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Review

The Potential of Various Nanotechnologies for Coronavirus Diagnosis/Treatment Highlighted through a Literature Analysis

Edouard Alphandéry*

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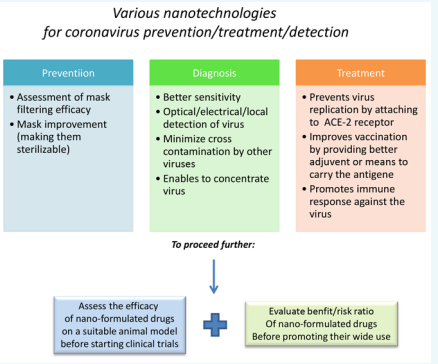
ABSTRACT: With the current COVID-19 outbreak, it has become essential to develop efficient methods for the treatment and detection of this virus. Among the new approaches that could be tested, that relying on nanotechnology finds one of its main grounds in the similarity between nanoparticle (NP) and coronavirus (COV) sizes, which promotes NP−COV interactions. Since COVID-19 is very recent, most studies in this field have focused on other types of coronavirus than COVID-19, such as those involved in MERS or SARS diseases. Although their number is limited, they have led to promising results on various COV using a wide range of different types of nanosystems, e.g., nanoparticles, quantum dos, or nanoassemblies of polymers/proteins. Additional efforts deserve to be spent in this field to consolidate these findings. Here, I first summarize the different nanotechnology-based methods used for COV detection, i.e., optical, electrical, or PCR ones, whose sensitivity was improved by the presence of nanoparticles. Furthermore, I present vaccination methods, which comprise nanoparticles used either as adjuvants or as active principles. They often yield a better-controlled immune response, possibly due to an improved antigen presentation/processing than in non-nanoformulated vaccines. Certain antiviral approaches also took advantage of nanoparticle uses, leading to specific mechanisms such as the blocking of virus replication at the cellular level or the reduction of a COV induced apoptotic cellular death.



INTRODUCTION

With the spread of the COVID-19 epidemic and the disorders that it has caused, i.e., an increased mortality rate, a saturation of the hospital infrastructures, and a sudden major slow-down of the world economy, it appears essential to better understand the behaviors of coronaviruses (COV) and to develop efficient methods for their detection and destruction. To this end, an enormous research effort has been implemented worldwide, which mainly relies on drug repositioning, i.e., testing as COV treatments drugs such as chloroquine and remdesivir or their derivatives, which have shown their efficacy against other diseases than COV, i.e., malaria and HIV.[1] Such an effort could be complemented by other approaches, e.g., in the field related to nanotechnologies for COV treatment. Indeed, the sizes of these viruses are similar to those of nanoparticles, hence promoting NP-COV interactions and potentially resulting in similar behaviors between NP and COV.[2] Thus, it has been suggested that certain drawbacks of standard attenuated/inactivated vaccines, such as their pathogenic virulence or weak immune responses, could be overcome by using nanoformulations (NF). This is due to sizes, shapes, functionalities, and antigen presentation/processing that could be favorably adjusted in NF, potentially yielding a more efficient and better-controlled immune system response for nanoformulated than non-nanoformulated drugs.[3,4] Here, methods using various types of nanotechnologies that have been tested for diagnosis and treatment of COV are reviewed. The different fields in which nanotechnologies could help to bring a solution linked to the COVID-19 crisis are (i) the development of a cheap and rapid test for diagnosing COVID 19 that could be deployed worldwide over the entire population,[1] (ii) the prevention of virus replication and viral RNA synthesis, for example, by using nanoparticles that block the interaction between COVID 19 and the cellular receptor ACE-2,[5] (iii) the development of new nanoparticle-based-vaccine,[3] and (iv) the restoration of innate immunity among infected patients.[3] Nanomaterial safety is a prerequisite for their administration to humans. To ensure this, regulatory agencies have set up specific regulations with dedicated biocompatibility tests.[6] Although it is difficult to consider nanomaterial safety in general terms due to the huge diversity of these materials, certain nanoparticles such as those composed of iron oxide have been granted authorization for human injection,[7] and could therefore potentially be tested clinically against COVID 19. Due to the

