International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 13, Issue 3, 2021

Review Article

STATE-OF-THE-ART NANOTECHNOLOGY BASED DRUG DELIVERY STRATEGIES TO COMBAT COVID-19

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Received: 23 Dec 2020, Revised and Accepted: 13 Mar 2021

ABSTRACT

The emerging Coronavirus Disease-19 (COVID-19) pandemic has had a global impact on all important aspects of our society. As it is known, SARS-Cov-2 can withstand up to 72 h in adverse environmental conditions, which can aid its rapid spread. Woefully, an efficacious and approbated vaccine for the SARS-CoV-2 virus remains unavailable, which makes the problem more frightening and presently more complicated bestowing forlorn medical care. Nevertheless, global clinical research is studying several over-the-counter (OTC) drugs approved for other indications to confront coronavirus. Over the past decade, therapeutic nanoparticles have been regarded as a felicitous tool for the efficient and persnickety delivery of therapeutic groups (i.e., drugs, vaccines, siRNAs, and peptides) to the site of infection. They can adequately convey the drug encapsulated nanoparticle to a designated locus without instigating unsought effects. Besides, they acquiesce the use of non-invasive imaging methods to monitor the surface of the infection and the response to treatment. The formulated nanoparticle is apposite for intranasal drug delivery which is a meritorious method to deliver therapeutic moiety for viral diseases affecting the lungs. Applying nanoparticles via intranasal route surmounted several demerits of mucosal administration like circumventing enzymatic degradation of the therapeutic moiety, upgrading and prolonging the action of the drug, etc., and can thus corroborate as an exceptional strategy to encounter respiratory viruses like coronavirus. In this article, we illuminate the promising role of nanoparticles as effective carriers of therapeutic or immunomodulatory agents to help combat COVID-19.

The search criteria used were Pubmed, Medscape, Google scholar, etc and the keywords are coronavirus, nanoformulations, nanoparticles, drug delivery, intranasal delivery, etc. The articles range from 2012 to 2020.

Keywords: COVID–19, SARS-CoV-2, Coronavirus, Nanoparticles, siRNA, Intranasal route

DOI: https://dx.doi.org/10.22159/ijap.2021v13i3.40865. Journal homepage: https://innovareacademics.in/journals/index.php/ijap
Speedy peer review was done as the subject of the manuscript was related with pandemic.

INTRODUCTION

Early the beginning of 2020, due to the continuing eruption of a novel Severe Acute Respiratory Syndrome, Coronavirus 2 (SARS-CoV-2), the global healthcare sector is undergoing a potentially hazardous condition. The World Health Organization (WHO) fired the sirens on 30 January 2020 against a rapidly disseminating infectious disease initiated by the newly discovered SARS-CoV-2 and stated the condition as Public Health Emergency of International Concern [1]. WHO named this disease COVID 19 on 10th February 2020. COVID-19 stands for COrona VIrus Disease 2019 as it was first reported on 31st December 2019 in Wuhan, China at the WHO Country Office as an unknown case of pneumonia. On 11th March 2020, it was declared a pandemic [2]. Coronavirus emerged in 2003 in China which was an epidemic that resulted in SARS and later in 2012 in Saudi Arabia it led to Middle East Respiratory Syndrome (MERS). This new virus, compared to the SARS-CoV in 2002 is much more virulent and pestilent and has spread rapidly worldwide. Transmission of SARS-CoV-2 from person to person is approximated to take place mainly through breathing droplets developed as a result of sneezing, coughing, and conversation and this is largely comparable to the spread of influenza. Transmission can, however, occur when a person infected with or without signs is near a normal person [3]. Also recently WHO has found COVID-19 to be an air-borne disease. The common types of symptoms, emergency conditions, and the associated complications of coronavirus infection are shown in table 1.

Table 1: Clinical manifestations of COVID-19

Clinical manifestations						
Common	**Less common**	**Early**	**Others**	**Emergency**	**Complications**	**Reference**
-Fever	-Rash	-Loss Of Smell	-Myalgia	-Trouble Breathing	-Pneumonia	4,5
-Cough	-Vomiting	-Loss Of Taste	-Apnea	-Blue Lips And Face	-Acute Kidney Injury	
-Tiredness	-Nausea		-Rhinorrhea	-Persistent Chest Pain	-Acute Respiratory Distress Syndrome	
-Weakness	-Diarrhea		-Sore Throat	-Inability To Stay Awake	-Heart Problems	
			-Chills	-Confusion	-Blood Clots	
			-Headache		-Organ Failure	
			-Chest Pain		-Additional Infections	

Patients with COVID-19 mainly develop cardiovascular disease/hemodynamics or breathing problems. Studies revealed that patients with underlying diseases such as neutrophilia, organ failure, coagulation dysfunction, hypertension, lung disease, diabetes, chronic respiratory illness, and heart disorders may be at greater mortality risk when compared to other patients [4]. Age is also a significant factor since patients above 60 and children below 10 y of age are more prone to get infected as their immunity has been weakened or not fully developed. While considering the mortality rate to date, it has been found that males have more death rate than females [5]. Various diagnostic tests involved in screening COVID-19 include RT-PCR (Reverse Trancriptase Polymerase Chain Reaction), nucleic acid tests, antibody testing, and sequencing of genomes. Even though at present there no definite management for COVID-19, quite a lot of drugs permitted for other indications have been explored in clinical studies. These treatments rely on the drugs that prevent the virus from entering the host cell to shut out the virus from multiplying and infecting other cells, or potentially inhibiting protease activity

(antiviral drug ritonavir/lopinavir). Other promising treatments in clinical research, employ nucleoside analogs that aim at RNA-dependent polymerase, which inhibits the production of viral RNA (i.e. Remdesivir), or directly affect the assembly of virions and buds, and modify the molecular crosstalk of SARS-CoV-2, while ultimately decreasing the generation and/or activation of pro-inflammatory cytokines anti-SARS-CoV-2 CD8+T cells (ie hydroxychloroquine). This article mainly focuses on the drug design strategies against COVID-19 virus especially nanoparticle-based treatment which emphasis on intranasal administration.

Structure of COVID-19 virus

A study conducted by Prasad *et al.* in March 2020 revealed that the electron microscopy image of SARS-CoV-2 manifested the appearance of stalk-like protuberance that ends in round peplomeric assembly which is prototypical for a particle-like coronavirus [6]. Coronavirus is an enveloped virus with positive-sense single-stranded RNA composed of 30,000-bases and has a mean diameter of approximately 125 nm (125 μm). The diameter of the envelope of the coronavirus particle is 85 nm and the length of the spike is 20 nm. They have a characteristic crown-like structure after which they are named. Coronaviruses are the largest among the known RNA virus and they belong to Coronaviridae and Nidovirales; family and order respectively [7]. There are 4 genera/subgroups of coronaviruses:-alpha coronaviruses, beta coronaviruses, gamma coronaviruses, and delta coronaviruses. Darwinism study or evolutionary analysis discovered the gene sources of these subgroups. The gene sources of alpha coronaviruses and beta coronaviruses are rodents and bats while avian species are the gene sources of gamma and delta coronaviruses. SARS-CoV-2 belongs to the beta subgroup of coronaviruses. Like other βCoVs, the SARS-CoV-2 genome contains two untranslated flanking regions and a long open sequence. The reading frame encodes a polyprotein. The SARS-CoV-2 genome sequence is the structural protein 5'replicase (orf1/ab) [S-E-M-N]-3 ', and the hemagglutinin esterase gene is missing which is the characteristics found in β-CoV type A.

