Molecular Therapy
Nucleic Acids
Review

Aptamer-decorated nanocarriers for viral adsorption: A special look at COVID-19

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Viral infections are one of the leading causes of death in the world. One main challenge in fighting against these diseases is the unavailability of effective eradicating drugs and specific treatments. Nanocarriers and aptamer-decorated nanocarriers are designed to attach to many targets, including viral particles. By lowering the viral infectivity and attachment capability, they add therapeutic values even without containing antiviral drugs. Nevertheless, the nanoparticles (NPs) with encapsulated antiviral drugs can display extra therapeutic effects. Furthermore, it has been shown that aptamers can bind to viral particles and nanocarriers, presenting promising approaches for the identification of viruses and treatment of viral infections. Although there is no satisfying literature revealing the strong therapeutic potential of nanotechnology against COVID-19, the following information can provide new perspectives for upcoming investigations pertaining to developing effective aptamer-nanocarrier agents against COVID-19.

INTRODUCTION

Nanotechnology is an interesting tool for designing nanocarriers in drug delivery approaches against different diseases, such as viral infections. Nanocarriers are generally designed in a way that releases the drug in the specific site with high precision and high concentrations. Although employing modified or decorated nanocarriers in the treatment of viral diseases is very interesting and potentially effective, this issue has less been addressed. In fact, when a nanocarrier is designed to adsorb or bind to a viral particle, it can display a sort of potential therapeutic effect on its own without containing an antiviral drug. However, nanocarriers with loaded antiviral drugs can exhibit extra therapeutic effects. Nanocarriers as drug delivery systems have widely been used for the improvement of therapeutic effects due to their particle size and large surface area-to-volume ratio, which alters the medication's pharmacokinetics and effectiveness.

Aptamers can reciprocally bind to viral particles and nanocarriers, providing promising strategies for detecting viruses and combating viral infections. Designed aptamers have been able to directly bind to viruses, exhibiting antiviral effects that resulted in the inhibition of virus entry into host cells.[1] Aptamers can bind specifically to viral targets,[2] enhancing the targeting efficiency more than blank nanocarriers. Aptamers can also be easily chemically modified,[3] allowing them to recognize and bind to target viruses more efficiently compared to blank nanocarriers. In addition, the combination of aptamers with nanocarriers can lead to a synergistic effect, enhancing the therapeutic efficacy of the loaded drugs. Aptamer-nanocarrier conjugates can increase cellular internalization via receptor-mediated endocytosis, leading to higher intracellular drug concentrations at the target site.[4] Several attempts have been made to use unloaded nanocarriers to adsorb and scavenge viruses due to their high capacity in targeting and development. For this purpose, numerous studies have been conducted on chitosan, dendrimers, cyclodextrin nanoparticles (NPs), and so on, and interesting results have been obtained. At the same time, when an aptamer is attached to an NP containing an antiviral drug, improved therapeutic effects may be achieved.

Several mechanisms have been proposed for how aptamers can internalize from cell membranes, including phagocytosis, macropinocytosis, and endocytosis mediated by clathrin and caveolae.[5] The metabolism of aptamers in the systemic administration occurs through rapid renal filtration, digestion with nucleases, and distribution from the plasma into tissues (Figure 1).[6] It has been reported that the half-life of aptamers in the plasma is 7 min, owing to the rapid digestion of oligonucleotides by nucleases.[7] Plasma exonucleases degrade nucleic acid through phosphodiester bonds at the 3′ or 5′ terminal, resulting in the cleavage of nucleotides.[8] The average diameter of aptamers (with a mass of 6–30 kDa) is less than 5 nm.[9] To increase metabolic stability and slow elimination and renal filtration, aptamers are usually modified with a moiety like cholesterol,[6] liposomes,[10] and polyethylene glycol (PEG).[11] It was found that cholesterol-conjugated aptamers exhibited higher plasma stability, which increased aptamer exposure to the body through binding to plasma lipoproteins and facilitated uptaking by hepatocytes via a receptor-mediated endocytosis. Moreover, it was observed that the intravenous route was more suitable than the intraperitoneal route because the cholesterol-conjugated aptamers remained longer in the serum. It has been shown that cholesterol-conjugated aptamers inhibit hepatitis C virus (HCV) replication without provoking any immune reaction.[6] After internalization, aptamers accumulate in endosomes, lysosomes,

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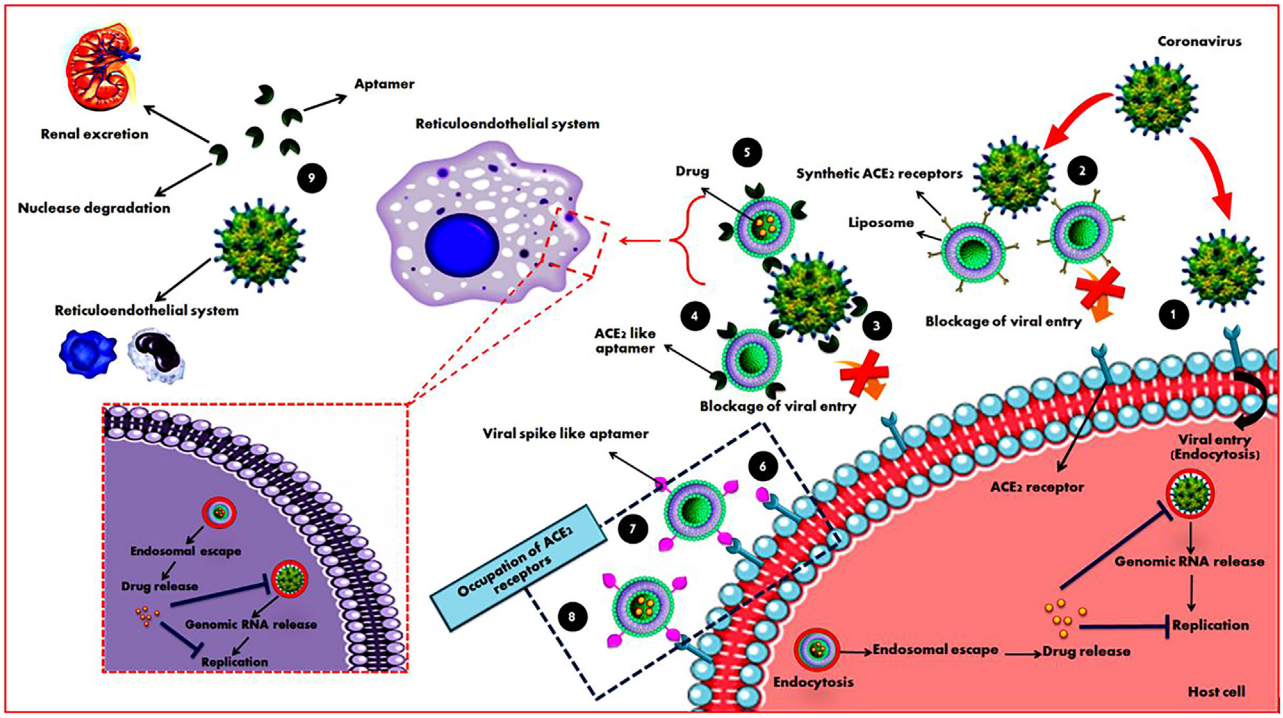

