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Nanotechnology Responses to COVID-19

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Dedicated to Dr. André Van Meerbeeck and to all the direct and indirect victims of the COVID-19 pandemic

Researchers, engineers, and medical doctors are made aware of the severity of the COVID-19 infection and act quickly against the coronavirus SARS-CoV-2 using a large variety of tools. In this review, a panoply of nanoscience and nanotechnology approaches show how these disciplines can help the medical, technical, and scientific communities to fight the pandemic, highlighting the development of nanomaterials for detection, sanitation, therapies, and vaccines. SARS-CoV-2, which can be regarded as a functional core–shell nanoparticle (NP), can interact with diverse materials in its vicinity and remains attached for variable times while preserving its bioactivity. These studies are critical for the appropriate use of controlled disinfection systems. Other nanotechnological approaches are also decisive for the development of improved novel testing and diagnosis kits of coronavirus that are urgently required. Therapeutics are based on nanotechnology strategies as well and focus on antiviral drug design and on new nanoarchitectured vaccines. A brief overview on patented work is presented that emphasizes nanotechnology applied to coronaviruses. Finally, some comments are made on patents of the initial technological responses to COVID-19 that have already been put in practice.

1. Introduction

At the end of December 2019, the World Health Organization (WHO) China Country Office was informed by the Chinese authorities of an infectious disease, later on called COVID-19 (coronavirus disease 2019), which was initially diagnosed in the city of Wuhan and caused by a new type of coronavirus, now named severe acute respiratory syndrome (SARS) coronavirus 2 (SARS-CoV-2).[1,2] The WHO declared COVID-19 as a pandemic on March 11, 2020.[3]

Presently, COVID-19 affects most countries on our planet with the result of several millions of infected individuals and hundreds of thousands of deaths around the world.[4] This is the most serious of the three pandemics caused by coronaviruses in the last two decades after the viral respiratory diseases caused by SARS-CoV in 2002–2003 and the Middle East respiratory syndrome (MERS-CoV) in 2012.[5]

The current impact of COVID-19 on global health is enormous, but in addition, the worldwide impact on the economy, employees, and companies is going to be considerable and may entail deep economic and negative social impacts as well as geopolitical repercussions as a further possible consequence.[6] This global emergency requires a response to the COVID-19 pandemic with science and technology means, wherein nanotechnology approaches may contribute with advanced solutions to this crisis. Nanotechnology comes into play when keeping in mind that SARS-CoV-2 has nanometric dimensions with a core–shell nanostructure (**Figure 1**A) and therefore, could be regarded as a functional nanomaterial.

Coronaviruses such as SARS-CoV, MERS-CoV and SARS-CoV-2 are enveloped viruses with a positive single-stranded RNA genome. SARS-CoV-2 viral particles (Figure 1) are spherical to pleomorphic and 65 to 125 nm in size. Inside the particle, the viral RNA, with 29,811 nucleotides, is tightly coiled and coated by the nucleocapsid (N) protein. Three glycoproteins, called spike (S), membrane (M), and envelope (E), are embedded in the lipid outer membrane. Spike proteins form homotrimers, which protrude from the lipid envelope and form the characteristic "corona". Spike proteins mediate viral entry into host cells by binding to angiotensin-converting enzyme 2 (ACE2) expressed on respiratory tract cells.[7] When these components change their

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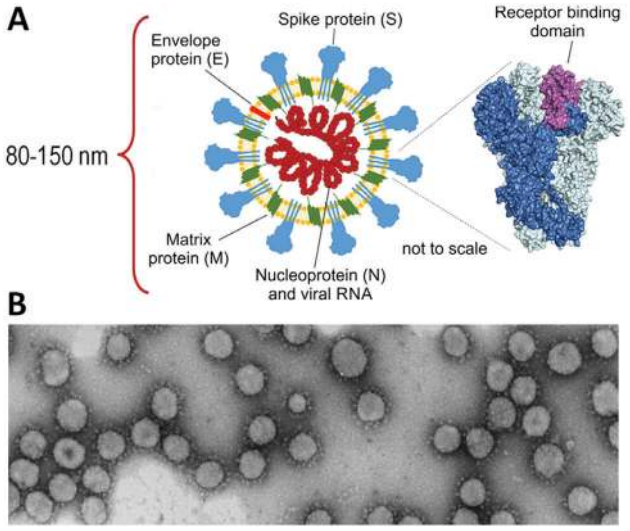

Figure 1. A) Schematic representation of SARS-CoV-2 as a core–shell nanoparticle (left) highlighting trimeric spike protein of SARS-CoV-2 (right). Reproduced under the terms of CC BY-NC-ND 4.0 license from Amanatet al.[132] Copyright 2020, Elsevier Co. B) TEM image of SARS-CoV-2 virions (from CNB-CSIC webpage[257]).

structural arrangements and spatial configurations, a loss of functionality could take place leading to the viral inactivation. In fact, viral disinfection can be produced either by physical means, as for instance, by heating at temperature above 65 °C, ultraviolet C (UVC) irradiation, varying the relative humidity, etc., or by chemical means, for example, by treatment with organic solvents (alcohols), chlorine (sodium hypochlorite), surfactants, transition/noble metal ions or metal/metal oxide nanoparticles, etc.[8,9]

COVID-19 is a very contagious disease and people get infected with SARS-CoV-2 mainly by inhaling virion particles contained in the respiratory fluid droplets expelled into the air by nearby infected individuals, for instance, generated by coughing, sneezing, or talking.[10] Lewis and his collaborators have also reported on the transmission of SARS-CoV-2 via aerosols, and it can be assumed that viral particles expelled into the air can easily cause infections from this medium.[11] According to van Doremalen and coworkers, SARS-CoV-2 in aerosols could stay active in air for up to 3 h.[12] Although stability of coronavirus in dispersions does not equal to the "transmission" of COVID-19 via aerosol,[13] Morawska and Milton presented evidence supporting the potential for airborne spread of COVID-19 by exposure to viruses in microdroplets at short to medium distances.[14] They advocate for the use of preventive measures (e.g., surgical masks) to mitigate this route of airborne transmission, especially acute in indoor environments and closed rooms such as intensive care units in hospitals and ambulances. It is crucial to avoid the propagation of virions in air by ultrafiltration and nanofiltration processes, using filters capable of efficient removal of the nanometric viral particles. Furthermore, it also appears possible that infections can happen by touching virus-laden surfaces and then touching the face (eyes, nose and mouth).[15] Infectious viruses, including different coronaviruses, can be longtime active on inanimate surfaces (i.e., fomite) including metals, plastics, cloths, and paper, for hours to days depending on various ambient parameters such as humidity, temperature, and the chemical and topological nature of the solid surface.[12,16] For instance, porous surfaces, such as paper and cardboard, exhibit less efficient transmission of viruses compared to non-porous surfaces.[17] Therefore, investigation of the interaction of viral nanoparticles with surfaces and porous solids at the nanometric level will be crucial to decrease the virus spreading as well as their persistency in air and on solid objects, which is discussed in depth in the next section of this review.

Theranostics aspects are another important point regarding nanotechnology and COVID-19.[18] The new coronavirus SARS-CoV-2 requires rapid detection and diagnostic in order to accelerate efficient therapy, ideally based on antiviral treatments and vaccines developments.[19,20] Despite some recent favorable results with antiviral treatments, it is urgent to maximize these investigations because the worldwide death figures provoked by COVID-19 continue to increase every day. In this context, nanotechnology approaches may offer efficient ways against this pandemic as nanoscale materials have recently emerged as effective platforms to impair the viral infection cycle.[21]

At the present moment, there are still no approved vaccines or efficient antiviral treatments to protect people against COVID-19. Consequently, significant and urgent efforts must extensively be devoted to develop reliable therapies. Around 100 groups worldwide are currently involved in specific research and clinical trials. Certain approaches to vaccine development consist in the assembly of fragments of the SARS-CoV-2 virion genome within a harmless virus with the intention of developing a safe viral particle that resembles the coronavirus and, consequently, could generate convenient immune responses. Another alternative is based on the use of DNA or RNA fragments able to express viral proteins that could activate the immune response. Nanotechnology concepts could be applicable here and diverse formulations based on synthetic nanoparticles have been considered as promising and precise carriers to administrate viral vaccines. For instance, according to Itani et al.,[18] intranasal administration is proposed as the preferred administration route for therapeutic agents against viral pulmonary diseases. Nanomaterial-based delivery formulations favor the concentration of active agents, such as vaccines, antibodies, silencing RNA (siRNA), and antiviral species at the targeted sites of infection producing also a general potentiation of the immune system.[22,23] Intranasal administration routes using nanoparticle-based formulations have already been applied for vaccination against respiratory viruses such as influenza and coronaviruses.[24–27] In this context, biohybrid nanomaterials based on the assembly of fibrous clays and polysaccharides show a favorable environment for influenza A viral particles, facilitating their binding to the hybrid material and preserving their bioactivity (**Figure 2**). Water dispersions of these supported viral particles can be directly used as intranasal or intramuscular vaccines inducing the formation of antibodies in mice.[25,28–30] In fact, these types of hybrid nanomaterials could be regarded as biomimetic mucous and the viral particles could be replaced by their surface antigens such as haemagglutinin (HA) and neuraminidase (NA).[29,31] The use of this type of nanostructured materials, easy to functionalize, as vector for intranasal administration vaccines should take into account the nature of the nanoparticle (including size and charge, specific surface area) for the new approaches that are urgently required to develop operative vaccines for COVID-19.

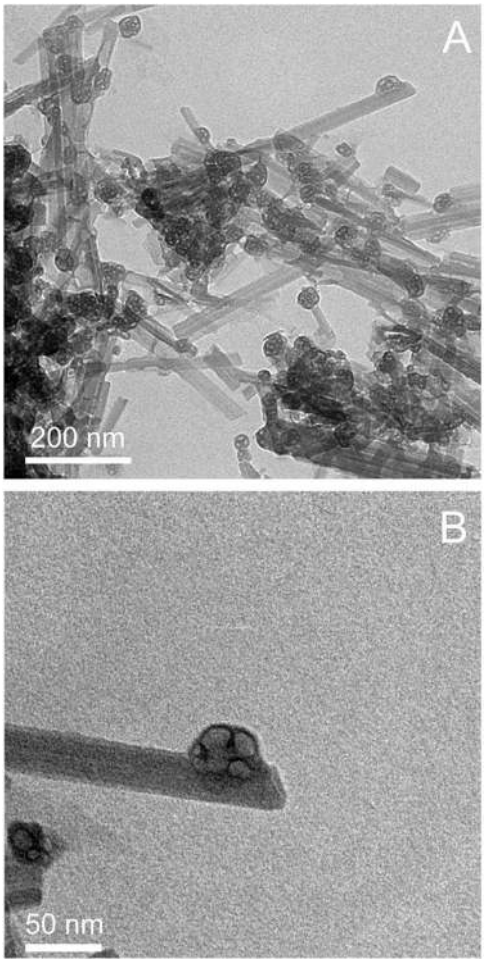

Figure 2. A) TEM images of influenza virus particles supported on xanthan–sepiolite bionanocomposites showing a homogeneous distribution and B) detail of a single viral particle supported on a xanthan-modified sepiolite fiber (Reproduced with permission.[25] Copyright 2009, Wiley Co.).

Very recent reviews by Sportelli and co-workers,[32] Huang and co-workers,[7] and Itani and co-workers,[18] among other excellent papers, have introduced diverse aspects in relation to SARS-CoV-2 and nanotechnology. The present communication aims to thoroughly review current research contributions on this topic, but including in addition a short overview of related patents to highlight the actual applicability of these strategies that ideally will contribute to solve the global problem caused by the COVID-19 disease.

2. General Aspects of the Interaction of Viral Particles with Solids

2.1. Stability on Inanimate Surfaces and Disinfection Methods

The most frequent transmission route for SARS-CoV-2 is through respiratory droplets and viral aerosols exhaled from COVID-19 patients (**Figure 3**). Nevertheless, these droplets may eventually settle as semi-dried "nuclei" on surfaces to form infectious fomites.[7] Therefore, self-inoculation through nose, mouth, or eyes after touching a contaminated surface in public areas is a potential route of SARS-CoV-2 transmission.[33,34] Van Doremalen and co-workers[12] and Chin and co-workers[16] have recently published experimental results about the stability of SARS-CoV-2 on surfaces of diverse nature (**Figure 4**). Furthermore, the persistence of other coronaviruses, such as SARS-CoV,

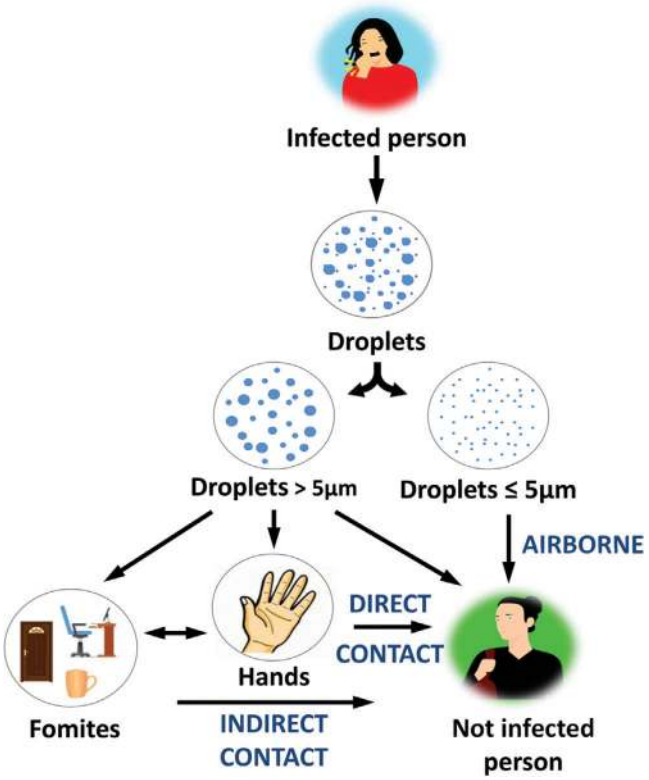

Figure 3. Common transmission routes for SARS-CoV-2: an infected person releases respiratory fluid droplets containing viable viruses which can infect other people within close proximity by inhaling airborne droplets or by direct contact with droplets or contaminated hand. These droplets can also contaminate different surfaces (fomites) and infect people by indirect contact (involving a combination of hand and surface).

