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Characteristics of SARS-CoV2 that may be useful for nanoparticle pulmonary drug delivery

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Abstract

As a noninvasive method of local and systemic drug delivery the administration of active pharmaceutical ingredients (APIs) via the pulmonary route represents an ideal approach for the therapeutic treatment of pulmonary diseases. The pulmonary route provides a number of advantages, including the rapid absorption which results from a high level of vascularization over a large surface area and the successful avoidance of first-pass metabolism. Aerosolization of nanoparticles (NPs) is presently under extensive investigation and exhibits a high potential for targeted delivery of therapeutic agents for the treatment of a wide range of diseases. NPs need to possess specific characteristics to facilitate their transport along the pulmonary tract and appropriately overcome the barriers presented by the pulmonary system. The most challenging aspect of delivering NP-based drugs via the pulmonary route is developing colloidal systems with the optimal physicochemical parameters for inhalation. The physiochemical properties of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) have been investigated as a template for synthesis of NPs to assist in the formulation of virus-like particles (VLPs) for pharmaceutical delivery, vaccine production and diagnosis assays.

Keywords: Pulmonary drug delivery; Covid-19; Aerosolization; nanoparticles (NPs); lung diseases

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Introduction

Before the emergence of the SARS-CoV-2 (Covid-19) pandemic, according to the world health organization (WHO) statistics, chronic obstructive pulmonary diseases (COPD), lower respiratory infection and lung cancer were the third, fourth and fifth causes of death in the world, respectively [1]. In late 2019 SARS-CoV2, which causes acute respiratory infections ultimately leading to pneumonia, and bronchitis in severe cases, was characterized as a pandemic by the world health organization. The pandemic infected millions of people all around the world, presenting an unprecedented challenge to the healthcare systems of the countries across the globe. Also, tuberculosis (TB), which is caused by *Mycobacterium tuberculosis*, accounts for one of the top 10 causes of death worldwide. TB affects the human lungs and spreads through the air from one infected person to others [2]. Efficient pulmonary drug delivery can, therefore, improve the quality of healthcare offered to a large number of patients suffering from pulmonary diseases.

Pulmonary drug delivery is primarily recommended for the treatment of pulmonary diseases and secondly, for other diseases which need penetration into the systemic circulation such as diabetes. If the target tissue is located within the lungs themselves, then targeted delivery via the pulmonary route reduces the systemic side effects, and delivers the dose directly to the site of action [3,4]. In general, the large surface area of the lung, fast absorption due to the presence of numerous vessels, and the ability to circumvent the first pass metabolism in the liver, are the main advantages offered by pulmonary drug delivery [5].

NPs may further enhance the advantages of drug delivery via the pulmonary route, because they can protect the incorporated APIs against degradation by chemical and biological agents and enhance drug penetration through biological barriers. The typical diameter associated with NPs permits potential transcellular transportation via endocytic pathways across the alveolar

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membranes. The main nano-sized systems considered for pulmonary drug delivery include polymeric NPs, dendrimers, liposomes, lipid-soluble, solid NPs, exosomes, micelle systems, nano-sized emulsions, as well as nanocomplexes [6,7].

VLPs as a biomimetic vehicle use the viral proteins on the NPs surface which can be used for 3 main purposes;

1. Vaccine design [8],
2. Positive control in the sensing tools [9] and
3. Pharmaceutical delivery [10].

The goal of vaccination, which uses viral envelope proteins, is to deliver antigens that stimulate the immune system for antibody production. Sensing tools can be used with positive controls for the molecular assay to validate each test and provide high accuracy that takes advantage of the unique features of viruses but is noninfectious and safe to use in a diagnostic assay. In the pharmaceutical delivery, viral capsids or envelope proteins derived from viruses are utilized to encapsulate drugs, genes, proteins and different molecules to deliver them to the special targeted cell with the aid of virus ligands which specifically bind to their receptors on the cell surface. SARS-CoV-2 with transmembrane glycoprotein known spike (S) protein binds to angiotensin-converting enzyme 2 (ACE2) in the alveolar epithelial type II cells has a high potential for VLPs synthesis [10]. In this review we try to compare the NPs for pulmonary drug delivery with the SARS-CoV-2 virus as a natural biological particle to dose a successful interaction, penetration and spreading in the lung tissue. By considering the limitations for penetrating the pulmonary system the successful SARS-CoV-2 virus uses the mechanism of binding to the surface receptor of the epithelial cells in pulmonary system especially with S proteins which act as a targeting moiety. As well as presenting surface proteins containing S proteins, membrane (M) and

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envelope (E) (illustrated in Fig. 1), the other characteristic of SARS-CoV-2 virus such as size, surface charge, hydrophilicity/hydrophobicity was investigated which has an active role in penetration to the pulmonary system which can help to designing the tunable NPs for pulmonary drug delivery based on mimicking viruses.

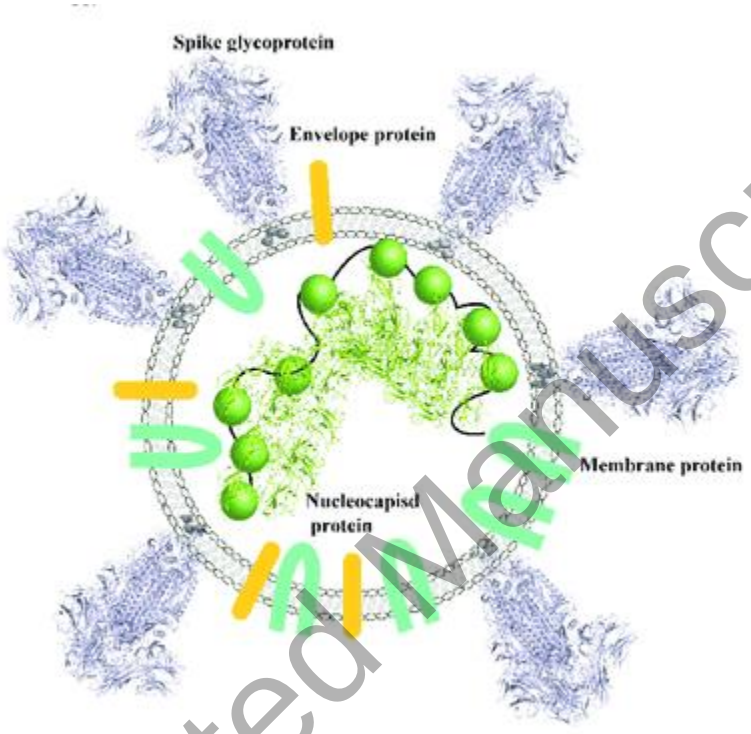


Figure. 1. Schematic representation of SARS-CoV2 virus with main proteins [11].

