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Review Article

Nanoagent-based theranostic strategies against human coronaviruses

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ABSTRACT

The emergence of human coronaviruses (HCoVs), especially the current pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), engender severe threats to public health globally. Despite the outstanding breakthrough of new vaccines and therapeutic medicines in the past years, HCoVs still undergo unpredictable mutations, thus demanding more effective diagnostic and therapeutic strategies. Benefitting from the unique physicochemical properties and multiple nano–bio interactions, nanomaterials hold promising potential to fight against various HCoVs, either by providing sensitive and economic nanosensors for rapid viral detection, or by developing translatable nanovaccines and broad-spectrum nanomedicines for HCoV treatment. Herein, we systemically summarized the recent applications of nanoagents in diagnostics and therapeutics for HCoV-induced diseases, as well as their limitations and perspectives against HCoV variants. We believe this review will promote the design of innovative theranostic nanoagents for the current and future HCoV-caused pandemics.

KEYWORDS

human coronaviruses, nanosensors, nanovaccines, nanomedicines, variants

1 Introduction

In the past decades, the emergence and evolution of human coronaviruses (HCoVs) that induced pandemics have been the most terrible threats for social health globally [1]. So far, three major HCoVs appeared around the world with high mortality rates, including the severe acute respiratory syndrome coronavirus (SARS-CoV) in 2002 [2], the middle east respiratory syndrome coronavirus (MERS-CoV) in 2012 [3], and the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that caused the current coronavirus disease in 2019 (COVID-19) [4]. It has been reported that the emerging SARS-CoV-2 presents high genetic similarity with previous SARS-CoV (79%) and MERS-CoV (50%), respectively [5]. Because the first HCoV only causes mild respiratory symptoms in humans since its discovery in the 1960s [6], the HCoVs are not treated as fatal pathogens until the eruption of SARS-CoV. Incredibly, the new HCoV strain, SARS-CoV-2, exhibits unprecedented infectivity in the COVID-19 pandemic [7], with around 238 million confirmed cases and a total of 4.85 million related deaths worldwide (from World Health Organization (WHO) on October 13, 2021) [8]. Most seriously, multiple SARS-CoV-2 variants with higher infectivity are circulating globally, especially for Alpha (B.1.1.7) from United Kingdom, Beta (B.1.351) from South Africa, Gamma (P.1) from Brazil, and Delta (B.1.617.2) from India as classified by the different lineage and component of spike mutations [9, 10]. Owing to these facts, effective treatment regimens are urgently desired for COVID-19 and future pandemics.

Typically, when a new infectious pathogen emerges, the tools for fast and precise diagnosis are considered as the foremost requirement to trace and isolate infected cases quickly [11]. The reverse transcription-polymerase chain reaction (RT-PCR) method serves as the well-known gold standard for viral detection. Briefly, the sample of a person's nose or throat is collected by the swab and treated with chemical solutions to extract the RNA. If HCoV is present, the viral RNA will be first reverse-transcribed to single-stranded complementary DNA (cDNA) using an enzyme (reverse transcriptase) and primer (to recognize unique RNA sequence). Then another enzyme, DNA polymerase, extends a second strand to produce a double-stranded DNA. This DNA copy undergoes successive cycles (~30–45) of amplification in an RT-PCR machine, which can produce up to a billion DNA copies of viral RNA within 6–8 h. Organic dyes are usually used as markers to attach with DNA strands and release fluorescence for real-time detection. When a certain level of fluorescence is surpassed, the patient is confirmed to be infected by HCoV. Despite the high sensitivity and accuracy, RT-PCR technique still suffers from a few drawbacks including the time-consuming processes and unavailability of equipment [12]. Effective

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medicines are also urgently required for the later stages of infected patients to attenuate immunologic complications like cytokine storm [13]. Unfortunately, there are no broad-spectrum antiviral agents to prevent or treat HCoV infections when the pandemic initially emerges [14]. Some existing drugs such as remdesivir (a nucleoside analog prodrug designed for Ebola virus) [15] and chloroquine (a medicine primarily used to treat malaria) [16], are repurposed to exploit their therapeutic efficacy against COVID-19, but limited by their unpredictable side effects in the patients [17]. Moreover, vaccination acts as the best way to eradicate pandemics by accelerating herd immunity without causing more deaths [18]. Up to date, several vaccines are approved by Food and Drug Administration (FDA) with high preventing efficiency for COVID-19, including the mRNA vaccine from Pfizer-BioNTech or Moderna, and the recombinant viral vector vaccine from Janssen [19]. Despite the inspiring success, the current vaccines have high risks to decline their protective efficacy against COVID-19 due to the unpredictable mutations of HCoVs [20]. For example, when comparing with the 95% protection efficacy against initial SARS-CoV-2 strain in clinical trial, the BNT162b2 mRNA vaccine from Pfizer-BioNTech only presented 90%–93% for Alpha, 75% for Beta, not available for Gamma, and 83%–88% for Delta variants [21]. Therefore, the development of innovative agents for the diagnostics and therapeutics against mutated HCoVs in the future, still remains an enormous challenge.

Recently, nanomedicine has attracted much attention to overcome the limitations of ongoing theranostic strategies against epidemics caused by HCoVs [22]. On the one hand, thanks to the nanoscale morphology of HCoVs, the synthesized nanoparticles present strong nano–bio interaction to *in situ* recognize viral structures [23, 24]. These properties provide irreplaceable benefits to developing economic nanosensors for sensitive and rapid detection of HCoVs [25, 26]. On the other hand, nanoagents can serve as smart carriers to deliver antiviral drugs in the desired region, which will minimize their side effects and degradation [27, 28]. Moreover, benefitting from their large surface area to volume ratio, nanoparticles can be surface-modified with functional moieties to capture the spike protein and block the viral entry, or act as nanovaccines to boost the prophylactic immune responses after vaccination [29, 30]. Thus, the exploration of novel nanoagents provides an advanced antiviral approach for HCoV-caused pandemics.

In this review, the crucial design of nanoagents as diagnostic and therapeutic tools for HCoV infections was systematically summarized (Fig. 1). We briefly introduced the biological properties and life cycles of HCoVs in the host, followed by the emerging nanoagents for detection and treatment against HCoVs including SARS-CoV, MERS-CoV, and SARS-CoV-2 in recent decades. Finally, the limitations and perspectives of these theranostic nanoagents were also discussed, which would inspire researchers to explore new strategies towards the unpredictable pandemics caused by HCoV variants.

2 Biology of HCoVs

Coronaviruses (CoVs) are enveloped viruses with crown-like surface proteins, which belong to the subfamily of Coronavirinae within the family of Coronaviridae and the order Nidovirales [31]. There are four genera in the Coronavirinae, including the α-, β-, γ-, and δ-CoVs, which are classified based on their phylogenetic relationships and genomic structures [32]. The α- and β-CoVs only infect mammals, γ-CoVs only infect avian species, and δ-CoVs infect both mammalian and avian species. Since the first discovery of HCoVs in the 1960s, only seven types of HCoVs were identified, including the α-genera (e.g., HCoV-229E and HCoV-NL63) and β-genera (e.g., HCoV-OC43, HCoV-HKU1, SARS-CoV, MERS-CoV, and SARS-CoV-2) [7]. Typically, CoVs are spherical particles with a diameter of 100–160 nm containing two basic components: (i) Genome: a single-stranded positive-sense ribonucleic acid ((+)ssRNA) with 27–32 kb in size. The 5'-terminal of the genome (about two-thirds) consists of two open reading frames (ORFs), ORF1a and ORF1b, to encode the polyproteins involved in genome transcription and replication [33]. Besides the structural proteins encoding gene, the genome also includes accessory genes that are species-specific and dispensable for HCoV replication [34]. (ii) Structural proteins, such as spike (S), envelope (E), membrane (M), and nucleocapsid (N) proteins (Fig. 2(a)) [1]. As the major immunodominant antigen on virus surface, S glycoprotein is considered to involve in the specific binding with host cells based on the S1 and S2 subdomains [35]. The S1 part mainly contributes to the recognition and adhesion on cell membrane by using the receptor-binding domain (RBD), while the S2 part mediates membrane fusion and entry of coronavirus into the host cells. It has been demonstrated that the SARS-CoV and SARS-CoV-2 bind to angiotensin-converting enzyme 2 (ACE2) and transmembrane serine protease 2 (TMPRSS2) receptors, and the MERS-CoV binds to dipeptidyl-peptidase 4 (DPP4 or CD26) [7]. More importantly, HCoVs are estimated to present higher mutation rates than other ssRNA viruses at two main gene loci, including the S protein gene and accessory gene of ORF region [36]. Due to the wide expression of ACE2 in most human organs (especially for the epithelial cells in the respiratory system), the extensive pulmonary invasion and multiple-organ failure are considered as the major reasons for the death cases caused by HCoVs.