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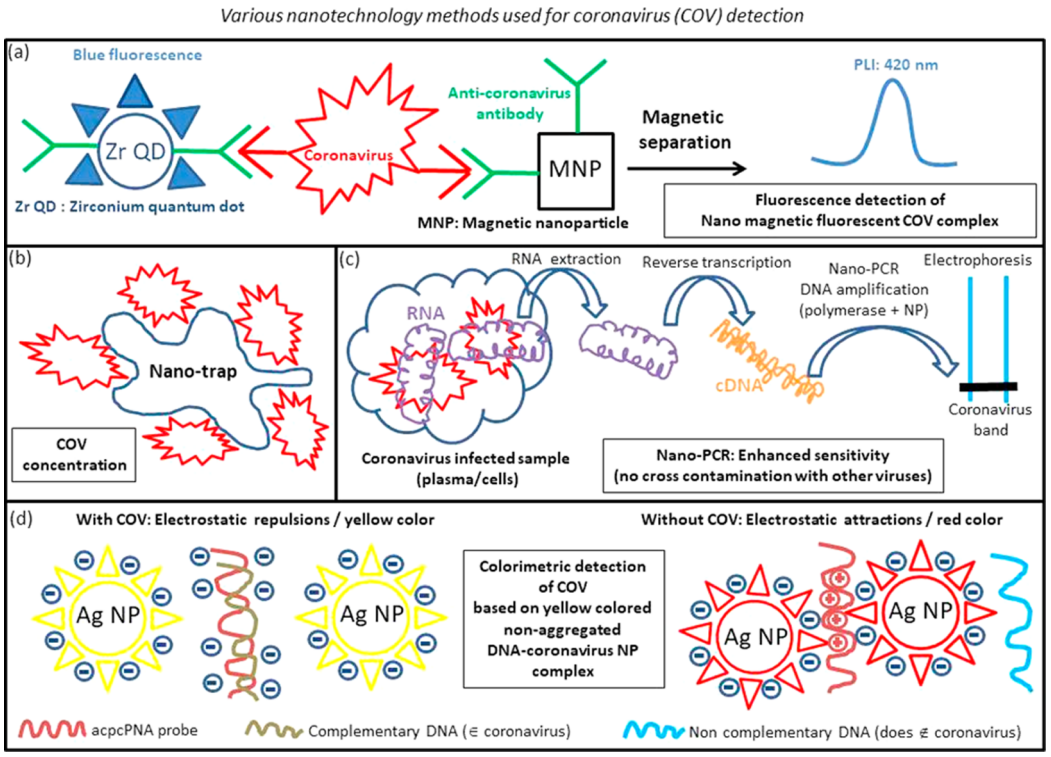

Figure 1. Schematic diagrams showing different examples of nanomaterial-based COV detection methods. (a) Fluorescent Zr QDs and magnetic nanoparticles are conjugated with antibodies that specifically bind to COV. In the presence of COV, a magnetic fluorescent complex is formed, which is isolated magnetically and detected by fluorescence measurements. (b) Nanotraps are used to concentrate COV and improve their stability, hence facilitating their detection. (c) Reverse transcription PCR is carried out in the presence nanoparticles, improving the efficacy of the polymerase chain reaction, and resulting in a better detection sensitivity of this method. (d) COV detection method, which is based on the interactions between complementary DNA originating from COV and acpnPNA probe at the surface of Ag NP, which results in a separation between Ag NPs, and a yellow color associated with the luminescence of well dispersed Ag NPs, further revealing COV presence.

recent outbreak of COVID-19, the majority of studies relates to other types of coronaviruses than COVID-19, i.e., human COV such as those associated with Middle East Respiratory Syndrome (MERS) and Severe Acute Respiratory Syndrome (SARS) diseases, and animal COV such as Feline Coronavirus (FCOV), Porcine Epidemic Diarrhea Viruses (PEDV), and Infectious Bronchitis Virus (IBV). The use of similar treatment strategies for COVID-19 than for these other COV may be foreseen, but even so, such therapies remain exploratory, and no effective MERS/SARS vaccine has yet been put in place to the author's knowledge.

I. GENERALITIES ABOUT CORONAVIRUS TREATED/DETECTED WITH NANOTECHNOLOGIES, I.E., SARS, MERS, IBV, FCOV, TEGV, AND PEDV

Coronavirus (COV) comprises 50−150 nm viruses, which are made of nucleocapsid (N) proteins attached to positive single-stranded RNA covered by an envelope, which consists of a lipid bilayer containing membrane (M), envelope (E), and spike (S) proteins.[8−10] Under simplified terms, coronavirus replication in the organism is characterized by the following chain of events: (i) attachment of viral spike (S) glycoprotein to its complementary host cell receptor, (ii) virus endocytosis in cells, (iii) virus uncoating, (iv) virus replication, and (v) virus release.[10] COV can infect humans (COVID-19, SARS, and MERS), chickens (IBV), cats (FCov), or pigs (PEDV and TGEV). It was suggested that some coronavirus could be transferred from one species to another. For example, it was reported that SARS and MERS could originate from bats or camels, which seems to be based on viral ARN similitudes observed between these different species.[11,12] Following an incubation period, coronaviruses affect specific parts of the organism such as tract or gastrointestinal system, potentially resulting in severe diseases such as pneumonia.[13] Coronavirus detection is usually carried out through ELISA, immunofluorescence, PCR, and/or chest X-rays.[14] While coronavirus infection can be avoided through vaccination for PEDV,[15] vaccines have not yet been developed for SARS and MERS.[16]