The characteristic of the pulmonary tissue

The pulmonary system possesses characteristics that make it suitable for local drug administration. In particular, these characteristics include the presence of 300 million alveoli, 280 billion blood vessels, a large area of approximately 70 m^2 associated with the blood-gas interface [5]. The respiratory endothelium comprises roughly 25% of the total vascular surface of the total body vessels [12]. Exchange of gases occurs across the epithelium lining the alveoli, the

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endothelium lining the capillaries and the intercellular layers. The epithelia lining the wall of the alveoli contains two types of cells, namely pneumonocytes type 1 and 2. A small distance on the order of 0.5 μm separates the alveoli and the blood capillaries forming the blood-gas interface where gas exchange occurs readily across the interface by diffusion. The alveoli are coated with a layer of surfactant composed of phospholipid and proteins which function to reduce the surface tension and decrease the friction exhibited by lung tissue and are therefore essential for effective breathing. The presence of pulmonary surfactant (PS) offers a number of advantages for pulmonary drug delivery. Specifically, they facilitate the dissolution of drugs with low solubility, effectively transport the drug throughout the pulmonary system based on capillary diffusion and offer protection against the extracellular barrier. Different cell types including macrophages and fibroblasts are found within the supporting connective tissue of the lower respiratory tract along with nerves and lymphatic vessels. The lower pulmonary tract is, therefore suitable for drug delivery given its access to both the pulmonary and lymphatic systems. In spite of, the desirable characteristic of the pulmonary system for drug delivery, the systems defensive mechanisms for prohibiting invasion by foreign materials need to be taken into account [5]. The pulmonary system consists of a central and peripheral region. In the central area the presence of mucosa layer and the motion of respiratory cilia as well as the thick epithelium impedes the entry of foreign substances. The mucosa removes the foreign particles before they reach the lower areas by inducing coughing and swallowing. In the peripheral regions of the lung the presence of alveolar macrophages limits absorption through phagocytosis [3]. Furthermore, in these regions there is a high density of lipids and proteins which form a barrier against further transport. These lines of defense associated with the alveoli and tight junction present between epithelial cells serve as the first barriers against drug transport [5]. Research on drug delivery using

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nano/microparticles has been conducted to overcome these limitations. **Fig. 2** shows a schematic of the central and peripheral structures of the pulmonary system [3].

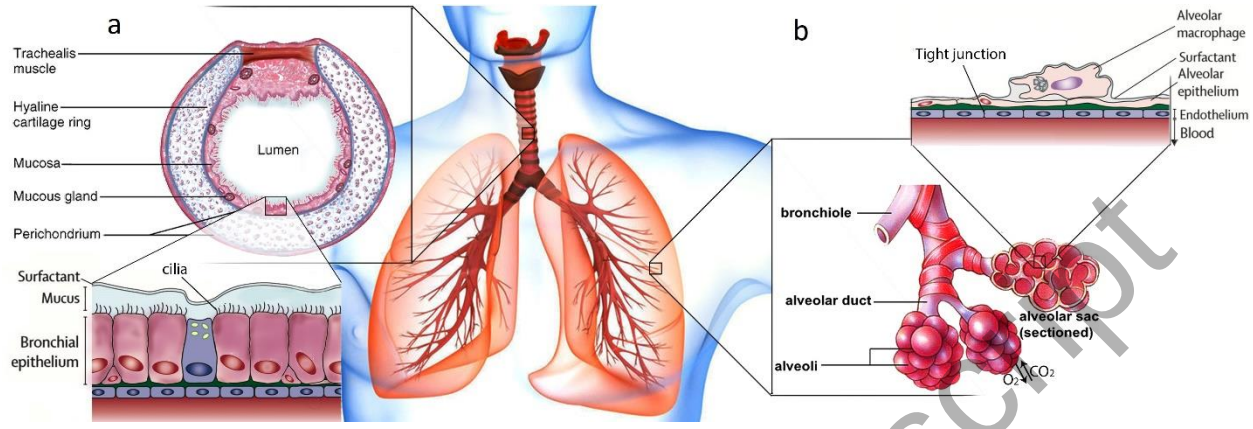


Figure. 2. Schematic illustration of: a) central pulmonary system, b) peripheral pulmonary system.

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Characteristic of drug formulation for pulmonary drug delivery

1. The size of particles

Drugs intended for delivery to the pulmonary tract may be in powder or suspension form so that they can propagate as either a dry powder or a wet aerosol. The size of particles in this case is very critical. The size of particles taken up by alveolar macrophages lies in the 0.5 - 5 μm range. Particles with diameters in the < 0.5 μm range are not effective in drug delivery, since they may become expelled during exhalation due to their light weight [3,5]. The relationship between the particle size and deposition site within the pulmonary system is outlined in **Fig. 3.**

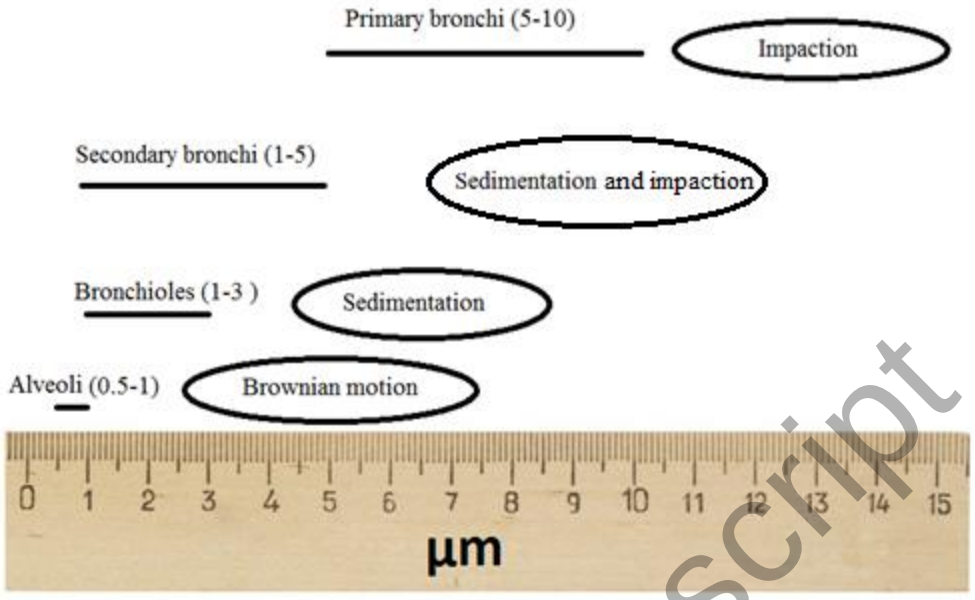


Figure. 3. Size and mechanism of particles deposition in the pulmonary system.