The genome of SARS-CoV-2 encodes four main structural proteins [spike (S), envelope (E), membrane (M), and Nucleocapsid (N)], approximately 16 non-structural proteins (nsp1-16), and 5 to 8 other proteins. Protein S binds to the virus, fuses, enters, and induces transmission. The spike on the surface of the coronavirus is a homotrimer of S protein, consisting of 2 subunits, the S1 subunit, and the S2 subunit. The N-terminal and C-terminal include the S1 subunit and the S2 subunits which are responsible for viral receptor binding. The homotrimeric protein S is a class I fusion protein that can mediate binding to receptors and fusion of membranes of the virus and the host cells. The head of the spike forms the S1 subunit and possesses a receptor-binding domain (RBD) and N-terminal domain (NTD). The S2 subunit forms the stem that holds the spike in the viral envelope and allows fusion during the activation of the protease enzyme. E and M proteins are the fundamental proteins required for the formation of viral envelopes and the preservation of their structural configuration. The viral envelope is made of a lipid bilayer, in which the structural proteins like a membrane (M), envelope (E), and spike (S) are anchored. The ratio E: S: M in the bilayer of lipids is about 1:20:300. Coronavirus particles have an average of 74 spikes at the surface [8, 9]. The genome has a methylated cap at the 5' end and a polyadenylated tail at the 3' end. The organization of the genomic material of coronavirus is 5′-leader-UTR-replicase (ORF1ab)-spike (S)-envelope (E)-membrane (M)-nucleocapsid (N)-3′UTR-poly (A) tail. Surprisingly, orf3b encodes for a new protein which is short. Additionally, the new orf8 can encode a secreted protein having an α spiral, then a six-chain beta plate. Using direct RNA sequencing and synthetic sequencing, Kim provided high-resolution maps of the transcriptome and the SARS-CoV-2 epitope transcriptome. In addition to viral genomic RNA and 9 canonical subgenomic RNAs, SARS-CoV-2 will also produce transcripts encoding unknown ORFs with fusions, deletions, and/or turn code. The researchers also established that there are about 41 alteration sites in RNA in viral transcription, the commonest reason being AAGAA [10]. In a study conducted by Paraskevis *et al.*, and Fahmi M *et al.*, it was described as SARS CoV-2 can be the result of the accumulation of mutations in response to changes in selective stress, or of the fickling RNA polymerases that continue to exist by replicating neutral mutations [11, 12]. The diagrammatic representation of the structure and genomic composition of the SARS CoV virus is shown in fig. 1.

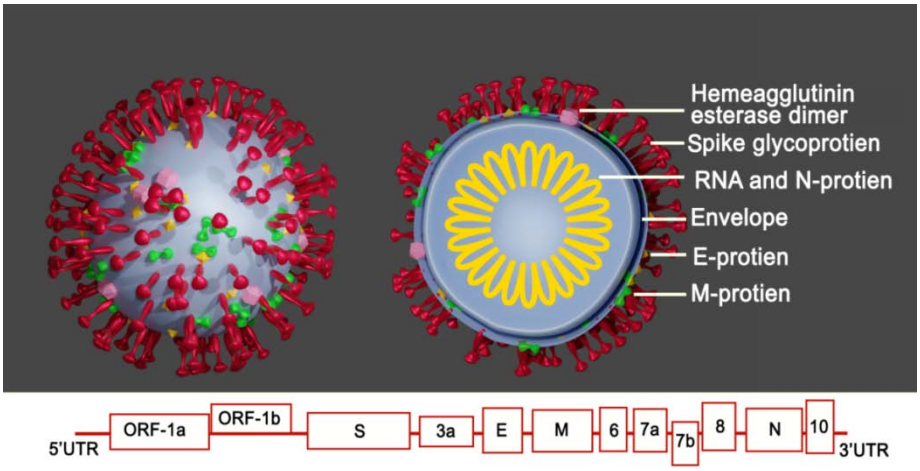

Fig. 1: Structure and genomic composition of SARS-CoV-2 virus

Pathophysiological mechanisms associated with SARS—COV2 infection

Covid 19 viruses are positively stranded enveloped nucleocapsid retroviruses. The synthesis of polyprotein 1a/1ab (pp1a/pp1ab) is recognized in the host beginning with the viral RNA. Once the virus-related genome of RNA is released into the cytosol, the RNA undergoes uncoating to enable transcription of the viral genome RNAs and genomic replication. The process of transcription occurs through the RCT complex known as the Replication transcription complex produced by the synthesis of subgenomic sequences of RNA. The termination process of transcription occurs in the regulatory transcription sequences which are housed between the open reading frames (ORF) that acts as the template for the viral genome mRNA assembly. About 6 ORFs are present in the atypical form of the genome of CoV [13].

The first step in the pathogenesis of COVID-19 infection is viral tropism. The presence of S protein is accountable for the access of the virus into the host cell and it occurs after attaching to the receptor of the cell. The angiotensin responsible for the conversion of enzyme 2 (ACE2) was considered as the cellular receptor of the

host for the spike glycoprotein SARS-CoV2 envelope. ACE2 is mainly found in blood vessels, kidneys, heart, gastrointestinal tract, and particularly the epithelial cells present in the pulmonary system are susceptible to infection. In the pulmonary system, the ACE2 are identified in the bronchus, alveoli, trachea, etc. Hoffmann, in a study, established that the virus makes use of the ACE2 receptor for its entry and also TMPRSS2, a serine protease used for priming of S protein [14]. Following the sequencing of a single cell, Meng discovered that in type II alveolar cells (AT2) there was a presence of increased genes linked with endocytosis indicating that the process of endocytosis allows the entry of the virus into the host cell. In the AT2 cells, the SARS CoV virus causes damage to alveoli as well as augment the surface tension in the alveoli leading to dyspnea [15]. Chen identified that the expression of ACE2 in the pulmonary system increased exponentially by age. Thus, a significant viral count in older patients may not only be linked with less immunity but also with strong ACE2 receptor expression. Therefore, these data concurrently suggest the increased level of serious disease in older SARS-CoV-2 patients. Following viral tropism, the next step in covid pathogenesis is the entry portal. ACE2 expression in salivary glands was larger when compared to the pulmonary system, inferring that salivary glands could be a possible target for SARS-CoV-2. Hui noticed that the epithelium of the conjunctiva and the conducting airways seem to be a plausible entrance for SARS-CoV-2 infection with the help of *in vitro* and ex vivo studies. The major targets for the virus are mucus cells, ciliated and club cells of the epithelium of bronchioles, conjunctival mucosa, and type I pneumocyte cells [16].