II. NANOTECHNOLOGIES TO PREVENT COVID-19 INFECTION

Transmission of COVID 19 occurs through droplets containing the virus that are emitted by an infected person through coughing, sneezing, or talking. These droplets are either directly transmitted to another person in proximity or are evaporated to end up as dried nuclei located at the surface of an object such as a knob, a button, a table, or a door. From a general standpoint, virus transmission can be prevented by isolating the infected person, by cleaning contaminated objects, by frequently washing hands, or by wearing personal protection equipment.[17,18] This latter aspect, notably the necessity (or not) to wear masks, has been the topic of contradictory recommendations from the authorities. This is essentially due the lack of knowledge on the efficacy of existing masks to filter COVID 19 virus. With the size of COVID 19 being nanometric, nanotechnological tools can help to clarify this issue. To this aim, a study has been led to compare the filtering efficacy of common masks made of cotton, silk, chiffon, flannel, and their combination.[19] It concluded that a

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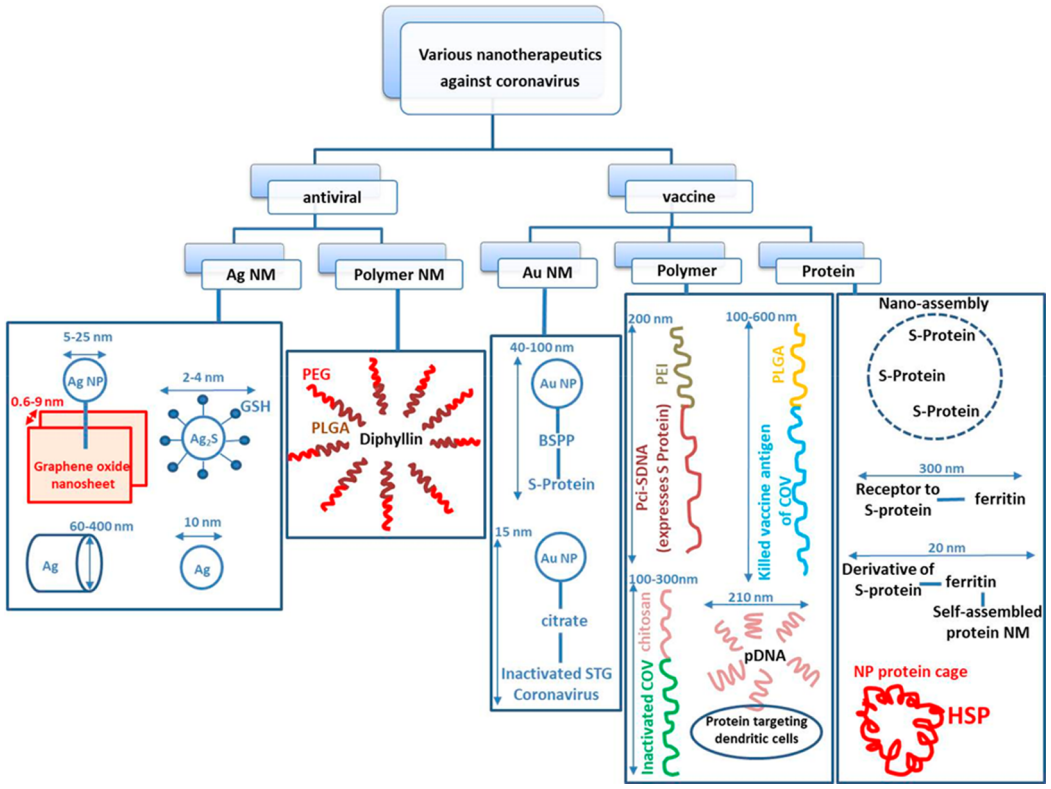

Figure 2. Schematic diagram presenting various types of nanotherapies used against COV, which are categorized as vaccines or antiviral drugs, and comprise various types of Ag nanomaterials, i.e., free NP/NW or NP attached to graphene nanosheets, diphyllin inserted within PEG−PLGA vesicles, various nanomaterials, i.e., Au NP, polymers such as PEI, PLGA, or chitosan, bound to COV antigens, nanoassemblies comprising COV antigens, as well as an interesting nanocage used as vaccine despite its lack of COV antigen.

mask made of a single fabric or containing holes was not optimal for filtering COVID 19, while that comprising a combination of cotton and silk, cotton and chiffon, or cotton and flannel could filter 80−90% of the virus, where such enhanced efficacy was attributed to a mechanism relying on a combination of mechanical and electrostatic interactions between the virus and the fabrics that could only be reached by combining materials. Another group used electrostatically charged PVDF nanofiber filters to capture the deadly airborne coronavirus.[20,21] A third approach consisted of depositing nanostructured graphene at the surface of surgical masks. Such masks became superhydrophobic with a temperature that could reach 80 °C under sunlight illumination, making them sterilizable and reusable, an important aspect given the necessity to be able to easily decontaminate masks to avoid virus spreading through mask misuses.[22]