The size of SARS-CoV-2 is approximately 40-160 nm which is not suitable for lung deposition, so how can it penetrate and affect the pulmonary system? Research show that particle size distribution emitted by coughing is 2 and 3 µm and contains a high amount of viruses [13]. Indeed, nearly 61% of particles are filtered by extra-thoracic airways through inertial impaction but 8.5% of particles can reach and deposit in the alveolar region. Hence, upper respiratory infection is the first symptom of SARS-CoV-2 infection. The probability of particle deposition in the lung decreases as their diameter decreases. The mechanisms of virus transfer from the throat to the lower airways is via re-inhalation of a person's own cough, as well as aerosol and droplet generation in the throat during inhalation resulting in virus particles moving on the surface of the bronchial airways, or gradual infection of neighboring cells expressing ACE2 towards the peripheral respiratory system [14].

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Different NPs such as polymeric NPs, micelles, dendrimers, nano-in-microparticles, liposome, transferosome and solid lipid NPs can incorporate drug payloads for pulmonary drug delivery. These NPs can be administered by three kinds of available devices; pressured metered-dose inhalers (pMDI), dry powder inhaler (DPI) or a nebulizer or soft mist inhaler (SMI). Nebulizers and pMDI's require the drug to be in solution and produce a liquid aerosol whereas the DPI uses powdered drug [15]. Inhalable particles in the MDI, SMI and nebulizer produce by the device, however, in the DPI previous produced particles powder poured into the devices [16]. Table 1 compares 4 kinds of pulmonary drug delivery devices based on their mechanism of action, particle size and NP formulation used.

Table 1 Comparison of four pulmonary drug delivery devices.

Device	**Mechanism of action**	**Particle size**	**NPs formulation**
Pressured meter dose inhaler (pMDI)	Using propellant such as chlorofluorocarbon (CFC) or hydrofluoroalkane (HFA)	Depending on propellent from nano (100-500 nm) to micrometer (0.5-5 μm)	Formulation containing the propellent, drug (encapsulated in NPs) and excipient, (Sometimes cosolvent such as ethanol for increasing the NPs solubility) [17]
Dry powder inhaler (DPI)	Respirable NPs in powder form	10-1000 nm particles in the in the nanocluster or nanoaggregate	Formulating NPs into inhalable microaggregate via spray drying or spray-freeze-drying with mannitol, PVA or leucine as matrix material [18]
Nebulizer	Production of liquid aerosol by external power (nozzle array, ultrasonics, vibrating meshes, and several novel approaches)	Depending on aerosol generator: ≤ 4μm	Homogenous NPs solution (Often applies for lipid-based NPs) [19]

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Soft mist inhaler (SMI)	A variety of principles are utilized, including forcing liquid under pressure through a nozzle array, ultrasonics, vibrating meshes, and several novel approaches, such as condensation of vapors to form particle dispersions.	≤ 5μm	High fine particle fraction [20]

Homogenous NPs in the suspension prevents the Ostwald ripening phenomenon of NPs in pMDI. This is assisted by controlling the zeta potential using various surface modifications. This is not possible in the dry state is complicated due to aggregation of NPs in the inhaler, inefficient redispersion in lung fluid and maintaining the biological activity of drug in the particle. To achieve the production of inhalable MPs/NPs they need to be made porous or hollow with a decrease particle surface energy [15].

The reduction of particle size to values in the nanometer range, is accompanied by a higher dissolution rate in the respiratory mucosa. To address the particle size issue, NPs can be designed such that upon separation from the aerosol they form micrometer-sized aggregates, with sufficiently high mass to allow for their deposition in the lungs. The presence of these aggregates within the bronchi for relatively long duration can produce the desired therapeutic effect [5]. Trojan particles may also be effective for this purpose. Trojan particles are porous particles of micrometer sized built using NPs. These particles are heavy enough to precipitate in the lung lining fluid where they may separate into their base NPs and propagate through the epithelium [3]. Another method uses a suitable matrix encompassing the NPs. For instance, NPs may be embedded within an excipient matrix such as mannitol forming suspension which is spray-dried

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to produce an inhalable powder [21]. Recently, new nano-in-microgel systems have been developed for the effective delivery of APIs to the respiratory system. Notably, nano-in-microgel systems consisting of microscale carriers containing 300 nm NPs have been demonstrated to reach the deep lung tissue triggering the release of the NPs in the diseased lung environment, while avoiding rapid clearance by alveolar macrophages [22]. In another recent development, microparticles embedding NPs have been employed for localized gene delivery via aerosols. These polyplexes incorporated polyethylenimine (PEI) and nucleic acids with mannitol and trehalose applied as matrix excipients and stabilizers. Analysis of the microparticles in powder indicated the presence of fused, slightly furrowed particles with aerodynamic diameters permitting deep lung deposition [23].

2. Hydrophilicity/Hydrophobicity

Another relevant factor to the absorption and transportation of NPs within the pulmonary system is whether or not the particles are hydrophilic in nature. NPs entering the deep structure of the pulmonary system interact with the PS, which is composed of lipids such as phospholipid and cholesterol which contain 90% and the remaining 10% made up proteins. As PS comes in contact with NPs a molecular corona forms around the NP. The formation of a PS-corona depends on the physiochemical properties such as hydrophilicity/hydrophobicity and the electric charge of the NP. The corona determines the fate of the NP in the pulmonary system. Studies show that the corona surrounding hydrophilic NPs permit easier absorption of the NP as compared with that of hydrophobic NPs [24]. Another investigation indicates that hydrophilic NPs are decorated with lipid molecules interact more efficiently with the PS. The resulting structure has the capacity to combine with the plasma membrane and then subsequently transport the hydrophilic NP into the

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secondary organ. **Fig. 4** shows molecular dynamic stimulation of interaction between PS and a) hydrophobic, b) hydrophilic and c) lipid coated NP [25]. Presence of phospholipid in the drug formulation facilitate the efficiency of drug delivery to the pulmonary tissue. Specifically a 30:70 ratio of phospholipid to triglyceride in the solid lipid NPs (SLN) is demonstrated to permit efficient delivery to the lung tissue [5].