The next step is enhanced expression of receptors in which a study conducted by Ziegler revealed that the production of interferon stimulates the ACE2 expression in the pulmonary tissues and nasal epithelium. This led to the conclusion that the pathway of interferon necessary to protect the host against virus leads to increased infection [17]. The next step is the infection occurring to immune cells. Wan demonstrated that T cells were infected with SARS CoV-2 and infection occurred through receptor-associated fusion which was facilitated by S protein [18]. Acute respiratory distress syndrome (ARDS) occurs which results in cytokines and chemokines upregulation leading to another syndrome known as cytokines release syndrome. The syndrome pattern is quite comparable to sHLH (secondary haemophagocytic lymphohistiocytosis). In most of COVID 19 affected people, a cytokine enhancement pattern similar to that of sHLH is observed. There is an increase in levels of cytokines like IL-1β, IL-6, IL-7, IL-8, etc, TNF and other chemokines in COVID-19 suspected individuals [19]. The mechanism of virus entry into the host cell and types of drugs used to prevent the different steps involved in the pathogenesis is shown in the following fig. 2.

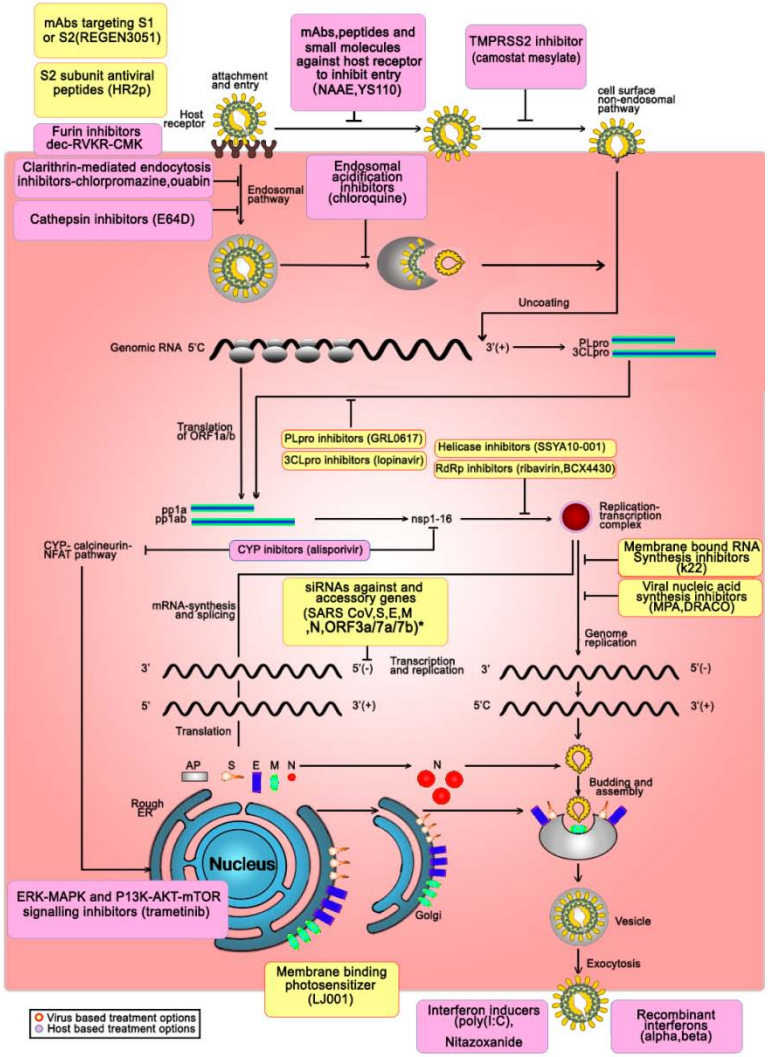

Fig. 2: Pathogenesis involved in SARS CoV2 infection and the drugs targeted to inhibit each step in the pathogenesis

Conventional treatment for the management of COVID-19

To achieve proper management of COVID-19, no clear and exact treatment with antiviral agents has been identified yet. About patients diagnosed with COVID-19, it was proposed that adequate symptomatic treatment and supportive cautions be pursued. The supportive measures include respiratory and cardiovascular support and helps to eliminate side effects and complications rather

than killing the virus [20]. Respiratory support seems to be the primary step taken care of by the healthcare team and the various respiratory support systems included are non-invasive positive pressure ventilation (NPVV), conventionally done oxygen therapies, and the use of high flow nasal cannula. An epidemiological study conducted on a group of COVID-19 affected patients showed that NPVV appears to be the commonly used respiratory support system for the treatment of acute respiratory failure. For patients suffering from respiratory failure with early COVID-19 stage, the use of NPVV is suggested for about 1 to 2 h and if the condition is still not improved, then intubation should be done [21].

Six clinical trials were recorded in both the Chinese Clinical Trial Registry and the International Clinical Trials to assess the effectiveness or safety of the focused medicines for COVID-19 prognosis. For finding out an effective treatment method for COVID-19, it is of prime importance to understand how the virus acts [22]. By using machine learning software, Richardson P. *et al.* proposed that AAK1 (AP2-associated protein kinase 1) linked drugs could disable the virus from reaching the target cells. Conversely, large levels of such inhibitors, such as chemotherapeutic drugs like Erlotinib and Sunitinib are needed, which may result in serious adverse effects [23]. Another drug Baricitinb belonging to the class Janus kinase (JAK) inhibitor can attach to cyclin G-linked kinase, another regulator of endocytosis, and can cause AAK1 inhibition thereby constraining the viral entry into the cell. Certain remarkable COVID-19 inhibitors comprise inhibitors of HIV protease, such as Lopinavir and Ritonavir, which counteract SARS and MERS 3-chymotrypsin-like proteases [24]. To determine the performance of these antiviral medications, several clinical experiments were instituted. Phase IV clinical trial study was demonstrated to test the success of antiviral drugs Lopinavir and Ritonavir in the therapy of viral pneumonia in COVID-19 affected individuals. A clinical study is scheduled to examine the safety profile and efficiency of Baricitinib with Lopinavir/ritonavir (together with other two additional medicines) in the treatment of serious COVID-19 infection in hospitalized individuals [25]. Also in a clinical uncontrolled trial, 12 patients with pneumonia having mild COVID-19 were provided with baricitinib at a dose of 4 mg/day through oral route along with the combination of lopinavir and ritonavir, and no clinical adverse effects were reported with augmented benefits in respiratory and clinical outcomes [26].