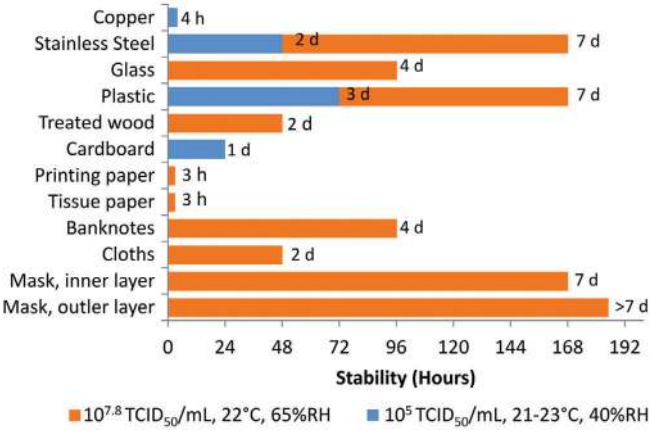

Figure 4. Stability of SARS-CoV-2 on different surfaces. Blue bars: Viral titer of inoculum 10^5 $TCID_{50}$ mL^{-1}, 21–23 °C, 40% RH (data from van Doremalen et al.[12]). Orange bars: Viral titer of inoculum $10^{7.8}$ $TCID_{50}$ mL^{-1}, 22 °C, 65% RH (data from Chin et al.[16]).

Table 1. Stability of coranoviruses on smooth surfaces.

Surface	Virus	Strain	Viral titer inoculum	T [°C]	RH [%]	Stability	Ref.
Metal	SARS-CoV	P9	10^6	RT	n.i.	5 d	[40]
Aluminium	HCoV	229E and OC43	5×10^3	21	55–70	3–12 h	[42]
Copper	SARS-CoV-2	Tor2	10^5	21–23	40	4 h	[12]
	SARS-CoV	nCoV-WA1-2020	10^5	21–23	40	8 h	[12]
Stainless steel	SARS-CoV-2	n.i.	$10^{7.8}$	22	65	7 d	[16]
	SARS-CoV-2	Tor2	10^5	21–23	40	2 d	[12]
	SARS-CoV	nCoV-WA1-2020	10^5	21–23	40	2 d	[12]
	HCoV	229E	10^3	21	30-40	5 d	[41]
	MERS-CoV	Isolated*	10^5	20	40	3 d	[37]
	MERS-CoV	Isolated*	10^5	30	30	2 d	[37]
	MERS-CoV	Isolated*	10^5	30	80	1 d	[37]
Glass	SARS-CoV-2	n.i.	$10^{7.8}$	22	65	4 d	[16]
	SARS-CoV	P9	10^6	RT	n.i.	4 d	[40]
	HCoV	229E	10^3	21	30–40	5 d	[41]
Ceramic	HCoV	229E	10^3	21	30–40	5 d	[41]
Plastic	SARS-CoV-2	n.i.	$10^{7.8}$	22	65	7 d	[16]
	SARS-CoV-2	Tor2	10^5	21–23	40	3 d	[12]
	SARS-CoV	nCoV-WA1-2020	10^5	21–23	40	3 d	[12]
	SARS-CoV	HKU39849	10^5	22–25	40–50	5 d	[38]
	SARS-CoV	HKU39849	10^5	38	80–90	1 d	[38]
	SARS-CoV	HKU39849	10^5	38	>95	3h	[38]
	SARS-CoV	P9	10^6	RT	n.i.	5 d	[40]
	SARS-CoV	FFM1	10^7	21–25	n.i.	9 d	[39]
	HCoV	229E	10^7	21–25	n.i.	3 d	[39]
	MERS-CoV	Isolated*	10^5	20	40	3 d	[37]
	MERS-CoV	Isolated*	10^5	30	30	2 d	[37]
	MERS-CoV	Isolated*	10^5	30	80	1 d	[37]
PTFE	HCoV	229E	10^3	21	30–40	5 d	[41]
PVC	HCoV	229E	10^3	21	30–40	5 d	[41]
Silicon rubber	HCoV	229E	10^3	21	30–40	3 d	[41]
Teflon	HCoV	229E	10^3	21	30–40	5 d	[41]
Treated wood	SARS-CoV-2	n.i.	$10^{7.8}$	22	65	2 d	[16]
Disposable gown	SARS-CoV	GVU6109	10^6	RT	n.i.	2 d	[36]
	SARS-CoV	GVU6109	10^5	RT	n.i.	1 d	[36]
	SARS-CoV	GVU6109	10^4	RT	n.i.	1 h	[36]
Surgical glove	HCoV	229E and OC43	5×10^3	21	55–70	1–6 h	[42]

Viral titers of inoculums are expressed in tissue culture infection dose ($TCID_{50}$) per mL. PTFE, polyfluorotetraethylene; PVC, polyvinyl chloride; HCoV, human coronavirus; SARS-CoV, severe acute respiratory syndrome coronavirus; MERS-CoV, Middle east respiratory syndrome coronavirus; n.i., not indicated; isolated*, isolated HCoV-EMC/2012; RT, room temperature.

MERS-CoV, or endemic human coronaviruses (HCoV), on different inanimate surfaces could be of great help in order to indirectly evaluate the potential risk of SARS-CoV-2 transmission.[33,34]

Tables 1 and **2** show the stability times reported for these coronaviruses on smooth and porous surfaces, respectively. It should be taken into account that direct comparison of persistency times from different studies could be controversial due to important methodological differences such as virus species and strain, applied titer and drop volume, suspending medium, deposition mode, temperature and relative humidity, nature of the surface, and the virus detection method.[35] Nevertheless, some tendencies can be extracted from Tables 1 and 2: i) the stability of coronaviruses decreases not only when inoculation doses are reduced[36] but also when both, temperature and relative humidity are increased,[37,38] and ii) higher persistency has been reported on smooth surfaces compared to porous surfaces.[12,36]

2.1.1. Smooth Surfaces

According to van Doremalen et al.,[12], SARS-CoV-2 showed similar stability on smooth surfaces than SARS-CoV: 2 days on stainless steel and 3 days on plastic surface. Similar stabilities have been reported for MERS-CoV on the same surfaces[37] and for HCoV on plastic.[39] However, when surfaces were inoculated

Table 2. Stability of coranoviruses on porous surfaces.

Surface	Virus	Strain	Viral titer inoculum	T [°C]	RH [%]	Stability	Ref.
Cardboard	SARS-CoV-2	Tor2	10^5	21–23	40	1 d	[12]
	SARS-CoV	nCoV-WA1-2020	10^5	21–23	40	8 h	[12]
Printing paper	SARS-CoV-2	n.i.	$10^{7.8}$	22	65	3 h	[16]
	SARS-CoV	GVU6109	10^6	RT	n.i.	1 d	[36]
	SARS-CoV	GVU6109	10^5	RT	n.i.	3 h	[36]
	SARS-CoV	GVU6109	10^4	RT	n.i.	<5 min	[36]
Press paper	SARS-CoV	P9	10^6	RT	n.i.	4 d	[40]
Filter paper	SARS-CoV	P9	10^6	RT	n.i.	5 d	[40]
Tissue papers	SARS-CoV-2	n.i.	$10^{7.8}$	22	65	3 h	[16]
Banknotes	SARS-CoV-2	n.i.	$10^{7.8}$	22	65	4 d	[16]
Cloth	SARS-CoV-2	n.i.	$10^{7.8}$	22	65	2 d	[16]
	SARS-CoV	P9	10^6	RT	n.i.	5 d	[40]
Cloth (cotton)	SARS-CoV	GVU6109	10^6	RT	n.i.	1 d	[36]
	SARS-CoV	GVU6109	10^5	RT	n.i.	1 h	[36]
	SARS-CoV	GVU6109	10^4	RT	n.i.	5 min	[36]
Cotton gauze	HCoV	229E and OC43	$5{\cdot}10^3$	21	55–70	1–12 h	[42]
Mask, inner layer	SARS-CoV-2	n.i.	$10^{7.8}$	22	65	7 d	[16]
Mask, outler layer	SARS-CoV-2	n.i.	$10^{7.8}$	22	65	> 7 d	[16]

Viral titers of inoculums are expressed in tissue culture infection dose ($TCID_{50}$) per mL. SARS-CoV, severe acute respiratory syndrome coronavirus; HCoV, human coronavirus; n.i., not indicated; RT, room temperature.

with higher virus titer ($10^{7.8}$ instead of 10^5 $TICD_{50}$ per mL), Chin et al.[16] reported longer persistency of SARS-CoV-2 on stainless steel (7 days), plastic (7 days), and glass (4 days). In line with these results, other authors have reported that SARS-CoV can persist on plastic surfaces up to 5 or even up to 9 days for high virus concentrations.[39,40] High stabilities (1–5 days) have also been reported for different coronaviruses on metal, ceramics, PTFE, PVC, silicon rubber, Teflon, treated wood, and disposable gown.[36,40,41] However, much lower survival times were found on aluminum (3–12 h) and surgical gloves (1–6 h) for HCoV[42] and on copper (4–8 h)[37] for SARS-CoV and SARS-CoV-2.

2.1.2. Porous Surfaces

In case of SARS-CoV-2, no infectious virus was recovered after 3 h of incubation on printing and tissue papers,[16] 1 day on cardboard,[12] and 2 days on cloth.[16] Other coronaviruses have also shown low persistency (≤1–2 days) on similar porous surfaces,[12,16,36,42] except for the P9 strain of SARS-CoV (4–5 persistency days),[40] which also presented higher stabilities on non-porous surfaces compared to other SARS-CoV strains. According to Lai et al.,[36], the risk of SARS-CoV infection through contact with a droplet-contaminated paper or clothes is low, since no virus infectivity remained in the porous surface after drying up (stability ≤ 5 min), even after contamination with higher virus titer (10^4 $TCID^{50}$ per mL) than the usual concentration in nasopharyngeal aspirate (NPA) samples ($10^{2.2}$ $TICD_{50}$ per mL). Similar low infection risk could be expected for SARS-CoV-2 through paper and clothes. However, Chin and co-workers[16] reported that SARS-CoV-2 was surprisingly detected even 6 and 7 days after inoculation on the inner and outer layer, respectively, of surgical masks. Nevertheless, the virus titer used in these assays for inoculation of masks ($10^{7.8}$ $TCID^{50}$ per mL) was much higher than the usual concentration in NPA. Therefore, significantly lower stability could be expected in masks worn by citizens, since persistency of the virus strongly depends on the virus titer, as mentioned above.

Although the effect of surface porosity is not well-understood, according to Huang et al.,[7] the faster deactivation observed on porous materials could be related to faster desiccation of the virus, since condensed water can be drained away from the virus into the surrounding porous surface. In addition, capillary compression can also mechanically squeeze the drying droplets and potentially deform and damage the virus particles inside.[7,43]

In order to determine the real risk of indirect transmission, not only the stability of coronaviruses but also the transfer efficiency of the virus from the surface to hands should be evaluated. Although data related to transfer of coronaviruses have not been found, transmissibility of other viruses could be useful. Lopez et al.[17] tested the transfer efficiency of MS2 coliphage and poliovirus-1 viruses from porous (cotton, polyester, and paper currency) and smooth fomites (acrylic, glass, ceramic tile, laminate, stainless steel, and granite) to fingers under different relative humidity conditions. These authors observed that smooth surfaces had a greater transfer efficiency (up to 22%) than porous surfaces (≤0.4%) under low humidity (15–32% RH), probably because infectious organisms may be entrapped within the porous matrix. Even at high humidity (40–64% RH), which prevents inocula from drying and boosts the virus transfer, virus transmission efficiency was lower on porous surfaces (below 2.3%) compared with non-porous surfaces (up to 78%). Similarly, several authors have reported lower transmissibility of influenza A virus from porous surfaces to hand (0.3–3%) than from smooth surfaces (7–7.9%).[44,45] By extrapolating these results to

coronaviruses, it could be concluded that indirect coronavirus transmission from porous surfaces such as paper and clothes is more unlikely than from smooth surfaces, especially at low relative humidity.[36]

Widespread apprehension over COVID-19 concerns cash money such as banknotes and coins. They might be regarded as potential transmission agent despite the lack of any clear scientific evidence reported until now.[46] Although more investigation on this topic will be needed for SARS-CoV-2 persistency on banknotes, Chin et al.[16] have recently reported a persistency of 4 days on banknotes for SARS-CoV-2 (Table 2), which is similar to that reported for influenza viruses.[47] All caution to save lives is mandatory even in case of doubt. Therefore, the Bank of China has ordered destruction of all banknotes collected at hospitals, wet markets, and buses in high-risk zones during the COVID-19 crisis during the last months. Alternatively, it would be necessary to find safe ways for disinfection of cash money by thermal treatments and UV irradiation among other procedures as recently patented.[48–50] A quarantine deposit of banknotes for 2 weeks is also a solution proposed during emergencies in pandemic times. Nevertheless, according to the WHO, people should not be warned against the use of paper money despite the possibility of infectious transmission with coronavirus.[51]

Finally, it should be mentioned that common household detergents and dilute hypochlorite solutions can be efficiently used as decontamination agents for textiles against coronaviruses such as SARS-CoV.[36] Nevertheless, innovative nanotechnological textiles with reduced levels of microbial persistence, for instance by incorporating graphene nanomaterials, could be more adequate even in personal protective equipment (PPE).[52,53]