III. NANOTECHNOLOGIES USED FOR CORONAVIRUS DIAGNOSIS

To avoid false positive/negative that can occur in standard detection methods of COV such as PCR, ultrasensitive nanodetection methods have been developed. Nanotechnology-based COV detection methods are summarized in Figure 1. They first rely on highly sensitive optical mechanisms. The latter can be a measurement of fluorescence at 412 nm of a complex made of Zr QDs bound to anti-COV antibodies linked to COV, following magnetic isolation.[23] It can also be fluorescence detection of green fluorescent protein (GFP) comprising a complex formed by SARS virus proteins attached to Au NP whose fluorescence is different from that of free GFP.[24] It can be based on several calorimetric assays enabling COV detection by using Au associated with various entities such as double-stranded DNA that specifically bind to COV,[25] antibodies acting against IBV spike proteins,[26] or monoclonal antibodies (mAbs) activated against PEDV,[27] or by using Ag NP attached to acpcPNA, which remain dispersed in the presence of complementary COV derived DNA, giving rise to a detectable color change.[28] Au nanoislands were also functionalized with specific DNA strands that bind to SARS-COV-2 nucleic acids under heating produced by laser illumination followed by Au NP plasmon absorption, enabling COVID-19 detection at virus concentration as low as 0.22 pM.[29] Another type of COV detection method relies on the use of nanotraps that capture COV, improving their stability and enabling their detection over a long period of time.[30] It was also reported that COV could be detected with a biosensor made of carbon electrodes that comprise Au NP associated with viral spike proteins, whose response, i.e., peak current position/intensity, depends on the presence (or not) of MERS-COV.[31] In another approach, a field-effect transistor (FET) coated with graphene sheets attached to antibodies that specifically recognize COVID 19 spike proteins enabled the detection of these proteins in different media (phosphate-buffered saline, culture medium, clinical samples) down to COVID 19 concentrations of 1 fg/mL.[32] Finally, the efficacy of PCR, which is the most widespread method of COV detection, can be improved by using nanoparticles, either through the simultaneous (duplex) detection of two virus types such as DNA and RNA viruses, e.g., using nanoparticles conjugated to specific probes of these viruses,[33,34] or by making the polymerase chain reaction more efficient in the presence than absence of nanoparticles.[35,36] In

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Table 1. Vaccines against COV Based on Various Nanotechnologies

VACCINE						
Nanomaterial backbone	Size (nm) Shape ZP[a]	Active substance	COV type	Admin route[b]	*In vivo* data	refs
Gold	16 nm sphere	Antigen of gastroenteritis COV	SARS	Subcutaneous	Mice immunized with Gold-COVantigen: ↑ activation of antigen presenting cells, γ-IFN, IL-1β, IL-6, macrophages, B cells	47
PLGA	100−600 nm sphere	Killed vaccine antigen (KVA)	PEDV	Intranasal	Pregnant sows inoculated with PLGA-Kag → passively immunized suckling piglets ↑ lymphocytes, IFN-γ, humoral immune response, IgA, IgG ↓ Mortality of suckling piglets	48
chitosan	286 nm sphere 20 mV	Inactivated infectious bronchitis virus (IBV)	IBV	Oculo-nasal	Chicken immunized with chitosan-IBV: ↑ Humoral and cellular immune response against IBV ↓ Viral load in trachea and kidney	49
chitosan	210 nm 10 mV	DNA expressing COV protein (DNA-COV)	SARS	Intranasal Intramuscular	Mice immunized with chitosan-DNA-COV: → DC targeting + both humoral and cellular immune responses	50
PEI	195 nm	pci-S: SARS DNA Vaccine	SARS	Intranasal	Mice immunized with PEI-pci-S: ↑ IgG1/IgA antibodies	51
chitosan	122 nm sphere 53 mV	Antigen of infectious bronchitis virus (A-IBV)	IBV	Intranasal	Chicken immunized with chitosan—A-IBV: ↑ IgG/IgA antibodies, lymphocyte proliferation, IL-2, IL-4, IFN-γ ↑ Humoral + cellular immune response	52
Nanoassembly of COV spike proteins (Nano-COV-P)	25 nm 160−180 kDa	Spike proteins of COV	MERS SARS	Intramuscular	Nano-COV-P inoculated into Balb/C mice: → Production of neutralizing antibodies	53, 54
Nanoassembly of COV spike proteins (Nano-COV-P)	80 nm 140 kDa	Spike proteins of COV	MERS	Intranasal	Mice immunized three times with (Nano-COV-P): → induces both Th1 and Th2 immune responses against COV	55
Ferritin (FR): scaffold of nanoassembly SSG: linker between FR and RBD	28−30 nm sphere 1080 kDa	Receptor binding domain (RBD) of COV	MERS	Intramuscular	Mice immunized 2 times with RBD-[SSG]-FR: ↑ RBD-specific antibody responses	56
Self-Assembling Protein Nanoparticle with flagellin adjuvant (COV-Flagellin-SAPN)	23 nm 38 kDa	Epitope of COV spike proteins	IBV	Intranasal	Chickens immunized with IBV-Flagellin-SAPN: → Antibody response (less tracheal lesion)	57
Spike protein nanoparticle vaccine (SPNV)	NA	COV spike protein	MERS	Intranasal	Bovines/mice immunized 1/5 times with SPNV → immunoglobulin IgG antibodies	58
Self-assembled polypeptide nanoparticle with epitote of spike COV protein (COV-SAPN)	25 nm sphere 1.4 MDa	COV spike protein	SARS	Interperitoneally	Mice immunized with COV-SAPN → anti-SARS antibodies were obtained without adjuvant	59
Incorporation of SARS peptide in synthetic NP (SARSP-N)	Sphere	COV peptide	SARS	Interperitoneally	Mice injected with SARSP-N → Targets T-cell receptor → Suppresses Collagen-Induced Arthritis	60
Protein cage nanoparticle (PCN)	12 nm hollow sphere	PCN (no antigen)	SARS	Intranasal	Mice inoclulated with PCN:	61