Figure 1. Schematic representation of COVID virus entry

Schematic representation of (1) viral entry, (2) decoy liposomes, (3) bindings of ACE2-like aptamer to virus, (4) bindings of liposomes decorated with ACE2-like aptamer to virus, (5) bindings of drug-loaded liposomes decorated with ACE2-like aptamer to virus, (6) bindings of viral spike-like aptamer to ACE2 receptor, (7) bindings of liposomes decorated with viral spike-like aptamer to ACE2 receptor, (8) bindings of drug-loaded liposomes decorated with viral spike-like aptamer to ACE2 receptor, and (9) aptamer and virus kinetics.

Golgi, mitochondria, and the endoplasmic reticulum, depending on their targets.[5]

In this review, we attempted to discuss and provide some strong evidence for aptamer-decorated nanocarriers. Because of the current COVID-19 pandemic, it seems that employing these approaches can have promising results and dealing with details can provide interesting insights. Compared with severe acute respiratory syndrome coronavirus (SARS-CoV), SARS-CoV-2 has more contagious capabilities and has rapidly spread worldwide. Since the beginning of the global problem of the COVID-19 disease, many efforts have been made to develop new preventive and therapeutic agents against COVID-19. Although a number of vaccines and drugs have become available in this field, more research is needed to find more effective agents.[12] Aptamers have previously been used to detect toxins[13,14] and also the SARS virus. For example, in 2009, there is a report showing that the SARS nucleocapsid is a suitable target for aptamer selection, which could be used in the exact direction of the virus. In this report, nucleocapsid proteins were detected at a concentration of 2 pg/mL with high sensitivity.[15] A complementary oligonucleotide aptasensor was designed as a probe for COVID-19 detection. The sensor displayed suitable selectivity against N protein in throat swabs and blood samples.[16] The two standard screening methods to confirm SARS-CoV-2 are the PCR method using viral RNA and the serology method using the detection of antibodies produced in the body due to viral infection, but due to the cost, time required, and complexity of the procedure, new technologies are needed. The use of aptamers can be very helpful in this field. Aptasensors can target different components of the virus, such as the spike (S) protein. Currently, aptamers fixed on NPs have been used. The aptamers are placed on the NPs with acceptable uniformity, and the incubation time with S proteins was 40 min. The LOD of this method is reported to be 66 pg/mL, which is lower than the LOD of the PCR method. The selectivity and sensitivity of the method also indicate the high quality of this technique.[17]

In addition to their identification, aptamers are explored as potential therapeutic agents for COVID-19. They have shown promise in blocking viral proteins and interfering with the SARS-CoV-2 infection process, making them candidates for treatment strategies against the virus. For example, an RNA aptamer with high affinity to the receptor-binding domain (RBD) has been designed, which, after binding, prevents the interaction of this part of the virus with the host receptor ACE2. In this study, aptamers are trimerized, so they can target more viruses with much lower concentrations. The results of this study showed that these aptamers can effectively inhibit viral infection. Moreover, these aptamers can be produced in large quantities and in a short time. This method is not complex and is reproducible.

Considering that these aptamers are resistant to nuclease degradation and have high stability in serum, they can be used for both treatment and diagnosis.[18]

Apart from identification and treatment, aptamers have the ability to be used in the control of complications related to COVID-19. In a study, an RNA aptamer was prepared in nanomolar and had high affinity to factor V (FV) and FVa, and therefore, they showed a strong anticoagulant effect in COVID-19. This anticoagulant effect is related to the role of the aptamer in the prevention of FV/FVa interactions with phospholipid membranes.[19]

BLANK VS. LOADED NANOCARRIERS WITH THERAPEUTIC EFFECTS

For a long time, nanocarriers have been used in the production of new formulations, and sometimes, these nanocarriers can show bioactive effects without containing any active ingredients. SPL7013 is a dendrimer that shows antiviral activity against HIV, human papillomavirus (HPV), and herpes simplex viruses (HSVs). The mechanism of the antiviral activity of SPL7013 is unknown, but it is likely to bind to envelope proteins of viruses, therefore blocking viral attachment to the host cell receptors and preventing the entry of viruses. Other antiviral mechanisms may include the interaction of SPL7013 with viral envelope proteins, which leads to envelope conformational changes that diminish the infectivity of the virus.[20]

The functionalization of dendrimers with DC-SIGN (dendritic cell-specific ICAM-3-grabbing nonintegrin), glycosphingolipids, and CD4 receptors can prevent HIV binding to host cells.[21] Employing T cell-mimicking NPs is another approach that can neutralize viral infectivity. It was observed that T cell membrane-coated Poly(lactic-co-glycolic acid) (PLGA) NPs (TNPs), NPs with HIV-binding T cell surface antigens such as CD4 receptor and CCR5 or CXCR4 as co-receptors, behaved as decoys and, consequently, inhibited the entry of virus into the T cells.[22] It has been reported that dendrimers with modified functional moieties can bind to the gp120 of HIV and the CD4 molecule of the host cell to prevent the attachment of HIV to host cells. PAMAM G4 (BRI2932 and BRI6195) suppresses the attachment of HIV to gp120. Dendrimers are able to prevent the replication of HIV by affecting the reverse transcription of the genetic materials. The attachment of the Tat protein to *trans*-acting responsive element (TAR) RNA of HIV is essential for the replication of HIV. It was found that PAMAM with a large number of NH_2 peripheral groups can interact with the phosphate groups of RNA through electrostatic forces, which hinders additional interactions between the TAR and Tat protein. Moreover, dendrimers are able to deliver antiviral drugs into the host cells, which further interfere with HIV activity.[23] Another study showed that sialyllactose-conjugated dendrimers displayed hemagglutination inhibition activity against influenza virus infections.[24] It was reported that CD4 molecules on the surface of exosomes (as nanodecoys) suppressed HIV-1 infection *in vitro*, preventing HIV-1 binding to $CD4^+$ T cells. $CD4^+$ T cell-originated exosomes contain the same membrane proteins, including CD4 and major histocompatibility complex (MHC) class I molecules.[25]