Other proposed alternative therapies include Remdesivir (RDV), an analog in the nucleoside group that can target specific RNA-dependent polymerase preventing the production of viral RNA in a large variety of viruses like the coronavirus [27]. Holshue M. *et al.* described healthy retrieval of a patient infected with SARS-CoV-2 who received Remdesivir through the IV route had a healthy recovery without adverse outcomes. Umefenovir, Favipiravir, Oseltamivir are other antiviral agents used alone or in combination with other drugs for treating coronavirus [28]. Chloroquine (CQ) or the use of hydroxy-chloroquine (HCQ) has been of significant importance in treating the infection with the COVID-19 virus. Guidelines that include the use of Hydroxy-Chloroquine strengthened by Azithromycin indicated supporting results for reliable COVID-19 treatment. But, the mechanism of action of chloroquine by which it can effectively treat the virus remains doubtful. Possibly, it acts by preventing the SARS virus from fusion with the host cells by lysosomal acidification and obstructs cathepsins that need smaller pH for breaking spike protein of SARS CoV-2 [29]. CQ has the potential to decrease proinflammatory cytokine production and also stimulates the action of anti-SARSCoV-2 CD8+cells. The combination of CQ and Remdesivir has been recognized as the treatment method for pregnant women with COVID-19 [30]. Remdesivir remains harmless in pregnant women while CQ and its metabolites cross the placenta but remains safe during all trimesters without any greater risk for perinatal negative events. The drug is used in women who are pregnant necessities to be based on conclusive evidence [31]. ARDS is one of the primary complications of COVID-19 which can lead to severe problems including mortality. Various therapeutic strategies involved in treating ARDS comprise anti-inflammatory drugs like steroids, anti-proteases, anti-oxidants, etc., and other agents like anticoagulants, diuretics, beta-2-agonists, etc. Diverse therapies are emanating to treat this complication and include macrolides, insulin, anti-interleukin-8, ACE inhibitors, colony-stimulating factors, etc [32, 33]. Some experts have proposed the use of heparin in COVID-19 because of the increased occurrence of venous thromboembolism and intravascular coagulation. The success of this intervention, however, remains to be confirmed [34].

Recently, dexamethasone was proved as the first drug to indicate the survival improvement in patients suffering from COVID-19. The treatment with this steroid was verified on patients using a randomized trial with a dose of 6 mg once daily through oral or IV route. It was shown that the use of dexamethasone minimized death by around 35% in ventilatory patients and by 20% in patients who received oxygen supplementation. Supportive measures currently under practice include the use of antibiotic Azithromycin in combination with hydroxychloroquine which showed better results compared with Azithromycin alone [35]. Other supportive measures include the use of Vitamin C, corticosteroids, and immunosuppressive agents like sirolimus which had shown to inhibit the activity of MERS-coronavirus. Tocilizumab, a monoclonal antibody was found to be effective in severely coronavirus-affected individuals resulting in the recovery of patients in a study issued in April this year. Convalescent plasma has gained good appreciation in treating people with this novel virus. In this method, blood is drawn from patients who had recovered from the infection and the obtained plasma is administered to the infected patient. The use of this plasma helps the infected patient to augment their capacity to attack the virus [36]. Some of the adjunctive therapies involved in treating SARS-CoV 2 are shown in table 2.

Table 2: Few of the adjunct therapies used for treating COVID-19

S. No.	Category	Drug	Dose	Benefits	References
1.	Corticosteroids	Dexamethasone	6 mg oral per day	Reduce the inflammatory response of host in the pulmonary system.	[35]
2.	Immunomodulatory agents	Tocilizumab	400 mg	Enhancement in clinical outcome with enhanced respiratory status with accelerated defervescence.	[32]
3.	Immunoglobulin therapy	Immunoglobulin	0.3 to 0.5g/kg/day IV	Helps in reducing mortality associated with the infection especially in high risk patients.	[32]
4.	Pulmonary vasodialators	Epoprostenol and nitric oxide	Maximum of 50ng/kg for epoprostenol and 30 ppm on first day, 20 ppm on second day and about 10 ppm for third day in case of nitric oxide.	They can significantly reduce arterial pressure in the pulmonary system and can augment oxygenation.	[36]
5.	Vitamins	Vitamin C	1500 mg	Aids in the growth of T lymphocytes and natural killer cells and thus leads to developed immune responses. Also helps in reducing the generation of reactive oxygen species	[33, 34]

Amid these emerging possible treatments, however, the death rate of patients diagnosed with this new coronavirus continues to rise alarmingly. Consequently, attempts should focus in parallel on an alternative proposal for successful therapy whilst minimizing adverse effects. Current treatment has limitations such as reversing of infectivity contributing to poor inflammatory response and limited or reduced immunogenicity. Higher doses of the therapeutic agents are necessary to provide optimum benefits which can lead to serious adverse outcomes. The unremitting growth of novel and mutated strains of coronavirus limits the benefits of the proposed treatments with antiviral agents and urges the application of specific antiviral agents or other innovative methods for these new mutated genes [37].

Existing old therapy for targeting coronavirus infection

The theory of reuse of drugs justifies attention in the treatment of the COVID-19 pandemic due to its exceptionally great infection pressure and the urgent necessity to satisfy unmet medical needs. The reused drugs are very useful because they can avoid preclinical research related to drug research and quickly benefit patients [38]. Guo concised the relevance of regenerative medicine in COVID-19 therapy and other related viral diseases [39]. Wang *et al.* assessed medications approved by FDA, comprising ribavirin, nitazoxanide, penciclovir, chloroquine (CQ), narfalimus, and other two recognized broad-spectrum antiviral medications, Lundvir (RDV, GS-5734) and Faviprevir (T-705) is a 2019 nCoV clinical isolate in a cell culture infection model. The authors found that the two compounds CQ (EC50 value = 1.13 μmol/l; CC50>100 μmol/l, SI>88.50) and RDV (EC50 = 0.77 μmol/l; CC50>100 μmol/l; SI>129.87) at low micromolar concentrations strongly block viral infections and show a large index of selectivity. RDV has been observed to be functional against multiple viruses and is currently being evaluated in clinical studies to assess its effectiveness to fight the infection from Ebola virus. The research provides further information about the use of this adenosine analog precursor to battle COVID-19 infection. Apart from this, CQ is recognized for its antimalarial infection and its anti-inflammatory effects [40]. Attributes additionally, CQ is accepted for the therapy of autoimmune diseases like lupus erythematosus and rheumatoid arthritis. And recently it was found that CQ has unveiled to subdue infections of various viruses including SARS coronavirus, MERS coronavirus, EBOV, chikungunya virus, influenza A virus, dengue virus, human immunodeficiency virus, fever virus hemorrhagic from Crimea Congo, West Nile virus, and hepatitis A virus [41]. Savarino *et al.* confirmed that the drug CQ could effectively pass into cells and accumulate in acidic compartments, like endosomes, suicidal bags, and vesicles of the anti-Golgi network [42].