2.1.3. Surface Disinfection Methods with a Nanotech-Twist

Indirect contact involving contaminated surfaces, especially with smooth surfaces as mentioned above, could be a secondary transmission route for SARS-CoV-2. Therefore, the disinfection of surfaces, but also of PPE materials, is of utmost importance in the control of pandemic spread of viruses including the current SARS-CoV-2.[33,32] Common methods consist in the application of formulations containing 80% or 75% alcohol (ethanol or isopropanol, respectively), 1.45% glycerin or 0.125% hydrogen peroxide as well as irradiation with UVC light, which is known for its effective germicidal disinfection activity against SARS-CoV and MERS-CoV with a >5 log reduction in 5 min[54] or ozone-based disinfection of hospital wastewater.[55]

However, nanotechnology may also help in the quest for effective and efficient disinfection and may add further features like inherent virucidity to nosocomial surfaces. For instance, self-sanitizing surfaces that release antimicrobial actives that encompass copperization or display surface topologies that promote fomite desiccation and self-deactivation of viral particles are just a few examples of how smart surfaces can alleviate the constant need for active disinfection.[7] In this vein, silver and copper are well-known since ancient Egyptian times for their antiviral and antibacterial activity, referred to as oligodynamic activity.[56] A key mechanism of these metals is the slow release of Cu^{2+} and Ag^{+} cations on the surface, which can damage the membrane and nucleotides of viruses. Nanotechnology provides also the means to incorporate Ag or Cu NPs on surfaces, PPE textiles, and air and water filters for efficient antiviral action while at the same time restricting their lixiviation into the environment, a common issue of public concern.[32,57] For instance, AgNP can be conveniently immobilized in polymer nanocomposites, on nonwoven nanofiber mats or coatings (see also Section 2.1.4).[58,59] Interestingly, Hasan and co-workers[60] have recently reported the antibacterial and antiviral properties of nanostructured aluminum surfaces inspired by insect wing architecture. They fabricated nanostructures randomly aligned as ridges on aluminum alloy surfaces by a wet-etching process and studied the persistency of respiratory syncytial virus (RSV) and rhinovirus (RV). These nanostructured surfaces were more effective against the nonenveloped virus (RV) but they also accelerated the natural degradation ofthe enveloped virus (RSV) by disrupting the envelope. Furthermore, they were able to reduce the potential for surface contact transmission of both viruses. Although no assays with coronaviruses were performed, it may be conjectured that aluminum nanostructuring could be a viable and effective treatment for surfaces against SARS-CoV-2.

2.1.4. Antiviral Polymer Coatings

The search for materials able to kill bacteria and viruses by contact is crucial to prevent the spreading of contagions. Polymers can be endowed with antimicrobial properties by covalent grafting of biocidal agents (quaternary ammonium and phosphonium groups, sulfonates…), leading to permanent or non-leaching sterile surfaces,[61–65] or simply by embedding biocidal agents (chlorine dioxide, alcohols, metal ions, or nanoparticles…) within the polymeric matrix from where they can be released.[66–69] Regarding the first group of non-leaching contact-killing polymers, an intense research has been carried out by Klibanov's group at the MIT on hydrophobic polyethyleneimine (PEI) derivatives.[61,70–72] These polymer coatings can be non-covalently deposited on a variety of solid surfaces (plastics, glass, fabrics, bandages, etc.) by spraying, brushing, or dipping, leading to virucidal materials.[70] Similar compositions have been reported based on analogous polymers containing also photoreactive groups to allow their covalent bonding to medical fabrics, which are then able to inactivate lipid-enveloped viruses including coronaviruses.[65] The mechanism for this contact-killing ability, studied with *N,N*-dodecyl,methyl-PEI deposited on glass or plastic slides, seems to be an irreversible adhesion of the viral particles onto the virucidal coating, provoking structural damage and release of their RNA (**Figure 5**).[71] Deposition of layer-by-layer films of *N,N*-dodecyl,methyl-PEI with poly(acrylic acid) also gave rise to antiviral coatings, reaching 100% virucidal efficacy above 7.5 layers that procure a total surface coverage.[73]

Quaternized chitosan, *N*-[(2-hydroxy-3-trimethylammonium) propyl] chitosan chloride (HTCC), also shows antiviral activity upon contact.[74] Other non-leaching virucidal polymers are prepared by covalent linking of quaternary phosphonium groups to a cationic polyacrylamide derivative. This results in a promising material for application in papermaking to produce hygiene products and in water purification with the ability to kill highly resistant non-enveloped adenovirus (ADV).[63]

In the second group of release-killing polymers, diverse disinfectant agents are encapsulated within the polymer matrix. For instance, chlorine dioxide (ClO_2) is a potent antimicrobial agent

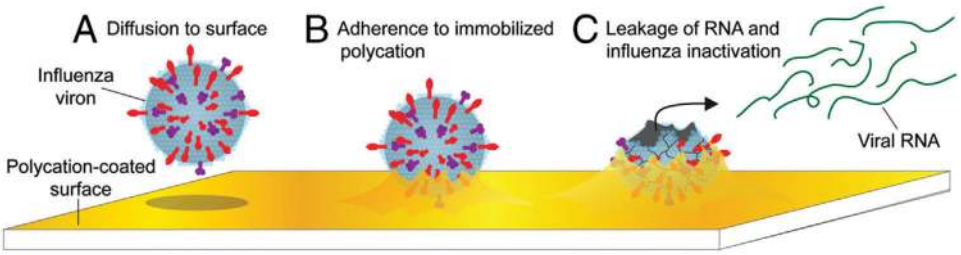

Figure 5. Possible mechanism of virus inactivation in polycationic PEI derivative coatings. Reproduced with permission.[71]).

that was encapsulated in micelles of pluronic P123 and pluronic F127, which are also modified with CuNP to impart stability and additional contact-killing behavior, to develop smart functional materials against several pathogens including H1N1 influenza virus.[66] The micelles reduce undesired evaporation of ClO_2 and enable its sustained release over 15 days.

Metal ions or nanoparticles and metal oxide nanoparticles have also been reported to impart virucidal properties to polymers. For instance, silver ions were incorporated in renewable polylactide (PLA) films and evaluated both in vitro and in contact with food against *Salmonella* and a human norovirus surrogate.[67] Migration of the silver ions from the film was evaluated after several washing cycles, confirming a good long-term antiviral activity in films loaded with 1% Ag^+. Silver can be also incorporated in the form of nanoparticles, for instance, homogeneously adsorbed on chitin nanofibers to produce functional wound dressings that are highly effective against H1N1 influenza A virus.[68] All these strategies that were successful to develop contact-killing or release-killing materials against a wide variety of viruses could be explored or serve as models to develop virucidal materials helpful against SARS-CoV-2.

2.2. Nanotechnology-Enabled PPE and Filter Systems

A special concern about COVID-19 surrounds the question of aerial infection via circulation of SARS-CoV-2 in aerosols.[10–12] There are empirical observations that in communities with obligation to wearing face masks (e.g., Hong Kong), the COVID-19 outbreak is significantly lower than in mask-off countries (e.g., Italy, Germany, US, Brazil, etc., at the time of writing that study[75]), which has been ascribed to the reduced transmission of virion-laden respiratory droplets.[75] This lays the focus on the filter efficiency of face masks and fabrics. Konda et al.[76] tested several common fabrics from cotton, silk, flannel, chiffon, and synthetic textiles for filter efficiency toward <300 and >300 nm aerosols. Interestingly, the combination of fabric layers affording mechanical filtration (cotton) and electrostatic filtration (silk, chiffon, flannel) showed >90% efficiency for <300 nm aerosols, albeit at much lower pressure drops and air flows than typically used in such tests. Nevertheless, this result may hint at the potential of natural fibers in wider PPE applications and may open a new research line for biodegradable PPE materials to mitigate the huge plastic disposal common to conventional protective gear. Functionalized cellulose fibers and other biopolymers are promising candidates for antimicrobial PPE.[77] An alternative approach to improve PPE fabrics was explored by Bhattacharjee et al.,[53] who reviewed graphene as functional additive for lending mechanical, antibacterial, barrier, UV protective, fire retardant, light-weight, and conductive properties to a wide range of woven and non-woven textiles used in PPEs.

Another crucial issue in pandemics caused by respiratory viruses is their transmission through ventilation systems, for instance, in hospitals, care homes, ambulances, aircraft cabins, or commercial buildings.[78] Also here, due to the small size of virus particles (50–130 nm), nanotechnology can provide solutions with ultra-fine glass fiber air filters (ultra low penetration air [ULPA] filter) with up to 99.99999% filter efficiency for 0.1 µm particles.[79,80] High-efficiency particle air (HEPA) filters are commonly employed in buildings and transportation and show high efficiency for larger (>0.3 µm) particles,[79] the typical size range of respiratory droplets.[78] These filters can also be modified with photocatalytic TiO_2 NP for combined filtration and destruction of retained microbes.[79] On the other hand, activated carbon in the form of granulated powder or fibers has been successfully tested for adsorption of bacteria and virus particles in conjunction with biocidal Ag and CuO NP for enhanced efficiency.[81]

Air filter may also be equipped with antimicrobial Ag, Ag-hybrid, or Zn-MOF nanoparticles for in situ inactivation of filtered pathogens, thus, minimizing the risk from filter handling.[57,82,83] Like air filters, water treatment membranes play an important role in disinfection of bacteria and virus contaminated hospital effluents and drinking water. Advanced filter systems may contain antimicrobial metal nanoparticles (Ag, Cu, CuO, Zn, etc.)[84,85] but also other nanomaterials like carbon nanotubes, graphene, or silver nanowires that enable physical disinfection methods like pulsed electric fields (PEF) leading to cell death by electroporation (**Figure 6**A).[86,87] In the latter case, the nanoscopic materials enhance the electrical field strength by the antenna effect, which makes inactivation of bacteria or viruses more effective. In view of the potential adversities of leached metal nanoparticles, several filter systems use antimicrobial chemical compounds adhered to the surface of electrospun nanofiber mats, for instance, N-halamine compounds against aerosolized influenza A virus[88,89] or benzophenones and polyphenols that produced day-light induced biocidal reactive oxygen species (ROS) species on filter and PPE fabrics against T7 bacteriophage, a non-enveloped double-stranded DNA virus (Figure 6B).[90] ROS species are created by light-induced excitation of electrons, which in turn lead to the generation of superoxide radicals ($^{\bullet}O_2^-$) and hydroperoxide radicals ($^{\bullet}HO_2$) from reaction with oxygen and hydroxyl ($^{\bullet}OH$) radicals from reaction with water.[91]

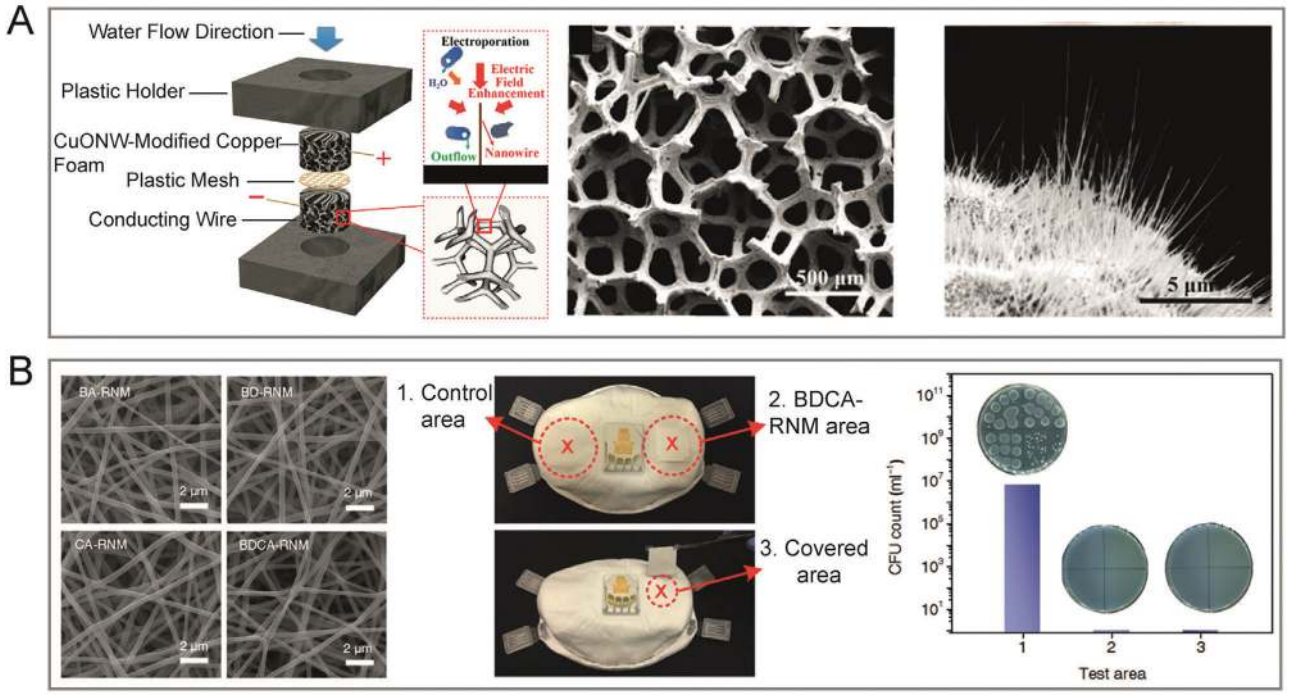

Figure 6. A) Electroporation–disinfection cells (EDCs) for water sterilization with copper oxide nanowire (CuONW)-modified copper foam electrodes. Reproduced with permission.[86] Copyright 2016, American Chemical Society. B) Electrospun antiviral filter based on ROS species production from benzophenone and polyphenol compounds and the bactericide effect on face masks. Reproduced under the terms of the CC BY-NC 4.0 license.[90] Copyright The Authors, some rights reserved; exclusive licensee American Association for the Advancement of Science. Distributed under a Creative Commons Attribution NonCommercial License 4.0 (CC BY-NC) http://creativecommons.org/licenses/by-nc/4.0/.