Despite the interaction with specific receptors on the cell membrane, different HCoVs share similar replication strategies during the infection process in the host cells, including attachment, viral entry, uncoating, biosynthesis, assembly, and excretion (Fig. 2(b)) [37]. The S glycoproteins of HCoVs first bind to the membrane receptors (e.g., ACE2, TMPRSS2, and DPP4) and enter the host cells by endocytosis. Following the fusion between the viral envelope and endosome membrane, the viral genome is released into the cytoplasm and translated to produce the RNA-dependent RNA polymerase (RdRP) for gene replication

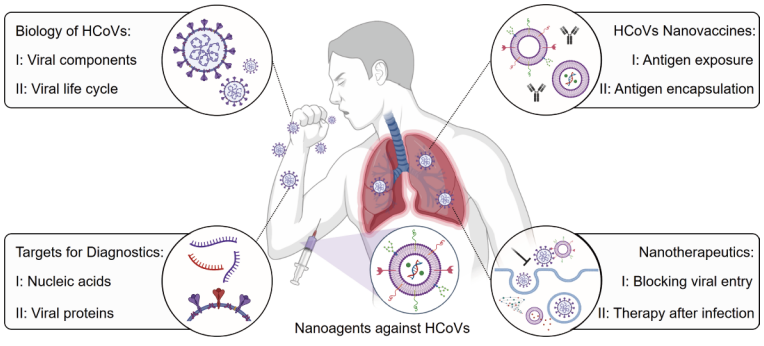

Figure 1 Schematic illustration of nanoagent-based strategies for the diagnostics and therapeutics of human coronaviruses (HCoVs).

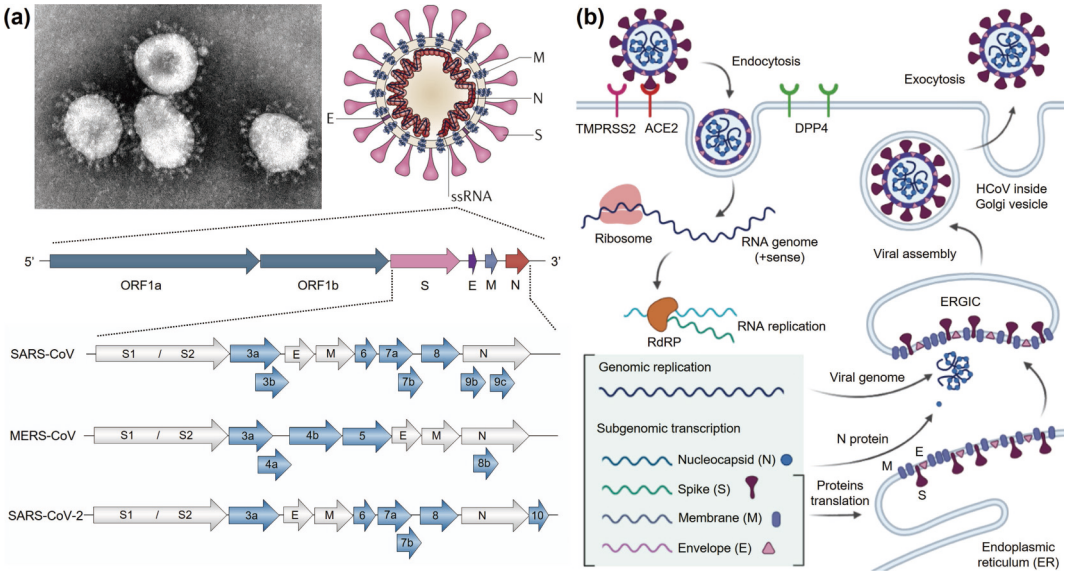

Figure 2 The biology and life cycle of HCoVs. (a) The transmission electron microscope (TEM) image of HCoVs, and schematic diagram of various structural proteins such as S, E, M, and N proteins, as well as non-structural proteins (nsps) translated from ORF1a, ORF1b, and accessory genes, including 3a, 3b, 6, 7a, 7b, 8, 9b, and 9c for SARS-CoV, 3a, 4a, 4b, 5, and 8b for MERS-CoV, and 3a, 6, 7a, 7b, 8, and 10 for SARS-CoV-2. Reproduced with permission from Refs. [32] and [42], © Nature Publishing Group 2019 and Cureus Inc 2020, respectively. (b) The life cycle of HCoVs. Upon S protein binding with the cellular receptors, HCoV enters the host cell and releases the genomic RNA for the replication of viral genome and translation of N, S, M, and E proteins. They are assembled to produce the mature HCoV and further released outside by exocytosis for another life cycle.

[38]. The S, E, M, and N proteins are further translated from viral genomic RNA, followed by budding into the lumen of the endoplasmic reticulum–Golgi intermediate compartment (ERGIC) and interacting with the N protein–RNA complex for viral assembly [39]. Finally, the mature HCoVs are released outside by exocytosis for another infection cycle.

According to the pathogenesis of SARS-CoV-2 infection in humans from World Health Organization (WHO), HCoVs can spread from person to person within 6 feet through respiratory droplets when an infected person coughs or sneezes. Once inside the body, HCoVs begin infecting epithelial cells in the upper respiratory tract and lung tissue based on the above infection process. It usually takes 2–14 days for a person to develop symptoms after initial exposure to HCoVs. The HCoV-infected patients are usually classified into mild, moderate, severe, and critical in clinics [40]. General symptoms for the mild or moderate infection include cough, fever, headache, nasal congestion, diarrhea, muscle pain, and shortness of breath. However, the patients with severe or critical disease present with pneumonia up to acute respiratory distress (ARDS), severe sepsis or septic shock caused by high amounts of inflammatory cytokine storm, and even multiple organ dysfunction [41]. Considering their nanoscale morphology, synthetic nanoparticles could strongly interact with HCoVs by mimicking the viral structures for therapeutic intervention. Therefore, the replication processes and clinical features of HCoVs in humans are significant for the development of nanoagents against current pandemics.

3 Nanoagents for HCoV diagnostics

Rapid diagnosis of HCoVs serves as the essential step to identify the infected patients in pandemics. The current tools are mainly based on computerized tomography (CT) scans of lung in patients and viral RNA testing by RT-PCR [43]. However, several limitations still need to be addressed, including the time-consuming processes, unavailability of equipment, low RNA extraction efficiency, and contamination-induced false positives [44]. Therefore, sensitive and affordable detection of HCoVs *in situ* is highly desirable against HCoV-induced infections. Thanks to the diversified physicochemical properties of nanomaterials, various strategies are proposed based on the optical, electronic, or magnetic properties of nanomaterials in the last decade. Herein, we summarized the advances of nanoagents to detect different HCoVs based on two basic components, nucleic acids and viral proteins (Table 1).

3.1 Nucleic acids testing

As alternative techniques of the conventional RT-PCR method, biosensors are more convenient and economic for HCoVs detection in real time. Colorimetric nanosensors provide attractive solutions in pandemics due to their visual outputs, low costs, and simplicity. So far, gold nanoparticles (AuNPs) based colorimetric biosensors have gained enormous attention owing to their outstanding optical properties, such as the incredible photostability, high molar extinction coefficient, and localized surface plasmon resonance (SPR) [62]. For example, Moitra et al. developed a colorimetric bioassay based on AuNPs in conjugation with thiol-modified antisense oligonucleotides (ASOs) to detect N gene (nucleocapsid phosphoprotein) of SARS-CoV-2 (Figs. 3(a) and 3(b)) [49]. In the presence of targeting RNA sequence, the ASO-capped AuNPs underwent selective agglomeration and obvious color change from violet to dark blue due to the SPR effect. The visual differences were further amplified with the addition of RNaseH, which cleaved the RNA strand from the RNA–DNA hybrid to induce naked-eye detectable precipitation among the AuNPs. This smart nanosensor not only had a low limit of detection (LOD) for SARS-CoV-2 viral RNA (0.18 ng/μL) and excellent selectivity with the interference of MERS-CoV RNA load, but also presented rapid naked-eye detection of positive COVID-19 cases within 10 min from the isolated RNA samples. With a similar approach, Kim et al. reported a colorimetric nanosensor by using the extended self-assembly of double-stranded DNA (dsDNA) shielded AuNPs for the detection of MERS-CoV [46]. They designed two thiol-modified single-stranded DNA (ssDNA) probes at the 5' or 3' ends for targeting partial genomic regions (30 bp) of MERS-CoV along with upstream E protein gene and encoding ORF1a. In the presence of the target viral gene, the ssDNA probes hybridized with the target to form a dsDNA–AuNP network, which inhibited the salt-