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Table 1. continued

Nanomaterial backbone	Size (nm) Shape ZP[a]	VACCINE Active substance	COV type	Admin route[b]	*In vivo* data	refs
					→ Development of bronchus-associated lymphoid tissue (iBALT) in lung → Apparent activation of T/B cell response ↑ Mouse survival	

[a]Zeta potential [b]Route of administration of nanomaterials.

general, compared to standard PCR, nano-PCR presents the advantages of requiring a simplified operating mode, i.e., DNA/RNA extraction, RNA purification, and reverse transcription may not be needed, of resulting in a better sensitivity, and of avoiding cross-contamination with other viruses.

IV. VACCINES CONTAINING NANOMATERIALS TESTED AGAINST COV INFECTION

A series of nanoformulated anticoronavirus vaccines, which are presented in Figure 2, were developed. They often resulted in a better efficacy than their non-nanoformulated counterparts. Such vaccines contain a backbone made of gold nanoparticles, polymers such as PLGA, chitosan, and PEI, or protein assemblies. This backbone is conjugated or associated with an active principle consisting of COV antigen, inactivated or destroyed COV, DNA expressing certain COV proteins, or specific COV proteins such as spike ones (Table 1). Although a nanoparticulate formulation usually refers to a material whose size is between 1 and 100 nm,[6] the sizes of these vaccines could exceed this upper limit, ranging from 12 to 600 nm (Table 1). Other specific properties of these vaccines include their charge, which is positive when it is measured apparently corresponding to the charge of their coating material, and their shape, which is most often reported as spherical (Table 1). When animals such as mice and chicken were immunized with vaccines, it led to a production of cytokines, i.e., essentially γ-IFN, IL-1β, IL-2, and IL-6, as well as an activity of certain immune cells such as macrophages, lymphocytes, and B cells, which were more important using formulated than non-nanoformulated vaccines. To the author'sknowledge, it was not reported whether the immune reaction was dominated by a humoral or cell mediated response. In fact, both types of responses seem to occur, resulting in some cases in the restoration of respiratory body activity and protection of specific body parts such as the trachea. Whereas in most cases, a specific antigen originating from COV was inserted in the nanoformulation to trigger a reaction against the virus, an interesting study presented a nanoformulated system, i.e., a protein cage nanoparticle (PCN), which was devoid of such antigens. Following its injection to mice, it resulted in the production of antiviral antibodies, and led to an increase in mouse survival. Beyond the explanation provided, i.e., the development of inducible bronchus-associated lymphoid tissue (iBALT) in the lung that triggered T and B cell responses, the most interesting aspect of this study seems to lie in the fact that a specific antigen may not be absolutely necessary for a nanoformulated COV vaccine to be efficient.[37] Very recently, a clinical trial was launched to study the efficacy of a vaccine against COVID 19 containing m-RNA lipid nanoparticles.[38] Its outcome is pending.