2.3. Interaction of Viral Particles with Functional Nanomaterials

2.3.1. Antiviral Semi-Conductors and Photoactive Compounds

Semi-conductors are an interesting class of materials for the fight against viral infections as they can produce virus-killing radicals through interaction with light. This process is commonly referred to as photodynamic inhibition (PDI) of viruses and other microbes and is enabled by both inorganic semi-conducting nanoparticles and organic photosensitizing (PS) compounds.[92] A common feature in both groups is the light-induced generation of ROS species.[91] These interactions may potentially damage viral components like the membrane, proteins, and DNA/RNA (**Figure 7**).

The fact that light, oxygen, and the photoactive agent need to be present at the same time and at the same locus makes PDI prone for surface (nosocomial, streets, pavements, banknotes, door handles, etc.) or liquid (wastewater, blood plasma, beverages, etc.) treatments. Organic PS compounds that display photodynamic inhibition over a wide range of viruses including coronaviruses are curcumins against feline coronavirus,[93] psoralen derivatives against SARS-CoV[94] and MERS-CoV,[95] respectively, and riboflavins against MERS-CoV.[96] These compounds were tested in blood plasma treatment for the security of transfusions against bloodborne viruses. The organic photosensitizers are also often conjugated with inorganic nanoparticles like silica, Au, or TiO_2 that act as carriers or co-actives, respectively.[97,98]

Inorganic photosensitizers like semi-conducting TiO_2, ZnO, SnO_2, inorganic azide and iodide salts, CdS and CdSe/ZnS quantum dots (QD), and graphitic carbon nitride (g-C_3N_4) exhibit strong photocatalytic properties with biocide effects on germs, bacteria, fungi, and viruses.[92,99–101] The target applications of these compounds are two-fold; disinfection of wastewater from waterborne viruses (e.g., poliovirus, hepatitis viruses A and E, coronavirus)[102] and impregnation of solid and textile surfaces

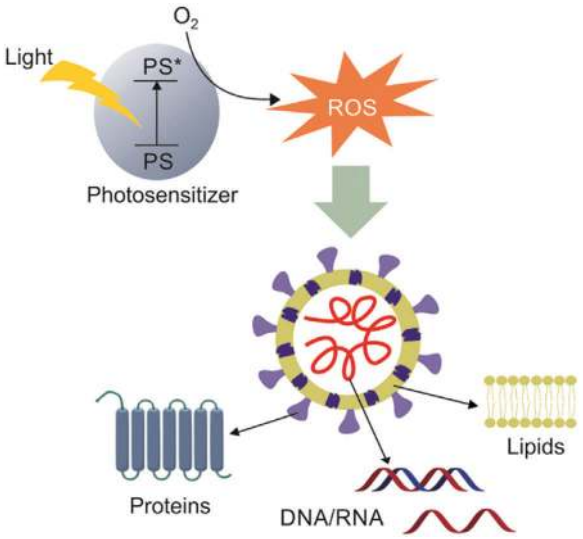

Figure 7. Photodynamic inhibition of viruses by reactive oxygen species (ROS) induced damage of the membrane, proteins and DBA/RNA (design based on Figure 5 in ref. [92]).

for self-sanitation,[103,104] respectively. In the former case, for instance, SnO_2 NP were encapsulated in a zeolitic imidazole framework and showed a 50–80% efficiency of photocatalytic reduction of chikungunya virus titer in water.[105] With respect to the recent COVID-19 outbreak, Barcelo[106] commented on the detection of SARS-CoV-2 in feces that remained active for 4–22 days and the possibility of fecal transmission to wastewater. It is known from other enveloped human viruses that they can survive for a number of days in human sewage,[107] which makes its effective disinfection all the more important to close down an alternative viral transfection route in addition to fomites and aerosols.

Self-sanitizing surfaces are another focal point in the prevention of viral transmission. The case of self-sterilization of the streets of Milan in the wake of the COVID-19 pandemic with a TiO_2/Ag mixture has caught media attention.[108] Yet, the fate of the deployed photocatalytic nanoparticles remains questionable, likely getting washed out over time into water bodies with all entailed environmental consequences. Therefore, photoactive nanoparticles also need to be immobilized firmly on surfaces and protected against lixiviation, which can be achieved by physical entrapment in membranes or matrices or by chemical attachment on coatings among other possibilities (see Sections 2.1.3 and 2.1.4). In addition, semi-conductors like TiO_2 and ZnO are also frequently doped with heteroatoms or conjugated with other metal NP like Ag or oxides like WO_3 and NiO for broadband light sensitivity and enhanced photodynamic inhibition.[91]

Zinc oxide and TiO_2 nanoparticles were also shown to be of therapeutic use in the treatment of viral infections. For instance, TiO_2 inactivated H3N2 influenza virus by direct contact with destruction of the viral membrane in the absence of light, suggesting that PDI is not involved in this mechanism.[109] On the other hand, bare and PEGylated ZnO NP inhibited the proliferation of H1N1 influenza virus in infected cells by a mechanism that is possibly related to dissolution of Zn^{2+} ions.[110] There are currently no comparable observations with ZnO NP reported for SARS-CoV-2, but similar inhibition effects are to be expected. Especially, since the role of zinc ions in antiviral immunity to many viruses including SARS-CoV-1 coronavirus is known[111,112] and new evidence concerning Zn^{2+}-induced RNA polymerase inhibition in SARS-CoV-2 is currently emerging.[113] The summary of the above-presented findings points to a potential dual use of photocatalytic ZnO and TiO_2 NP both as facilitating self-sanitizing surfaces and as antiviral agent interfering with the viral replication cycle.

2.3.2. Interaction of Viral Particles with Carbon-Based Materials

Nanotechnology counts with an extraordinary cast of carbon nanoparticles that can also be modified to further increase their potential use as functional nanomaterials.[114] They are functionalized by assembly to metal nanoparticles or polymers via surface functional groups such as hydroxyls, lactones and carboxylic acids among others.[115] There are selected examples of viral particle interactions with different types of carbonaceous nanomaterials like activated carbons, carbon (quantum) dots (CD), nanodiamonds (ND), multiwall or singlewall carbon nanotubes (MWCNT or SWCNT), graphene, and graphene oxide (GO). Their potential applications against the COVID-19 disease range from the removal of viral particles from air or water to efficient antiviral agents through diverse virucidal mechanisms.

Activated carbon materials can play an important role in the current COVID-19 pandemic due to their elevated capacity to retain viruses. Commercially available powdered activated carbons remove viral particles by entrapment in their nanopores as well as by hydrophobic interactions with the virus surface.[116]Activated carbons are applied in water purification by filtration and adsorption processes, which is a safe and efficient method for the elimination of diverse pathogens including viruses.[116–118] Considering the ability of SARS-CoV-2 to spread through aqueous media, these results represent a good approach for its controlled removal. As mentioned above (see Section 2.2), activated carbon can complement both HEPA air filters and face masks, which can be useful to capture SARS-CoV-2 viral particles from the atmosphere in closed rooms. Further studies are required to design the specific pore size distribution to fit the dimensions of coronaviruses with the appropriate carbon adsorption sites.

On the other hand, CD are another member of the carbon nanoparticles family (approximately 10 nm in diameter) that have an extraordinarily high surface-to-volume ratio, high ability to form stable and homogeneous water dispersions, as well as the ability to assemble to other nanomaterials useful to establish different strategies in the fight against viruses. The role of these carbon materials as antiviral agents can be based on different mechanisms interfering with the viral replication cycle. In this context, earlier studies demonstrated that CD can inhibit viral replication by activation of the interferon response for the pseudorabies virus(PRV) as well as for the PRRS virus (DNA and RNA viruses, respectively).[119] Uniform and stable cationic CD prepared from curcumin exhibit antiviral properties against coronaviruses, like the porcine epidemic diarrhea virus (PEDV). Ting and co-workers[120] reported that this type of CD significantly inhibits PEDV viral entry, the synthesis of negative-strand RNA, and the budding of these viruses. Hence, these results are of potential interest in the fight against other coronaviruses such as SARS-CoV-2. Loczechin and co-workers reported on a tenfold enhancement of the antiviral response to the human coronavirus 229E (HCoV-229E) , responsible for mild respiratory infection, by incorporating 4-aminophenylboronic acid and phenylboronic acid groups via click chemistry on the carbon surface (**Figure 8**).[121] The mechanism of action of these functionalized CD is attributed to the interaction of the boronic acid functions with the S protein of HCoV-229E. Similar values of inhibition activity were observed at the viral replication step. Nevertheless, the mechanism involved in this case still remains unclear.[121] Nevertheless, this approach could be valuable to be extrapolated to other coronaviruses, such as SARS-CoV-2.

Nanodiamonds interact with enveloped viruses, such as influenza A and B viruses, leading to adsorption processes even in a more effective manner than MWCNT, as reported by Ivanova and co-workers.[122] These nanocarbon allotropes also show the possibility to introduce boronic functions by anchorage reactions on these carbon nanoparticles,[123] and therefore, it would be possible to use ND for inhibition of viral replication of SARS-CoV-2 and potential new therapeutics for COVID-19.

Different carbon nanomaterials have been studied as nanocarriers for a variety of drugs applied in therapy for several

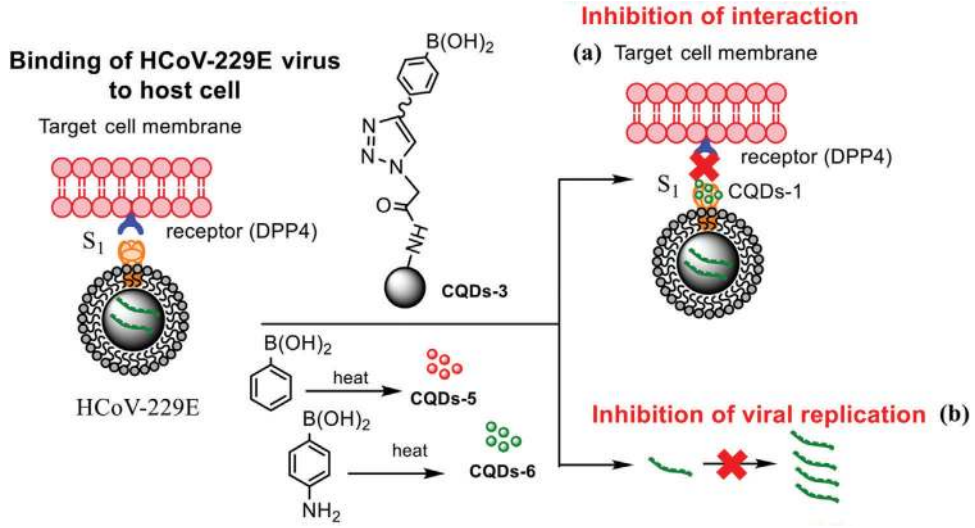

Figure 8. Scheme of carbon dots functionalized with boronic functions indicating the inhibition of protein S and viral RNA genome replication in experiments with HCoV-229E coronavirus. Reproduced with permission,[121] https://pubs.acs.org/doi/10.1021/acsami.9b15032. Copyright 2019, American Chemical Society.

diseases, although still scarcely in viral infections.[124] For instance, they can boost the antiretroviral therapy against the human immunodeficiency virus (HIV), for example, graphene QD and oxidized MWCNT combined with several retroviral molecules such as CHI499, CDF119, CHI360, and CHI415,[125,126] which represent good alternatives as efficient viral treatments with potential application to coronaviruses.

Surprisingly, bare carbons without coupled drugs or inhibition agents have been described in literature for the use of virus blockage. For instance, differences in physical characteristics, such as size, charge, and stacking, produce changes in the antiviral activity of graphite, GO, and GO functionalized with polymers, which inactivate the virus prior to the entrance into the cell.[127] However, there are still significant points that need to be clarified to better understand the mechanisms of action in order to extend the application of these carbonaceous nanomaterials to other viral infections, including the COVID-19 disease.

The antiviral properties of GO and other related nanomaterials can also be useful in protective PPE clothes. Herein, textiles are functionalized with nanocomposites based on GO and different polymers such as polyester, cotton, and polyamide.[53] Although this technology is promising, the scale-up, however, at this moment shows important handicaps for commercial production. In the same context, there are intelligent paints capable to deliver potent disinfectants, like peracetic acid from a composite of MWCNT and perhydrolase with polyethylene glycol, reaching 99.99% of quick viral inactivation when tested with influenza viruses.[128] This represents a promising option for application in SARS-CoV-2 disinfection processes.

Another approach potentially useful in the design of new vectors for specific antiviral drug delivery in the context of COVID-19 could be based on carbon NP assembled to materials that are easily functionalizable. These materials in turn can serve as additional anchor sites for antiviral compounds. This is the case of MWCNT and graphene nanoplatelets (GNP), which can be assembled to micro- or nano-fibrous sepiolite leading to hybrid materials capable of incorporating diverse molecular species and biopolymers.[129–131]

3. Nanomaterials in Detection Technologies for SARS-CoV-2 in the COVID-19 Diagnosis

A wide variety of technologies is available as diagnostic tools in the fight against SARS-CoV-2, including nucleic acid tests mainly based on polymerase chain reaction (PCR), and serological assays that can detect the presence of antibodies produced during the respiratory infection.[132–135] Diagnosis in the early stages of the disease mainly focuses on viral genome detection by real-time (RT)-PCR, while the determination of immuoglobulin (Ig) G and IgM antibody levels starts after 5–7 days or more than 10 days, respectively, by means of serological tests such as enzyme-linked immunosorbent assay, chemiluminescence assay, immunofluorescence assay, or immunochromatographic test (ICT), among others.[133–135] Nanomaterials are an important component in some of these technologies, being a key factor in the detection or transduction of the biochemical interactions as detailed below. Recently, a rapid and reliable colorimetric bioassay has been developed by modifying plasmonic gold nanoparticles (AuNP) with designed antisense oligonucleotides specific for two of the N-gene regions of SARS-CoV-2.[136] The nanoparticles agglomerate in the presence of the target viral RNA allowing a naked-eye detection in about 10 min.