Therefore, they increase pH and many viruses require acid endocytosis at certain phases of replication, like viral membrane removal and cell entry through membrane fusion. Consequently, the well-described CQ action needs a lot of consideration [43]. Tony provided the most likely pharmacological action for CQ against SARS-CoV-2, namely, inhibition of binding of phosphatidylinositol to clathrin assembly protein (PICALM), which hinders the endocytosis mediated ingestion of SARS-CoV-2 cells [44]. Gao and others reported that in a multicenter clinical study in China, chloroquine phosphate was very efficient and secure for pneumonia linked with COVID-19. Due to its activity against SARS-CoV-2, hydroxychloroquine (HCQ), a hydroxylated derivative of CQ, has also been recently reported [45]. Zhou *et al.* proposed that HCQ can reduce the serious progress of COVID-19, subdue cytokine storm by inhibiting activation of T cell, and has the benefit of clinical safety, particularly appropriate for females who are pregnant [46]. Gautret *et al.*, efficaciously established the synergistic effect of hydroxycholine and the antibiotic azithromycin for about 20 cases of COVID-19, with detection of effective virus clearance. Similarly, Xu and others also testified the usefulness of the broad-spectrum antiviral drug Nilosamide on COVID-19. This work is also an important instance of drug reuse, as it extends its possibility to clinical trials [47]. Fan *et al.* made use of the pangolin coronavirus GX_P2V as a viable model to assess the value of drugs recovered in nCoV treatment in 2019. This study showed that cefuroxime (CEP), selamectin, and mefloquine hydrochloride showed complete prevention of cytopathic events in cell cultures of 10 μmol/l, of which CEP had the strongest inhibitory effect on GX_P2V infection. The concentration is 50% of the maximum effect [EC50 [0.98 μmol/l] [48]. Hui *et al.* recommended a combination of rifaxivir, ritonavir or lopinavir, lopinavir or ritonavir and interferon-beta, for patients with recovery plasma nCoV pneumonia and monoclonal antibody in 2019. Cao *et al.* noted the controversial results in the treatment with lopinavir-ritonavir was found to be in no way superior to normal treatment [49]. Wang *et al.* conveyed that patients who received antiviral therapy for symptoms related to pneumonia, including lopinavir/ritonavir. The treatment also significantly reduced the viral load of the β-coronavirus [50]. Russel and others reported that corticosteroids are ineffective in treating nCoV lung injury in 2019. All of these observations suggest extensive research to understand the effect of conventional antiviral drugs on COVID-19 [51]. Liu *et al.* observed that, as shown in *in vitro* studies, anticoagulant dipyridamole (DIP) inhibited HCoV-19 replication at an EC50 of 100 nM. The authors carefully chose DIP by examining the drug approved by the FDA and determined that patients infected with HCoV-19 may gain improvement from DIP adjuvant therapy by dropping multiplication of virus, inhibiting thrombophilia, and improving immune repair [52].

Sang *et al.* confirmed the hypothesis of using HIV-1 protease inhibitors as anti-SARS drugs by attacking SARS-Co-V 3CLpro. The author used six approved anti-HIV-1 medications to study their binding interaction between 3CLpro. Molecular coupling and MM-PBSA binding free energy calculations show that, of all inhibitors, Darunavir has the greatest binding affinity for SARS-Co-V-2 and SARS-Co-V 3CLpro, specifying that it can be converted into an anti-COVID-19 drug [53]. Chen and others used a 3CL (pro) molecular model for virtual screening, noting that the antiviral agent's ledipasvir or velpatasvir are particularly striking therapeutic agents, having double inhibitory effects on two viral enzymes with insignificant adverse events. Antiviral effects present with phytochemicals must also attract a great deal of attention [54]. Shaghaghi testified on the usefulness of terpenoids as fewer hazard drugs through molecular coupling experiments on the new protease COVID-19 [55]. Elfiky recommends Sofosbuvir, IDX-184, Ribavirin, and Remidisvir as effective alternative medications for HCoV disease, which is a worthy example of the reuse of drugs [56]. Baron *et al.* stated teicoplanin, an antimicrobial agent employed in the management of *staph* infections, can be used as an alternative medicine for patients affected by SARS-Cov-2. This observation must be confirmed by animal studies because it is effective against preceding coronaviruses [57].

Nanoparticles to confront respiratory virus and treatment modalities

The implementation of NPs in the field of medicine is very significant in the establishment of innovative theranostic and diagnostic approaches for COVID-19 management. Nano platform suggests innovative answers to a wide range of aspects linked to the diagnosis, prevention, and management of the COVID-19 pandemic. Since the virus particle causing COVID-19 has a width of approximately 125 nanometres, nanoparticles with this identical width range can be very useful to identify and incapacitate this unique coronavirus through several interventions [58]. The low toxicity, smaller size, charge, and chemical alteration of the nanoparticles enable these materials to resolve the numerous constraints that hinder their passage through several methods of administration [59]. The introduction of treatment interventions centered on nanoparticles (NPs) will overcome the drawbacks of current therapy with features such as size, shape regulation, and surface modification that eventually result in high immunogenicity and increased presentation of antigen. The implementation of nanoparticles as carriers for drug delivery can enhance the targeting of drugs to desired regions thus reducing the amount of dose needed, eliminating undesirable adverse effects, and improving compliance of patients [60]. Integration of nanoparticles with the therapeutic agent protects the molecule against enzymatic degradation, and thus the concentration of the therapeutic agent is many times higher than the expected concentration at the target site. NPs offer an excellent drug delivery with the capability to cross the barriers and carefully release drugs to the suitable target [61]. These particles also can improve the therapeutic efficacy of antiviral drugs as well as augment their pharmacokinetic profile of the drug [62]. The most important nanoparticles used for combating the COVID-19 pandemic are polymeric nanoparticles, liposomes, (both

of which belong to organic NPs), gold NPs belonging to inorganic NPs, nanoparticles of virus-like and self-assembling proteins, and peptide-based NPs [63]. The commonly used nanoparticles with their classification are described in fig. 3.

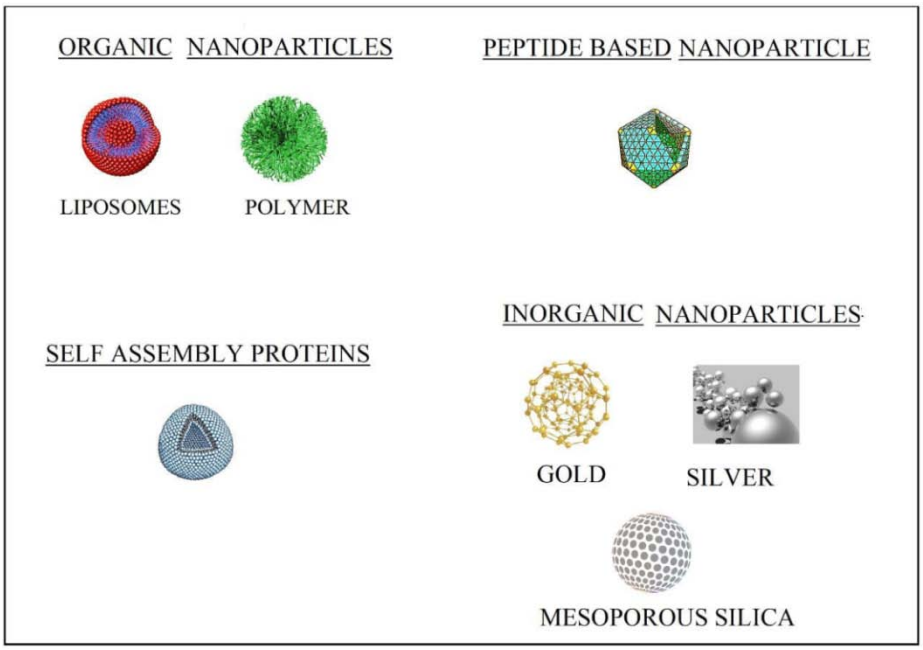

Fig. 3: Classification of nanoparticles based on their nature

Polymeric nanoparticles (PNPs)