Considering these results, it seems that employing nanocarriers containing ACE2 synthetically derived receptors as nanodecoys is an effective strategy to bind and scavenge COVID-19 virus. These receptors conjugated on the NPs can bind irreversibly to the viral S, consequently altering the viral configuration and blocking receptor-mediated entry into the cell (Figure 1).

In this regard, poly(N-vinylcaprolactam) nanogels can exhibit antiviral activity against HIV-1 infection through blocking the viral replication in TZM-bl cells.[26] It was reported that the chemical modification of chitosan using glycidyl trimethylammonium chloride is potentially active against coronaviruses by inhibiting their replication (patent: EP2849763B1).[27] Synthetic virus-like particles (sVLPs) are another novel approach to provoking antiviral immune responses against coronavirus infection. Vaccination with sVLPs prepared in optimized mixtures containing viral proteins (S glycoprotein derived from an avian infectious bronchitis virus [IBV]) and gold NPs through spontaneous protein corona formation improved cellular and humoral immune responses against an avian model of coronavirus infection.[28]

Unloaded nanocarriers can not only be effective against microbes and microbial resistance but can also be used as carriers for antimicrobial agents (Figure 1). Blank nanocarriers can exhibit antiviral activity through several mechanisms, including disrupting viral envelopes,[29] binding to viral surface proteins and preventing the virus from attaching to and entering host cells,[30] interacting with viral proteins and forming reactive oxygen species (ROS),[31] and modulating the immune response to reduce inflammation and lung damage caused by COVID-19.[32]

It was observed that poly-β-cyclodextrins (pβCDs) are effective agents against *Mycobacterium tuberculosis*. Empty pβCD NPs disrupt cell surface lipid rafts and, consequently, inhibit bacterial uptake after pulmonary administration in mice. In fact, pβCD inhibits macrophage colonization by inducing cholesterol depletion in the plasma membrane. Cholesterol is needed for the entry of mycobacteria into macrophages. Furthermore, pβCD NPs induce apoptosis and subsequently deplete alveolar macrophages, which serve as a major reservoir for bacterial pathogens. On the other hand, pβCD NPs allow for the release of encapsulated anti-*Mycobacterium* drugs directly into the lung.[33] Therefore, both unloaded and loaded pβCD NPs have direct antibacterial effects and can be employed as drug nanocarriers against tuberculosis. Cyclodextrins were suggested to be active against coronavirus. Cholesterol increases the binding of SARS-CoV2 to the cell membrane and facilitating its entry into cells through endocytosis.[34] Li et al. reported that methyl-β-cyclodextrin (MβCD), by depleting cholesterol, affects the expression of ACE2 on the membrane of Vero E6 cells and inhibits the release of SARS-CoV from the host cells.[35]

Ribavirin, a nucleoside analog, was extensively used for the treatment of SARS-CoV infection. However, its medical application against SARS-CoV is limited due to considerable hemolysis in many

patients.[36] It has been reported that red blood cells (RBCs) are deficient in endocytic uptake mechanisms, the main pathway for NP uptake by cells.[37] Using NPs as a drug delivery system for ribavirin can improve its action and reduce hemolysis in patients.

A two-dimensional CuInP2S6 (copper indium thiophosphate [CIPS]) nanosheet can bind to eight of the eleven ACE2-binding amino acid residues on the SARS-CoV-2 S protein RBD and prevents the virus from attaching to and entering host cells. The binding between CIPS and the RBD is stronger than the affinity of the virus for ACE2. Moreover, CIPS is biodegradable and biocompatible, making it a promising agent for the elimination of SARS-CoV-2.[30]

Chitosan-based nanocarriers have been developed for the delivery of remdesivir, an antiviral drug used to treat human coronavirus HCoV-OC43. According to the obtained results, these nanocarriers can be a promising approach for remdesivir delivery due to their ability to modulate drug loading and release while having low cytotoxicity and strong antiviral activity.[38] Moreover, a novel biomimetic niosomal NP (BNN) was developed for controlled-release aspirin injections for COVID-19 and cardiovascular treatment. The formulation displayed significant suppression of platelet aggregation with minimal hemolysis and did not exhibit significant cytotoxicity on cells.[39]

Specific delivery of RNA interference (RNAi) using S aptamer-functionalized lipid NPs (LNPs) to target SARS-CoV-2 as a potential therapeutic strategy has been investigated in a clinical case study. A 36-year-old man with severe SARS-CoV-2 infection was treated with inhalation of 10 mg of Apt-LNPs-RNAi daily for 6 days. Clinical evaluations, including computed tomography (CT) scans and laboratory tests, displayed improvement in the patient's condition after treatment. This aptamer-functionalized LNP formulation can block SARS-CoV-2 virus from infecting cells by binding to the viral S protein.[40]

APTAMER AS AN EFFICIENT COATING SYSTEM AGAINST VIRUSES

Aptamers (also known as chemical antibodies) are single-stranded oligonucleotide (DNA or RNA) with high binding affinity and specificity to their targets, including proteins, peptides, and nucleic acids, via molecular conformation adaptation.[41,42] Nanocarriers should resist aggregation, be stable, and maintain the drug until it reaches the target site. It is very important to consider these issues because they can disrupt the efficiency of nanocarriers as drug delivery systems.[43] Aptamers can prevent their aggregation by changing the surface of nanocarriers.[44,45] In addition, factors such as size and surface charge can play important roles in the toxicity of nanocarriers; therefore, safety is a crucial issue for the use of nanocarriers.[46] Aptamers can reduce the toxicity of nanocarriers by targeting them to specific cells or tissues. This minimizes off-target effects and reduces adverse reactions.[47,48] Unmodified aptamers are vulnerable to degradation by nucleases, which significantly limits their half-life *in vivo*. The small size of aptamers can also lead to their rapid renal filtration, which limits their clinical application.[9] The stability of aptamers can be improved through conjugation with nanocarriers, which leads to an increase in their lifetime in the bloodstream. This approach facilitates their absorption by the target cells and increases their therapeutic potential.[49,50]

Aptamers possess several advantages over antibodies that make them attractive alternatives in various biomedical applications, including easy *in vitro* development without the need for complex cellular or animal experiments[51] and lower immunogenicity and high stability at room temperature.[52] Due to these unique features, aptamers can have an important place in the diagnosis and treatment of various infectious diseases and cancers in the future.