Typically, nanoparticles are also the detection components in ICT, also known as lateral flow immunoassays (LFIA), which are mainly applied for detection of antigens or antibodies. These rapid point-of-care tests can be very valuable for diagnosis when laboratory facilities are not available, as they are easy to use, do not require trained staff, operate with small amounts of sample (around 10–20 µL), and provide a result in typically less than 20 min.[133,137] Since the beginning of the pandemic, a large number of rapid tests have been developed by research groups and in vitro diagnostics companies for detection of IgM and IgG

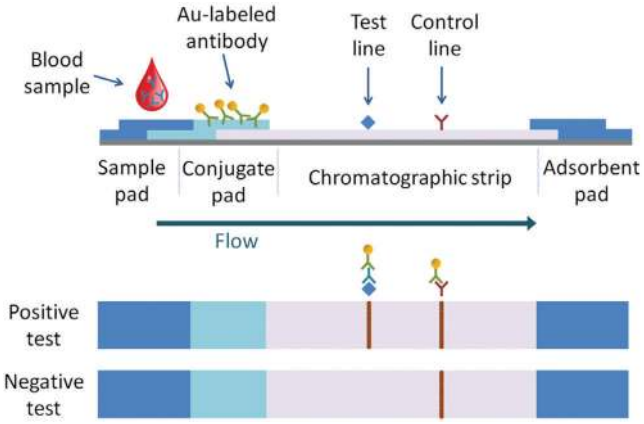

Figure 9. Schematic representation of an immunochromatographic test for detection of antibodies in blood or serum samples, using AuNP as label for direct visualization.

antibodies in patients with COVID-19. Intense research continues aiming at increasing the sensitivity and specificity of these diagnostic tools.[137–142] As schematized in **Figure 9**, a typical ICT configuration for detection of IgG and IgM antibodies against SARS-CoV-2 consists of: i) a sample pad, where the sample and buffer are added; ii) the conjugate pad containing the antibodies or antigens labeled with colloidal AuNP (diameter around 20–40 nm); iii) the chromatographic strip, which is a porous polymer membrane, where the captured biomolecules are immobilized in the test line and a suitable antibody in the control line; and iv) the liquid adsorbent pad. The Au-labeled molecules bind to the antibodies present in the sample, and are dragged through the chromatographic strip by capillary action, reaching the test and control lines, where they concentrate developing a color that can be seen with the naked eye. The absence of color in the test line indicates the absence of the target antibodies in the blood sample. Synthetic antigens of the S, M, and N proteins of SARS-CoV-2 are immobilized in the test line to detect IgM and IgG specific to this coronavirus.[138–140] Li and co-workers[141] reported a new ICT configuration with two test lines that can determine simultaneously IgM and IgG antibodies in the same test within 15 min, with sensitivity of 88.7% and specificity of 90.6% evaluated in blood samples from both PCR-confirmed COVID-19 patients and negative patients.

Compared to the large amount of ICT developed for serological assays, a smaller number of ICT are produced for direct viral antigen detection. A recent work reports the development of a half strip LFIA for detection of SARS-CoV-2 antigen.[143] This is a simple configuration used in assay development that comprises only the chromatographic strip. In this case, red latex beads (400 nm) conjugated to polyclonal antibodies and blue latex beads (400 nm) conjugated to an antibody are used for the test and control lines, respectively. After 20 min, the appearance of color is observed with the naked eye or with an optical reader for semi-quantitative determination. The advantage of this type of ICT is that detection of the SARS-CoV-2 antigen can provide information in the early stages of the disease, but the specificity and sensitivity of these tests still need to be improved to become a suitable alternative to RT-PCR.

Immunochromatographic tests can also be configured to detect nucleic acids. Broughton and co-workers[144] reported the detection of viral RNA extracts from nasopharyngeal swabs using a highly specific Cas12 protein in CRISPR, a powerful gene-editing tool, combined with lateral flow assay using AuNP as label. The specificity for SARS-CoV-2 detection with this system is very high, with no cross-reactivity for related coronavirus strains. Similarly, a portable integrated microdevice combining RT-PCR and ICT was reported some years ago for the genetic analysis of influenza A H1N1 virus by colorimetric detection,[145] and such technology could also be useful at present for SARS-CoV-2 detection.

Intense research is being carried out to improve the reliability and sensitivity of these rapid tests for SARS-CoV-2. Fluorescence-based ICT are being developed for quantitative or semi-quantitative detection, using fluorescent labels instead of AuNP. A recent work makes use of lanthanide-doped polystyrene nanoparticles, and detection of fluorescence is carried out in a portable fluorescence reader with good agreement with results obtained by RT-PCR.[142] Once an anti-SARS-CoV-2 IgG standard is available, this rapid test could be optimized to provide accurate quantification instead of the current semi-quantitative detection.

Several biosensor platforms have been reported to date as diagnostic tools for SARS-CoV-2. Biosensors are devices that make use of specific biochemical reactions and their conversion into a measurable readout in the form of electrical, thermal, or optical signals by means of a transducer.[146] The main parameters in the design of biosensor platforms for SARS-CoV-2 detection involve the target analyte (viral RNA, antigens or antibodies), the receptor, which are nucleic acid probes, antibodies or other biomolecules that will interact specifically with the target analyte, and the transducer that will monitor this specific interaction.[147]

Seo and co-workers have recently reported a field-effect transistor (FET)-based biosensor platform that provides rapid detection of SARS-CoV-2.[148] This biosensor consists of graphene nanomaterial as the transducer or sensing material, and a SARS-CoV-2 spike antibody that was used as the receptor biomolecule immobilized on the graphene layer (**Figure 10**). The biosensor performance was evaluated in clinical samples of COVID-19 patients using antigen protein, cultured virus, and nasopharyngeal swab specimens, allowing a rapid and highly responsive detection of SARS-CoV-2. It also showed high specificity, distinguishing the SARS-CoV-2 antigen protein from those of MERS-CoV.

Surface plasmon resonance (SPR)-based biosensor platforms make use of this highly sensitive optical technique, which detects the changes in refractive index occurring at the metal interface, allowing to monitor the biochemical interactions in real time. Based on the successful performance of SPR-based biosensors developed for SARS-CoV detection,[149,150] an improved device has been recently reported for SARS-CoV-2.[5] This is a dual-functional plasmonic photothermal (PPT) biosensor that combines the PPT effect and localized surface plasmon resonance. It consists of 2D gold nanoislands functionalized with complementary DNA receptors that can hybridize selected sequences from SARS-CoV-2. The hybridization temperature is increased in situ with the thermoplasmonic heat generated by illumination of the gold nanoislands at their plasmonic resonance frequency, which helps to discriminate similar gene sequences and to increase the specificity of the biosensor.

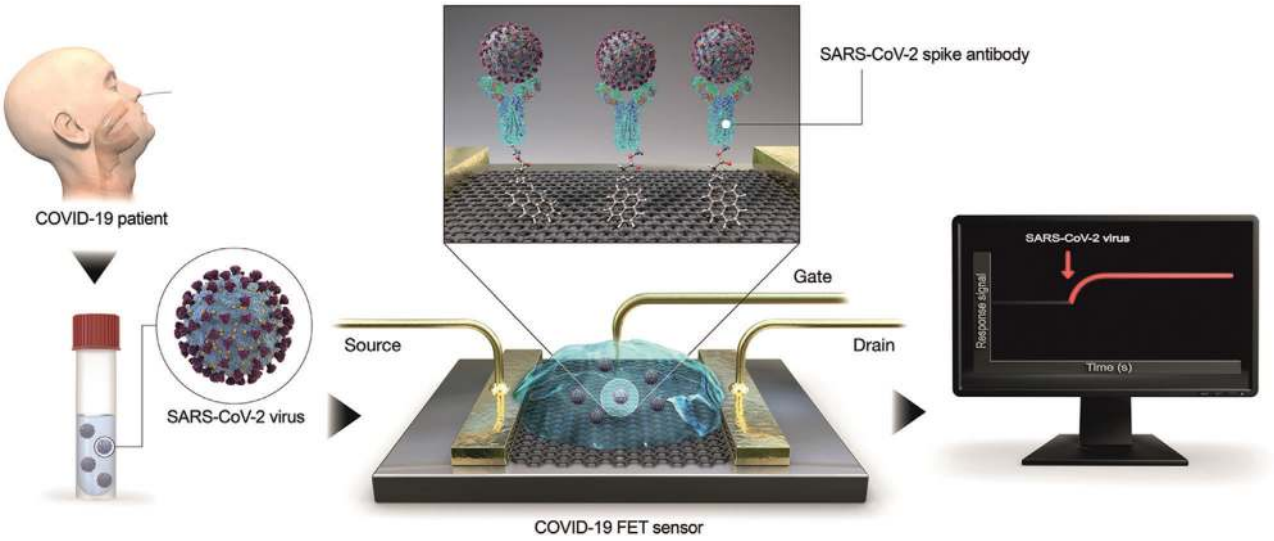

Figure 10. Schematic diagram of COVID-19 FET sensor operation procedure, showing the SARS-CoV-2 spike antibody conjugated onto the graphene sheet used as sensing material. Conjugation is carried out by means of 1-pyrenebutyric acid *N*-hydroxysuccinimide ester as a probe linker. Reproduced with permission,[148] https://pubs.acs.org/doi/10.1021/acsnano.0c02823. Copyright 2020, American Chemical Society.

Together with FET- and SPR-based devices, other biosensor designs involving nanomaterials were reported for detection of other coronaviruses, as for instance, electrochemical immunosensors based on an array of AuNP-modified carbon electrodes for detection of MERS-CoV[151] or an optical biosensor chip modified with QD-conjugated RNA aptamer for detection of SARS-CoV.[152] Other alternatives for future biosensors developments for detection of SARS-CoV-2 could be based on the use of biomimetic nanoarchitectures as Wicklein and co-workers applied for the impedimetric detection of influenza A virus.[153] In this case, sialic acid-galactose receptor molecules mimicking those found on the membrane of target cells of the virus act as the sensing entity, which can be transduced by the impedimetric gold detector.

The above-mentioned designs can open ways to the development of new biosensor platforms for rapid, sensitive, and specific detection of SARS-CoV-2.

4. COVID-19 Therapeutics: Antiviral Treatments and Vaccines

4.1. Antiviral Treatments for COVID-19

4.1.1. Virucidal Nanoparticles and Metal Cations in COVID-19 Therapy

Nanoparticles can make an effective contribution in the fight against the COVID-19 pandemic in all four key areas of action,[154] that is, point-of-care detection, surveillance and monitoring, COVID-19 therapeutics, and SARS-CoV-2 vaccines. The broad applicability of inorganic nanoparticles in these areas relies on their wide range of chemical composition, size and shape, biocompatibility, adjustable biological and physical (functional) properties, and ease of production.[155,156] Certain nanoparticles also display antiviral activity against a range of viruses like influenza, herpes, hepatitis, or HIV. The virucidity of nanoparticles depends first on their physical properties like small size, high specific surface area, and surface charge, which enable membrane penetration, high antiviral payload uptake, and binding, respectively. Second, they possess biomimetic properties that are important for binding to viral particles or host cells. Third, nanoparticles may encapsulate antiviral actives and release the payload at the desired site, which improve dosing, bioavailability, circulation time, and drug stability.[155] Organic nanoparticles including liposomes, dendrimers, and polymer (polylactic acid, polyglycolides, etc.) are the most researched antiviral nanomaterials to date, and some formulations are already approved for clinical use against influenza, HIV, and HBV.[155] On the other hand, inorganic nanoparticles like transition metal NP (e.g., Ag, Cu, Zn), metal oxides (e.g., Fe(II), TiO_2, ZnO_2, ZrO_2), and QD (e.g., CdTe, carbon, GO) as well as metal cations (e.g., Ag^+, Cu^{2+}, Zn^{2+}) have shown intrinsic virucidal activity and are currently researched intensely, yet much of the understanding of the underlying mechanism is still in its infancy.[156] What is common to most employed nanoparticles is that they interfere in one or more of the four stages of the viral life cycle, that is, binding to a host cell, internalization, replication inside the cell, and release (budding). For instance, the key mechanism of silver and copper is the release of Ag^+ and Cu^{2+} cations that can damage the viral genome (e.g., stage 3) and may also provoke viral membrane disruption.[155] The exact mechanism depends greatly on the virus. Concerning the applicability against SARS-CoV-2, there are promising results of nanoparticles against related coronaviruses like the porcine epidemic diarrhea virus (PEDV)[157,158] (**Figure 11**). On the other hand, zinc, silver, and copper cations also showed antiviral activity against different coronaviruses with characteristics similar to SARS-CoV-2.[112,159] Velthuis et al.[112] showed that intracellular zinc ions can inhibit the RNA polymerase function in cell cultured SARS-CoV, which is a vital process in the replication step of various RNA viruses, including influenza virus, respiratory syncytial virus, and several picornaviruses. Zinc ions were shuttled into the cells with the help