Table 1 Nanosensors for the detection of various HCoVs

Type	Virus	Target	NPs	Size (nm)	Mechanism	LOD	Assay time (min)	Ref.
Nucleic acids	SARS-CoV	N gene	AuNPs	70	Electrochemical	2.5 pmol/L	—	[45]
	MERS-CoV	ORF1a and E gene	AuNPs	19	Colorimetric	1 pmol/μL	10	[46]
		—	AgNPs	19	Colorimetric	1.53 nM	—	[47]
	SARS-CoV-2	RdRp gene	AuNPs	5	LSPR	0.22 pM	20	[48]
		N gene	ASO–AuNPs	<30	Colorimetric	0.18 ng/μL	10	[49]
		ORF1a and N gene	Polymer NPs	129	mRT-LAMP and LFB	12 copies	60	[50]
		N gene	MNPs	10	RT-PCR	10 copies	20	[51]
Viral proteins	SARS-CoV	N protein	AuNPs	20	Optical	0.1 pg/mL	—	[52]
		N protein	In_2O_3 nanowire	—	Electrochemical	—	—	[53]
		N protein	QDs	—	Fluorescent	0.1 pg/mL	60	[54]
	MERS-CoV	S protein	AuNPs	50	Electrochemical	1.0 pg/mL	20	[55]
	SARS-CoV-2	S protein	Graphene	—	FET	242 copies/mL	—	[56]
		S protein	Au nanostars	100	Electrochemical	38 copies/mL	1	[57]
		IgM and IgG	AuNPs	40	LFB	—	15	[58]
		IgG	AuNPs	30	LFB	—	15–20	[59]
		S, E, M proteins	AuNPs	20	Colorimetric	—	3	[60]
		N protein	AuNPs	50	Electrochemical	0.4 pg/mL	15	[61]

Abbreviations. QDs: quantum dots; AgNPs: silver nanoparticles; LSPR: localized surface plasmon resonance.

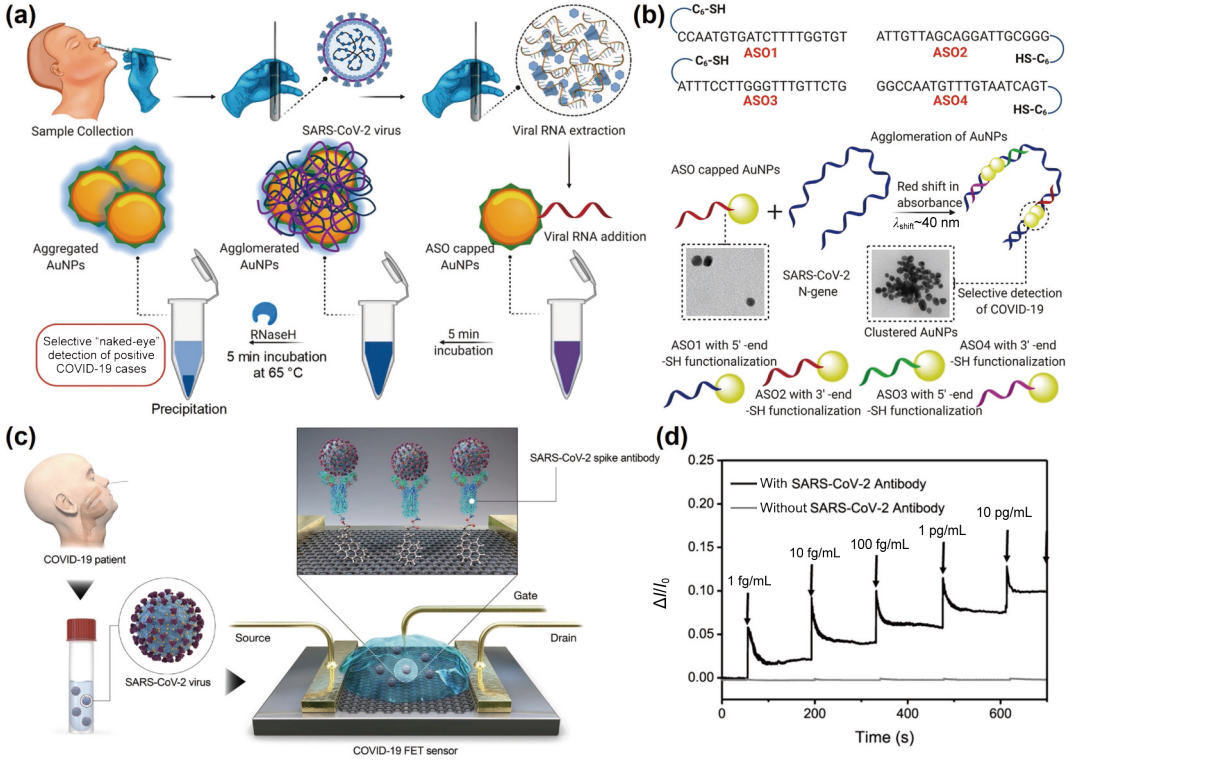

Figure 3 The diagnostics of HCoVs by nanosensors. (a) and (b) Nucleic acids-based nanosensor. (a) Schematic representation of antisense oligonucleotides (ASOs)-capped AuNPs for the naked-eye detection of SARS-CoV-2 N gene. The targeting viral RNA sequences induced the selective aggregation of AuNPs, resulting in the visible color change and boosted detection upon RNaseH treatment. (b) The specific sequences of thiol-functionalized ASOs and detection procedures of N gene. Reproduced with permission from Ref. [49]. © American Chemical Society 2020. (c) and (d) Viral proteins-based nanosensor. (c) Schematic illustration of FET-based SARS-CoV-2 nanosensor by using S protein-immobilized graphene on the electrode layer. (d) Real-time response of FET sensor toward SARS-CoV-2 antigen protein in PBS. A graphene-based FET device without SARS-CoV-2 antibody was presented as a negative control. Reproduced with permission from Ref. [56]. © American Chemical Society 2020.

induced aggregation of AuNPs and color change. This biosensor could rapidly and sensitively detect the MERS-CoV gene in 10 min (LOD: 1 pmol/μL) without electrophoresis or other amplification procedures.

To improve the sensitivity of HCoV diagnostics, other nanoagents are also utilized to simplify the nucleic acid extraction and determination procedures. For example, Zhao et al. reported a simple method for SARS-CoV-2 viral RNA isolation based on magnetic nanoparticles (MNPs) and further detected it by RT-PCR [51]. The MNPs were modified with carboxyl group-

containing polymer to achieve a strong bind with nucleic acid for the effective enrichment of viral RNA. This simplified protocol also enabled the high sensitivity (10-copy) and rapid extraction of SARS-CoV-2 pseudovirus RNA from multiple samples within 20 min, which could reduce the operation time and contamination risk for the diagnosis of COVID-19 in a high-throughput manner. In addition, by utilizing the multiplex reverse transcription loop-mediated isothermal amplification (mRT-LAMP) technique, Zhu et al. developed a lateral flow biosensor (LFB) for the simultaneous diagnosis of N gene and ORF1a/b gene in the SARS-CoV-2 genome [50]. The streptavidin-dye-loaded polymer nanoparticles (SA-DNPs) could evaluate the amplified copies of viral RNA through the LFB assay, which presented high sensitivity and specificity for COVID-19 diagnostics.

3.2 Viral proteins testing

Discrimination of the specific proteins on virus surface provides another feasible strategy for HCoV detection. Due to the essential functions of S protein to bind with host cells, it is considered as the major pathogenic component for the diagnosis of HCoVs [63]. For instance, Seo et al. developed a field-effect transistor (FET)-based biosensing device with graphene sheets and SARS-CoV-2 spike antibody modification for COVID-19 test (Figs. 3(c) and 3(d)) [56]. The antibody was firstly immobilized on the graphene surface of FET biosensor through a coupling agent. With the addition of clinical samples from COVID-19 patients, the real-time current change ($\Delta I/I_0$) increased owing to the specific interaction of antibody and viral S protein. This FET device had excellent selectivity against SARS-CoV-2, and a low LOD value for S protein in phosphate buffer (1 fg/mL) and nasopharyngeal swab specimens from COVID-19 patients (242 copies/mL). Except for the S protein, other structural components such as M, E, and N proteins are also utilized for the HCoV determination. Ventura et al. reported a colorimetric method based on antibodies-labeled AuNPs for rapid detection of SARS-CoV-2 in a few minutes [60]. The multiple antibodies (S, M, and E) modified AuNPs underwent a visual color change from red to purple when mixed with a solution containing the target virus. Ishikawa et al. established an In_2O_3 nanowire FET device modified with antibody mimic proteins (AMPs), a kind of polypeptides that bound to SARS-CoV N protein with high affinity and specificity [53]. This biosensor presented a specifical test of N protein at 0.6–10 nM range upon addition of 44 μM bovine serum albumin (BSA) as an interference.

