viruses similar to SARS-CoV-2, which suggest that CBNs are promising nanomaterials as alternative antiviral agents against this pathogen. The viruses tested against the CBNs are shown in Table 2 with all their characteristics such as name, abbreviation, genus, family, viral affection and disease/action and references.

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Table 2. Information of the enveloped viruses tested to study the antiviral properties of CBNs belonging to the same Baltimore classification of SARS-CoV-2 (Group IV ((+)ssRNA[116]): single-stranded positive-sense RNA virus).

Virus name	Abbreviation	Genus	Family	Infects	Disease/action	References
Human coronavirus	HCoV	Alphacoronavirus	Coronaviridae	Humans	Common cold, pneumonia and bronchiolitis	[23]
Porcine reproductive and respiratory syndrome virus	PRRSV	Betaarterivirus	Arteriviridae	Pigs	Porcine reproductive and respiratory syndrome	[54,55,109]
Porcine epidemic diarrhea virus	PEDV	Aphacoronavirus	Coronaviridae	Pigs	Porcine diarrhea	[29,55,109]
Human immunodeficiency virus type 1	HIV-1	Lentivirus	Retroviridae	Humans	AIDS	[27,52,99,101–103,106,107,110]
Human immunodeficiency virus type 2	HIV-2	Lentivirus	Retroviridae	Humans	AIDS	[27,101–103]
Feline coronavirus	FCoV	Alphacoronavirus	Coronaviridae	Cats	Feline infectious peritonitis	[108]
Japanese encephalitis virus	JEV	Flavivirus	Flaviviridae	Humans through Culex mosquitoes	Inflammation of the brain occurs	[105]
Simian immunodeficiency virus	SIV	Lentivirus	Retroviridae	Non-human primates	Simian AIDS	[100]
Moloney murine leukemia virus	M-MuLV	Gammaretrovirus	Retroviridae	Mouse	Cancer	[100]
Zika virus	ZIKV	Flavivirus	Flaviviridae	Humans through Aedes mosquitoes	Zika fever	[25,105]
Dengue virus	DENV	Flavivirus	Flaviviridae	Humans through *Aedes* mosquitoes	Dengue fever	[25,105]
Hepatitis C virus	HCV	Hepacivirus	Flaviviridae	Humans	Hepatitis C	[104]
Severe acute respiratory syndrome coronavirus 2	SARS-CoV-2	Betacoronavirus	Coronaviridae	Humans	COVID-19	Not studied

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4. Toxicological aspects of carbon-based nanomaterials

There are widespread concerns on the toxicological aspects of CBNs because some studies have reported that depending on the type of CBNs, dimensions, oxidation degree, functionalization, concentration, and exposure time, CBNs may exert cytotoxicity effects on host cells [73–80]. Nevertheless, we focused our attention on the toxicological studies performed with the CBNs tested against the 12 enveloped single-stranded positive-sense RNA viruses analyzed in this review (Table 1). Details of these studies are discussed below.

4.1. Fullerene and derivatives

An anti- HIV-1 fullerene derivative (compound 1) showed no cytotoxicity on human peripheral blood mononuclear cells in 1993 [99]. However, a series of anti-HIV-1 bis-functionalized fullerene derivatives were developed after that in 2003 and only one of them (derivative 1) exhibited moderate toxicity [101]. Nonetheless, low cytotoxic properties were reported for anti-HIV cationic fullerene derivatives and anti-HIV water-soluble fullerene derivatives in 2005 and 2007, respectively [102,103]. A later study in 2011 showed that anti-HIV derivatives of C70-fullerene also exhibited low toxicity *in vitro* and *in vivo* [27]. More recently, an anti-HCV fullerene derivative (derivative 1a, Figure 2) showed no cytotoxicity in the low micromolar range [26]. Furthermore, very recently, anti-ZIKV and anti-DENV tridecafullerenes appended with up to 360 1,2-mannobiose showed no cytotoxicity in the picomolar range [25].

4.2. Carbon dots and derivatives

CDs are highly promising nanomedicine tools for antiviral applications due to their low toxicity and potent antiviral activity [112]. Thus, CDs showed antiviral activity against PRRSV and low cytotoxicity in MARC-145 cells [54]. Furthermore, the modified CDs or their combination with other compounds significantly decreased the cytotoxicity of these carbon-based antiviral nanomaterials [23,52,55,105]. For example, in the cytotoxicity tests of both Cdots and CBBA-Cdots at high concentrations (up to 300 $mg \cdot mL^{-1}$) proliferation of human cells was as fast as that of the untreated cells (control) after 24 hours incubation suggesting the absence of cytotoxic effects for both types of nanomaterials [52]. However, this study did not assess ROS or cytokine generation, immunomodulatory effects that are known to be induced by other carbon-based nanoparticles such as graphene (see next paragraph). Anti-HCoV CDs produced from 4-aminophenylboronic acid, BZM-CDs with anti-JEV, anti-ZIKV and anti-DENV properties, and anti-PEDV and anti-PRRSV Gly-CDs manifested no cytotoxicity [23,55,105].