Aptamers are produced through systematic evolution of ligands by exponential enrichment (SELEX).[42] SELEX is a traditional way to generate selective aptamers for the virus. Modifications have been made to this method over time, including cell-SELEX, where the selection target is an inactivated virus particle.[53] Important advantages of aptamers are their ease of synthesis, good stability, no immunogenicity or toxicity, and flexible structure.[42] Consequently, they are considered as biomolecules with multiple functions, such as controlled drug release, diagnosis, targeting of drug delivery systems, and as biomarkers.[41] Peptide aptamers are small combinatorial proteins that consist of a short residue peptide (5–20 amino acids). Nucleotide aptamers bind more strongly since they provide efficacious surface contact with targets by using adaptive conformational changes.[54] Both types of aptamers contain a variable domain and an area for attaching to the specific target.[55]

In recent years, a large number of aptamers have been developed against human viral infections such as influenza virus, HCV, HIV-1, seasonal coronavirus (SCoV), HBV, HPV, and HSV (Figure 1).[56] Two specific aptamers (ApPABP7 and ApPABP11) were determined to recognize poly(A)-binding protein 1 (PABP1) and have anti-influenza capability. These aptamers decreased viral replication and production of new influenza virus.[57] The A12-16 aptamer is an RNA aptamer that binds to glycosylated hemagglutinin (HA) of influenza A virus and prevents influenza infection.[58] It was reported that aptamer-AuNPs can be used as antiviral agents or for imaging purposes. Aptamers conjugated to AuNPs displayed appropriate binding affinity for human influenza virus. These conjugated aptamers aggregated upon attachment to viral surface, and finally, a golden shell formed around the viral envelope, which enabled viral imaging.[59] Also, a small DNA aptamer was designed against the surface glycoprotein D (gD) of HSV type 1 (HSV-1). Glycoprotein gD is necessary for the entry and spread of HSV into host cells. The findings showed that the DNA aptamer exhibited high affinity for HSV-1 gD and prevented viral entry and replication *in vitro* and *in vivo*.[60] HIV Rev is a crucial regulatory protein that is expressed at the beginning of viral replication. It was observed that RNA aptamers attached to the arginine-rich helix of Rev and blocked Rev self-assembly, which resulted in the inhibition of HIV replication.[61] In parallel, the SCoV helicase is essential for viral replication, and anti-SCoV aptamers attracted much attention. This helicase contains an unwinding double-stranded nucleic acid as the functional group with ATPase activities. The nucleic acid unwinding activity

of the enzyme is repressed by aptamers, while the ATPase activity is slightly affected.[62,63]

Aptamer-based targeted drug delivery systems are another approach for improving the efficiency of therapeutic agents without damaging normal cells. It was observed that aptamer-conjugated doxorubicin encapsulated PLGA-poly(N-vinylpyrrolidone) (PVP) NPs enhanced the anticancer activity of doxorubicin via nucleolin receptor endocytosis and induced apoptosis in a mice model.[64] Aptamer A6-conjugated hybrid NPs (liposome/PLGA) were employed for the treatment of breast cancer. Aptamer A6 displays affinity to bind HER-2 receptors. According to the obtained results, the conjugation of NPs with aptamers enhanced the selective delivery of small interfering RNA (siRNA) into tumor cells, which led to increased knockdown of P-glycoprotein (P-gp). Furthermore, the aptamer-conjugated P-gp siRNA-loaded NPs enhanced the cellular internalization of doxorubicin in cancer cells.[65] Epithelial cell adhesion molecule (EpCAM) is overexpressed on the surface of different tumor cell lines compared to normal cells. EpCAM aptamer-conjugated PEG-PLGA NPs were designed for the targeted delivery of doxorubicin to breast cancer cell lines. The encapsulated drug-aptamer-decorated NPs showed efficient cellular uptake and exhibited significant cytotoxic activity on cancer cells compared to non-targeted NPs.[66]

An RNA aptamer (Sc5-c3) was designed against HPV-16 L1 VLPs. This aptamer showed the highest specificity and affinity for HPV-16 L1, which makes it a promising candidate for HPV diagnostics and potential antiviral applications.[67]

A specific determination of HBV was developed using an aptamer-based molecular imprinting polymer sensor with a ratiometric metal-organic framework (MOF). The sensor combines an aptamer that specifically binds to the HBV with a molecularly imprinted polymer based on a MOF carrier. The detection limit for HBV was low, indicating high sensitivity. The unique features of this method can provide a promising strategy for the detection of this virus.[68]

In an *in silico* study by Cleri et al., the interaction of two aptamers with the S protein was investigated. The affinity of these aptamers for binding to the S1 domain was also evaluated. The possibility was assayed that these aptamers may be able to modify S1 domain conformations in such a way that access to cell-specific receptors for the S protein is reduced. The results showed that DNA aptamers can be efficiently attached to the RBD of the S portion. On the other hand, by establishing a stable bond with the subdomains of adjacent promoters, they prevent the opening of the RBD to connect to the receptor.[16]

Nucleic acid aptamers can bind to their own target with high affinity and high selectivity. They have little batch-to-batch variability. Recently, the use of these aptamers in various platforms to detect SARS-CoV-2 virus has been considered. Functionalization of aptamers with components such as biotin fluorophore, biotin, nanomaterials have made them an attractive tool for this purpose. In the study of Gupta at al., DNA aptamer was examined for the diagnosis of SARS-CoV-2 by binding to the protein S virus. This aptamer demonstrated high efficiency in detecting the virus. When tested on samples from suspected COVID-19 patients, it exhibited significant specificity, yielding results comparable to those of RT-PCR in terms of effectiveness.[69]

Research in the field of vaccine production and medication for COVID-19 is ongoing. Many vaccines are now available for COVID-19. In addition to remdesivir, which has already been approved for COVID-19, two drugs from the Merck (molnupiravir) and Pfizer (paxlovid) attained emergency-use authorization for mild to moderate COVID-19. Moreover, monoclonal antibodies displayed a special position in the treatment of COVID-19. Drugs and monoclonal antibodies have their own limitations. Therapeutic antibodies are generally immunogenic. For this reason, the use of other treatment options, including aptamers, which are not immunogenic and can rapidly be prepared in large quantities, attracted more attention. Antiviral aptamers have also been used before the onset of the coronavirus disease, including the anti-influenza A22 aptamer.