Moreover, the diagnosis of various viral biomarkers, such as the immunoglobulin M (IgM) and IgG antibodies in the serum, are complementary to the HCoV assays in pandemics. It is well known that IgM serves as the first line of defense against HCoVs in the innate immune system, and the generation of IgG implies the activation of adaptive immune response after viral infection in the patients [64]. Wen et al. developed a point-of-care testing (POCT) method to detect IgG antibodies against SARS-CoV-2 [59]. They prepared a lateral flow immunoassay strip (LFIAs) with surface modification of N-protein and anti-human IgG monoclonal antibody coupled AuNPs (mAbs–AuNPs). After adding blood samples in the well, the IgG bound with mAbs–AuNPs and further moved forward along the test card under capillary force. The entire testing procedure was less than 20 min with 10 μL of serum and reliable stability. Li et al. also presented a simple POCT kit by using the colloid AuNPs modified LFIAs for the diagnosis of IgM and IgG simultaneously against SARS-CoV-2 in human blood within 15 min [58]. They further validated the outstanding sensitivity and specificity in the blood samples from 397 COVID-19 patients and 128 negative patients in clinical trials.

4 Nanoagents for anti-HCoV treatment

Benefitting from the development of nanomedicines in recent decades, innovative nanoagents are proposed for the effective treatment against HCoVs, either by presenting nanovaccines to activate the immune response, or by delivering therapeutic drugs to interfere with viral infection in targeted regions [27]. Moreover, these nanoagents have tunable capabilities including the size, shape, charge, drug loading, or surface modification against different types of viruses, and are also suitable for multiple therapeutic routes such as subcutaneous, intramuscular, intranasal, oral, or inhalational administration [65]. Herein, we summarized the advances of nanoagents for anti-HCoV treatment based on the prophylactic and therapeutic strategies.

4.1 Nanovaccines preventing HCoV infections

Vaccines remain the most powerful strategy to provide long-lasting protection of humans against HCoV infections. Generally, vaccines introduce major viral antigens such as S proteins to the human body in a safe manner [66]. After administration, the antigens are captured by the antigen-presenting cells (APCs) including dendritic cells, then delivered to nearby lymph nodes, and presented to other immune cells such as T and B cells for the recognization of invaders. The memory B cells further develop HCoV-specific antibodies in the serum, which will trigger a rapid immune response to recognize and eliminate the invaded HCoVs after the first contact [67]. Recently, different types of vaccines, including the inactivated vaccines from Sinovac and Bharat Biotech, the subunit vaccines from Medigen, the non-replicating viral vector vaccines from Oxford-AstraZeneca and Janssen, and the nucleic acid-based mRNA vaccines from Pfizer-BioNTech and Moderna, have been authorized for clinical use to fight against COVID-19 pandemic globally [68].

Viruses are usually considered as naturally occurring nanoparticles due to their sizes. Therefore, nanovaccines have great potential to mimic the structures and functions of HCoVs, thus providing prolonged antigen presentation and enhanced immunogenicity against current and future pandemics. In general, based on the locations of antigens, the nanovaccines can be mainly divided into two categories: (i) antigen-exposed nanovaccines where the antigens are directly exposed onto the nanoagents surface; (ii) antigen-encapsulated nanovaccines where the antigens are encapsulated inside of nanocarriers (Table 2).

4.1.1 Antigen-exposed nanovaccines

In this strategy, the antigens are anchored on the nanovaccine surface, aiming to mimic viral structures. S proteins are considered as the main antigens to trigger an adaptive immune response against HCoVs. For example, Walls et al. reported a self-assembling protein nanovaccine to prevent SARS-CoV-2 infection (Figs. 4(a)–4(d)) [75]. To overcome the limited immunogenicity of monomeric antigen, they displayed multiple RBD on the nanovaccine surface for multivalent antigen presentation. The RBD was first fused to trimeric I53-50A using flexible linkers with 8, 12, or 16 glycine and serine residues (termed as RBD-8GS-, RBD-12GS-, or RBD-16GS-I53-50A), and then self-assembled with pentameric I53-50B in a 1:1 molar ratio to prepare the nanovaccine with a diameter of 30–40 nm. Surprisingly, the anti-SARS-CoV-2 S antibody titers induced by nanovaccine were significantly 10-fold higher than the monomeric RBD and prefusion-stabilized spike (S-2P trimer) despite at a 5-fold lower dose after vaccination, which should be attributed to its similar morphology and nanoscale size with a virus. The RBD-8GS-I53-50 and RBD-12GS-I53-50 nanovaccines could completely prevent the replication of a mouse-adapted SARS-CoV-2 in mouse lung

Table 2 Nanovaccines for the prevention of different HCoV infections

Type	Virus	Antigen	Nanocarrier	Size (nm)	Adjuvant	Route	Ref.
Antigen-exposed nanovaccine	SARS-CoV	Ectodomain of S protein	Au NPs	25	TLR agonists	s.c.	[69]
		HRC1 of S2 protein	Polypeptide NPs	25	—	i.p.	[70]
		S protein	S protein NPs	—	Matrix-M1	i.m.	[71]
	MERS-CoV	RBD of S protein	Ferritin-based NPs	30	Alum/MF59	i.m.	[72]
		RBD of S protein	Hollow PLGA NPs	114	STING agonist	s.c.	[73]
		RBD of S protein	CoPoP liposomes	100–200	MPLA/QS-21	i.m.	[74]
	SARS-CoV-2	RBD of S protein	Protein NPs	30–40	AddaVax	i.m.	[75]
		RBD of S protein	Particulate Alum	< 4000	Alum	i.m.	[76]
		S, M, E protein cocktail	Virus-like particles	100	—	i.m.	[77]
		RBD/HR of S protein	Ferritin-based NPs	—	SAS	s.c./i.m.	[78]
		RBD of S protein	Virus-like particle	20	AddaVax	i.m.	[79]
	SARS-CoV	Plasmid of S protein	PEI NPs	195	—	i.n.	[80]
		Plasmid of N protein	Chitosan NPs	210	Anti-CD40 mAb	i.n./i.m.	[81]
Antigen-encapsulated nanovaccine	SARS-CoV-2	mRNA of S protein	LNPs	90	LNPs	i.m.	[82]
		mRNA of RBD	LNPs	—	—	i.m./s.c.	[83–85]
		mRNA of RBD/S protein	LNPs	—	—	i.m.	[86, 87]
		saRNA of S protein	LNPs	75	—	i.m.	[88]
		repRNA of S protein	LION	90	Squalene	i.m.	[89]

Abbreviations. TLRs: Toll-like receptors; HRC1: C-terminal heptad repeat region 1; MPLA: monophosphoryl lipid A; QS-21: quillaja saponaria 21; i.p.: intraperitoneal; s.c.: subcutaneous; i.m.: intramuscular; i.n.: intranasal; SAS: Sigma adjuvant system; PEI: polyethylenimine; LION: lipid inorganic nanoparticle (cationic squalene emulsion with Fe_3O_4 NPs).

and nasal turbinates. Lin et al. developed a hollow nanovaccine with a diameter of 114 nm for the prevention of MERS-CoV infection [73]. The RBD antigen of MERS-CoV was conjugated on the nanovaccine surface via thiol–maleimide linkage for antigen presentation. The soluble adjuvant cdGMP, a new class of stimulator of interferon genes (STING) agonist, was encapsulated in the nanocarrier for cellular uptake and immune activation. For *in vivo* studies, the level of anti-RBD titers reached a higher peak at two weeks than other control groups, and was sustained for 300 days after vaccination in mice. The increased antibodies significantly neutralized the amount of MERS-CoV in the lung and induced effective prophylaxis against lethal MERS-CoV challenges in the DPP4-overexpressed transgenic mice. Moreover, Huang et al. also demonstrated the rapid fabrication of SARS-CoV-2 nanovaccine by chelating polyhistidine (His)-tagged RBD antigen with liposomes containing cobaltporphyrin-phospholipid (CoPoP) [74]. The displayed RBD on the surface of adjuvant-contained liposome induced effective activation of antigen-specific populations of immune cells such as macrophage, dendritic cell, T cell, and B cell. In the animal studies, the nanovaccine produced 100-fold higher levels of anti-RBD titers than other vaccine formulations, which could greatly block the interaction of RBD-ACE2 and inhibit live SARS-CoV-2 viral replication.