V. ANTIVIRAL NANOMATERIAL DRUGS STUDIED TO FIGHT CORONAVIRUS

Even so, nanoformulated vaccines aimed at triggering an immune response against COV, while antiviral nanoformulations should prevent certain interactions between virus and cells or their consequences, these two types of mechanisms, whose details are provided in Figure 3, can coexist together. The study of the response of the immune system was carried out *in vivo* to be able to monitor the production of specific antibodies acting against COV, whereas antiviral behaviors were examined *in vitro* to decipher the mechanisms of virus interactions with cells. As presented in Figure 2 and Table 2, antiviral nanoformulations

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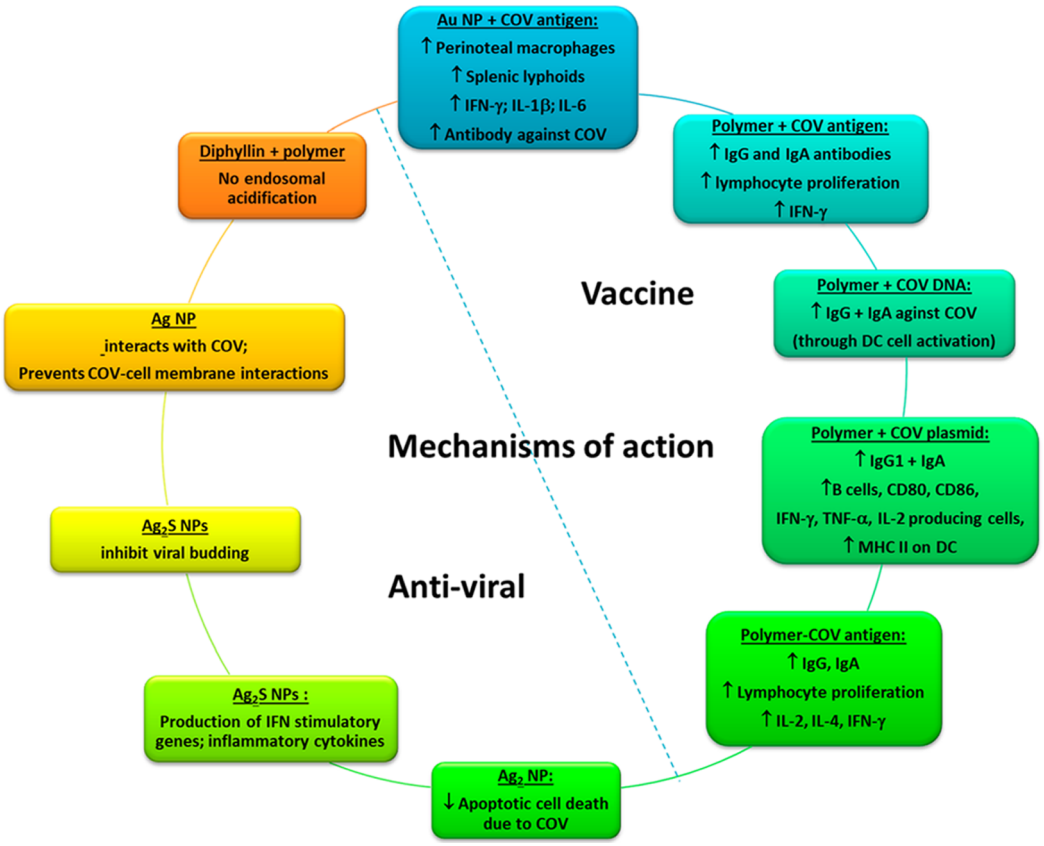

Figure 3. Schematic diagram presenting the proposed mechanisms of action of the various nanomaterials, which are mainly based on the stimulation of various parts of the immune system against COV for vaccines and on the inhibition of COV replication at cellular level or the prevention of COV-induced apoptotic cellular death for antiviral drugs.

Table 2. Antiviral Drugs against COV Relying on Various Nanotechnologies

Nanomaterial backbone	Size (nm) shape ZP[a]	Active substance	COV type	Admin route[b]	*In vitro/in vivo* data	refs
PEG−PLGA	40 nm	diphyllin	FCoV	NA	In fcwf-4 cells, PEG−PLGA-diphyllin reduces endosomal acidification: → Inhibits V-ATPase and virus replication	39, 40
Graphene-Ag	7.5 nm Ag NP	Ag NP	FCoV	NA	In fcwf-4 cells, GO-Ag inhibits virus infection of FCoV → Mechanism warrants further studies	41
Ag_2S Nanoclusters	3.2 nm Sphere	Ag_2S nanoclusters	PEDV	NA	In Vero cells, Ag_2S NC reduce PEDV infection → inhibits production of viral RNA and viral budding → Activates IFN-stimulating genes pro-inflammation cytokines	42
Ag NP and NW	60−400 diam (NW) <20 nm (NP)	Ag NP/NW	TGEV	NA	In ST cells, Ag NP/NW reduce TGEV infection ↓ number of apoptotic cells induced by TGEV → Regulation of p38/mitochondria-caspase-3 signaling pathway	43