Currently, there are many clinical studies about available drug repositioning to treat COVID-19. Moreover, repositioning has even been suggested for aptamers. In a study by Weisshoff et al., aptamer BC 007 has been evaluated for this purpose. These aptamers were previously designed to neutralize pathogenic autoantibodies in cardiovascular disease and are currently in phase 2 of clinical trial. Previous phases have shown that these aptamers have an appropriate profile safety. The above study showed that aptamer BC 007 has efficient binding to RNA polymerase and RBD-related S proteins, and therefore, it can counteract virus growth in *in vitro* conditions.[70] Yang et al. introduced DNA aptamers that can specifically detect SARS-CoV-2 in human serum and prevent infection by inhibiting the S protein's RBD. The S protein has two subunits, S1 and S2. S1 is an important site for host neutralizing antibodies and is also a target for vaccine design. This subunit interacts with ACE2 and the respiratory epithelial cells. In the above study, S1 subunits have been investigated to identify the appropriate aptamer, and among them, 6 aptamers were selected based on the SELEX method and entered the next phase for more evaluations. Affinity studies for RBD and ACE2 were performed on these aptamers, one of which was able to have excellent neutralization activity. This aptamer can be used in the treatment or prevention of COVID-19. This type of aptamer can be designed as fusion inhibitors, neutralizing oligonucleotides, and targets for drug delivery to fight against COVID-19.[71]

A serological aptamer-assisted proximity ligation assay was developed for the sensitive and specific detection of COVID-19-associated antigens in serum samples. This method enables the quantitative detection of serum nucleocapsid protein by converting the protein detection into a detectable qPCR signal within 2 h using a simple and homogeneous workflow.[72]

A rapid aptamer-based screening assay for the detection of COVID-19 variants was designed. This assay allows visual differentiation

between different types of SARS-CoV-2 based on the color change observed on the surface of the sensing cotton swab. This capability is very important to track the emergence and spread of new variants.[73]

A new DNA aptamer has been developed that specifically binds to the SARS-CoV-2 S protein and prevents its interaction with the human ACE2 receptor, therefore reducing the inflammatory response.[74]

The addition of aptamers to the surface of NPs can alter their size and the surface charge, which can impact their cellular uptake, the concentration of drugs in cells, biodistribution, metabolism, and excretion of drugs.[44,45] On the other hand, the small size of aptamers can lead to their rapid clearance through the kidneys, making their use *in vivo* a challenge. However, by improving the stability of aptamers through conjugation with nanocarriers, their lifetime in the bloodstream can be extended. This approach facilitates the uptake of aptamers by their target cells, as a result increasing their therapeutic potential.[49,50]

According to these results, it seems that NPs containing antiviral drugs decorated with aptamers can improve the activity of antiviral agents against coronavirus (Figure 1).

Conclusion

According to the above discussion, it seems that the use of nanocarriers as nanodecoys can be an effective approach to capture coronaviruses. Aptamers can directly attach to viruses and exhibit antiviral effects by inhibiting virus entry into host cells. In addition, aptamer-conjugated nanocarriers can improve the pharmacokinetics of designed free aptamers and the antiviral effect and safety of drugs against coronavirus.

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AUTHOR CONTRIBUTIONS

All authors wrote, read, and approved the final manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES

1. Singh, L., Kruger, H.G., Maguire, G.E.M., Govender, T., and Parboosing, R. (2017). The role of nanotechnology in the treatment of viral infections. Ther. Adv. Infect. Dis. *4*, 105–131.
2. Yang, L.F., Ling, M., Kacherovsky, N., and Pun, S.H. (2023). Aptamers 101: aptamer discovery and in vitro applications in biosensors and separations. Chem. Sci. *14*, 4961–4978.
3. Chandola, C., Kalme, S., Casteleijn, M.G., Urtti, A., and Neerathilingam, M. (2016). Application of aptamers in diagnostics, drug-delivery and imaging. J. Biosci. *41*, 535–561.
4. Szymanowski, W., Szymanowska, A., Bielawska, A., Lopez-Berestein, G., Rodriguez-Aguayo, C., and Amero, P. (2023). Aptamers as potential therapeutic tools for ovarian cancer: Advancements and challenges. Cancers *15*, 5300.
5. Yoon, S., and Rossi, J.J. (2018). Aptamers: Uptake mechanisms and intracellular applications. Adv. Drug Deliv. Rev. *134*, 22–35.
6. Lee, C.H., Lee, S.-H., Kim, J.H., Noh, Y.-H., Noh, G.-J., and Lee, S.-W. (2015). Pharmacokinetics of a cholesterol-conjugated aptamer against the hepatitis C virus (HCV) NS5B protein. Mol. Ther. Nucleic Acids *4*, e254.
7. Zavyalova, E., Samoylenkova, N., Revishchin, A., Turashev, A., Gordeychuk, I., Golovin, A., Kopylov, A., and Pavlova, G. (2017). The evaluation of pharmacodynamics and pharmacokinetics of anti-thrombin DNA aptamer RA-36. Front. Pharmacol. *8*, 922.
8. Odeh, F., Nsairat, H., Alshaer, W., Ismail, M.A., Esawi, E., Qaqish, B., Bawab, A.A., and Ismail, S.I. (2019). Aptamers chemistry: Chemical modifications and conjugation strategies. Molecules *25*, 3.
9. Zhou, J., and Rossi, J. (2017). Aptamers as targeted therapeutics: current potential and challenges. Nat. Rev. Drug Discov. *16*, 181–202.
10. Willis, M.C., Collins, B.D., Zhang, T., Green, L.S., Sebesta, D.P., Bell, C., Kellogg, E., Gill, S.C., Magallanez, A., Knauer, S., et al. (1998). Liposome-anchored vascular endothelial growth factor aptamers. Bioconjug. Chem. *9*, 573–582.
11. Yang, Y., Zhu, W., Cheng, L., Cai, R., Yi, X., He, J., Pan, X., Yang, L., Yang, K., Liu, Z., et al. (2020). Tumor microenvironment (TME)-activatable circular aptamer-PEG as an effective hierarchical-targeting molecular medicine for photodynamic therapy. Biomaterials *246*, 119971.
12. Sivasankarapillai, V.S., Pillai, A.M., Rahdar, A., Sobha, A.P., Das, S.S., Mitropoulos, A.C., Mokarrar, M.H., and Kyzas, G.Z. (2020). On Facing the SARS-CoV-2 (COVID-19) with Combination of Nanomaterials and Medicine: Possible Strategies and First Challenges. Nanomaterials *10*, 852.
13. Jafari, M., Rezaei, M., Kalantari, H., Tabarzad, M., and Daraei, B. (2018). DNAzyme–aptamer or aptamer–DNAzyme paradigm: Biochemical approach for aflatoxin analysis. Biotechnol. Appl. Biochem. *65*, 274–280.
14. Jafari, M., Rezaei, M., Kalantari, H., Tabarzad, M., and Daraei, B. (2017). Optimization of aflatoxin B1 aptasensing. J. Toxicol. *2017*, 2461354.
15. Ahn, D.-G., Jeon, I.-J., Kim, J.D., Song, M.-S., Han, S.-R., Lee, S.-W., Jung, H., and Oh, J.-W. (2009). RNA aptamer-based sensitive detection of SARS coronavirus nucleocapsid protein. Analyst *134*, 1896–1901.
16. Yu, M., Zhang, X., Zhang, X., Zahra, Q.U.A., Huang, Z., Chen, Y., Song, C., Song, M., Jiang, H., Luo, Z., and Lu, Y. (2022). An electrochemical aptasensor with N protein binding aptamer-complementary oligonucleotides as probe for ultra-sensitive detection of COVID-19. Biosens. Bioelectron. *213*, 114436.
17. Abrego-Martinez, J.C., Jafari, M., Chergui, S., Pavel, C., Che, D., and Siaj, M. (2022). Aptamer-based electrochemical biosensor for rapid detection of SARS-CoV-2: Nanoscale electrode-aptamer-SARS-CoV-2 imaging by photo-induced force microscopy. Biosens. Bioelectron. *195*, 113595.
18. Valero, J., Civit, L., Dupont, D.M., Selnihhin, D., Reinert, L.S., Idorn, M., Israels, B.A., Bednarz, A.M., Bus, C., Asbach, B., et al. (2021). A serum-stable RNA aptamer specific for SARS-CoV-2 neutralizes viral entry. Proc. Natl. Acad. Sci. USA *118*, e2112942118.
19. Soule, E.E., Yu, H., Olson, L., Naqvi, I., Kumar, S., Krishnaswamy, S., and Sullenger, B.A. (2022). Generation of an anticoagulant aptamer that targets factor V/Va and disrupts the FVa-membrane interaction in normal and COVID-19 patient samples. Cell Chem. Biol. *29*, 215–225.e5.
20. Telwatte, S., Moore, K., Johnson, A., Tyssen, D., Sterjovski, J., Aldunate, M., Gorry, P.R., Ramsland, P.A., Lewis, G.R., Paull, J.R.A., et al. (2011). Virucidal activity of the dendrimer microbicide SPL7013 against HIV-1. Antiviral Res. *90*, 195–199.
21. Moura, L.I.F., Malfanti, A., Peres, C., Matos, A.I., Guegain, E., Sainz, V., Zloh, M., Vicent, M.J., and Florindo, H.F. (2019). Functionalized branched polymers: promising immunomodulatory tools for the treatment of cancer and immune disorders. Mater. Horiz. *6*, 1956–1973.
22. Wei, X., Zhang, G., Ran, D., Krishnan, N., Fang, R.H., Gao, W., Spector, S.A., and Zhang, L. (2018). T-Cell-Mimicking Nanoparticles Can Neutralize HIV Infectivity. Adv. Mater. *30*, 1802233.
23. Peng, J., Wu, Z., Qi, X., Chen, Y., and Li, X. (2013). Dendrimers as potential therapeutic tools in HIV inhibition. Molecules *18*, 7912–7929.

24. Günther, S.C., Maier, J.D., Vetter, J., Podvalnyy, N., Khanzhin, N., Hennet, T., and Stertz, S. (2020). Antiviral potential of 3′-sialyllactose-and 6′-sialyllactose-conjugated dendritic polymers against human and avian influenza viruses. Sci. Rep. *10*, 1–9.

25. de Carvalho, J.V., de Castro, R.O., da Silva, E.Z.M., Silveira, P.P., da Silva-Januário, M.E., Arruda, E., Jamur, M.C., Oliver, C., Aguiar, R.S., and daSilva, L.L.P. (2014). Nef neutralizes the ability of exosomes from CD4+ T cells to act as decoys during HIV-1 infection. PLoS One *9*, e113691.

26. Macchione, M.A., Guerrero-Beltrán, C., Rosso, A.P., Euti, E.M., Martinelli, M., Strumia, M.C., and Muñoz-Fernández, M.Á. (2019). poly (N-vinylcaprolactam) Nanogels with Antiviral Behavior against HIV-1 Infection. Sci. Rep. *9*, 1–10.

27. Pyrc, K., Milewska, A., Nowakowska, M., Szczubialka, K., and Kaminski, K. (2015). The use of chitosan polymer in the treatment and prevention of infections caused by coronaviruses. Google Patents.

28. Chen, H.-W., Huang, C.-Y., Lin, S.-Y., Fang, Z.-S., Hsu, C.-H., Lin, J.-C., Chen, Y.-I., Yao, B.-Y., and Hu, C.-M.J. (2016). Synthetic virus-like particles prepared via protein corona formation enable effective vaccination in an avian model of coronavirus infection. Biomaterials *106*, 111–118.

29. Xu, C., Lei, C., Hosseinpour, S., Ivanovski, S., Walsh, L.J., and Khademhosseini, A. (2022). Nanotechnology for the management of COVID-19 during the pandemic and in the post-pandemic era. Natl. Sci. Rev. *9*, nwac124.