Polymeric Nanoparticles prove to be excellent carriers for drug delivery due to their biocompatible nature, and the likelihood of altering their functions and characteristics for specific delivery of drug molecules [64]. PLGA (Poly-lactic-co-glycolic acid) is the frequently used polymer in this category with biodegradable and biocompatible features. These types of NPs can be produced by the addition of numerous monomeric molecules into different forms like 3 dimensional, linear, branched forms, etc [65]. The natural or synthetic type of PNPs are used for carriers of drugs or genes and can load siRNA (small interfering RNAs) and these are described as effective molecules to target the virus and have the ability to attack mutations in the virus. Different studies have revealed the effectiveness of siRNA in preventing replication of the virus, especially in Hepatitis C. The specificity and selectivity of this molecule are the reason for the success of the site-specific treatment approach. The delivery of this RNA without any protective cover makes it more susceptible to enzymatic degradation along with subsequent effects like instability, toxicity, clearance by the reticuloendothelial system and kidneys. Thus, siRNA can be tagged with NPs, especially polymeric NPs either with chitosan or with polyethyleneimine to deliver this delicate molecule to the target site. Chitosan-type NPs have confirmed their potential in providing immunity against various viral infections and were also examined for incorporation into different types of vaccines like the HBV vaccine [66].

Liposomes

Liposomes are lipid-based nanoparticles having numerous applications in the biomedical field due to their biocompatible nature. Liposomes are characterized by having a spherical shape with an outer bilayer consisting of phospholipids and an inner core that is hydrophilic so that it can load hydrophilic therapeutic moiety [67]. These nanoparticles offer advantages like they can effectively enclose the therapeutic moiety and can undergo simple alterations thus enhancing the biocompatibility and enabling easy drug delivery to the target site. Apart from PNPs, lipid nanoparticles also offer effective delivery of siRNA against numerous viral diseases. Diverse formulations of lipid nanoparticles like semi-solid particles, solid, or even liquid form were assessed for targeting viral infections. siRNA loaded with lipid nanoparticles are also targeted for treating MERS-coronavirus [68]. The lipid-based nanoparticles guard siRNA from enzymatic degradation by the nuclease enzyme thus augmenting their distribution and enhancing the bioavailability of low soluble drugs and ensuring that the therapeutic agent reaches desired region [69].

Inorganic nanoparticles

Inorganic nanoparticles gain attention in the biomedical field due to their capability to convey the nanoparticle to the specified target site and also permit stimuli-responsive properties, with biocompatibility and optical characteristics [70]. Although inorganic NPs are widely researched in clinical and preclinical settings in the identification, diagnosis, and treatment of various diseases they still raise issues about their effective medical applications regarding factors such as bio-compatibility and specificity. To resolve this, research teams are focusing on inorganic functionalization of the nanoparticles with different kinds of biocompatible constituents thus providing promising benefits of organic as well as inorganic nanoparticles [71]. Gold nanoparticles, being an inorganic nanoparticle offer a great advantage in the treatment of COVID-19. Along with gold nanoparticles, various other inorganic nanoparticles like silica nanoparticles, magnetic iron oxide, carbon nanotubes, and quantum dots were assessed as promising transporters of siRNA [72]. Gold NPs were emanated as impressive nanosystems and were evaluated for target-specific gene silencing and as a carrier of siRNA against neoplasm due to their incomparable biocompatibility, adaptable designs, and surface adjustments and thus lead to efficient distribution mechanisms [73]. Important research done by Loczechin A *et al.* revealed that carbon quantum dot conjugated ligands of boronic acid disturbed the function of the S protein of the coronavirus and significantly prevented the entry of the virus into the host cell. The addition of inorganic NPs to the cell culture medium confirmed that the rate of infection of the cells dropped dramatically during and before infection with coronavirus [74]. A significant inhibitory activity of the virus was identified at the phase of viral replication after the end of one cycle of viral life. This nanomedicine with outstanding water solubility is a potential candidate for attacking coronavirus as it can efficiently enter cellular components via endocytosis and intermingle with the protein of the virus thus inhibiting the replication process of the virus.

Nanoparticles made of silica propose safe and efficient therapeutic delivery mechanisms and may aid in formulating a vaccine for treating coronavirus [75]. Nanoparticles formulated with silica provide stable and biocompatible candidates to load DNA, RNA,siRNAs. The silica-based NP are conjugated with polyethyleneimine and can accurately deliver nucleic acids. This formulation shelters the nucleic acids from degradation by nuclease and once the nucleic acid is delivered to the cell, it results in the stimulation of the immune system causing humoral and cell-mediated immunity [76]. The cell-mediated reaction will identify and destroy the cells infected with the virus and the antibodies produced from the humoral response can bind to the virus, impeding its ability to attach to the cell and rapidly eliminating it by phagocytic processes.

Virus-like nanoparticles (VLNPs) and self-assembling protein

VLNPs exist as spherical nanoforms composed of multiple components having a size range between 20 to 200 nm. They are the products of the self-assembly of viral capsid-derived proteins. These were implemented as desirable nanoparticles which do not consist of genomic elements but can appropriately impersonate the original virus based on its structure and epitope. This allows the nanoparticles to stand out to antigen-producing cells which can easily detect them and induce an immune response. VLNP can act as a vector in the transfer of human genes in which could be regarded as one of the brilliant methods for appropriately transporting a transgene to the mutated site to alter the expression of a gene or protein-encoding [77]. With the great improvements in vaccine research, massive attempts were concentrated on designing new vaccines using VLNP which can imitate the viruses. These NPs can also stop the degradation by enzymes when compared to the unprotected administration of viroids [78]. Self-assembling nanoparticles are an innovative form of nanomedicine generated from the monomeric proteins oligomerization reactions (with a diameter of approximately 20 to 100 nanometres). They are an excellent form of drug delivery wherein they can easily cross cell membranes and safely release drugs, nucleic acids, and genetic components into the nucleus of the cell [79]. These nanoparticles can not only act as drug carriers but can also activate the immune system thus carrying synergistic actions. Chen H. *et al.* [80] revealed that in an experiment of combining gold nanoparticles with the S protein of a virus causing infectious bronchitis; the stability was enhanced after VLNP was used. This also had substantial S protein retention when compared to virus-related antigens. Also, it was established that the use of VLNP caused higher levels of IgG because of improved gold NP drug delivery leading to enhanced cell uptake and strengthened fixation of complement.

Peptide-based nanoparticles

Nanoparticles made of peptides are a newly evolving multipurpose tool meant for medical applications and facilitates the management, prevention, and diagnosis of various diseases [81]. The covering of this NP with peptides or proteins enhances the stability of NPs biologically and can modify and control the accumulation of NP in tissue by allowing interaction with biomolecules and the cell structures of the host [82]. In a study done by Han Y and Kral P, it was revealed that peptide inhibitors taken out from the ACE2 gave encouraging results for inhibiting the SARS-CoV2. These inhibitors are produced from alpha-helices which are extracted from ACE2 and it was shown that the peptides of alpha-helices sustained their structure and enabled stable and definite binding to the viral structure. To facilitate multivalent binding to the receptors of the virus, different kinds of peptide moiety can be linked to the NP surface. Thus, the peptide-based NPs can deliver effective therapeutic assistance to attack the COVID-19 disease [83].