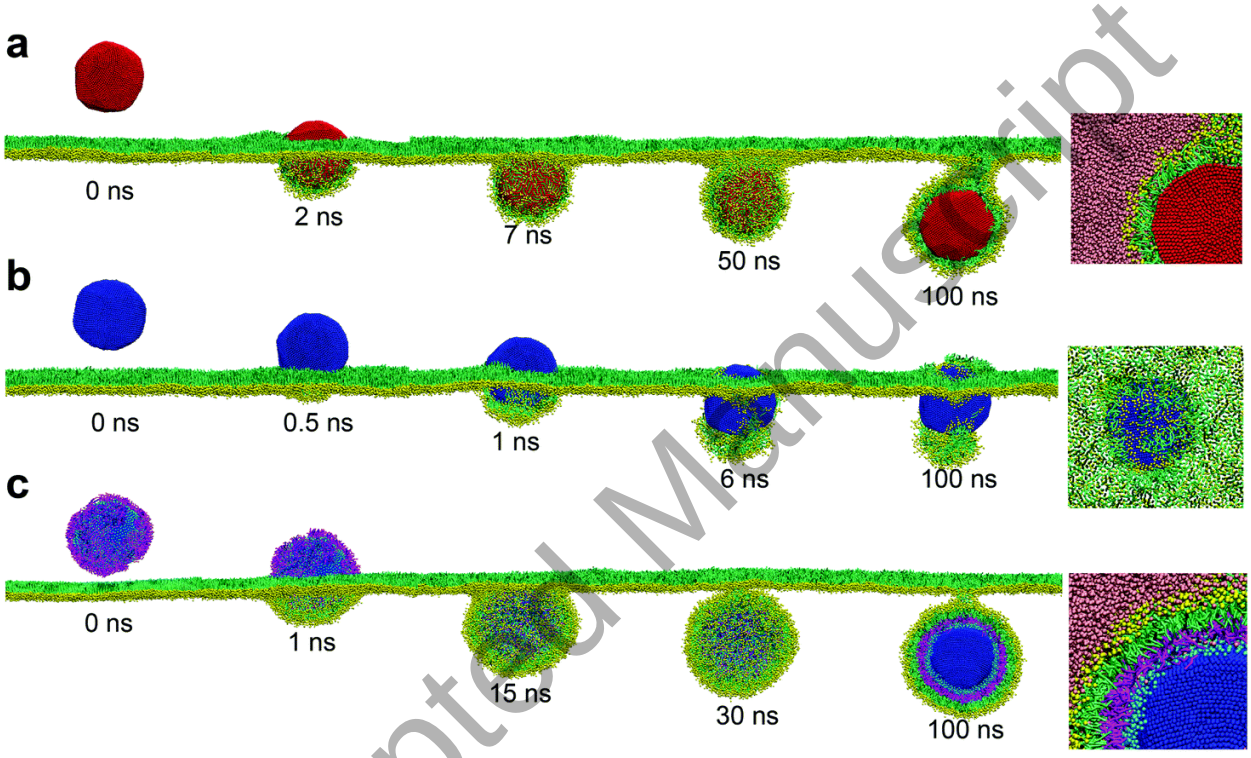


Figure. 4. Interaction between pulmonary surfactant monolayer with three types of NPs: a) hydrophobic NP, b) hydrophilic NP and c) lipid coated hydrophilic NP. The right column showing the distinct lipid arrangements of pulmonary surfactant covering the different NP surfaces. Reprinted with permission from Ref. [25]. Copyright (2017), RSC.

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The high affinity of SARS-CoV-2 contribute to higher infection and transmission of virus which initiate from the hydrophobic interaction of RBD of S protein with ACE2 in the aqueous

environment. The hydrophobicity of the protein surface is the main factor that stabilizes the protein–protein binding. As shown in Fig.5 from the docking of ACE2 with RBD of SARS-CoV-2 hydrophobic interaction enables hydrophilic side chains to shed their original hydrogen-bounded water molecules, so that the hydrophilic side chains can participate in the hydrophobic interaction via their hydrophobic portions, namely, enthalpy– entropy compensation occurs during the docking [26].

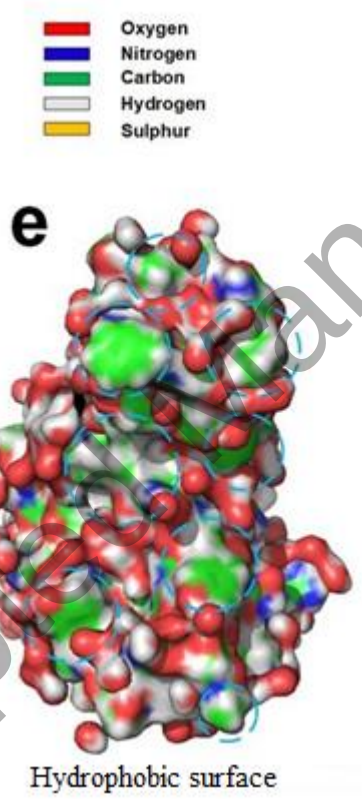


Figure. 5. Distribution of hydrogen-bonded water molecules at docking site of the RBD of SARS-CoV-2 with ACE2; the exposed hydrophobic surface is marked with dashed lines [26].

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3. Charge of NPs

The location of the surface electrical charge of the NP can determine the efficiency of its transport through the PS. NPs with face surface charge can penetrate the PS. While NPs with edge surface charge does not possess this ability. Compared with anionic NPs, cationic NPs have a higher probability of being absorbed via the PS. The presence of cationic NPs causes a reduction in the thickness of the PS corona, thereby reducing the distance between the charged NP and the PS [27]. Investigations show neutral particles have higher average transport rate than negatively charged particles [28].