[a]Zeta potential. [b]Route of administration of nanomaterials.

were reported to be of essentially four types: (i) the antiviral agent diphyllin encapsulated in PEG−PLGA copolymers of 40 nm,[39,40] (ii) Ag nanoparticles of 7.5 nm bound to graphene nanosheets,[41] (iii) Ag_2S nanoclusters of 3.2 nm,[42] and (iv) several Ag based nanomaterials including Ag nanoparticles of 20 nm and Ag nanowires of 60 and 400 nm diameters.[43] The antiviral activity of these nanoformulations against COV was first achieved by blocking cellular endosomal acidification, which seems to be an essential step in COV replication, using an ATPase blocker, diphyllin, which is nanoformulated to yield a good solubility and be released in a sustainable manner. It resulted in efficient COV replication blockage in fcwf-4 cells.[39,40] Second, inhibition of the synthesis of viral RNA was reached by using the various types of Ag nanomaterials mentioned above.[41−43] Third, antiviral activity could be associated with a certain level of cellular immune response, as highlighted by the expression of pro-inflammation cytokines following cellular exposure to Ag NP.[42] Fourth, it could correspond to a reduction of COV-induced apoptotic cellular death in the presence of Ag nanomaterials.[43] Finally, for the treatment of COVID 19, it has

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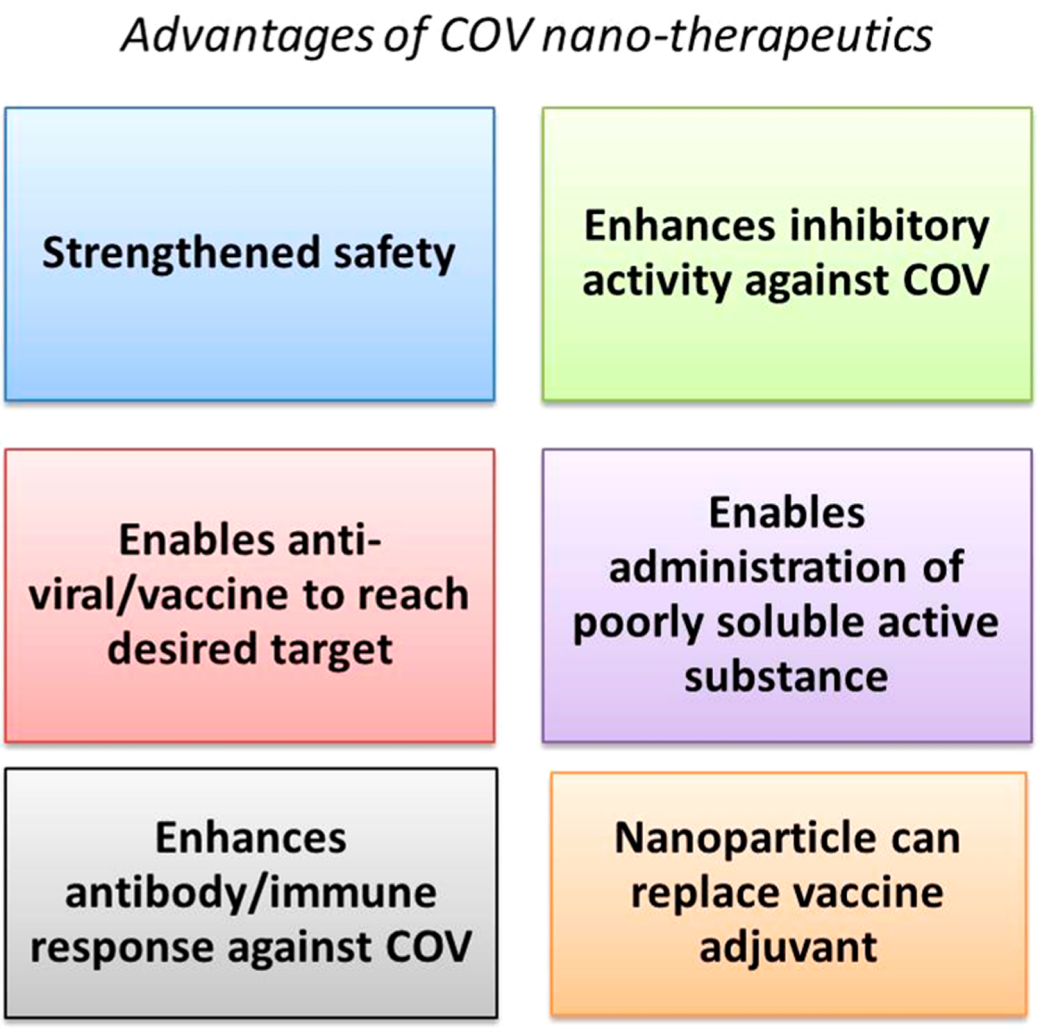