Nanoparticles formulated in supporting agents

Presently, copper or silver salt conjugated with disinfectants are easily available as these nanomedicine-related products can incapacitate the SARS Cov-2, reduce opportunistic microbes and prevent viruses present on the surfaces [84]. Nanoparticles are also used to enhance the utility of air filters and can also minimize the spread of viruses. Nanotechnology is also applied in producing wound dressings because it can defend the viral infections and enhance the rate of healing speed [85]. Nanomaterials can minimize microbial biofilms resulting in a decreased opportunity for secondary microbial infectious diseases [86]. Thus they can augment the potency of antimicrobial drugs and thus resist secondary microbial infections. Nanotechnology makes use of nanofibers and other nanomaterials which can be integrated into respiratory masks enabling great filtration rates and breathing efficiency along with washing capability and antibacterial, antiviral properties [87]. ACE-2 conjugated nanoparticles like quantum dots and nanoflowers are formulated in nose filters, masks, gloves, clothes, etc., and thus can block the entry of the virus into the host [88]. Recently, the demonstration was done on the use of a replaceable nanoporous membrane conjugated with silicon for its incorporation in N-95 masks. This type of mask can overcome problems associated with normal N-95 masks like the possibility of the formation of cakes that occur due to the collection of particles on the membrane and thus inhibiting the further passage of additional particles. The porous membrane present on this is made of hydrophobic molecules and when the respiratory droplets come in contact with the mask, it gets rolled and slipped over the mask because of the high angle of inclination of the membrane when it is present on the mask [89]. Other applications under consideration are enhancement of detection kits since it is considered as one of the foremost requirements of the present approaches to face and tackle the spread of coronavirus [90].

Intranasal delivery through nanocarriers

The different types of nanoparticles loaded with drugs, vaccines, antibiotics, siRNA can be targeted to the desired region effectively via the intranasal route [91]. Intranasal drug delivery has already been evaluated against respiratory infections causing virus and coronaviruses. There are several benefits for drug delivery via a mucosal route which include guarding the drug against degradation by enzymes, prolonging the release and time of residence of the drug, enhancing the amount of loaded drug in the target region, guaranteeing the release of the drug along with adjuvants and stimulating the immune system [92]. Intranasal delivery of drugs is highly beneficial for infective diseases as most invading organisms commence their action at the mucosal surfaces. This therapy offers modest, cost-effective, and non-invasive delivery mechanisms [93]. The increased surface area and the abundance of the capillary bed offer rapid absorption of drug moiety. The nanoparticle-related factors that play an important role in enhancing intranasal drug delivery are size, the surface charge of the nanoparticle, characteristic features, etc [94]. A variety of studies have been carried out to determine the desired characteristics of nanoparticles for intranasal administration and have been tested recently [95]. For an ideal delivery of a drug through the pulmonary system, the nanoparticles should have a size of less than 100 to 200 nanometres to have an augmented immune response [96]. It is to be noted that most of the studies done to evaluate the benefits of using nanoparticles for intranasal delivery are centered on preclinical studies carried out in animals and cannot be readily applied to humans [97].

The variety of nanoparticles mentioned in the previous sections can be targeted via the intranasal route. Liposomes, being organic nanoparticles, are readily suited for intranasal delivery as they can easily encapsulate the required drug and are available for cellular and mucosal uptake [98]. In a study carried on cationic liposomes, it was concluded that the nanoparticle showed a greater rate of absorption and bioavailability about its negatively charged complements [99]. It occurs because the membrane of the mucosa, being charged negatively tends to attract to the positively charged nanoparticle and subsequently prevents the mucosal cilia clearance of the drug [100]. Further, liposomes can be successfully formulated as mucosal vaccination since their maintenance in the nasal cavity stimulates the immune system causing large amounts of immunoglobulin production. Polymeric nanoparticles, especially those produced from chitosan forms an appropriate molecule for intranasal delivery as they are non-toxic, biodegradable, and can open the tight junctions present between the cells of the epithelium [101].

A new treatment strategy presently under study is the use of nanoparticles made of silver through the intranasal route to combat coronavirus. Nanoparticles made of silver with a size range of approximately 10 nm are applied and the nanoparticles can destroy the viruses in the pulmonary epithelium. The silver particle which is released from the formulated nanoparticle induces a change in pH conditions in the epithelium of the pulmonary system to basic. This medium is unfriendly for the survival of the virus and subsequently results in the diminished viral count and thus prevents the possibility of the virus to transmit from a diseased person to a healthy person [102]. The existence of antiviral effects of this nanoparticle is caused by the binding of this NP to the virus's surface glycoprotein thus circumventing the fusion of coronavirus to the cell structures of the host. The use of silver nanoparticles has resulted in a substantial decrease in the production of pro-inflammatory cytokines in mice induced with the syncytial virus. These results recommend that the use of silver nanoparticles will be efficient therapy against the coronavirus. Currently, there are no approved trials or animal models induced with coronavirus to conclude the use of this NP to treat coronavirus but can certainly be investigated for tackling coronavirus [103].

Nanoparticles made of gold have gained distinct attention in the expansion of vaccine invention since they can activate the immune system through internalization by the accessory cells. The strategies of synthesis, notable progress and future opportunities for using gold NP for intranasal vaccinations are being analyzed. Nanoparticles made of gold can be effortlessly tailored for mucosal administration and have the benefits of easily getting diffused into the lymph nodes with the stimulation of killer T-cells mediated immune reactions. With the high atomic number, gold NPs can act as greatly constant and biologically compatible X-ray contrast agents for imaging purposes particularly in computed tomography [104]. VLNPs targeted for intranasal delivery against influenza virus revealed that there was an improved immune response against this specific virus. Thus it can act as a vaccine that can avoid infections by generating T-cells and antibodies [105]. An experiment conducted by Kanekiyo M. *et al.*, showed that self-assembling protein NPs aggravate efficient immune response than conventional influenza vaccine after mucosal administration thus enabling a potential platform for establishing vaccine development against a variety of viruses and other pathogens [106]. The schematic illustration of intranasal delivery of different nanoparticles is described in fig. 4.