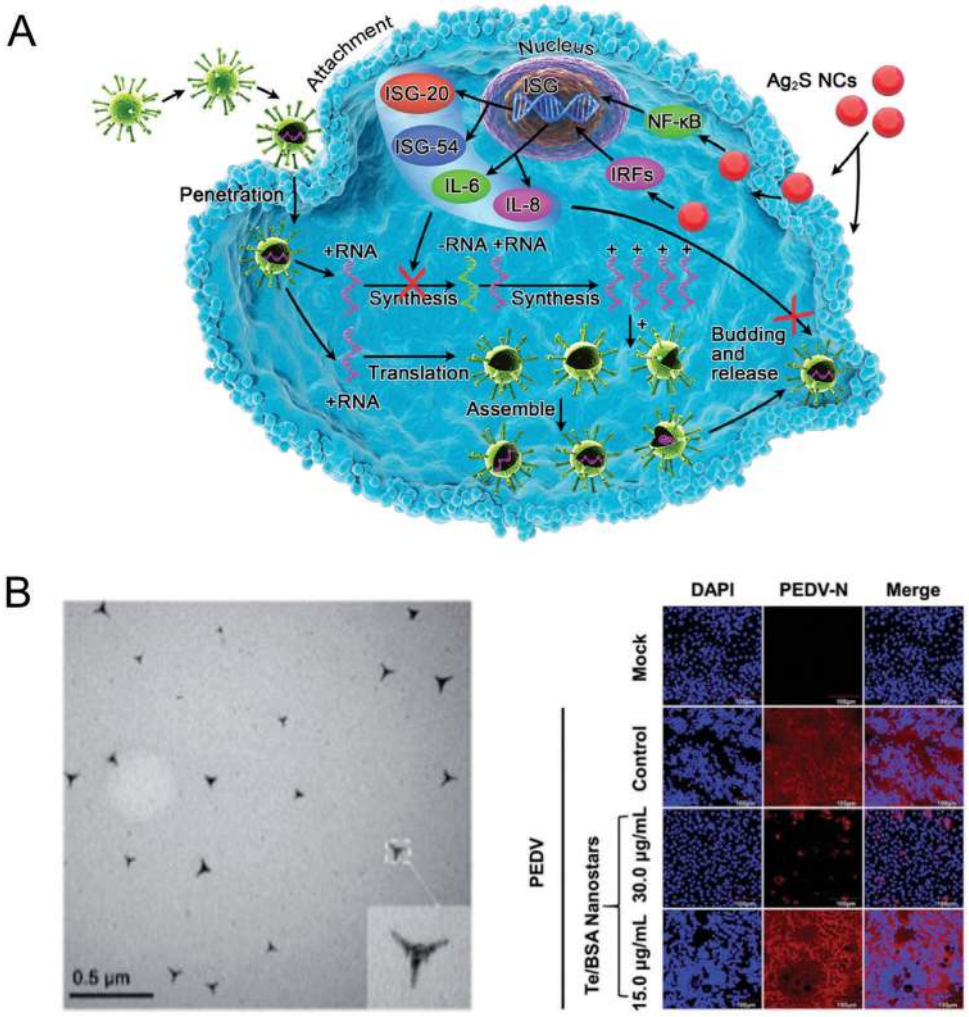

Figure 11. A) Possible mechanisms of the antiviral activity of Ag_2S nanocrystals. Reproduced with permission.[157] Copyright 2018, American Chemical Society. B) TEM image of Te nanostars and the antiviral effect of Te/BSA nanostars on PEDV in Vero cells. Reproduced under the terms of CC BY-NC 3.0 license.[158] Copyright 2020, The Royal Society of Chemistry.

of ionophores. It could be imagined that this service is provided by nanoparticle carriers like sepiolite nanoclay that are efficient cellular vectors[160] with high loading capacity for Zn and Cu cations.[161]

4.1.2. Antiviral Activity of Polymers and Their Derivatives Against Viruses

Among the antiviral agents aimed to prevent or treat infections especially relevant in the current pandemic due to SARS-CoV-2, there are several natural and synthetic polymer compounds with virucidal properties. For example, the biopolymer chitosan has been reported to induce resistance against diseases caused by different mosaic viruses in plants, or to stimulate the immune response to viral antigens in animals, most likely by inducing interferon production.[162] Along with natural polymers, a variety of synthetic polymers have been shown to be effective against the Marburg virus, SARS-CoV, HIV, and other viruses. Especially, poly(vinylphosphonic acid) shows an outstanding antiviral activity against SARS-CoV.[163] In certain cases, the antiviral properties are developed or enhanced after appropriate modification of the polymer, for instance, enhancing its net positive charge[164] or incorporating sulfate groups.[165] Other functional groups that provide polymers with antiviral properties are quaternary ammonium groups, sialyl groups, and certain peptides. For instance, HTCC is able to inhibit the common cold pathogen HCoV-NL63, most likely through interaction with the S protein, which is critical for the coronavirus entry.[166] The experimental research in the development of antiviral agents against SARS-CoV-2 is also supported by theoretical studies and computational tools that can help to find optimal formulations for prevention and treatment of the infection as recently reported by Parks and Smith.[167] Another recent contribution concerns a computational model of the HR1/2 regions of the surface spike protein of SARS-CoV-2 and the study of its binding energy with designed antiviral peptides by molecular dynamics simulation. The aim is to find the best peptide to competitively bind the host cell and block the virus entry.[168]

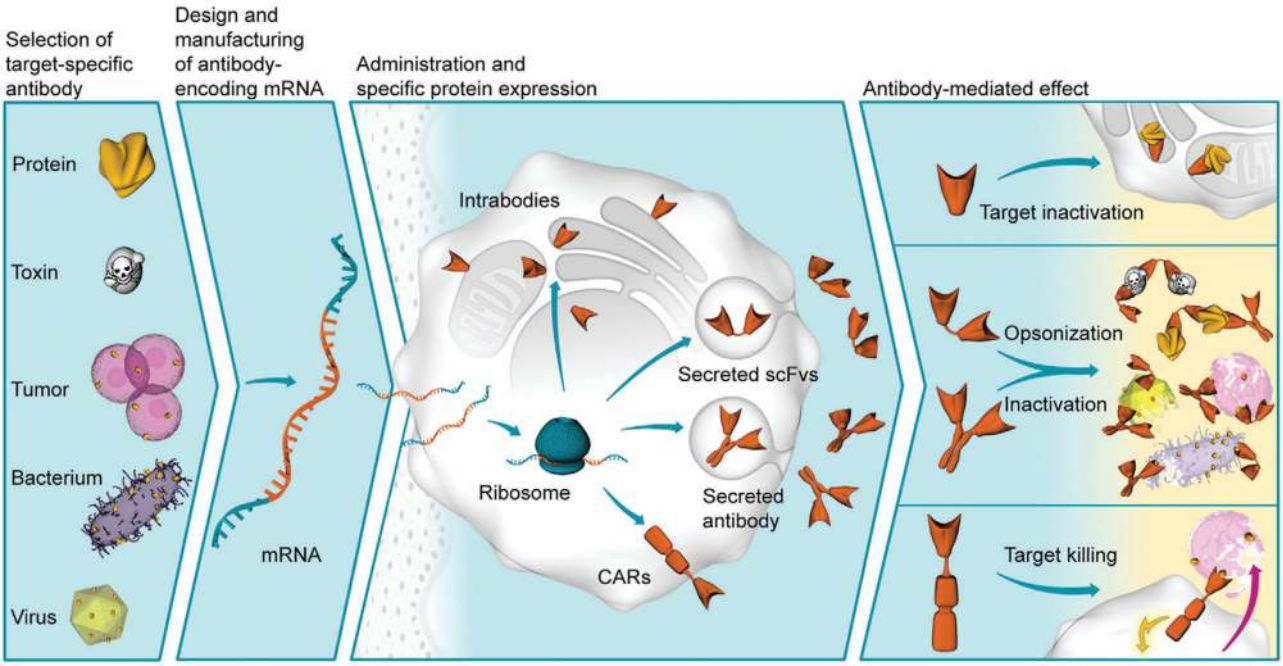

Figure 12. Scheme indicating the mechanism of mRNA-based antibody treatment. Reproduced with permission.[258] Copyright 2019, The American Society of Gene and Cell Therapy.

4.1.3. Nanotechnology and COVID-19 Therapeutics

Currently, there are no specific therapeutic agents licensed for COVID-19.[169] Most available treatments are supportive but do not efficiently stop the infection and eliminate the virus.[170] Therefore, there is an urgent need to identify and develop new efficient antiviral drugs that could reduce mortality and morbidity of COVID-19. The candidate agents include immunotherapies with monoclonal antibodies or convalescent sera, antiviral drugs such as protease inhibitors and nucleoside analogues, and immune mediators like Interferon β.[171] Some of them are broad antiviral drugs like remdesivir (used against the Ebola virus), and others are repurposed such as HIV protease inhibitors.[172,173] In this context, nanotechnology can offer extremely useful solutions to develop new therapeutic tools or help to improve the antiviral effect of candidate agents. In fact, novel antiviral drug delivery platforms benefit from the unique physicochemical properties of NP.[25] Nanoparticle delivery systems offer multiple pharmacologic advantages: i) protection of therapeutic compounds from enzyme degradation; ii) preservation of the native molecule conformation; iii) control of releasing kinetics; iv) co-delivery of different agents or adjuvants; v) specific cell/tissue/organ targeting of agents; vi) high concentration of the therapeutic compound; vii) acting as immunostimulator itself.[21]

One of the most promising therapeutic candidates against COVID-19, currently in clinical trials, consists of messenger RNAs (mRNAs) coding for neutralizing monoclonal antibodies (MAbs) against different epitopes of SARS-CoV-2, which are delivered directly to the mucosa of the respiratory tract and into the lungs of symptomatic patients via nebulizers with NP aerosols.[174] A similar approach was developed by another biotechnology company, but in this case the administration is done via intravenous infusion.[175]

This passive immunization approach, that is, the delivery of antibody-encoding mRNA (**Figure 12**), is especially indicated for diseases, for which there are no vaccines or effective drugs available such as for COVID-19. Similar to the administration of convalescent sera, the strategy of passive immunity provides immediate, short-term immunization achieved by the transfer of specific neutralizing antibodies.[176] Traditionally, mRNA has not been used as a therapeutic agent because it is highly unstable and activates the innate immune system when injected. In addition, to get into the target cells, mRNA requires a carrier system to cross the cell membrane. Therefore, investigators have designed a delivery system that consists of lipid nanoparticles (LNP) that encapsulate the mRNAs coding for the MAbs.[177] This RNA-therapy platform stabilizes the mRNA and can be administered repeatedly leading to sustained production of antibodies evading the effect of the innate immunity against exogenous RNA.[178] In addition, the LNPs enhance their mucosal and cellular uptake and improve their biocompatibility. Moreover, the positively charged LNP leads to electrostatic attraction to the negative charge of the mucosal membranes, reducing their clearance by the mucosal cilia.[12] Another mRNA-based therapeutic approach against coronaviruses is the use of siRNA. Since coronaviruses are positive-sense single-stranded RNA (ssRNA) viruses, RNA interference could be an efficient approach to control the virus by silencing the viral mRNA at particular stages of infection. The delivery of siRNAs was investigated for treating MERS-CoV and several formulations of LNPs have been evaluated.[179] LNPs protected siRNAs from RNases, improved their bioavailability, and delivered the compound to the target sites.[180]

Table 3. The four candidates of COVID-19 nanoparticle based vaccines in clinical evaluation (as of July 2020).

Platform	Type of vaccine	Developer	Clinical trial status
RNA	LNP encapsulated mRNA	Moderna/NIAID	Phase 3 (NCT04470427)
RNA	LNP encapsulated mRNA	BioNJTech/Fosum Pharma/Pfizer	Phase 1/2 (2020-001038-36) (NCT04368728)
RNA	LNP-nCoVsaRNA	Imperial College London	Phase 1 (ISRCTN17072692)
Protein subunit	Full length recombinant SARS-CoV-2 glycoprotein nanoparticle	Novavax	Phase ½ (NCT04368988)

In summary, the versatility of nanotechnology makes it an effective tool to design new or improve existing therapies against emerging infectious diseases like the COVID-19 pandemic.

4.2. Nanotechnology and COVID-19 Vaccines

Preliminary epidemiological data from COVID-19 pandemics in several countries reveal that the seroprevalence of the population is quite low due to strict measures of confinement, ranging from 5–15% after more than 4 months since the advent of the pandemic.[181] Assuming that all of the seropositive individuals are immune resistant to the coronavirus,[182] still 85–95% of population would be susceptible to infection and vulnerable to the disease. Therefore, an effective vaccine is essential to achieve immunity in 60–70% of the population to the virus in order to reach the desired "herd immunity" and control of the pandemic. Additional tools to contribute to the control of the pandemic such as specific therapeutic agents are not available yet, and in the meantime, drugs developed for other diseases are being used with variable efficacy.

Vaccination is the most successful approach to infectious diseases prevention and control. Currently, there are no specific vaccines against SARS-CoV-2, but since the onset of the outbreak in December 2019 and the fast propagation of the virus around the world, the scientific community, biotechnology companies, and global health authorities have fueled the development of a vaccine against the SARS-CoV-2.

The fundamental goals of a COVID-19 vaccine are the prevention of severe illness and the spread of virus at the population level. It is generally accepted that protection will largely come from neutralizing antibodies, which primarily prevent viruses from entering cells by blocking the interaction between the receptor binding domain (RBD) of the SARS-CoV-2 spike protein with the ACE receptors on the membrane of target cells. However, other researchers emphasize the role of T cells in the protective immune responses by clearing infected cells. Probably, the best approach for an effective vaccine is having a balance of antibody and T cell responses. Apart from the immunological aspects, the ideal vaccine should also take into account other essential posologic and logistic factors: easy to administer, preferably oral or intranasal and in a single dose, easy and fast to produce and scale-up, and long-term stability at room temperature to facilitate storage and transport in non-developed countries.

Vaccine development usually takes decades. However, the development of vaccine candidates against COVID-19 is being accelerated immensely. Nevertheless, for instance, there are still no licensed vaccines for previous epidemic diseases caused by other coronaviruses (SARS-CoV in 2002 and MERS-CoV in 2012) despite strong initial efforts.

Some health organizations such as the WHO maintain an overview of the global landscape of vaccines against COVID-19. To date, there are more than 240 candidate vaccines (as of July 2020) at different stages of development, including nucleic acid (mRNA and DNA) vaccines, inactivated or attenuated virus vaccines, replicating or non-replicating viral-based vaccines, and autologous dendritic cell-based vaccines. Out of 14 candidates in clinical trials, four of them are based on nanotechnology (**Table 3**) and have recently published promising results in terms of safety and immunogenicity.[183–186] Many of these vaccine platforms are based on next-generation approaches and are not licensed, but the positive experience in other fields such as oncology motivates manufacturers to speed up their development.