Figure 4. Summary of the various advantages of COV nanotherapies, which could be deduced from literature analysis.

been suggested to use certain nanomaterials with a structure similar to that of ACE-2 receptor, which plays a central role in COVID 19 cellular internalization and replication. Such materials consisted of peptid inhibitors derived from the protease domain of ACE-2, which specifically bind to COVID-19 receptor binding domains, hence potentially preventing COVID 19 multiplication in cells.[5]

VI. INDIRECT TREATMENT/DETECTION METHODS OF COVID-19

In addition to methods of direct virus destruction presented above, the bases of other therapeutic approaches have been introduced, whose mechanisms of action rely on the correction of certain dysfunctions of the organism due to the virus. For example, the modification of the microbiote induced by COVID 19, which is suspected through several signs among infected patients such as diarrhea, a relatively long incubation time of 2−5 days of the virus, the transmission of the virus through fecal route, but is not formally demonstrated, could be examined. On one hand, nanomaterials could be used for the detection of compounds or inflammatory proteins originating from a disturbed microbiota, which could indirectly reveal the presence of COVID 19. On the other hand, nanomaterials could serve to improve the local delivery of drugs in the gut, which would fight against COVID 19 by restoring healthy activity of the microbiome.[44]

CONCLUSION

In this topical review, I have highlighted the potential of certain nanotechnologies for improving the detection and destruction of coronavirus. Concerning COV detection using nanomaterials, it either allows specific COV binding at NP surface or improves PCR efficacy, generally leading to better sensitivity compared with other detection methods. Compared with their non-nanoformulated counterparts, nanodrugs display a series of advantages in the fight against COVID 19 such as an improved drug safety and solubility,[45] better virus inhibition,[5] and more efficient immune response and organ targeting,[3,4] as summarized in Figure 4. In addition, when they are included in vaccines, they can replace standard adjuvants. The appealing properties of these nanomaterials have been highlighted not only through their use in new vaccination methods but also in the context of specific antiviral treatments, where nanoformulated antiviral drugs could block virus replication, trigger an anti-COV immune response, or prevent COV-induced apoptotic cellular death.

In order to develop nanotechnology based treatments for COVID 19, certain additional aspects that have not been mentioned in the literature should be examined. They notably concern the interactions between nanomaterials and cells or cell

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Bioconjugate Chem. XXXX, XXX, XXX−XXX

receptors that should not only prevent virus replication but also yield minimal cellular toxicity. These two aspects should probably be studied in parallel to evaluate the benefit/risk ratio of nanomaterial-based treatment against COVID 19. Furthermore, the efficacy of nanotechnology-based vaccines or antiviral drugs against COVID 19 should be tested on suitable animal models such as hACE2 transgenic mice,[46] a task that has not been undertaken to the author's knowledge. The fact that we urgently need a treatment against COVID 19 should not make us skip some essential steps in drug development. It appears insufficient to start a human clinical trial based only on the observation of cellular drug efficacy. Drug assessment on animals is essential to examine the conditions under which a drug is efficient, notably to determine preclinically the dose or administration route of anti-COV 19 nanoformulated drugs that could potentially result in treatment efficacy on humans.

AUTHOR INFORMATION

Corresponding Author

Edouard Alphandéry − *Paris Sorbonne Université, Muséum National d'Histoire Naturelle, UMR CNRS 7590, IRD, Institut de Minéralogie, de Physique des Matériaux et de Cosmochimie, IMPMC, 75005 Paris, France; Nanobacterie SARL, 75116 Paris, France; Institute of Anatomy, UZH University of Zurich, CH-8057 Zurich, Switzerland;* orcid.org/0000-0002-2952-2270; Phone: 0033632697020; Email: edouardalphandery@hotmail.com

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.bioconjchem.0c00287

Notes

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ABBREVIATIONS

ACE-2, Angiotensin-converting enzyme 2; COV, Coronavirus; COVD or COVID, Coronavirus disease; COVID 19, Coronavirus disease appearing in 2019.; ELISA, Enzyme linked immune-sorbent assays; FCOV, Feline coronavirus; HIV, Human immunodeficiency virus; IBV, infectious bronchitis virus; IMA, Immuno-chromatic assay; MERS, Middle East Respiratory Syndrome; NM, Nanomaterial; NF, Nanoformulation; NP, Nanoparticle; PCR, Polymerase chain reaction; PEDV, Porcine epidemic diarrhea virus; PEG, Polyethylene glycol; PEI, Polyethylenimine; PLGA, Poly(lactic-*co*-glycolic acid); QD, quantum dot; SARS, Severe Acute Respiratory Syndrome; SEP, Self-assembled proteins; TGEV, Transmissible gastroenteritis coronavirus

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Bioconjugate Chem. XXXX, XXX, XXX−XXX