Research on SARS-CoV-2 charge indicates that there is no significant relationship between virus charge and the ability to penetrate the lungs. Data about virus charge gained from the isoelectric point (pI) at which the pH of the virus is net zero charge. The evaluation on pI based on ProtParam tool and protein sequence identification shows a range of 1.9 to 8.4, but mostly be found between 3.5 and 7 [29]. Hence, the SARS-CoV-2 is stable at a wide range of pH levels. However, the proteins which play a critical role in entry to host cell namely, S glycoprotein, membrane proteins and envelope small membrane proteins have pI values of 6.24, 9.51 and 8.57, respectively [30]. When considering the pulmonary surfactant pH (5-7.4) the charge of these proteins are likely to present as neutral or positive in the mucus. The pulmonary mucus which possesses a negatively charged group therefore has higher tendency to interact with the positive charge particles and inevitably immobilize the particles in the mucus network via electrostatic interactions [28].

4. Mucosal adhesion

Yet another important consideration when formulating pulmonary drugs is the mucosal adhesion capacity. A high capacity for adhesion to the mucosa is observed in certain natural polymers

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such as chitosan [31]. In addition to high mucosal adhesion due to the positive charge of its network, chitosan exhibits several other properties such as; a capacity to swell on contact with fluids; self-assembly; ability to load hydrophobic and hydrophilic drugs; and interactions with cell membranes derived from the amphiphilic nature of the polymer chains and facilitating release of the drug [32,33].

Mucin is one of the mucus components containing glycoproteins which forms a gel-like network that is critical for mucoadhesion. Investigations about mucoadhesive materials show many polymers possess mucoadhesive properties [34]. As well as polymers, large polymeric proteins such as silk with, repetitive structural elements, have strong mucoadhesive properties. Highly charged and polar amino-acid in the silk protein structure assist in generating its strong its mucoadhesion, by creating high contact areas of hydrogen bonding. In addition to protein organization, aggregate silk has a viscous glycoprotein core surrounded by aqueous solution of slats which make it similar to mucin molecules. Additionally, the charged block of silk is similar to chitin-binding protein and should react similarly to changes in pH [35]. Based on the abovementioned points, S of SARS-CoV-2, having the 99 positively charged amino acids and 111 negatively charged amino acids provides enough strength for mucoadhesion. Additionally, negatively charged amino acids residues associate with the Ca2+ cations present in the mucus, further strengthening the binding interaction [36]. Lastly, the carbohydrate moiety of the glycoprotein in the virus envelope provides higher interaction with the mucin carbohydrate which further enhances virus-cell attachment [37].

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5. Other factors

Other factors relevant to the formulation of drugs for delivery to the pulmonary system include; prolonging residence time; protecting against enzymatic degradation or efflux transport; reducing clearance to the systemic circulation; targeting specific cells using anchoring ligands and/or masking odor or aftertaste [4]. Recently several drug loaded NPs systems have been reported for the treatment of TB, for example, maleate gellan gum- silk sericin- chitosan nanocomposite loaded with rifampicin and pyrazinamide [38] and alginate-chitosan NP containing rifampicin and ascorbic acid. Niosome's may incorporate antibiotics for the treatment of infectious diseases which affect the lungs. For example, the delivery of antibiotics such as Amphotericin B, Beclomethasone dipropionate, and Ciprofloxacin as well as Glucocorticoids have been evaluated for delivery by niosomes via the pulmonary route. This technique allows the preparation of the drug as a powder, with niosomes formed by hydration prior to administration [40].

In addition to size, charge, hydrophilicity/hydrophobicity and muco-adhesion, there are other effects of other proteins of SARS-CoV-2 in the attachment and stability to the lung tissue. These include envelope protein (E) and nucleocapsid (N) protein. N protein elicits a response to host cells and engages in the replication cycle while the E protein is hydrophobic and can integrate into phospholipids which also facilitates interactions with host cell [41].

The S protein of SARS-CoV-2 contains a furin-like cleavage site. Furin is an enzyme from the convertase family which is highly expressed in the lungs and operates to activate proteins by cleavage. Similarity the cleavage site of furin and a specific sequence in S protein facilitate the virus fusion and entry in the host cells. The cleavage of S protein generates the S1 and S2 subunit which drive two different actions. The N-terminal S1-ectodomain recognizes the host cell surface receptor and the C-terminal S2-membrane-anchored protein drives the viral entry

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into the host cell. Hence, the presence of the furin-like sequence increase the spreading and infection rate of SARS-CoV-2 in human lungs [42].