These platforms are formulated as nanoarchitectured particles that allow antigens to be properly exposed to the immune system, to be protected from proteases and nucleases, or to associate with other compounds with adjuvant activity.[25] Nanotechnological solutions are also applicable to vaccines to ensure high thermostability in order to enhance vaccine distribution and availability.[31] Their low-scale composition can also influence uptake and localization in desired types of cells or tissues that favor the induction of optimal protective immune responses against the pathogen.[18,187] **Table 4** shows diverse platforms and nanotechnology approaches for COVID-19 vaccines currently under investigation.

The use of LNP is one of the most promising vaccine platforms. LNPs are highly efficient in encapsulating DNA- or RNA-based immunogens or antibodies by using a microfluidic mixer.[188] At least ten vaccine prototypes make use of LNP among the global landscape of vaccines of the WHO.

The use of LNP has contributed to solve some of the reported disadvantages of the RNA-vaccines: susceptibility to ubiquitous RNases and low thermal stability that hamper long-term storage and transportation. Additionally, the use of LNPs allows for the co-encapsulation of immunostimulating molecules that could enhance and modulate the immune response, thereby avoiding booster dosing. The induction of inflammatory reactions is another potential hurdle of RNA-based vaccines but encapsulation in NPs helps to mitigate these adverse effects.[189] Functionalized NPs, including polymer-based NP and LNP, can act as efficient carriers for the delivery of antigens to immune cells such as dendritic cells.[190] The composition of adjuvant LNP is variable but typically contains cationic lipids, cholesterol, and polyethylene glycol conjugated with lipids.[191] The mechanism of the

Table 4. Vaccine platforms and nanotechnologies for COVID-19.

Vaccine platform	Nanotechnology	Advantages
RNA	LNP-encapsulated mRNA or self-amplifying RNA encoding SARS-CoV-2 glycoproteins, the receptor binding domain or encoding VLP	Protection RNA from RNases; adjuvant effect; induction of potent T helper response and high number of germinal center B cells; production of high affinity neutralizing antibodies; induction of local innate immunity
Subunit vaccines (CoV-2 recombinant proteins, peptides)	Full length recombinant SARS-CoV-2 glycoprotein nanoparticle vaccine adjuvanted with Matrix M; saponine-based adjuvant, cholesterol and phospholipid particles	Stimulates strong and long-term humoral and cellular neutralizing immune responses to SARS-CoV-2 S-protein, reduces the antigen dose
	Capsid VLP display of SARS-CoV-2	Generates dense antigen display and elicits strong neutralizing antibody responses, high safety and efficacy
	Peptide antigens formulated in LNP	Allows a specific, robust, and sustained humoral immune response to non-overlapping neutralizing epitopes of the spike-protein; high safety profile
	Recombinant-protein, nanoparticles (based on spike-protein and other epitopes)	Induces strong neutralizing antibodies
	Adjuvanted microsphere peptide	Induces a strong immune response; generates neutralizing antibodies
	ADDomerTM multiepitope display (VLP); self-assembling protein-based nanoparticles encapsulating multiple peptide antigens	Trigger B cell receptor clustering and cross-presentation inducing a strong immune response

adjuvant effects of LNP remains unclear, but the lipid composition seems to be determinant as some cationic lipids could activate TLR2 (Toll-like receptor) and TLR4 mediators of innate immune response and induce the production of cytokines by antigen-presenting cells.[192] The size of LNP (up to 100 nm) is also a determinant for the efficient transport of antigens to dendritic cells of draining lymph nodes.[193]

4.2.1. Inactivated Vaccines

Inactivated vaccines are made with whole viral particles, mainly inducing specific humoral immune responses with antibodies capable to block virus entry into target cells. However, sometimes this approach fails to work in viruses prone to antigenic escape by mutation of their surface molecules implicated in cellular entry. Inactivated vaccines are usually associated with adjuvants; molecules that improve the release of the antigen at the site of the administration (depot effect) and/or induce the synthesis of immunostimulating mediators such as cytokines, TLRs.[194,195] Several inactivated vaccines against SARS-CoV-2 are under Phase 1/2 clinical evaluation (ChiCTR2000031809, ChiCTR2000032459, NCT04352608).[186]

4.2.2. Subunit Vaccines: Protein and Peptide Vaccines

This type of vaccines is composed of peptide epitopes or structural proteins of the virus, which trigger an immune response without exposing the body to the whole virus. They are less effective at eliciting a robust CD8+ immune response, which is important for intracellular pathogens.[195] However, the addition of appropriate immunostimulatory molecules such as vaccine adjuvants helps to overcome this limitation.[196] The transmembrane spike glycoprotein (S) is the main antigen of SARS-CoV-2 present at the viral surface and is the target of neutralizing antibodies during infection. The S protein is highly immunogenic, with the receptor-binding domain being the target of many neutralizing antibodies (Figure 1). Most subunit COVID-19 vaccines use the S-protein, in whole or in different fragments, especially the RBD. However, the SARS-CoV-2 nucleoprotein is more conserved than the S protein among different strains of coronavirus and is expected to induce more cross-reactivity. A candidate vaccine is made using a patented nanoparticle with a saponin-based adjuvant (Matrix-M, NOVAVAX, NVX-CoV2373) that is well-tolerated and stimulates strong and long-term antibody and cell-mediated immune responses to the spike protein of SARS-CoV-2. Saponins are steroid or triterpenoid glycosides present in many plants. In Matrix-M, saponin is mixed with synthetic cholesterol and a phospholipid to form stable particles that can be readily formulated with a variety of vaccine antigens. This adjuvant allows to reduce the antigen dose and the cost of production and increasing the manufacturing capacity in rapidly emergency threats. In preclinical studies, NVX-CoV2373 demonstrated high immunogenicity and stimulated high levels of neutralizing antibodies in animal models.[197]

4.2.3. Outer Membrane Vesicle-Based Vaccines

Four outer membrane vesicle (OMV)-based candidate vaccine have also been included in the WHO landscape of candidate COVID-19 vaccines.[198] Nanoparticle-sized OMV are present in Gram-negative bacteria and consist of proteins, lipids, and periplasmic contents.[199,200] They are able to adhere, enter, and deliver the content into host cells. OMV contains a variety of compounds acting as pathogen-associated molecular patterns that bind to pattern recognition receptors of the antigen presenting cells (APC) and activate the immune system.[201] OMV-based vaccines present unique and significant advantages: safety, non-live organisms, mucosa targeted via oral or nasal routes, easy and rapid adaptation to potential virus variants, and thermostability.

Other artificial lipid vesicles such as cationic liposomes contain a peptide antigen of SARS-CoV-2 and a combination of three adjuvants that are being tested to enhance the mucosal immunity following nasal administration.[202,203]

4.2.4. Live Attenuated Virus

These vaccines are composed of the whole virus, however the infectivity has been weakened so that it can replicate and stimulate an immune response without causing disease. However, these vaccines raise concerns such as the potential reversion to a virulent form by mutation or recombination with infectious wild-type strains.[204] In addition, they could cause disease in immunocompromised persons. Live attenuated virus vaccines based in codon de-optimized SARS-CoV-2 are in preclinical stage.[205] A promising replicating-defective SARS-CoV-2 vaccine candidate produces non-infective and highly immunogenic strains by deleting virulence genes and introducing attenuating mutations by using reverse genetics techniques.[206] This approach was previously used for SARS-CoV and MERS-CoV vaccines.[207]

4.2.5. Non-Replicating Viral Vector Vaccines

These vaccines use a well-established inactivated viral vector such as "modified vaccinia virus Ankara" (MVA) or adenovirus to express proteins of SARS-CoV-2, so that the proteins can be recognized by the immune system to elicit an immune response. The MVA vector has already been used to successfully develop several vaccines, including one against the MERS coronavirus, which is closely related to SARS-CoV-2.[208,209] A clinical trial on this MERS vaccine has already been completed and a clinical development is currently ongoing for SARS-CoV-2.[209] Another candidate vaccine based on MVA expresses several viral antigens from SARS-CoV-2.[206]

A further candidate is an adenoviral vector-based vaccine expressing the S-protein of SARS-CoV-2. This vaccine is based on a novel simian adenoviral vector with strong immunological potency and low pre-existing immunity in humans. This type of vaccines have been extensively evaluated in Phase 1 and 2 clinical trials and proved to be safe and immunogenic.[210]

CanSino Company is testing another well-positioned vaccine based on a non-replicating version of adenovirus-5 (Ad5), an agent causing the common cold, as a vector to carry the gene for the SARS-CoV-2 S-protein. However, the potential presence of anti-Ad5 immunity in many individuals that could prevent the expression of the S-protein and even cause harm could be experienced in a previous trial of an Ad5-based HIV vaccine. However, recent clinical Phase-1 trials have shown that is tolerable and the Ad5-based HIV vaccine induces immunogenicity at 28 days post-vaccination with high levels of humoral and T-cell immune responses.[211]

4.2.6. Replicating Viral Vector Vaccines

These are strong stimulators of innate immune responses and of T and B cell immunity. Undesirable features are the induction of anti-vector immunity and cell-based manufacturing. Live, recombinant viral vaccines incorporating genes from SARS-CoV-2 are inserted into the backbone of another virus (so-called "chimeric virus vaccines": yellow fever, influenza A, horsepox, VSV, measles).[212,213]

4.2.7. Virus-Like Particles

This vaccine platform is made of many copies of epitopes such as peptides, which are otherwise non-immunogenic enough per se, to elicit a protective immune response. In addition, virus-like particles (VLP) act as self-adjuvant NPs avoiding the need to complement vaccines with additional adjuvant compounds with potential adverse side effects.[214,215] A candidate VLP vaccine is the ADDomer multiepitope display (Imophoron Ltd and Bristol University's Max Planck Centre).[216] It consists of self-assembling protein-based spherical NPs that encapsulate a central cavity to carry the antigens. They are formed spontaneously from simple precursor monomers. In this platform, displayed peptide epitopes can reach very high densities promoting strong B cell immune responses.[216]

4.2.8. Nucleic Acid-Based Vaccines

These vaccines mimics infection or immunization with live pathogens and stimulate potent T and B immune responses.[217,218] Furthermore, nucleic acid-based vaccine manufacturing is fast and safe without the risk of growing highly pathogenic organisms at a large scale. That feature is relevant for most emerging infectious diseases whose main obstacle is obtaining a stockpile in a short period of time.[219]

DNA Vaccines: These types of vaccines use the DNA of SARS-CoV-2 generally expressed in a plasmid that is injected into the body (**Figure 13**). DNA vaccines show a number of advantages: non-infectious platform, stability, free of egg and cell components, rapid and scalable production, capability of stimulating the innate immunity, and the induction of T and B immune responses. However, they induce poor immunogenicity in humans and have potential risk of integration into genome. Enhanced delivery technologies, such as electroporation, have increased the efficacy of DNA vaccines in humans,[218] but have not reduced the potential risk of integration of exogenous DNA into the host genome, which may cause severe mutagenesis and induce new diseases.[220,221] Like RNA-based vaccines, DNA can also be encapsulated in lipid-nanoparticles.

RNA Vaccines: Several RNA-based vaccines as prophylactic or therapeutic agents against infectious diseases have been developed.[222–224] Like DNA vaccines, these use RNA of SARS-CoV-2 to be injected into the host. They can be replicating-defective viral RNA or, more often, mRNA that can be translated into viral proteins. Similar to DNA vaccines, RNA vaccines are non-infectious, egg and cell free, with rapid and scalable production and capable of stimulate innate immunity and T and B cell responses. Additionally, they do not integrate in the genome and are naturally degraded. There are concerns, however, about instability and low immunogenicity. Despite the fact that mRNA vaccine technology needs further optimization, experts predict

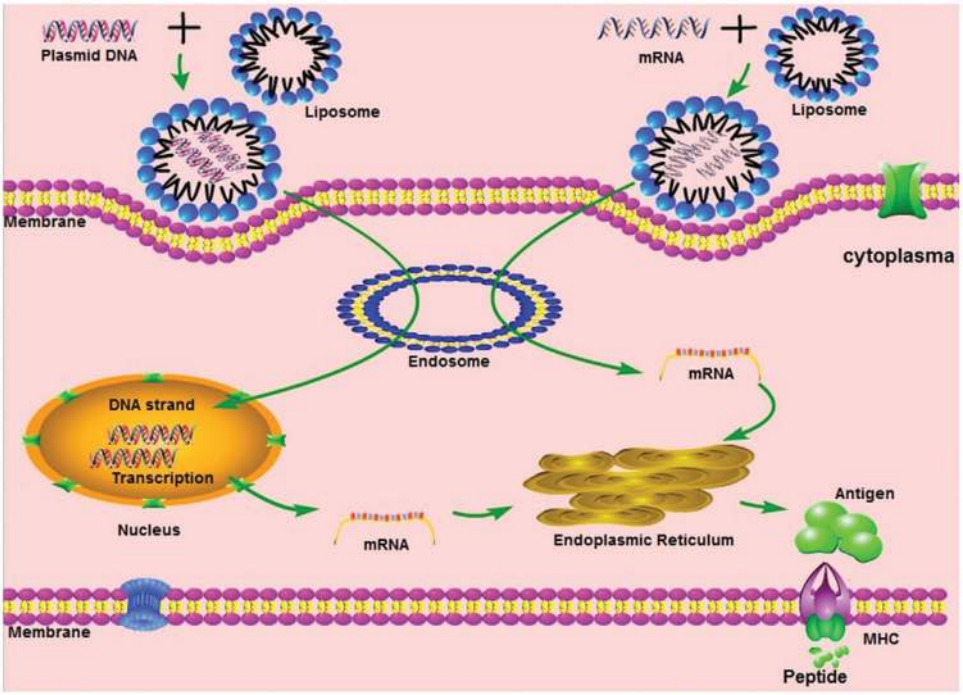

Figure 13. Antigen expression and presentation by nucleic acid (DNA and mRNA) vaccines. Reproduced under the terms of CC BY 4.0 license,[259] https://www.frontiersin.org/articles/10.3389/fimmu.2019.00594/full.