4.1.2 *Antigen-encapsulated nanovaccines*

In another strategy, the antigen-encoding nucleic acids are protected by the nanocarrier to avoid degradation and to target nanovaccine towards APCs for cellular uptake. These APCs could translate the encapsulated nucleic acids to the relevant viral antigens, and implement them on the cell membrane by major histocompatibility complex (MHC) I and II for T cell or B cell activation [67]. In one example, Zhang et al. developed a messenger RNA (mRNA)-based vaccine (termed ARCoV) against SARS-CoV-2 infection (Figs. 4(e)–4(g)) [82]. The mRNA encoding SARS-CoV-2 RBD was encapsulated in the lipid nanoparticle (LNP) of ARCoV with a diameter of ~90 nm. Following intramuscular (i.m.) administration, the ARCoV vaccine mainly expressed RBD antigen in the liver of mice, and a slight expression in the spleen and muscle tissues. Remarkably, two immunizations with ARCoV vaccine at 2 or 10 mg resulted in significant elevation of IgG and neutralizing antibodies in mice serum, which could cross-neutralize different epidemic strains of SARS-CoV-2. More importantly, the ARCoV vaccine had high thermostability at room temperature for at least one week, suggesting the potential use in clinical trials. McKay et al. designed a self-amplifying RNA (saRNA) to encode the SARS-CoV-2 S protein, and encapsulate it within an LNP as a nanovaccine [88]. Different from the mRNA, saRNA encoded the alphaviral replicase and a gene of interest as antigen to enable the self-replication of the desired RNA upon delivery to the cytoplasm [90]. They further demonstrated that saRNA LNP immunizations could induce a Th1-based proinflammatory response in mice after vaccination. Under two i.m. injections, SARS-CoV-2 specific IgG was remarkably determined in mouse sera with a dose-responsive manner, which could effectively neutralize both a pseudo-type and wild-type SARS-CoV-2.

4.2 Nanoagents for anti-HCoV therapeutics

In the initial stage of HCoV-induced pandemics, the rapid development of new antiviral drugs is challenging due to the lack of genetic and structural knowledge of emergent viruses [14]. Although several existing drugs such as remdesivir and chloroquine have the potential to inhibit COVID-19 progression, they are originally designed for other diseases treatment, thereby suffering from limited therapeutic effects and undesired side effects such as hepatotoxicity and allergic reactions [15–17]. Recently, various antiviral nanocarriers have been extensively explored as a promising approach against COVID-19. Moreover, nanoagents could be surface modified by specific receptors for enhanced HCoV binding, which could inhibit viral infection in

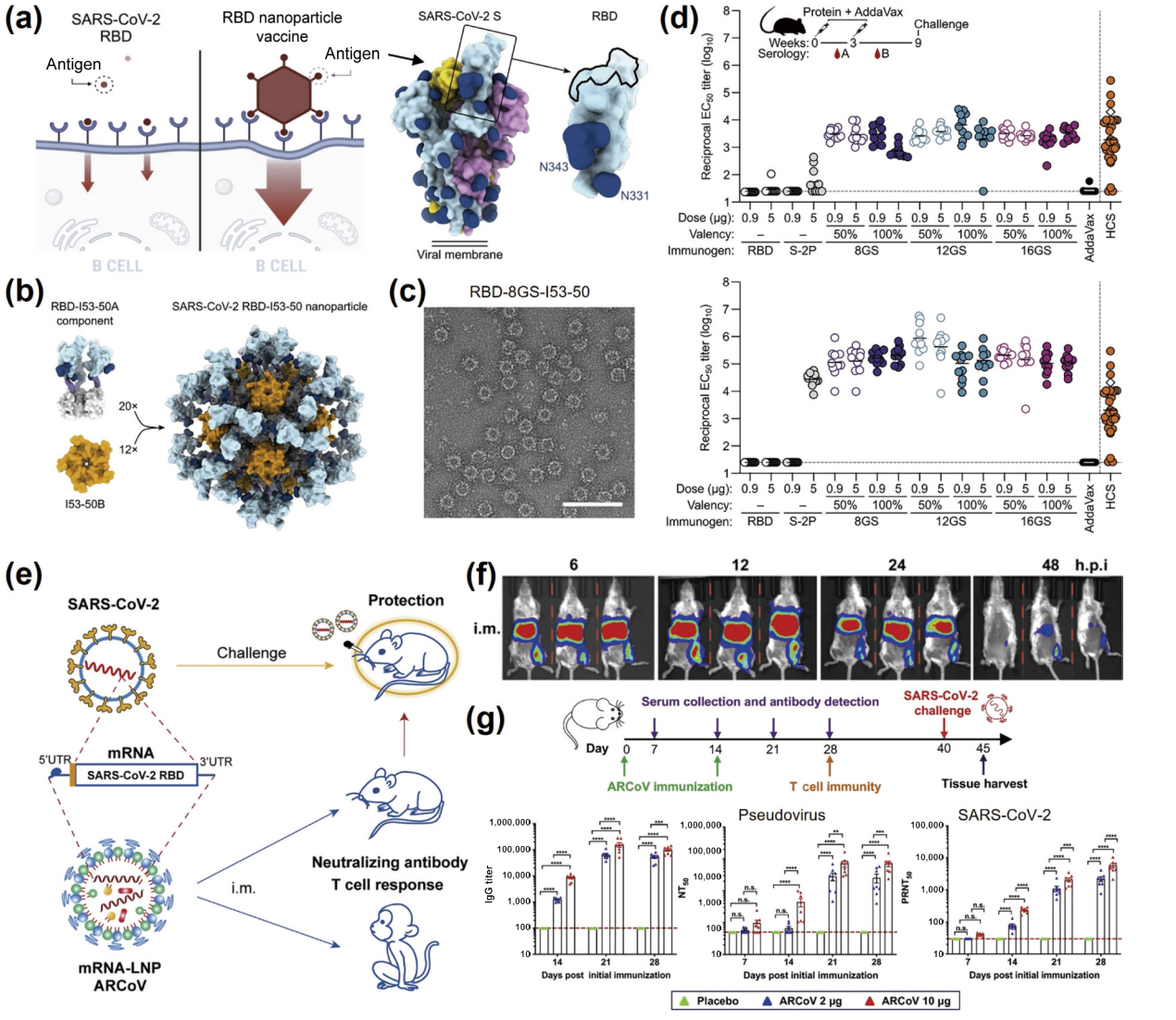

Figure 4 The prevention of HCoV infections by nanovaccines. (a)–(d) Antigen-exposed nanovaccine. (a) Schematic diagram of RBD-based nanovaccine to elicit higher antibody response against SARS-CoV-2 S protein than the spike ectodomain trimer. The three-dimensional (3D) molecular surface represented the SARS-CoV-2 S protein and RBD, including the N-glycosylated sites at positions N331 and N343 (native antigenic glycans). The ACE2 receptor-binding site was indicated with a black outline. (b) Structural models of RBD-I53-50A (cyan: RBD, gray: I53-50A) and I53-50B (orange) components. Upon mixing *in vitro*, RBD-I53-50A and I53-50B components self-assembled to form nanovaccine (ratio: 20:12). (c) Representative TEM image of RBD-8GS-I53-50A nanovaccine. Scale bar: 100 nm. (d) Anti-S antibody binding titers in mice measured by enzyme linked immunosorbent assay (ELISA) at week 2 (top) and week 5 (bottom) after vaccination. The adjuvant (AddaVax) and clinical samples from 30 COVID-19 human convalescent sera (HCS) were used as controls. Reproduced with permission from Ref. [75], © Elsevier B. V. 2020. (e)–(g) Antigen-exposed nanovaccine. (e) Scheme of ARCoV, a lipid nanoparticle (LNP)-encapsulated mRNA nanovaccine, to protect mice and primates under SARS-CoV-2 challenging. (f) *In vivo* bioluminescence imaging of mice when administrated with firefly luciferase (FLuc) reporter encoding mRNA-LNP. (g) Study timeline of immunization, sample collection, and challenge schedule. The SARS-CoV-2-specific IgG antibody titer (left), the 50% neutralization titer (NT50, middle), and plaque reduction NT50 (PRNT50, right). Dashed lines indicated the detection limit of the assay. Reproduced with permission from Ref. [82], © Elsevier B. V. 2020.

the host cells. Generally, these nanoagents are divided into three types based on their mechanisms for anti-HCoV therapeutics: (i) blocking viral entry, (ii) interfering viral replication, and (iii) modulating inflammatory responses (Table 3).