NP-based gene delivery via the pulmonary route

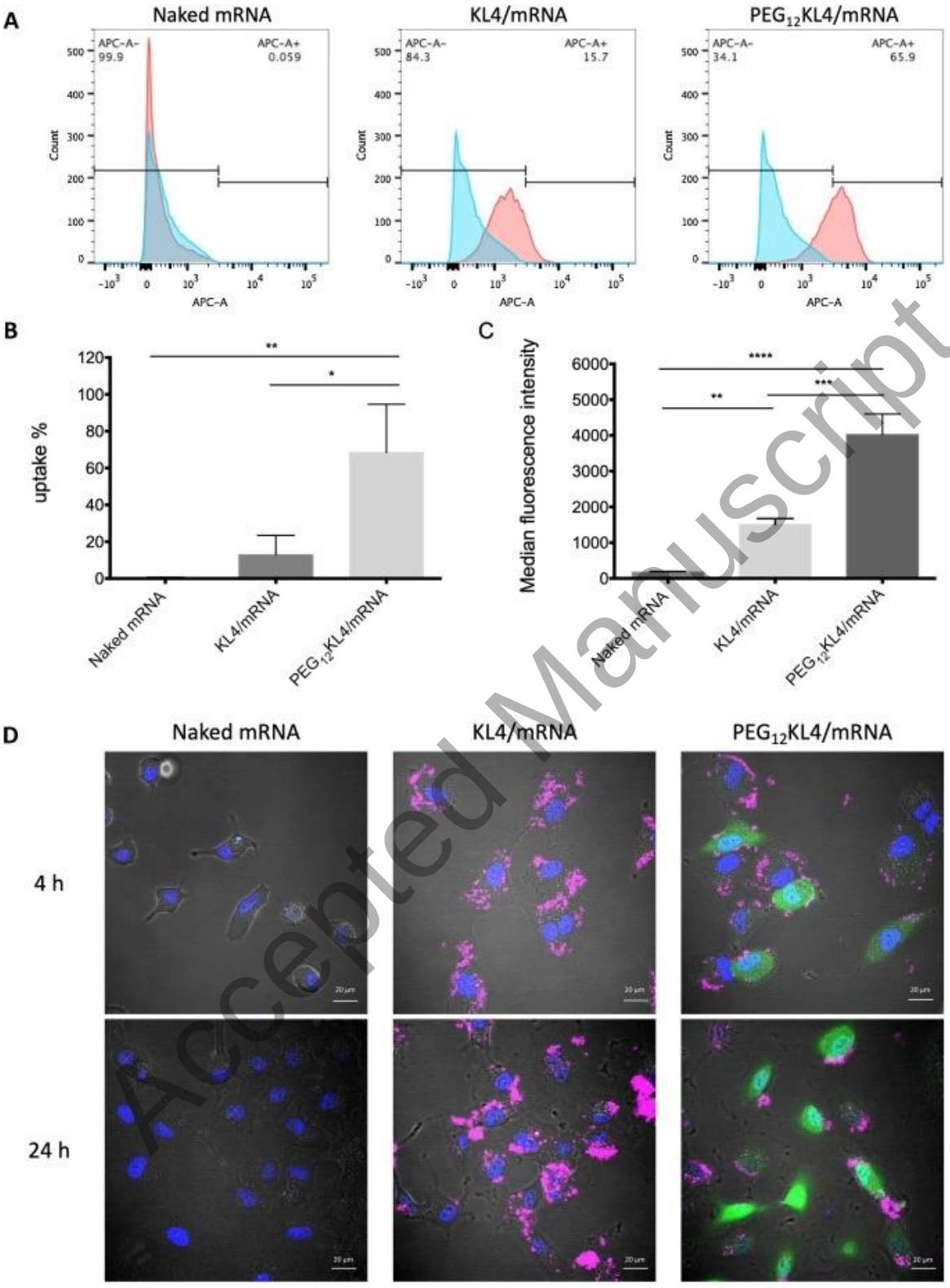
Of the multitude of conventional therapies developed to locally target lung diseases, gene therapy has emerged as one of the most promising treatments addressing severe pathologies affecting the lungs. For instance, small interfering RNA (siRNA), enables the silencing of pathologic genes causing the specific disorders. A number of lung pathologies including COPD, asthma, tuberculosis, cystic fibrosis, viral infections, and neoplasms affecting the lung have been evaluated as potential candidates for siRNA therapy. Notwithstanding their potential, siRNA-based therapies present several challenges restricting their efficiency and safety for treatment of lung diseases. To surmount the impediments related to the delivery of therapeutic siRNA, NP-based systems have served as a preferred method to encapsulate and protect nucleic acids for delivery [23].

Polymeric NPs exhibit the potential for controlled drug release with highly targeted specificity and superior therapeutic indices related to the biocompatibility and biodegradability of the polymer. Specifically chitosan can function as a carrier in polyplex NPs for delivery of oligonucleotide drugs such as DNA, small interfering locked nucleic acid (siLNAs), pDNA, siRNA, and plasmid DNA. In addition to chitosan other polymers can serve as a suitable pulmonary carriers in NPs formulations. Polymers such as poly (lactide-co-glycolide) (PLGA) represents another class of biocompatible and biodegradable polymers investigated for efficient pulmonary drug delivery [43]. Furthermore, PEGylation of messenger RNA (mRNA) exhibits

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significant promise as therapeutic agent or vaccine for a number of lung pathologies. PEG12KL4 has also been introduced as a novel RNA delivery vector with a synthetic cationic KL4 peptide bonded to a uniform, linear PEG of 12-mers. The PEG12KL4 polymer can form nanosized complexes causing adequate transfection on the epithelial tissue of the human lung as illustrated in **Fig. 6**. The efficiency of transfection proved to be better than that of naked mRNA or lipoplexes with no evidence of toxic effects or inflammatory response observed for PEG12KL4/mRNA complexes following a single administration via the intratracheal rate. In general, PEG12KL4 can serve as an excellent agent for mRNA transfection via the pulmonary route [44]. In addition, cationic polymers are representative of a non-viral vector category for a multitude of NPs under development to serve as vehicles for efficient delivery of siRNA. As an alternative, Polyethylenimine (PEI) is among the most extensively investigated vectors for gene delivery owing to the readily modifiable amine-rich structure of this compound. The high incidence of positively charged amines enables PEI to engage in electrostatic interactions with nucleic acids forming polyplexes facilitating internalization of siRNA by the cells. However, incorporation of the nanosized polyplexes within a powder-based matrix of microparticles, with aerodynamic diameters in the 1–5 μm, is required. Following inhalation, the nano-embedded microparticles (NEMs) must be dissolved in the fluid lining the lung tissue so that the embedded polyplexes may be released for cellular uptake [23].

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Figure. 6. A549 cells were treated with naked mRNA, KL4/mRNA and PEG12KL4/mRNA prepared at 10:1 ratio (w/w) with cyanine-5 labeled mRNA. Cells were examined at 4 h post-transfection using flow cytometry. (A) representative histograms showing the population of cyanine-5 positive cells (red) compared to the untreated control (blue); (B) percentage of cells with mRNA uptake; and (C) median fluorescence intensity of the cells. (D) Confocal images of cells transfected with cyanine-5 (red) labelled mRNA with EGFP (green) expression; the nuclei (blue) were stained with Hoechst. Images were taken at 4 h and 24 h post-transfection. Scale bar = 20 μm. Reprinted with permission from Ref. [44]. Copyright (2019), Elsevier.

The complex structure of SARS-CoV-2 prevents the direct comparison of this virus with a polyplex for carrying genes. Nevertheless, recently VLPs that are capable of mimicking the viral membrane proteins have provided higher transfection for their cargos when compared to other polymeric NP options. VLPs are assembled using capsids or envelope protein derived from viruses and can carry a range of different drug and genes [10]. VLPs can be produced by the incorporation of membrane (M) protein and envelope (E) protein from SARS-CoV-2 in the bilayer membrane [45]. VLPs have applications in vaccine and sensor synthesis [9], as well as targeted RNA delivery to component of the innate immune system. For example, Novavax developed a VLP based vaccine with full length glycoprotein adjuvanted Matrix M1 (saponin based adjuvant). The S protein of SARS-CoV-2 in the VLP stimulates the production of anti-SARS antibody [8] with the VLPs also being able to be modified with hydrophobic polymers to increase stability, prolong circulation time, reduce non-specific adsorption and attenuate immune response [10].