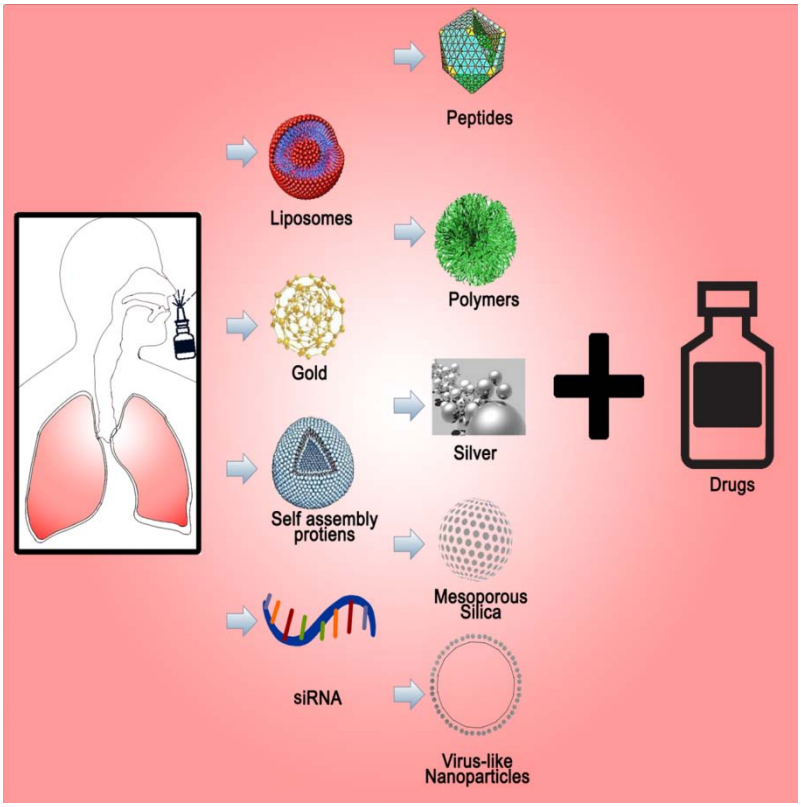

Fig. 4: Intranasal delivery of nanoparticles for the purpose of treating SARS-CoV2 infection

Safety and limitations

NP-based methods of administration have shown enormous potential applications, but studies have shown that these methods can cause serious damage to the respiratory system and even affect lung function. Within NP and related methods, four major pathological and biological aspects need to be considered, including oxidative stress, genotoxicity, inflammation, and fibrosis [107]. Oxidative stress is usually caused by the difference between the generation of reactive oxygen species (ROS) and the capability of biological mechanisms to eradicate active debris voluntarily.

Sometimes it can be directly caused by the production of ROS in the cell, or it can be caused indirectly by altering mitochondrial respiration or by decreasing the antioxidant residue in the cellular environment. The oxidative stress barrier can be an important stage in initiating a small amount of harmful pathological and biological activities in the cell microenvironment [108]. Also, the effect of NP on oxidative stress environments in animal models or at the cellular level to sense the harmfulness of NPs is usually done by a general termination study. The *in vivo* and *in vitro* experiments take part in considering the mechanism of NPs that cause oxidative stress. For instance, researches have shown that after 90 d of continuous administration of TiO2 NP in the tube, a large amount of titanium dioxide NP (TiO2-NP) accumulated in the lungs of mice. TiO2-NP significantly increases the pile-up of ROS levels, inflammation, and lipid peroxidation levels and also decreases antioxidant competition in the lungs. NPs can produce ROS and subsequently oxidize antioxidant residues, which can affect the respiratory system and related pathological and biological events, including pneumonia and gene-toxicity [109].

In some instances, the NPs administered through the nose have been observed to cause processes mediated by prolonged or acute inflammation, such as the uptake of inflammatory cells and the announcement of cytokines [110]. Note that the application of graphene oxide (GO) solution directly into the pulmonary system of C57BL/6 mice can cause alveolar exudate to cause severe pneumonia. NPs have been implicated in eliciting some pro-inflammatory pathways, along with mitogen-activated protein kinase (MAP). Cells treated with NP showed higher levels of the transcription factors AP-1 (activator protein 1) and NF-KB (nuclear factor activated kappa B cells), which influence the transcription process of DNA and cytokine generation and cell survival [111]. Primary or secondary genotoxicity is the major problem related to NP-mediated delivery mechanisms [112]. Genotoxicity or NP residues are directly affected by DNA structure or cell division components (like the spindle of microtubule or centromere). Carbon nanotubes (CNT) interact easily with components of DNA. This indicates that CNT can cause harm to genes *in vivo* (in an animal model) or at the cellular level. Studies have shown that the application of many-walled CNTs through the pulmonary route induces toxicity to genes by encouraging chronic inflammation, resulting in persistent oxidative stress [113]. Fibrosis is considered an indicator of the accumulation of inhaled NP in the lungs and causes rare pneumonia, such as eosinophilia. In one study, in the treated C57BL/6 male mouse model, inhaled single-walled CNTs caused multifocal granulomatous pneumonia and fibrosis [114]. Several major and important efforts are currently underway to develop a scientific research structure for risk management. The U. S. National Nanotechnology Program or Research Strategy for Environment, Health, and Safety is one of the major structures [115] which focus on setting up measuring instruments that can effectively determine the physical and chemical properties of nanotechnology-based or nano-drug delivery systems [116]. Therefore, effective management of respiratory infections, diseases, or conditions needs to emphasize the above pathological and biological processes to define restrictions and improve the protection of NP-centred methods [117].

CONCLUSION

The severe acute respiratory syndrome called coronavirus 2 caused by (SARS-CoV-2) poses a dangerous situation to the world population and is the greatest pandemic in recent history. To date, the greatly contagious SARS-CoV-2 coronavirus has attacked more than 111 million individuals in 216 countries and has caused an unparalleled financial disaster due to forced blockages to restrain transmission and left the lives of many affected people in danger worldwide. Even though the suggested conventional treatment is primarily administered intravenously, due to intranasal administration, it faces several mucosal challenges. Also, the conventional detection methods are based on the detection of nucleic acid and contain the following disadvantages like labor-intensive and low-sensitivity experimental procedures; long time between assembling of sample and elucidation of results; greater false-negative rate; and absence of specificity leading to misdiagnosis of people infected with viruses. Additionally, the use of NP-linked delivery systems can provide efficient treatment while diminishing the side effects of therapeutic moiety and also guarantee that therapeutic residues such as medications, vaccine preparations, siRNAs, and peptides reach their targets. Some studies have shown that these nanotechnology methods can cause serious damage to the airways and even affect lung function. Within NP and related methods, four major pathological and biological aspects need to be considered, including oxidative stress, genotoxicity, inflammation, and fibrosis. Therefore, the fundamental assumption is that COVID-19 is a novel disease and may primarily encounter some known nanomaterials that have been utilized in preceding SARS-CoV or similar viruses.

FUTURE PERSPECTIVES

Intranasal administration of biocompatible therapeutic nanoparticles may be a promising method to combat this new SARS CoV-2, as different methods have previously been used against different viral infections (including SARS or MERS coronavirus). Therapeutic NPs can improve the distribution of drugs, ensure the careful and definite administration of siRNA, effectively administer peptide inhibitors, and avoid the virus from entering and trigger the immune system of the cell. In the coming years, clinical experiments should permit drug therapy combination for tackling the coronavirus and also experiments or clinical trials involving models of animals must develop beyond usual organisms. Research into several promising therapeutic agents for SARS-CoV-2 conveyed via intranasal biocompatible drug therapeutic nanoparticles is likely to take place shortly which would be more effective than any other treatment with COVID-19.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICTS OF INTERESTS

There is no conflict of interest.

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