4.2.1 *Nanoagents blocking viral entry*

The most efficient strategy to suppress viral infection is to inactivate and damage viral infectivity before their binding with acceptors on the host cell membrane. Thanks to the high surface-to-volume ratio and tunable surface functionalization of nanoagents, they are employed to compete with the host cells on HCoV attachment for antiviral therapeutics. For example, Huang et al. reported an α-helix peptide, named pregnancy-induced hypertension (PIH)-modified gold nanorods (PIH–AuNRs) for the inhibition of S2 protein-mediated membrane fusion during MERS-CoV infection (Figs. 5(a)–5(c)) [91]. The S2 protein mainly includes three domains, heptad repeat 1 (HR1), HR2, and fusion peptides (FP). The FP inserts into the host cell membrane, and then the HR1 triplex and HR2 triplex bind together to form a 6-helix bundle (6-HB). The 6-HB promotes the fusion of MERS-CoV envelope and host cell membrane, resulting in the release of the viral RNA into the cells for infection. The PIH peptide had a similar conformation structure with the HR2 domain, which could selectively bind with HR1 to block the generation of 6-HB and viral fusion in host cells. Interestingly, PIH–AuNRs presented a 10-fold higher inhibitory activity than PIH alone due to the multivalent effects of modified PIH on nanoparticle surfaces. Cai et al. also reported a novel method by conjugating neutralizing antibodies with photothermal nanoparticles (Ab-PTT-NPs) to selectively capture and inactivate SARS-CoV-2 [100]. The monoclonal anti-SARS-CoV-2 neutralizing antibody enabled efficient binding with S protein and capturing of SARS-CoV-2

Table 3 Nanotherapeutics against HCoV infections and antiviral mechanisms

Type	Virus	Nanoagent	Size (nm)	Mechanism	Ref.
Blocking viral entry	MERS-CoV	PIH-AuNRs	54 × 18	Inhibit S2 protein-mediated cell fusion	[91]
		PAMAM dendrimers	<5	Damage viral outer membrane	[92]
	SARS-CoV-2	Cell membrane-coated PLGA NPs	100	Block S protein by membrane receptors	[93]
		Yeast-produced nanobody	—	Block S protein by ACE2	[94]
		ACE2-rich cell membrane vesicle	100	Block S protein by ACE2	[95]
		Lung spheroid cells-derived nanodecoy	300	Block S protein by ACE2	[107]
		ACE2-engineered nanodecoy	150	Block S protein by ACE2	[108]
		mRNA-encapsulated LNPs	80	Encode ACE2 to block S protein	[96]
		Silica/inorganic polyphosphate NPs	150–200	Interact with RBD to block S protein	[97]
		Iron oxide NPs	—	Interact with RBD to block S protein	[98]
		Ag NPs	10	Disrupt the disulfide bonds of S protein	[99]
		Antibody-conjugated polymer NPs	90	Capture virus and inactivate by photothermal	[100]
		TiO_2 NPs	—	UV light-induced oxidative damage	[101]
Interfering viral replication	SARS-CoV-2	IVM-loaded PLGA NPs	70–80	Releas IVM to downregulate ACE2 and IMPα/β1	[102]
		Lisinopril/remdesivir-loaded PLGA NPs	—	Inhibit ACE2 and block RdRp	[103]
Modulating inflammatory responses	SARS-CoV-2	Fused vesicle from MΦ and ACE2-rich cell	100	Neutralize cytokines (IL-6 and GM-CSF)	[104]
		Dexamethasone-loaded leukosomes	150	Modulate cytokines, chemokines, and complement factors	[105]
		Dnase-1-coated PD-PEG NPs	220	Suppress neutrophil activities and cytokine storm	[106]
		Nanodepot of manganese (Mn^{2+})	35	Release Mn^{2+} to promote IFN response	[111]

Abbreviations. PD-PEG: polydopamine-poly(ethylene glycol); Dnase-1: deoxyribonuclease I; PAMAM: polyamidoamine.

with high affinity (0.07 nM) when labeled on the surface of nanoagents. Upon 650 nm light irradiation, the semiconducting polymer core of Ab-PTT-NPs could selectively induce local heating to damage the viral integrality, thus leading to the effective inhibition of SARS-CoV-2 entry into host cells.

Another impressive approach for antiviral treatment is to focus on the targeted host cell membrane rather than the causative pathogens. The viral infection of SARS-CoV-2 must rely on the specific binding with some receptors, either known or unknown, on the host cell membrane. Inspired by this fact, Zhang et al. first created a cellular nanosponge, the host cell membrane-coated nanoparticle, for the capture and neutralization of SARS-CoV-2 (Figs. 5(d)–5(f)) [93]. The nanosponges inherited the natural protein receptors, such as ACE2, TMPRSS2, and DPP4 on human lung epithelial cells, or the ACE2, C-type lectin domain family 10 (CLEC10), and CD147 on the macrophage. These receptors on nanosponges displayed almost complete inhibition of authentic SARS-CoV-2 infectivity on Vero E6 cells due to the block of viral entry. Recently, the nanosponges or nanodecoys acted as inhalable nanoagents to neutralize SARS-CoV-2 in the lung tissue of cynomolgus macaques and mice without observed toxicity, which could serve as potential therapeutic nanoagents to treat COVID-19 and its variants [107, 108]. In principle, as long as the receptors of these mutated viruses remained on the host cell membrane, the nanosponges would be able to block the viral entry, leading to broad-spectrum antiviral nanoagents against any emerging HCoVs in the future.

4.2.2 *Nanoagents interfering viral replication*

Besides the blocking of viral entry, alternative pharmacological strategies are also desired when the viruses have entered inside of host cells at the middle or late stage of infection. So far, few antiviral drugs are clinically available against COVID-19 based on different mechanisms to interfere with viral replication, either by inhibiting the RdRP activities (e.g., remdesivir, favipiravir) or by blocking viral proteases (e.g., ivermectin, darunavir) [109]. Owing to the undesired systematic side effects of these drugs, nanocarriers were developed recently by embedding the drugs into the nanoparticles for targeting delivery against SARS-CoV-2 infection. For instance, Surnar et al. reported promising ivermectin (IVM)-loaded poly(lactic-co-glycolic acid) (PLGA)-based nanoparticles (IVM-NPs) for orally administrable COVID-19 treatment (Figs. 6(a)–6(d)) [102]. The IVM-NPs were first conjugated with an Fc immunoglobulin fragment through thiol–ene chemistry to produce targeting nanoparticles (T-Fc-IVM-NPs), which could recognize the neonatal Fc receptor (FcRn) to cross the intestinal epithelial barrier and gradually release the IVM into the bloodstream. They further demonstrated that the released IVM drug could block the activities of nuclear transport proteins such as importin α and β1 (IMPα/β1) heterodimer, thus downregulating the expression of viral spike protein and its ACE2 receptor on the infected host cell membrane. By comparing with the control groups including non-targeted IVM-NPs (NT-IVM-NPs) and free IVM drug, the T-Fc-IVM-NPs presented higher inhibition efficiency of pseudotyped SARS-CoV-2 infection in HEK293T host cells through both therapeutic and preventative regimens. Wu et al. reported a systematic computational investigation on the potential design of drug-loaded PLGA nanoparticles against COVID-19 [103]. Lisinopril (a hydrophilic inhibitor of ACE2) and remdesivir (a hydrophobic inhibitor of RdRp) are desired to be synchronously delivered using the core–shell PLGA as nanocarrier. To theoretically optimize this antiviral nanoformulation, they used molecular docking and dissipative particle dynamics (DPD) simulations to computationally investigate the drug-carrier interactions and their assembly process. They verified the strong interactions between lisinopril and ACE2, as well as remdesivir and RdRp. The DPD simulations showed that spherical nanodrug could be formed with