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NPs targeting in pulmonary drug delivery

Pulmonary drug delivery methods employing NPs can be divided into active and passive categories. In the passive delivery methods, the hydrodynamic radius of the aerosol is critical, whereas in active delivery, the targeting of NPs toward specific cells within the lung is more significant. For example in tuberculosis, alveolar macrophages can be targeted using NPs functionalized with mannose ligands [3]. In lung cancer, pH-sensitive lipids are employed so that pH-dependent release may occur near the tumor. One of these lipids is the 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) which may be used to build nano-vesicles. DOPE is both an incorporating and a pH-sensitive lipid, i.e. it can be incorporated more readily in the plasma membrane of the tumor cell and lysosomes under acidic pH condition [46]. Laminin peptide (YIGSR) has been shown to have a high affinity for lung cells and may be used to target NPs toward lung tissue [47]. Numerous ligands e.g., lectins may be employed for the modification of NPs to increase their retention in nasal mucosa, thereby facilitating their absorption. Lectins are proteins and/or glycoproteins containing a minimum of one non-catalytic domain capable of binding to specific monosaccharides and/or oligosaccharides in a reversible manner. Notably, they are capable of specific binding to carbohydrate residues present on the cell surface initiating vesicular transport across the plasma membrane [6].

Designing VLPs with special ligands to provide site-specific drug delivery is possible, for example VLPs containing S protein targeting cells with the ACE2 receptor expressed in alveolar epithelial type II cells of the human lungs. In addition to attachment, ACE2 has the potential for gene regulation in viral process, lifecycle, assembly and genome replication [10]. However, the ACE2 receptor is also express highly on other tissues such as vascular endothelial, renal and

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cardiovascular tissue [48]. Hence, the ACE2 receptor use for targeting of VLPs can be used more widely than just the lungs.

Table 2. Specifies the diseases whose treatment using pulmonary drug delivery have been investigated.

Table 2. Diseases treated by pulmonary drug delivery

Diseases	Drug/NP complex	Effects	References
Pulmonary Arterial Hypertension (PAH)	Tacrolimus/Nanocomposite Microparticles (nCmP)	Provide targeted pulmonary delivery, improved solubility of tacrolimus, the potential of penetration through mucus barrier, and controlled drug release	[21]
Diarrhea	Loperamide/calcium carbonate particles modified with mucoadhesive biocompatible polymer, HA, poly-l-lysine as well as polysorbate 80	Bypassing the BBB	[49]
Asthma and COPD	Beclomethasone dipropionate/ PHEA-PEG2000-EDA-LA micelles	Protect drug from hydrolysis, controlled and prolonged release, enhanced mucosal penetration, more biocompatibility.	[50]
Pulmonary Arterial Hypertension (PAH)	Cinaciguat/ Chitosan	Adhesion to mucosa, controlled release by chitosan swelling, protection against esterase	[32]
Cystic Fibrosis	Ivacaftor/ PEGylated and Tat-decorated fluorescent NPs (FNPs)	Enhanced diffusion from pulmonary mucosal barrier and promoting ivacaftor lung cellular uptake	[51]

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Pseudomonas aeruginosa Infections in Cystic Fibrosis	Tobramycin/, nano into micro formulations (NiMs) made of PVA, NAC, Arg, Cyst and helping materials	Facilitate diffusion of drug through the mucus and sustained delivery, pronounced antibacterial activity compared with TOBIPodhaler	[52]
HIV	SiRNA against Beclin1 gene /PEI	Direct delivery of the PEI-siRNA nanocomplex to the CNS	[53]
Lung cancer	Paclitaxel/ DOPE and DPPC	No signs of interstitial pulmonary fibrosis, chronic inflammation and any other pulmonary toxicity. Improved fusogenicity and cytosolic drug release	[46]
Lung cancer	Doxorubicin/Nano-in-microparticles (NIMs) containing SPIONs and fluorescent nanospheres in a lactose matrix.	Better accumulation in lung using presence of magnetic field, maintain the therapeutic cytotoxicity of doxorubicin	[54]
Lung cancer	Doxorubicin/PEGylated poly(amidoamine) dendrimer	Delivery of DOX to deep lungs, the modulation of DOX interaction with respiratory epithelium, and temporal/spatial control of DOX release from the dendrimer conjugates.	[55]
Lung cancer	AuNPs functionalized with oligo(2-oxazoline)-based optically stable fluorescent coatings, and conjugated with a laminin peptide (YIGSR) in chitosan matrix	Enhanced cellular uptake, sustained and controlled release of the embedded NPs.	[47]

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Lung cancer	Docetaxel-loaded folic acidconjugated liposomes (LPs-DTX-FA)	Tracheal administration resulted in a 45-fold higher concentration of docetaxel in the lung of Sprague Dawley rats.	[56]
Lung cancer	Paclitaxel-loaded micro or nano transfersome	*In vitro* studies show safty in normal cells and toxicity in cancer cells, *in vivo* studies show good deposition in animal lungs.	[57]
Lung cancer	Methotrexate in gelatin NPs	4-fold reduction in methotrexate IC50. Good aerosolization properties enabling lung deposition in the respirable airways.	[58]
Metastatic lung cancer	Doxorubicin and cisplatin in methoxy poly(ethylene glycol)-poly(ethylenimine)-poly(L-glutamate) (mPEG-OEI-PLG) copolymers	Higher accumulation and anti-tumor activity in comparison to doxorubicin and cisplatin alone in cancerous mice.	[59]
Lung infection in cystic fibrosis	Ciprofloxacin-loaded PLGA NP	Enhanced antibacterial activity, NPs' colloidal stability in mucus.	[60]
TB	Rifampicin and pyrazinamide in maleate gellan gum-silk sericin chitosan nanocopmosite	Higher antimycobacterial activity and rapid delivery of drugs at TB infected macrophages.	[38]
TB	Rifampicin and ascorbic acid in sodium alginate coated with chitosan and Tween 80 nanoparticle	Increasing the local drug concentrations, reducing the risk of systemic toxicity and hence improving the patient compliance,	[39]
Respiratory infection	Curcumin nanoparticles	Significant reduction in the viability	[61]

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	embedded in a mannitol matrix forming microparticles accompanied by photothermal therapy	of *Staphylococcus saprophyticus* subsp. bovis and *Escherichia coli* DH5 alpha.