30. Zhang, G., Cong, Y., Liu, F.-L., Sun, J., Zhang, J., Cao, G., Zhou, L., Yang, W., Song, Q., Wang, F., et al. (2022). A nanomaterial targeting the spike protein captures SARS-CoV-2 variants and promotes viral elimination. Nat. Nanotechnol. *17*, 993–1003.

31. Fox, C.R., Kedarinath, K., Neal, C.J., Sheiber, J., Kolanthai, E., Kumar, U., Drake, C., Seal, S., and Parks, G.D. (2023). Broad-spectrum, potent, and durable ceria nanoparticles inactivate RNA virus infectivity by targeting virion surfaces and disrupting virus–receptor interactions. Molecules *28*, 5190.

32. Allawadhi, P., Singh, V., Khurana, A., Khurana, I., Allwadhi, S., Kumar, P., Banothu, A.K., Thalugula, S., Barani, P.J., Naik, R.R., and Bharani, K.K. (2021). Silver nanoparticle based multifunctional approach for combating COVID-19. Sens. Int. *2*, 100101.

33. Machelart, A., Salzano, G., Li, X., Demars, A., Debrie, A.-S., Menendez-Miranda, M., Pancani, E., Jouny, S., Hoffmann, E., Deboosere, N., et al. (2019). Intrinsic antibacterial activity of nanoparticles made of β-cyclodextrins potentiates their effect as drug nanocarriers against tuberculosis. ACS Nano *13*, 3992–4007.

34. Wang, H., Yuan, Z., Pavel, M.A., and Hansen, S.B. (2020). Cholesterol and COVID19 lethality in elderly. Preprint at bioRxiv. https://doi.org/10.1016/j.jbc.2023.104763.

35. Li, G.-M., Li, Y.-G., Yamate, M., Li, S.-M., and Ikuta, K. (2007). Lipid rafts play an important role in the early stage of severe acute respiratory syndrome-coronavirus life cycle. Microbes Infect. *9*, 96–102.

36. Hui, D.S.C., and Zumla, A. (2019). Severe Acute Respiratory Syndrome: Historical, Epidemiologic, and Clinical Features. Infect. Dis. Clin. North Am. *33*, 869–889.

37. Barkur, S., Lukose, J., and Chidangil, S. (2020). Probing Nanoparticle–Cell Interaction Using Micro-Raman Spectroscopy: Silver and Gold Nanoparticle-Induced Stress Effects on Optically Trapped Live Red Blood Cells. ACS Omega *5*, 1439–1447.

38. Milkova, V., Kamburova, K., Martinov, P., Vilhelmova-Ilieva, N., and Rashev, V. (2023). Chitosan-based nanocarriers for delivery of remdesivir. Sci. Pharm. *91*, 37.

39. Rajeev, J., Kamalasanan, K., Sapa, H., Sabitha, M., and Abhi, C. (2023). Controlled release nanomedicine (CRNM) of aspirin using "biomimetic niosomal nanoparticles (BNNs)" for Covid-19 and cardiovascular treatment: DOE based optimization. OpenNano *9*, 100119.

40. Saify Nabiabad, H., Amini, M., and Demirdas, S. (2022). Specific delivering of RNAi using Spike's aptamer-functionalized lipid nanoparticles for targeting SARS-CoV-2: A strong anti-Covid drug in a clinical case study. Chem. Biol. Drug Des. *99*, 233–246.

41. Wang, T., Chen, W.-X., Xu, G.-F., Zhang, B., Wei, S.-Q., Hu, J., and Zhang, K.-H. (2019). A universal strategy designed for selecting bench-to-bedside aptamers to serum and validated in hepatocellular carcinoma diagnosis. Microchem. J. *150*, 104152.

42. Zhang, G.-X., Liu, Y.-L., Yang, M., Huang, W.-S., and Xu, J.-H. (2020). An aptamer-based, fluorescent and radionuclide dual-modality probe. Biochimie *171–172*, 55–62. https://doi.org/10.1016/j.biochi.2020.02.007.

43. Alshawwa, S.Z., Kassem, A.A., Farid, R.M., Mostafa, S.K., and Labib, G.S. (2022). Nanocarrier drug delivery systems: characterization, limitations, future perspectives and implementation of artificial intelligence. Pharmaceutics *14*, 883.

44. Liu, M., Wang, L., Lo, Y., Shiu, S.C.-C., Kinghorn, A.B., and Tanner, J.A. (2022). Aptamer-enabled nanomaterials for therapeutics, drug targeting and imaging. Cells *11*, 159.

45. Kovacevic, K.D., Gilbert, J.C., and Jilma, B. (2018). Pharmacokinetics, pharmacodynamics and safety of aptamers. Adv. Drug Deliv. Rev. *134*, 36–50.

46. Xuan, L., Ju, Z., Skonieczna, M., Zhou, P.K., and Huang, R. (2023). Nanoparticles-induced potential toxicity on human health: Applications, toxicity mechanisms, and evaluation models. MedComm *4*, e327.

47. He, S., Du, Y., Tao, H., and Duan, H. (2023). Advances in aptamer-mediated targeted delivery system for cancer treatment. Int. J. Biol. Macromol. *238*, 124173.

48. Deng, G., Zha, H., Luo, H., and Zhou, Y. (2023). Aptamer-conjugated gold nanoparticles and their diagnostic and therapeutic roles in cancer. Front. Bioeng. Biotechnol. *11*, 1118546.

49. Mahmoudi, A., Alavizadeh, S.H., Hosseini, S.A., Meidany, P., Doagooyan, M., Abolhasani, Y., Saadat, Z., Amani, F., Kesharwani, P., Gheybi, F., and Sahebkar, A. (2023). Harnessing aptamers against COVID-19: A therapeutic strategy. Drug Discov. Today *28*, 103663.

50. Dzuvor, C.K.O., Tettey, E.L., and Danquah, M.K. (2022). Aptamers as promising nanotheranostic tools in the COVID-19 pandemic era. Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol. *14*, e1785.

51. Ning, Y., Hu, J., and Lu, F. (2020). Aptamers used for biosensors and targeted therapy. Biomed. Pharmacother. *132*, 110902.

52. Dhar, P., Samarasinghe, R.M., and Shigdar, S. (2020). Antibodies, nanobodies, or aptamers—which is best for deciphering the proteomes of non-model species? Int. J. Mol. Sci. *21*, 2485.