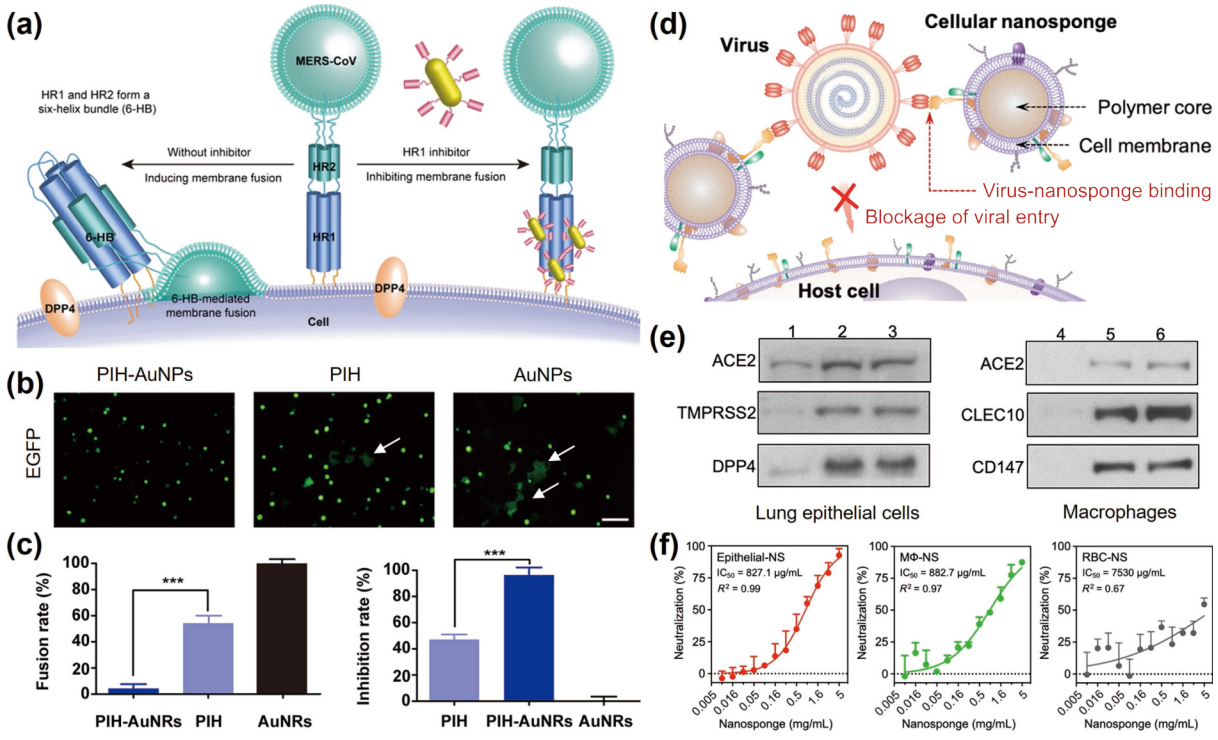

Figure 5 Nanoagents inhibiting HCoV infections by blocking viral entry. (a)–(c) Inactivation of viral infectivity. (a) Schematic diagram of PIH modified gold nanorods (PIH-AuNRs) to inhibit MERS-COV S2 subunit-mediated membrane fusion and infection. (b) Fluorescence images of fused cells (white arrows) between HEK293T cells ($MERS^+$/$EGFP^+$) and Huh-7 cells ($DPP4^+$) in the presence of PIH-AuNRs, PIH, and AuNRs. Scale bar: 100 μm. (c) Quantification of cell fusion rate (left) and inhibition activity (right) with PIH, AuNRs, and PIH–AuNRs treatment, respectively. Reproduced with permission from Ref. [91], ©American Chemical Society 2019. (d)–(f) Mimicking of the cell membrane. (d) Scheme of membrane-coated nanoparticle, cellular nanosponge (NS), to bind with SARS-CoV-2 S protein and inhibit the viral infection. (e) Western blotting analysis of membrane receptors from the cell lysate (1, 4), cell membrane vesicles (2, 5), and cellular nanosponges (3, 6) on the lung epithelial cells and macrophages (MΦs). (f) The neutralization efficiency against authentic SARS-CoV-2 infection by Epithelial-NS (left), MΦ-NS (middle), and red blood cell (RBC) membrane coated nanosponges (RBC-NS, right) on Vero E6 host cells. Reproduced with permission from Ref. [93], ©American Chemical Society 2020.

the ratios of PLGA to drug at 5 : 1 and 10 : 1, where the remdesivir was loaded in the hydrophobic PLGA core and lisinopril in the thin hydrophilic shell of designed nanocarriers. This study provided a promising strategy based on computational simulation for the design of hydrophilic or hydrophobic drug loading in the nanocarrier for COVID-19 treatment.

4.2.3 *Nanoagents attenuating inflammatory response*

Usually, the patients infected by HCoVs undergo exceedingly enhanced levels of inflammatory cytokines and chemokines in clinics, which will cause long-lasting lung injury and a high risk for the reduction of life quality [110]. Therefore, effective treatment approaches to attenuate the inflammatory response are highly demanded against HCoV infections. Although various methods such as immunoglobulins (e.g., eculizumab and nivolumab) and immunomodulatory drugs (e.g., dexamethasone and colchicine) were reported for COVID-19 treatment recently [66], they suffer from a single target in a specific cytokine pathway and unwanted side effects after systemic administration. As an alternative strategy, nanocarriers are employed to deliver anti-inflammatory drugs towards immune cells to avert ARDS. For example, Molinaro et al. prepared leukocyte-based biomimetic nanoparticles, termed leukosomes, to deliver Dexamethasone as an anti-inflammatory medicine for the regulation of uncontrolled inflammation *in vivo* [105]. Upon intravenous administration, the drug-loaded leukosomes could effectively reduce the levels of pro-inflammatory cytokines (e.g., TNF-α, IL-6, and IL-1β) and improve the anti-inflammatory cytokines (e.g., IL-10) in lipopolysaccharide (LPS)-induced endotoxemia model, which was applicable for the attenuation of cytokine storm syndrome observed in COVID-19 infection. Sun et al. reported a nanodepot of manganese (nanoMn) to release Mn^{2+} in a pH-sensitive manner, which could activate M1 macrophage to promote interferon (IFN) response and to reduce the HCoV-induced tissue damage [111]. Rao et al. also reported a smart decoy nanoparticle for the treatment of COVID-19 based on specific virus capture and cytokines neutralization (Figs. 6(e) and 6(f)) [104]. The nanodecoys were made by fusing two cell membrane vesicles isolated from human macrophage (THP-1) and ACE2-engineered HEK293T cells. In the presence of pseudoviruses and authentic SARS-CoV-2, the nanodecoys could capture the virus and protect host cells from viral infection. More importantly, the nanodecoys could efficiently neutralize multiple inflammatory cytokines, such as IL-6 and granulocyte-macrophage colony-stimulating factor (GM-CSF), mainly attributed to the natural cytokine receptors on their surface. In the acute pneumonia mouse model, the nanodecoys also greatly suppressed the severe inflammation and lung injury, suggesting a safe and powerful antiviral nanomedicine against HCoV-caused pandemics.

5 Conclusions and perspectives

The emergence and spread of HCoV-caused pandemics, especially COVID-19, represent unprecedented crises with massive people deaths and deep socio-economic damages globally. Despite the presence of new vaccines and therapeutic medicines in the past years, HCoVs still undergo unpredictable mutation and evolution, thus demanding more effective diagnostic and therapeutic methods, such as translatable vaccines and broad-spectrum anti-HCoV drugs [112]. The nanoagents provide advanced approaches to overcome the drawbacks of conventional vaccines or drugs against the COVID-19 pandemic, especially for the liposome-based mRNA nanovaccines from Pfizer-BioNTech and Moderna, which represent a milestone of nanomedicine in clinics. However,