Toxicity of NPs for pulmonary drug delivery

Reports have indicated that NPs employed for pulmonary drug delivery may produce toxicity leading to side effects such as the inflammation of bronchi or interstitial fibrosis. Commonly, oxidative stress can be caused by inorganic NP such as carbon nanotubes (CNTs) and silica and diastolic and mitochondrial malfunction induced by TiO_2 NPs [62]. Although polymer and lipid-based NPs are safer, they may also result in similar side effects. NP parameters such as aspect ratio, degradability, and surface charge may also be associated with the generation of these adverse effects [46]. The toxicity of silver NPs (AgNPs) coated with tannic acid has been investigated. Specifically, AgNPs were added to a two-chamber co-culture system consisting of Calu-3 human bronchial epithelial cells, EAhy926 endothelial cells, as well as differentiated Thp-1 macrophage-like cells to monitor their movement and the resulting toxicity. Analytical techniques were employed to verify cellular uptake and transport of AgNPs across a model barrier. The given dosage levels were observed to produce moderate cytotoxicity along with diminished release of interleukin-6 (IL-6), interleukin-8 (IL-8), and tumor necrosis factor-alpha (TNF-α). The human airway model employed can serve as an alternative, *in vitro* technique to assess uptake, transport and systemic toxicity caused by aerosol contaminants incorporating nanoscale delivery systems [63]. Another investigation indicated that that silica NPs (SiO2-NPs) and mesoporous silica NPs (mSiO2-NPs) reduce the cell viability while increasing the rate of apoptosis of HPAEpiC alveolar epithelial cells in humans. Overall, mSiO2-NPs produced less

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toxicity than SiO2-NPs with the same therapeutic dose, confirming the potential of mSiO2-NPs for biomedical applications. Given the uptake of SiO2-NPs into the cells and their accumulation within the endoplasmic reticulum (ER), the observed pathological changes and subcellular organelle damage may be attributed to the ER stress response leading to NP-induced apoptosis [64]. Hence, consideration of the level of NPs toxicity produced by any drug delivery through the pulmonary pathway is an important design consideration..

Although the toxicity of organic NPs is lower than the inorganic NPs, it should be noticed that multiple doses of organic NPs and long residence time in the lung can lead to accumulation of NPs in the lung and a local inflammatory response. Approximately, 30% of ^{3}H-labled liposome and SLN pulmonary dosing remain in the bronchoalveolar lavage fluid after 7 days [65], as well as non-polymerized micelles which accumulate in the cell membrane an induce membrane permeability. The weak permeability of the membrane was assayed with lactate dehydrogenase (LDH) enzyme measurements. LDH is a cytosolic enzyme which is increased in the extracellular medium after membrane damage [66]. Cationic solid lipid nanoparticle (cSLN) as an effective nanocarrier especially for the genetic materials leads to agglomeration of inflammatory cells in the lung tissue [67]. The interaction of cationic dendrimers with cells results membrane disruption via nanohole formation, membrane thinning, and erosion followed by leakage of cytosolic enzymes and cell death. Hence, surface engineering of organic NPs and reconsideration of precursor materials can help to attenuate the toxicity of organic NPs [68].

In the designing of VLP based on SARS-CoV-2 virus, two consideration are needed:

The toxicity of basic nanoparticles used, and

The toxicity of the virus proteins in the VLP structure

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An example of the base NP toxicity is the metallic nanoparticles and quantum dots (QD) applying for the VLP synthesis should be assayed cytotoxically. The potential toxicity of QD's has limited their application in biological environments. The toxicity of QD's associated with release of heavy metal ions or ROS generation in vitro. However, in vivo studies show interaction of QD with blood component, accumulation in non-desired organ and generation of immune response [69].

BioNTech/Pfizer's and Moderna's mRNA vaccines both use lipid nanoparticles (LNP) as mRNA carriers [70]. The potential toxicity of LNP related to disruption of membrane structures, which may cause cytoplasm vacuolization, cell lysis and necrosis. Interference with gene expression may be an adverse effect of the cationic LNP. Charge reduction of cationic LNPs may attenuate its toxicity, however, reducing the nucleic acid encapsulation efficiency and transfection efficiency. Nevertheless, LNPs represent the largest portion of NP-mediated nucleic acid vaccine delivery and have found many preclinical uses in treating a very wide variety of diseases. [71].

In consideration of the toxicity of the virus proteins; the SARS-CoV-2 spike 1 (S1) protein can cross the blood brain barrier (BBB) and induce neuroinflammation and impaired CNS function [72]. The acute respiratory distress syndrome (ARDS) is the general inflammatory reaction in Covid-19 diseases. During the ARDS the activation of toll-like receptor (TLR), such as TLR4 via lipopolysaccharide (LPS) stimulation take place which induce other inflammatory responses. The interaction between the LPS and S protein of SARS-CoV-2 is the initiator of ARDS and is related to the level of LPS in the blood stream [73]. So, the S protein alone can induce the inflammatory response in body and thus the toxicity of virus protein in the VLP designation should be considered.

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Conclusion

Recent development in NP-based technology for drug delivery has favored new methods of drug administration. NP-based technology, however, has necessitated investigations of new drug formulations, optimizing physiochemical factors such as particle size, hydrophilicity/hydrophobicity, surface charge, and mucoadhesive properties. Successful optimization of the new formulations may render delivery via the pulmonary route more effective through better deposition of particles, avoidance of mucosal removal and alveolar macrophages, thereby enabling enhanced absorption by the tissue suffering from infection, and even specific targeting of diseased tissues. However, the physiochemical and structural investigation of SARS-CoV-2 virus helps to know the reasons of its success in high level of transfection and spread in the lung tissue and mimicking the characteristic of virus for design of drug delivery systems and vaccines. Despite the benefits of NP-based delivery via the pulmonary route, investigations of the degree of toxicity caused by the NPs constitutes a major concern. Specifically, in VLP design, toxicity related to NPs and virus proteins is vitally important.

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