53. Liu, Q., Zhang, W., Chen, S., Zhuang, Z., Zhang, Y., Jiang, L., and Lin, J.S. (2020). SELEX tool: a novel and convenient gel-based diffusion method for monitoring of aptamer-target binding. J. Biol. Eng. *14*, 1–13.

54. Reverdatto, S., Burz, D.S., and Shekhtman, A. (2015). Peptide aptamers: development and applications. Curr. Top. Med. Chem. *15*, 1082–1101.

55. Jo, H., and Ban, C. (2016). Aptamer–nanoparticle complexes as powerful diagnostic and therapeutic tools. Exp. Mol. Med. *48*, e230.

56. Shum, K.-T., Zhou, J., and Rossi, J.J. (2013). Aptamer-based therapeutics: new approaches to combat human viral diseases. Pharmaceuticals *6*, 1507–1542.

57. Rodriguez, P., Pérez-Morgado, M.I., Gonzalez, V.M., Martín, M.E., and Nieto, A. (2016). Inhibition of influenza virus replication by DNA aptamers targeting a cellular component of translation initiation. Mol. Ther. Nucleic Acids *5*, e308.

58. Pan, Q., Luo, F., Liu, M., and Zhang, X.-L. (2018). Oligonucleotide aptamers: promising and powerful diagnostic and therapeutic tools for infectious diseases. J. Infect. *77*, 83–98. https://doi.org/10.1016/j.jinf.2018.04.007.

59. Wang, C.C., Wu, S.M., Li, H.W., and Chang, H.T. (2016). Biomedical Applications of DNA-Conjugated Gold Nanoparticles. Chembiochem *17*, 1052–1062.

60. Yadavalli, T., Agelidis, A., Jaishankar, D., Mangano, K., Thakkar, N., Penmetcha, K., and Shukla, D. (2017). Targeting herpes simplex virus-1 gD by a DNA aptamer can be an effective new strategy to curb viral infection. Mol. Ther. Nucleic Acids *9*, 365–378.

61. Dearborn, A.D., Eren, E., Watts, N.R., Palmer, I.W., Kaufman, J.D., Steven, A.C., and Wingfield, P.T. (2018). Structure of an RNA aptamer that can inhibit HIV-1 by blocking Rev-cognate RNA (RRE) binding and Rev-Rev association. Structure *26*, 1187–1195.e4.

62. Jang, K.J., Lee, N.-R., Yeo, W.-S., Jeong, Y.-J., and Kim, D.-E. (2008). Isolation of inhibitory RNA aptamers against severe acute respiratory syndrome (SARS) coronavirus NTPase/Helicase. Biochem. Biophys. Res. Commun. *366*, 738–744.

63. Shum, K.T., and Tanner, J.A. (2008). Differential inhibitory activities and stabilisation of DNA aptamers against the SARS coronavirus helicase. Chembiochem *9*, 3037–3045.

64. Saravanakumar, K., Hu, X., Shanmugam, S., Chelliah, R., Sekar, P., Oh, D.-H., Vijayakumar, S., Kathiresan, K., and Wang, M.-H. (2019). Enhanced cancer therapy

with pH-dependent and aptamer functionalized doxorubicin loaded polymeric (poly D, L-lactic-co-glycolic acid) nanoparticles. Arch. Biochem. Biophys. *671*, 143–151.

65. Powell, D., Chandra, S., Dodson, K., Shaheen, F., Wiltz, K., Ireland, S., Syed, M., Dash, S., Wiese, T., Mandal, T., and Kundu, A. (2017). Aptamer-functionalized hybrid nanoparticle for the treatment of breast cancer. Eur. J. Pharm. Biopharm. *114*, 108–118.
66. Alibolandi, M., Ramezani, M., Sadeghi, F., Abnous, K., and Hadizadeh, F. (2015). Epithelial cell adhesion molecule aptamer conjugated PEG–PLGA nanopolymersomes for targeted delivery of doxorubicin to human breast adenocarcinoma cell line in vitro. Int. J. Pharm. *479*, 241–251.
67. Leija-Montoya, A.G., Benítez-Hess, M.L., Toscano-Garibay, J.D., and Alvarez-Salas, L.M. (2014). Characterization of an RNA aptamer against HPV-16 L1 virus-like particles. Nucleic Acid Ther. *24*, 344–355.
68. Wang, L., Yang, J., Tang, L., Luo, L., Chen, C., Gong, H., and Cai, C. (2021). Specific determination of HBV using a viral aptamer molecular imprinting polymer sensor based on ratiometric metal organic framework. Mikrochim. Acta *188*, 221.
69. Gupta, A., Anand, A., Jain, N., Goswami, S., Anantharaj, A., Patil, S., Singh, R., Kumar, A., Shrivastava, T., Bhatnagar, S., et al. (2021). A novel G-quadruplex aptamer-based spike trimeric antigen test for the detection of SARS-CoV-2. Mol. Ther. Nucleic Acids *26*, 321–332.
70. Weisshoff, H., Krylova, O., Nikolenko, H., Düngen, H.-D., Dallmann, A., Becker, S., Göttel, P., Müller, J., and Haberland, A. (2020). Aptamer BC 007-Efficient binder of spreading-crucial SARS-CoV-2 proteins. Heliyon *6*, e05421.
71. Yang, G., Li, Z., Mohammed, I., Zhao, L., Wei, W., Xiao, H., Guo, W., Zhao, Y., Qu, F., and Huang, Y. (2021). Identification of SARS-CoV-2-against aptamer with high neutralization activity by blocking the RBD domain of spike protein 1. Signal Transduct. Target. Ther. *6*, 227.
72. Liu, R., He, L., Hu, Y., Luo, Z., and Zhang, J. (2020). A serological aptamer-assisted proximity ligation assay for COVID-19 diagnosis and seeking neutralizing aptamers. Chem. Sci. *11*, 12157–12164.
73. Aloraij, Y.M., Suaifan, G.A.R.Y., Shibl, A., Al-Kattan, K., and Zourob, M.M. (2023). Development of Rapid Aptamer-Based Screening Assay for the Detection of Covid-19 Variants. ACS Omega *8*, 32877–32883.
74. Kim, W., Song, E.S., Lee, S.H., Yang, S.H., Cho, J., and Kim, S.-J. (2024). A new DNA aptamer which binds to SARS-CoV-2 spike protein and reduces pro-inflammatory response. Sci. Rep. *14*, 7516.