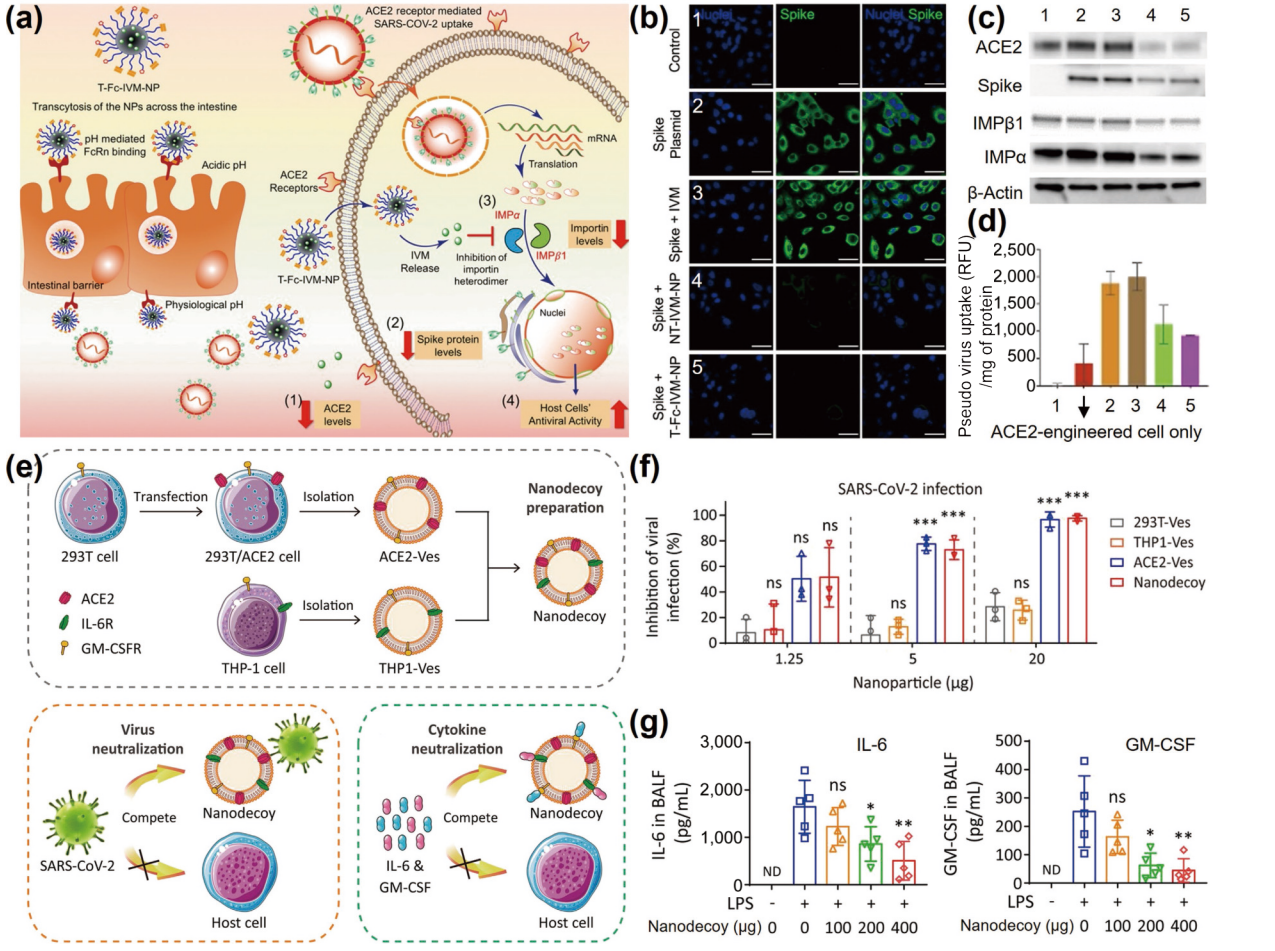

Figure 6 Therapeutic nanoagents against HCoVs. (a) and (d) Interference of viral replication. (a) Scheme of orally administrable IVM-loaded nanoparticles (T-Fc-IVM-NPs) to cross the intestinal barrier by transcytosis after binding with FcRn receptors in the acidic gut. The released IVM could down-regulate the levels of (1) ACE2 receptor, (2) SARS-CoV-2 spike protein, and (3) nuclear transport proteins, importin α and β1 (IMPα/β1), resulting in (4) an improved antiviral activity in the infected cells. (b) Immunofluorescence staining of spike protein engineered HEK293T cells (2) with the treatment of IVM (3), NT-IVM-NPs (4), or T-Fc-IVM-NPs (5). Naive cells were used as control (1). (c) Western blot analysis of ACE2, spike protein, and IMP α and β1 levels in different groups. (d) Inhibition efficacy of SARS-CoV-2 pseudovirus uptake by ACE2-engineered HEK293T cells in different groups. Reproduced with permission from Ref. [102], ©American Chemical Society 2020. (e)–(g) Attenuation of inflammatory response. (e) Scheme of nanodecoys prepared by fusing ACE2-engineered HEK293T cell and macrophage (THP-1) vesicles. The nanodecoys inherited the ACE2 and cytokine receptors and competitively protected the host cells from SARS-CoV-2 based on virus and cytokine neutralization. (f) Inhibition efficiency of authentic SARS-CoV-2 infection on Vero-E6 cells in the presence of nanodecoy and various membrane vesicles. (g) Neutralization of inflammatory cytokines including IL-6 and GM-CSF in the lung bronchoalveolar lavage fluid (BALF) of LPS-stimulated acute pneumonia mice model. Reproduced with permission from Ref. [104], © US National Academy of Sciences 2020.

several challenges still occur in developing innovative nanoagents against the HCoV variants.

(i) Nanosensors for HCoV diagnosis. So far, only AuNPs-based lateral flow immunoassays are commercially available as disposable point-of-care tests for SARS-CoV-2 detection [113]. Further efforts should be addressed towards the new nanosensors based on electrochemical and FET transduction, which are reusable and applicable for the simultaneous detection of multiple viral strains. On the other hand, some clinical biomarkers present significant sensitivity to distinguish the mild or severe patients under HCoV infection, including the C-reactive protein, serum amyloid A, inflammatory cytokines (e.g., IL-6, TNF-α, and IL-1β), lactate dehydrogenase, procalcitonin, neutrophil-to-lymphocyte ratio, cardiac troponin, lymphocytes, and platelet count [114]. The early prediction of severe cases by using smart nanosensors to detect these relevant biomarkers is critical for HCoV-infected patient management in clinics. In addition, more efforts could be focused on the development of wearable nanosensors, such as microneedles or nanorobots, to continuously monitor antigens, antibodies, or nucleic acids of HCoVs in the body. These smart nanodevices would real-time track the infected cases in pandemics, and also monitor the dynamic immune response after vaccination, which are beneficial to the health of communities.

(ii) Nanovaccines against HCoVs. Despite the success of various FDA-approved COVID-19 vaccines, a major challenge is the continuous evolution of SARS-CoV-2 under immune selective pressure to reduce the protection efficiency of vaccines. Delta variant, one of the most contagious SARS-CoV-2 strains, has attracted great attention recently due to its remarkable global transmission and strong immune escape ability in patients [115]. It has 8-fold less sensitivity to vaccine-elicited antibodies than the wild-type SARS-CoV-2 (D614G strain) [116]. Benefitting from the feasible abilities to load or conjugate mutable antigens on nanoagents, nanovaccines have excellent advantages for prolonged antigen presentation and enhanced immunogenicity against the Delta variant. Moreover, thanks to the tunable surface modification, nanovaccines can mimic the respirovirus and activate the mucosal immune system for an effective prevention of viral infection. It has been reported that the single-dose intranasal vaccination of liposomal S protein subunit nanovaccine could induce systemic neutralizing IgG and IgA antibodies *in vivo* [117], while current intramuscular vaccines in clinics are only designed

to elicit IgG rather than mucosal IgA in the nasal compartment which acts as the first barrier against mutable HCoVs [118].

(iii) Nanotherapeutics towards HCoVs. Currently, the clinical management of COVID-19 mainly emphasizes infection prevention, control measures, and supportive care including supplemental oxygen and mechanical ventilation. The FDA-approved drug (remdesivir) for SARS-CoV-2 inhibition still suffers from undesired side effects in clinics. Therefore, the drug-embedded nanocarriers are highly desirable for targeting delivery and improved bioactivity to fight against COVID-19. Moreover, a primary hurdle for the treatment of HCoV infections is the lack of broad-spectrum antiviral drugs due to their varied infection processes. The metal-based nanomaterials, including the gold or silver nanoparticles and quantum dots, present broad-spectrum antiviral properties by blocking the viral entry, replication, and diffusion regardless of the different viral infection mechanisms [119]. However, their long-term biosafety becomes the main concern for their clinical translation. The cell membrane-coated nanoparticles could act as a new generation of broad-spectrum nanotherapeutics against various HCoV strains [120]. The multiple binding receptors of these biomimetic nanosponges are able to capture any HCoV mutations before their cellular entry [121]. Moreover, the membrane-coated nanodecoys from natural immune cells such as the macrophages and neutrophils are the potential to neutralize overexpressed cytokines and chemokines [122]. These promising antiviral nanoagents may provide flexible and broad-spectrum candidates against HCoV variants.

(iv) Clinical challenges and limitations. Despite the initial success of nanoagents against HCoV infections in the laboratory, great challenges still exist for their subsequent translation in clinics. A major hurdle is how to ensure the safe use of nanoagents in patients with long-term exposure. Due to the formation of protein corona on the different particle surfaces, the pharmacokinetic behavior and therapeutic effect of nanoagents are flexible in the blood circulation, which are currently being studied [123]. It has been demonstrated the toxic effect of nondegradable silver nanoparticles through inhalation is due to generating acute neutrophilic inflammation and producing chemokines in the lung [124]. In addition, the high mutation rate and genetic diversity of HCoVs constitute the main obstacles of nanoagents for viral treatment in clinics. One example is the RBD-based nanovaccines. Although the RBD structure on SARS-CoV-2 is rapidly identified by scientists to design the vaccines, it still has high risks to attenuate their prevention efficiency against COVID-19 due to the variable RBD compositions in HCoV genomes [125]. Finally, the reliable techniques to scale up the nanoagents production are still limited in industry. Benefitting from the large-scale production of Good Manufacturing Practice (GMP)-grade mRNAs and lipids, the Pfizer-BioNTech and Moderna could manufacture liposomal mRNA vaccines in standardized, rapid, and controlled conditions [126]. Therefore, more attention and investments should be done to optimize scale-up procedures of other nanoagents in clinical practice.

In summary, diagnostic and therapeutic nanoagents play significant roles in the fight against HCoVs. Owing to the morphological similarities of synthetic nanoparticles with naive HCoVs, these nanoagents can perform selective interaction and inhibition of viral infectivity upon modification with functional moieties. Additionally, nanoagents are also desirable to make translatable nanovaccines and broad-spectrum antiviral drugs to protect humans from potential HCoV variants. The successive progress of nanoagents will facilitate the rapid development of promising theranostics against HCoV-induced pandemics in the future.

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