that the use of mRNA vaccines in humans and animals is only a matter of time.[178,225] Due to the intense development of RNA-based vaccine research[220] a substantial number of mRNA vaccines have been included in the WHO landscape of candidate vaccines and three of them are in Phase 1, 2, and 3 of clinical evaluation (Table 3). There are two types of mRNA vaccines: conventional mRNAs vaccines and self-amplifying mRNA vaccines derived from positive-sense/single-stranded (+) ssRNA viruses. Moreover, synthetic mRNA can now be produced in high quantities and purity by a cell-free enzymatic transcription reaction. mRNA vaccines are safer than DNA vaccines because they cannot potentially integrate into the host genome and will be degraded during the process of translation to yield the antigen.[226] Other RNA manipulations such as nucleoside modification of mRNA have increased its resistance to RNases. Once mRNA is introduced into the cells, there is a transient expression of the antigens that have shown to induce broadly protective humoral and T-cell immune responses.[227,228] Efficacy of mRNA vaccines has been improved by several delivery systems not only to protect the RNA from degradation but also to concentrate mRNA molecules at the site of injection and favor cellular uptake. Encapsulation with cationic liposome or cell penetrating peptide protected mRNA from RNase degradation.[177] Others encapsulate mRNA in LNPs and alternative approaches trap the RNA in different polymers through charge interactions.[225,229] At least 12 LNP-encapsulated mRNA vaccines against COVID-19 are included in the WHO list of promising vaccine candidates and one of them has completed a Phase 3 clinical trial with good safety and immunogenicity records suggesting that it may be effective.[230]

The eTheRNA Company is developing a novel intranasal vaccine against SARS-CoV-2 using its proprietary mRNA TriMix platform and preclinical assays have started.[231] The mRNA encodes a combination of T cell epitopes, including conserved epitopes of the virus that would protect against potential future variants of the SARS-CoV-2. It also integrates adjuvant elements (caTLR4, CD40L, and CD70) that stimulate dendritic cells to induce strong cellular immune responses. The intranasal administration stimulates the induction or mucosal immune responses to inhibit the progression of virus in the respiratory tract. Novartis designed a vaccine based on a cationic nanoemulsion using the adjuvant MF59 that retain mRNA on the surface of an emulsion droplet and protects it from RNase degradation. Researchers at BioNTech developed an LNP with specific characteristics of charge, size, and lipid composition that had affinity for lymphoid tissues after intravenous injection. There, APCs uptake the negative-charged nanoparticles and develop a strong cellular immune response against the antigen coded by the mRNA.[232] Nano-complexes consisting of poly(lactic acid) and cationic penetrating peptides as mRNA condensing agent were able to trigger activation of dendritic cells and induced a strong innate immune response.[233] Self-amplifying (sa) mRNA coding Influenza A hemagglutinin formulated in PEI stimulated high antibody titers[234] or the vaccine encapsulated into oil-in-water nanoemulsion conferred protection against homologous and heterologous influenza virus.[235] Nanoparticles made with chitosan and PEI were used to deliver sa-mRNA to dendritic cells.[236] Other researchers developed a dendrimer-based nanoparticle containing molecules of high amine density with branching structures that retain sa-mRNA molecules at high concentration. This vaccine was able to protect mice from a lethal dose of Influenza, Ebola, and Toxoplasma after a single intramuscular injection.[228] Nowadays, novel prototypes of nanoengineered particles such as

polyplexes, nanoplexes, and porous scaffold-mediated delivery are being investigated.[237–240]

5. Development of COVID-19 Nanotechnology: Recent Patents

The huge social and economic impacts of the COVID-19 pandemic have determined that numerous entities belonging to academia and industry as well as governmental and international institutions have made numerous documents and information available that can help in the fight against this disease. On the contrary, only a few of these initiatives try to provide data and analysis on relevant patented work that could be useful to both researchers and companies in the search of specific solutions against specific issues. In this way, the Chemical Abstract Service Division of the American Chemical Society published in March 2020 a general overview on patented work dealing with technologies focused on treatment and prevention of coronavirus infections, mainly centered on SARS-CoV and MERS-CoV.[241] This interesting review analyses patents covering therapeutic strategies using antiviral agents and other promising drugs for possible treatment of COVID-19 as well as biologic agents against COVID-19, including antibodies, cytokines, and RNA therapies. The large section focused on vaccines developed against SARS-CoV and MERS-CoV is useful, since the SARS-CoV-2 virus shares significant homology with two of the beta-coronaviruses responsible for those diseases and may be the basis toward upcoming vaccines against COVID-19.

Another interesting and helpful initiative has been recently launched by the Spanish Patent and Trademark Office (SPTO) with specific trimestral bulletins[242] and technological alerts[243] to provide updated information on recent patents related to "coronavirus: diagnosis and therapy in humans." The first SPTO Technology Watch Bulletin[244] on this topic was published in March 2020 and includes a list of the international patents published since 2018 within four main technological areas of interest to COVID-19, that is: i) antiviral and other agents for treatment of pathologies associated with the viral disease; ii) vaccines; iii) diagnosis; and iv) devices for the treatment and control of the viral infection. There is also a statistical analysis included of patents on "coronavirus"-related issues published since 2004, with information on applicants and countries. The technological alert[243] on "coronavirus" allows to keep updated information of ongoing patents related to this topic published in the last 365 days with direct links of patents at the European Patent Office and other patent sources for retrieving additional information. Thus, for instance, at the date of writing this review (May 20, 2020), there is a list of 43 patents published during the previous year, covering technologies related to coronavirus. Out of them, 20 are related to biological agents, including monoclonal antibodies, 13 to antiviral or virucide agents, 5 to detection, 3 to new vaccines, and 2 address other issues.

Using the ESPACENET patent search tool provided by the European Patent Office,[245] it is possible to carry out a systematic search of patents to analyze which are the most developed areas of nanotechnology dealing with coronavirus topics. In the middle of May 2020, from a total of 13 379 patents related to "coronavirus," 1370 of them were also related to "nano" topics. The ratio decreases to 699 out of 5563 patents when the topic is centered only on SARS and MERS coronaviruses, and just to 98 out of 1161 when the search is circumscribed to "SARS-CoV" (comprising SARS-CoV and SARS-CoV-2). **Figure 14** shows the distribution of patents related to SARS-CoV viruses within the "nano AND coronavirus" search when refined with keywords related to the topics treated in this article: "disinfectant," "virucide," "sterilization," "filter," "membrane," "coating," "detection," "biosensor," "antiviral," and "vaccine." Publication of patents is led by the USA, followed by the World Intellectual Property Organization (WIPO), China, Japan, and South Korea.

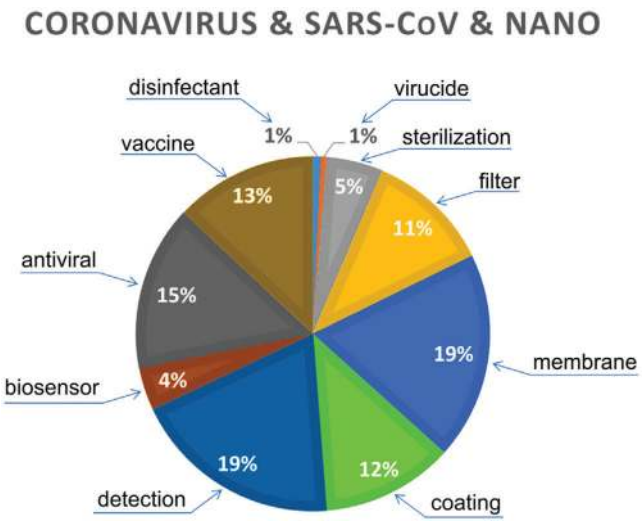

Figure 14. Distribution of patents dealing with SARS-CoV viruses within the "coronavirus and nano" search refined with other keywords (data obtained from ESPACENET[245]).

Despite the very short time that has elapsed since the COVID-19 outbreak, much research has been developed and various registered patents in relation to this disease have already been reported.[246–252] Most of them have been registered in China, where the pandemic disease started, and they mainly deal with SARS-CoV-2 detection and therapeutic issues. Thus, antiviral compositions based on benzylisoquinoline alkaloid and trans-resveratrol for treatment of the infection are protected in the patent CN110960532A,[247] in which also the combination of them with diverse pharmaceutical carriers is protected. Though, the use of nano-carriers is not stated in specific examples, the use of liposomes and nanoparticles might be helpful in the therapeutic application of these compositions. In this way, the KR20200032050A patent[246] relies on the use of nanotechnology to fight against COVID-19 and other coronaviruses using liposomes to transport a complementary single-stranded DNA oligomer developed for targeting infected cells.

In the search of effective vaccines against COVID-19, two protected methodologies have already been reported. In the CN110951756A patent,[248] the SARS-CoV-2 virus has been sequenced and analyzed to provide a nucleic acid sequence expressing a specific antigen peptide that may induce immune response in the human body. This patent also protects the use of expression vectors and compositions involving such nucleic acid sequences envisaging diverse strategies of producing vaccines

against COVID-19, where nanotechnology may be relevant. The CN110974950A patent[249] deals with the use of an adenovirus as carrier of a DNA plasmid or an RNA expression plasmid which, after transfection of human cell lines, can express and produce more S protein. This can be used as an antigen gene in nucleic acid vaccines or as a recombinant virus vaccine to prevent SARS-CoV-2 infection. This patent also claims similar vaccines in which the use of pharmaceutically acceptable adjuvants, carriers, diluents, and other excipients is involved.

Special interest has been focused on the protection of technology related to the detection of SARS-CoV-2. For instance, in the CN110982945A patent,[250] a nucleic acid composition is introduced together with a kit and a method for detecting SARS-CoV-2 with improved sensitivity and specificity by applying PCR techniques. In the CN111041089A patent,[251] a gene is employed as a host marker to prepare a COVID-19 infection detection reagent or a detection device, based on the analysis of different gene expression between patients with COVID-19 pneumonia and patients with pneumonia not caused by COVID-19 infection. An interesting approach using nanotechnology is claimed in the CN111024954A patent[252] in relation to the production of antigen/antibody detection tests. The immunochromatography device uses AuNP in the 55–65 nm diameter range for labeling coronavirus specific protein monoclonal antibodies that are used in the antibody detection test strips typically prepared with nitrocellulose membranes.

As mentioned above, the very short time elapsed since the occurrence of the COVID-19 pandemic is the reason for the still little public evidence of other patents, except for public announcements. This is the case of a recent patent applied by the Spanish National Research Council and Bioinicia S.L. (Spain) to produce biodegradable antiviral filters for application in face masks and respirators.[253] This company is already producing disposable filters for FFP2 and FFP3 protection masks, which can be easily replaced and eliminated by biodegradation. In the same way, Moderna Inc. (USA) announced beginning of May 2020 the initiation of the Phase 3 protocol for testing its mRNA-1273 vaccine,[254] developed in collaboration with the U.S. National Institutes of Health. Though, there is no information on any patent related to this vaccine, the company has protected diverse technologies dealing with nucleic acid-based vaccines,[255] some of them based on the use of mRNA sequences of coronavirus encapsulated into LNPs for treatment of respiratory virus infections.[256]

6. Conclusions

Academia and industry around the world are working from basic research to advanced technology to alleviate the effects of the COVID-19 health crisis. The application of nanoscience and nanotechnology concepts and tools is nowadays a good approach within the current global priority. We have summarized the present state of knowledge about the interaction of nanomaterials with diverse viral particles, mainly focusing on SARS-CoV-2, emphasizing as much as possible on prevention, diagnosis, and treatment of the COVID-19 disease under the nanotechnology umbrella.

It can be assumed that basic research, from computational simulation to the study of interactions with nanomaterials, will be further necessary to obtain fundamental information on the nanostructure of viral particles, as well as their intrinsic functionality and mechanisms of infection. These aspects need to be critically accelerated toward the discovery and deployment of the most convenient approaches and means in the prevention, diagnosis, and treatment of this infectious disease.

Important issues and potential benefits will derive from the study of the interaction mechanisms between different solids and coronaviruses, and particularly SARS-CoV-2. It would be recommended to expand research on this topic to ascertain persistency of viruses on common smooth and rough surfaces, trying to improve the current knowledge on this matter to avoid the spread of viruses and for clarifying some discrepancies found in different recently published studies.

Advances in diagnostics and treatment of COVID-19 is progressing more and more in a very quick manner, but this disease is much more complex and aggressive than typical seasonal flu infections. The effect of the initially promising drugs, such as chloroquine and hydroxychloroquine, is still not clear for COVID-19 therapeutics. At the present time, treatments could include cocktails of antiviral drugs addressed to patients with mild-to-moderate COVID-19 symptoms to inhibit the multiplication of the virus. However, the final objective is to develop a new generation of drugs that specifically could target SARS-CoV-2. Probably, the use of recently reported nanocarriers facilitating the transport of these drugs, and probably their administration via the nasal route, could contribute to find a cure for COVID-19 or at least to save as many lives as possible. Also, advances in detection systems based on nanotechnology are at this moment under rapid development and, for instance, antigen and antibody test kits allowing self-diagnosis using blood or exudates like mucus and saliva will represent an easy way to enable fast identification of asymptomatic patients and patients with mild symptoms and, hence, accelerating their isolation in quarantine.

As indicated above, there are currently many attempts in progress to develop COVID-19 vaccines, including clinical trials. Here also nanotechnology approaches could be of help in creating innovative nanoarchitectonics-based vaccines to prevent the disease. Just as an example in this context, there are vaccines based on spike protein nanoparticles or on virus-like particles mimicking nanovesicles recently developed for MERS-CoV, which could be potentially extrapolated to COVID-19 vaccine development. In short, nanotechnology is a powerful multidisciplinary tool that offers various approaches and strategies that could contribute strongly to promoting research projects around the world against this lethal infectious coronavirus disease.

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Conflict of Interest

The authors declare no conflict of interest.

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coronavirus, COVID-19, diagnosis, nanomaterials, nanotechnology, treatment, vaccines

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