4.3. Graphene and related carbon-based nanomaterials

The toxicological analysis of pristine MWCNT, ox-MWCNT and MWCNT-C-CHI36 with anti-HIV-1 properties showed opposite results [107]. Thus, ox-MWCNT and MWCNT-C-CHI36 showed low cytotoxicity contrary to pristine MWCNT which exhibited high cytotoxicity. However, GO showed potent anti-PEDV using a non-cytotoxic concentration (≤ 6 μg/mL) [29]. Anti-FCoV GO-AgNPs nanocomposites and neat GO could also be prepared at very low or non-cytotoxic concentrations [108]. In the same way, anti-PRRSV GO-AgNPs nanocomposites could be prepared at a non-cytotoxic

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concentration (≤ 4 μg/mL) [109]. Finally, a water-soluble GQD with a conjugated antiretroviral agent (GQD-CHI499) was selected as a good potential candidate due to its high antiviral activity against HIV and low cytotoxicity [110]. Therefore, the lack of toxicity could provide great potential in the development of safe therapeutics based on carbon-based nanomaterials to combat COVID-19 in the microbial resistant era.

5. Conclusions

Carbon-based nanomaterials have been evaluated for their antiviral activity against 12 enveloped viruses (HCoV, PRRSV, PEDV, HIV-1, HIV-2, FCoV, JEV, SIV, M-MuLV, ZIKV, DENV, HCV), all single-stranded positive-sense RNA viruses belonging to the same Baltimore group IV as SARS-CoV-2. Most of the studies have shown a potent antiviral activity and from low to no toxicity supporting the potential for the use of CBNs for the treatment of SARS-CoV-2. As a radically novel technology approach for the treatment of COVID-19, these carbon-based therapeutics can provide a significant breakthrough as these nanomaterials allow the targeting of microbial resistance issues and can potentially induce tissue regeneration at the same time. Furthermore, these novel antimicrobial nanoweapons could be employed to deal with SARS-CoV-2 alone or in coexistence with other types of viruses, bacteria, or fungi, causing pneumonia, including multidrug-resistant strains. The chance of success applying these wide-spectrum antimicrobial nanomaterials is very high because of the promising preliminary antiviral results reported for 12 viruses and the fact that the proposed approach could be extended to other types of pneumonia caused by other important pathogens. Current methods will provide the means for short-term research, which could already save lives, but more resources will be needed to secure a wider range of research for a larger long-term solution

Abbreviations

A549 — Human lung cancer cell
AgNPs — Silver nanoparticles
BHK-21 — Baby hamster Syrian kidney 21 cells
BZM-CDs — Benzoxazine monomer derived carbon dots
CBBA — Carboxyl phenylboronic acid
CBBA-Cdots — Carboxyl phenylboronic acid conjugated on Cdots
CBNs — Carbon-based nanomaterials
CDots — Carbon dots
CDs — Carbon dots
CCM-CDs — Cationic carbon dots
CEM — Lymphoblastic cells derived from a child with acute lymphoblastic leukemia
COVID-19 — Coronavirus disease 2019
CoVs — Coronavirus
CQDs — Carbon quantum dots
CNFs — Carbon nanofibers
CNFs/calcium alginate — Carbon nanofibers in calcium alginate
EC50 — Half maximal effective concentration
FCoV — Feline coronavirus
fcwf-4 — Felis catus whole fetus-4 cells
GBNs — Graphene-based nanomaterials

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Gly-CDs — Glycyrrhizic-Acid-Based Carbon Dots
GO — Graphene oxide
GO-AgNPs — Silver nanoparticle-modified graphene oxide nanocomposites
GQD — Graphene quantum dots
Gt — Graphite
GtO —Graphite oxide
HIV — Human immunodeficiency virus
HIV-1 — Human immunodeficiency virus type 1
HIV-2 — Human immunodeficiency virus type 1
HCoV— Human coronavirus
HELF — Human embryo lung diploid fibroblasts
Huh-7 — Human hepatic cell 7
JEV — Japanese encephalitis
Jurkat — Lymphocyte T CD4 immortalized cells
MARC-145 — Monkey kidney cells
MOLT-4 — Human T lymphoblast; acute lymphoblastic leukemia cells
MDCK — Madin Darby canine kidney cells
MT-2 — Human T cell leukemia cells
MT-4 — Human T-cells
MT-4/HIV-1 — HIV-1 infected MT-4 cells
MWCNT — Multiwall carbon nanotubes
MWCNT-C-CHI36 — CHI36 drug-conjugated MWCNT
ox-MWCNT — Carboxylated multiwall carbon nanotubes
PDDA — Poly(diallyl dimethyl ammonium chloride)
PEDV — Porcine epidemic diarrhea virus
PK-15 — Porcine kidney cells
PRRSV — Porcine reproductive and respiratory syndrome virus
PVP — Polyvinyl pyrrolidone
rGO — Reduced graphene oxide
rGO−SO3 — Partially reduced sulfonated graphene oxide
RSV — Respiratory syncytial virus
ROS — Reactive oxygen species
SARS-CoV-2 — Acute Respiratory Syndrome Coronavirus 2
SMRGO — Sulfonated magnetic nanoparticles functionalized with reduced graphene oxide
SWCNTs — Single-walled carbon nanotubes
Vero cells — African green monkey cells
WSL — Wild boar lung cells

Author Contributions

ÁSA conceived the idea of this work, wrote the draft manuscript and major editing. KT, ATM, MS, VNU, KL, PA, AAAA, GC, RK, BDU and AL edited the manuscript, and SSH, PPC, GP, MMT and AMB proof-read the manuscript.

Conflict of Interests

The authors do not have any conflicts of interest to declare.

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